

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/383864397>

# Medicinal Phytochemicals: Exploring Nature's Pharmacy Editors

Chapter · September 2024

---

CITATIONS

0

READS

810

5 authors, including:



Subhashini Ramakrishnan

Dr G R Damodaran college of Science

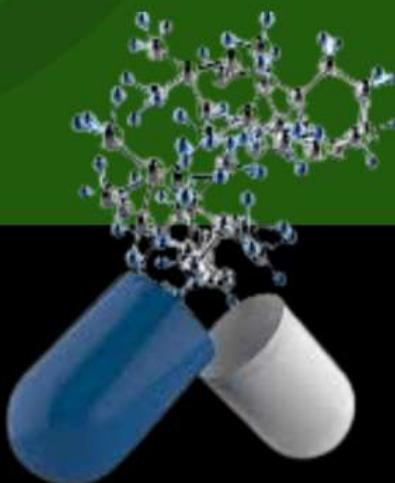
19 PUBLICATIONS 99 CITATIONS

[SEE PROFILE](#)

# MEDICINAL PHYTOCHEMICALS: EXPLORING NATURE'S PHARMACY



**Dr. M.N.Kathiravan**  
**Dr. M.Shanmugavadi**  
**Dr. P.Chidambara Rajan**  
**Dr. P. Arun**  
**Ms. K.Thilagavathi**



# **Medicinal Phytochemicals: Exploring Nature's Pharmacy**

## **Editors**

**Dr. M.N. Kathiravan.**

**Dr. M. Shanmugavadi.**

**Dr. P. Chidambaram Rajan.**

**Dr. P.Arun.**

**Ms. Thilagavathi Kumaran.**

*Professor(s)*

*Department of Biotechnology,*

*Dr. N.G.P. Arts and Science College,*

*Coimbatore.*

# **Medicinal Phytochemicals: Exploring Nature's Pharmacy**

**© Dr. M.N. Kathiravan.**

**Dr. M. Shanmugavadi.**

**Dr. P. Chidambaram Rajan.**

**Dr. P.Arun.**

**Ms. Thilagavathi Kumaran.**

**First Edition : September 2024**

**Size : 1/8 demy**

**Pages : 401**

**Prices : Rs.750**

**ISBN : 978-93-95137-80-5**

**Published By : INAM Pathipagam**

Coimbatore, Tamil Nadu, India

9600370671

**Printed by : Real Impact solution,**

Chennai - 600004

# Preface

In recent years, the field of ethnopharmacology and the study of medicinal plants have witnessed an unprecedented surge in interest and innovation. This edited volume brings together a collection of chapters that explore the intricate relationship between traditional knowledge and modern scientific research, focusing on the therapeutic potential of phytochemicals. Our objective is to bridge the gap between Indian Knowledge System (IKS) and modern medical practices, providing a comprehensive overview of the recent developments and opportunities in the field of medicinal phytochemicals.

This book explores into various aspects of ethnopharmacology, offering insights into the traditional uses of medicinal plants and their relevance in today's medical landscape. The chapters present an array of research findings on bioactive phytochemicals, highlighting recent developments in their extraction, isolation, and characterization. Readers will find an in-depth analysis of the pharmacognosy and phytochemistry of flavonoids, as well as the mechanisms of action of phytochemicals in disease prevention and treatment. The therapeutic potential of phytochemicals in combating diseases such as cancer and tuberculosis is examined, emphasizing their role in prevention and management. Additionally, this book explores the global markets and commercial opportunities in medicinal phytochemicals, providing a comprehensive view of the economic potential within this burgeoning field.

From the vegetation computation of specific regions to the screening of marine seaweeds for antimicrobial properties, this volume showcases diverse research methodologies and

innovative approaches in the study of phytochemicals. The formulation and evaluation of herbal products, such as toothpaste and other medicinal applications, are discussed, offering practical insights into the application of traditional knowledge in modern product development.

Furthermore, cutting-edge topics such as nano herbal medicines, AI, and machine learning in plant-derived medicine design are explored, signifying the future direction of research and development in this field. The integration of computational techniques, such as docking and ADMET analysis, is highlighted, underscoring the potential for discovering new therapeutic agents from Indian medicinal plants.

We hope that this volume will serve as a valuable resource for researchers, practitioners, and students interested in the field of ethnopharmacology and medicinal plants. By fostering a deeper understanding of the potential of phytochemicals, we aim to inspire further research and innovation, ultimately contributing to the advancement of global healthcare.

## Acknowledgments

We extend our sincere gratitude to all the contributors for their valuable insights and dedication to this work. We express our special thanks to our reviewers for their constructive comments and feedback, and to the publishing team for their unwavering support in bringing this volume to completion.

## Editors

## Editors



**Dr. M.N. Kathiravan.** M.Sc., M.Phil., Ph.D., PDF (South Korea), DMLT, PGDBI, is a professor in the Department of Biotechnology at Dr. N.G.P Arts and Science College, Coimbatore. His groundbreaking research interests span several domains of life sciences, including, drug design Green Energy and role of AI, ML, ANN & IOT in Agriculture Biotechnology, as well as innovative teaching and learning pedagogy. As a prolific researcher, he published over 60 research articles in SCI & SCIE indexed peer reviewed Journals with total impact factor of 120 and H-index 13. He authored 10 books, 15 book chapters, 5 lab manuals and 15 Scopus indexed conference proceedings. He also received 2 research funds and seed funds from various funding agencies. Dr. Kathiravan received many prestigious awards including "India top 100 Professors-2022" from India Prime Awards, New Delhi., "Education Leadership Award-2022" from Hindustan Times, New Delhi., "Rotary Club Award" from Rotary Club of Coimbatore Meridian RI Dist-320, Coimbatore, NPTEL Motivated Learner-2022 and NPTEL Belivers-2022 and NPTEL Enthusiasts Star-2024 awards from IIT, Madras. Dr. Kathiravan is an inspiring academician and visionary, recognized globally for his research, teaching, and leadership, motivating excellence.





**Dr. M. Shanmugavadi**, M.Sc., M.Phil., Ph.D., PGDCRDM., SET, is currently serves as Professor, Department of Biotechnology at Dr. N.G.P. Arts and Science College, Coimbatore, Tamil Nadu. Boasting a strong academic background and 16 years of experience in both teaching and research, she demonstrates exceptional proficiency in several domains including environmental waste management, molecular biology, nanotechnology, and contemporary pedagogy. Thus far, Dr. Shanmugavadi has authored 7 comprehensive books, 10 book chapters, 25 conference proceedings, and 35 research publications featured in esteemed, peer-reviewed international journals. As a respected scholar and academician, she has delivered presentations on her work at various prestigious national and international conferences. Furthermore, over 35 PG students have greatly benefitted from her erudite supervision. In addition to these responsibilities, she is diligently oversees the coordination of the Intellectual Property Rights (IPR) cell under the support of the Institution's Innovation Council (IIC).





**Dr. P. Chidambaram Rajan**, M.Sc., M.Sc (IT),, M.Phil., Ph.D., PGDBI, is currently working as Professor and Head, Department of Biotechnology, Dr. N.G.P Arts and Science College, Coimbatore, Tamil Nadu. He received his Doctoral Degree from Bharathiar University, Coimbatore. He has around 23 years of teaching and research experience. His specialized areas of research are Bioprospecting, Biomaterials, Cancer Biology and Smart Agriculture. He has published over 40 research articles in Scopus indexed & peer reviewed international journals and authored many books, book chapters and conference proceedings. He has filed a patent in the field of *in-silico* based diseases analysis. Dr. Rajan guided 4 Ph.D, 10 M.Phil research scholars more than 100 post graduate students. He also received a Summer Research Fellow (SRF-2019) award from Indian Science Academy, New Delhi. Demonstrating yet another facet of his commitment to research and innovation, Dr. Rajan has been recognized three times with awards for student projects from Tamil Nadu State Council for Science and Technology (TNSCST), Government of Tamil Nadu, India.





**Dr. Arun P.** M.Sc., M.Phil., Ph.D., MBA., PGDCRDM., is working as a Professor, Department of Biotechnology, Dr. N.G.P Arts and Science College, Coimbatore. He received his PG., Doctoral Degree in Biotechnology from Bharathiar University, Coimbatore, Tamil Nadu, India M.Phil., in Biotechnology from Periyar University, Salem, Tamil Nadu, India.

He has more than 17 years of teaching and research experience in various fields of biotechnology. His field of specialization is Bioprospecting. In addition, he published more than 30 research and review article in reputed peer reviewed journals. He supervised around 60 PG and M.Phil., research scholars.





**Ms. Thilagavathi Kumaran** is completed her Master's Degree in Nanoscience and Technology from Bharathiar University, Coimbatore, Tamil Nadu, India, where she has gained a keen interest in the fields such as metal-based and polymer-based hybrid-nanorobots. Ms. Thilagavathi published more than 5 research articles in reputed journals, and authored many textbooks, book chapters and conference proceedings. Her research focuses on the Development of Copper Oxide-Carbon Nanotube Composite as Binder Free Supercapacitor Electrode, Development of Functionalized Multi-walled Carbon Nanotubes (FMWCNTs) Hydrogen Fuel Cell for Green Energy Transportation. With her hardworking and dedicated approach, Ms. Thilagavathi is a promising researcher and dedicated to making noteworthy strides in the field of nanotechnology.



# Table of Contents

<b>Chapter No.</b>	<b>Title of the Chapter</b>	<b>Page No.</b>
1.	<b>Ethnopharmacology and Traditional Uses of Medicinal Plants</b> Vibash Kalyaan V. L. <sup>1</sup> , Gopinath S. <sup>1</sup> , Anju Ishwarya R. <sup>1</sup> , Priya G.*	01
2.	<b>Ethnopharmacology: Bridging Traditional Knowledge with Modern Medicine</b> KV Hridhya <sup>1</sup> , P.Srinivasan <sup>1*</sup> , M.Kulandhaivel <sup>2</sup> , M.Rajalakshmi <sup>1</sup> , R Kavya <sup>1</sup>	18
3.	<b>Bioactive Phytochemicals: Recent Developments in Extraction, Isolation, Characterization and Applications</b> Ramachandran A M*, Paul Abiaa J, Arivishnu G. Joel J S Vijay and Vishmitha M	32
4.	<b>Isolation, Characterization and Extraction of Bioactive Compounds from Plant Extracts</b> Sarumathi, S,*Senthil Kumar, V., Abdhul, K., Karthik, S., & Poonkothai, M	43
5.	<b>Pharmacognosy and Phytochemistry of Flavonoids</b> S.Ambika	55
6.	<b>Bioactive Phytochemicals: Isolation, Characterization and Synthesis</b> Senthilkumar P*, Anagha A. and Jishiga Jigeesh	67
7.	<b>Mechanisms of Action of Phytochemical in Disease Prevention and Treatment</b> Dr. R. Ravi <sup>1</sup> , Dr. M. Murugan <sup>2</sup> and Dr. B. Shanmugapriya <sup>3</sup>	81

<b>Chapter No.</b>	<b>Title of the Chapter</b>	<b>Page No.</b>
8.	<b>Mechanism of Action of Phytochemicals in Disease Prevention and Management</b> Gowri S, Vidhya shree M, KanageshwaranV, Rajalakshmi M, Akilan A	103
9.	<b>Mechanism of Action of Phytochemicals in Disease (Cancer) Prevention and Treatment</b> Karthika B <sup>1</sup> , Pradeepan K <sup>1</sup> , Sharumathy U <sup>1</sup> , Arunavarsini K <sup>2</sup> & Mahenthiran R <sup>3</sup>	116
10.	<b>Exploring the Therapeutic Potential of Phytochemicals in Tuberculosis Treatment</b> Dr. Saritha E <sup>*1</sup> , Saranya M <sup>2</sup> , Hemasri M S <sup>2</sup> , Roshini S A <sup>2</sup>	130
11.	<b>Global Markets and Commercial Opportunities in Medicinal Phytochemicals</b> Supriya L <sup>1</sup> , Sakthikokilambal.S <sup>2</sup> , Maneeshaa K <sup>3</sup> , Durga Devi L <sup>4</sup> & Mahenthiran.R <sup>5</sup>	143
12.	<b>Vegetation computation of Perundurai, Erode District, Tamil Nadu and Phytochemical screening of aqueousLeaf extract of <i>Costus Pictus</i> D.Don.</b> <sup>1</sup> M. Bhuvaneswari, <sup>2</sup> C. Chitra Vadivu, <sup>3</sup> K. Keerthishree, <sup>4</sup> S. Sakthi and A. Marthupandian	159
13.	<b>Phytochemical Screening of Marine Seaweeds Collected from Gulf of Mannar and Its Evaluation of Antimicrobial Poetential Using Solvent Extraction Method</b> P.Sagadevan <sup>1</sup> , S. Theyaneshvar <sup>1</sup> , M.Raghunath <sup>1</sup> , M. Nagarajan <sup>1</sup> and P. Janarthanan <sup>2</sup>	194

<b>Chapter No.</b>	<b>Title of the Chapter</b>	<b>Page No.</b>
14.	<b>Antibacterial Activity of a Traditional Medicinal Plant <i>Pentatropis Microphylla</i> Wight &amp; Arn (<i>Apocynaceae</i>)</b> Dr. R. Prema	212
15.	<b>Formulation and Evaluation of Herbal Toothpaste from <i>Mimosa pudica</i> for Gingivitis</b> Keerthiga S and Shanmugavadivu M	224
16.	<b>Unveiling the Comprehensive Role of <math>\beta</math>-Carotene in Human Health</b> Dencili Verginiya L <sup>1</sup> and Dr. Jancy Rani D <sup>2</sup> and Dr. Sridevi D <sup>3</sup>	241
17.	<b>An Overview of Bioactive Phytochemicals in <i>Hordeum vulgare</i> (Barley)</b> Jenisha. W, Sathya Devi. V, Yogeetha. G, Sasikala C& Vidhya N	252
18.	<b>Targeting Anthranilate Synthase from <i>Malassezia Globosa</i>from Tryptophan Pathway with the Chemical Constituents from <i>Albizia amara</i> Extract</b> R Subhashini, S Rithik, N Tharun Kumar, R Loganathan, and M Jeyam	271
19.	<b>Comprehensive Review of PCOS: Genetic Roots, Gut Health, and Cutting-Edge Treatments in the Indian Context</b> Sreesaila N P <sup>1</sup> , Dr Nimmi O S <sup>2</sup>	311
20.	<b>Cutting-Edge Nano Herbal Medicines, Phytochemical Products, Innovations and Applications</b> Dr. K. Kavithaa <sup>1*</sup> , Dr. P. Senthilkumar <sup>2</sup> and Aadersh Shibu <sup>3</sup>	327

Chapter No.	Title of the Chapter	Page No.
21.	<b>Nano Herbal Medicines, Phytochemical Products and Applications</b> Dr. M. Velammal	345
22.	<b>Exploring Potential Herpes Simplex Virus Inhibitors using Docking and ADMET Analysis of Phytocompounds from the Indian Medicinal Plants</b> G. Manigandan <sup>1</sup> , T. Raja <sup>2</sup> , S. Santhiya <sup>3</sup> , P. Ravikumar <sup>4*</sup>	357
23.	<b>In Silico Analysis of Phytocompounds from Indian Medicinal Plants for Treating Blood Cancer</b> P. Ravikumar <sup>1*</sup> · R. Haripriya <sup>2</sup> · S. Malini <sup>3</sup> · T. Raja <sup>4</sup>	380
24.	<b>Crafting Future Pharmaceuticals: The Fusion of AI, ML, and Plant-Derived Medicine Design</b> Kruthiga Natarajan <sup>1</sup> , Rajkuberan Chandrasekaran	401

## Ethnopharmacology and Traditional Uses of Medicinal Plants.

Vibash Kalyaan V. L.<sup>1</sup>, Gopinath S.<sup>1</sup>,

Anju Ishwarya R.<sup>1</sup>, Priya G.\*

<sup>1,\*</sup>Department of Biotechnology,

College of Science and Humanities

SRM Institute of Science and Technology (Ramapuram Campus),  
Ramapuram, Chennai 600089, Tamil Nadu, India.

\*Corresponding author e-mail id: priyag3@srmist.edu.in

### Abstract

It delves into the ethnopharmacological techniques that have created traditional medicine systems across the world, as well as the rich heritage of medicinal plant knowledge shared between nations. The article examines the historical development of medicinal plant use, tracing its origins from ancient civilizations to modern practices. It investigates the wide variety of medicinal plants used in traditional medicine, emphasizing their therapeutic benefits and cultural value. The essay offers light on the holistic approach to healthcare that many traditional medicine systems take by meticulously documenting indigenous knowledge and practices. Ethnopharmacological research methods and their applications in verifying traditional medical practices are also reviewed, highlighting the significance of connecting traditional knowledge to current scientific procedures. The article, which focuses on certain medicinal plants and their ethnopharmacological characteristics, provides insights into natural possible therapeutic applications of the substances. Overall, it provides a thorough examination of the junction of traditional medicine, ethnopharmacology, and plant medicinal

characteristics, resulting in a better knowledge of nature's pharmacy's therapeutic potential.

**Keywords:** *Ethnopharmacological techniques, Historical development, Medicinal plants, Therapeutic benefits, Cultural value, Traditional medicine.*

## 1. Introduction:

Ethnopharmacology, a branch of inquiry covering just approximately 50 years, has quickly grown into a major area of study, clarifying the traditional applications of medicinal plants, fungi, and other creatures within local contexts (Yeung *et al.*, 2018). Despite its brief history, ethnopharmacology has sparked widespread interest due to its substantial implications for medication research and healthcare. Historically, many research have looked into the biological, pharmacological, and medicinal use of plants and other creatures in traditional medicine. Many regularly used pharmacological compounds, such as aspirin and morphine, owe their roots to medicinal plants (Gilani & Atta-ur-Rahman, 2005). Furthermore, plants continue to play an important role in current drug development, with novel medicines being produced from natural chemicals (Atanasov *et al.*, 2015). Furthermore, plants continue to play an important role in current drug development, with novel medicines being produced from natural chemicals (Vogl *et al.*, 2013). Research into Asian, African, and Native American traditional medicine has made substantial contributions to ethnopharmacology, enhancing our understanding of plant-based medicines (Sheng-Ji, 2001; Steenkamp *et al.*, 2004).

The World Health Organization (WHO) encourages scientists conduct ethnopharmacological and experimental investigations to record folk knowledge, construct databases, and confirm scientifically traditional claims with the goal of generating new treatments (World Health Organization (WHO), 2013). According to WHO, 80% of people rely on traditional medicine to

address their basic health care requirements, with the majority using plant-based medicines (Surya *et al.*, 2014). Medicinal plants utilized as complementary/alternative medicines (CAM) to address various ailments present a genuine possibility in both developed and developing countries. Medicinal plants play an important part in world healthcare, with around 60-80% of people relying on herbal medication for their main healthcare requirements (Krupa *et al.*, 2019). The cost and accessibility of traditional medicine, particularly in resource-poor places like Asia and Africa, has resulted in a rising variety of plants recommended for herbal therapy (Sen & Chakraborty, 2015). This dependency emphasizes ethnopharmacology's importance in reducing healthcare inequities. Ethnopharmacology investigates traditional medical practices to gain insights into the therapeutic potential of natural substances, which is critical for serving varied healthcare demands and addressing global health concerns.

## **1. Ethnobotanical knowledge and methodologies:**

Medicinal plant use dates back millennia, from ancient China (5000-4000 BC) to indigenous societies in the Americas. Ethnobotanical knowledge, enhanced by documenting of plant applications by many civilizations, has made substantial contributions to contemporary medicine (Mengistu & Kindie, 2022). Methodological openness and replication are critical in ethnopharmacological research. Researchers must justify the study regions, participants, and data collection procedures to ensure relevance and accuracy. Clear selection criteria and species identification standards are required, with voucher specimens submitted for verification. Reporting citation frequencies by species facilitates the translation of field data to laboratory experiments. Best techniques include responding to particular research questions, including local populations as equal collaborators, and confirming data through participant observation. Comprehensive investigations that last at least a year capture seasonal fluctuations and evaluate globalization's

influence on medicinal flora. The documentation of dissemination strategies and benefit-sharing arrangements guarantees that research is conducted ethically. Including herbarium voucher photos improves data accessibility and verification, encouraging strong ethnopharmacological research (Weckerle *et al.*, 2018).

## 1. Traditional uses of Medicinal plants:

Plants have been employed for their health benefits since ancient times, with the oldest recorded evidence finding on a 5000-year-old Sumerian clay slab from Nagpur (Süntar, 2020). Historical records of Mesopotamia, Egypt, Greek, and Islamic civilizations (Petrovska, 2012) Document the therapeutic usage of nearly 1000 plants, including *Cedrus duhamiensis*, *Commiphora myrrha* Engl., *Cupressus sempervirens* L., *Glycyrrhiza glabra* L., and *Papaver somniferum* L. Initially, these medications were administered in primitive forms like as powders, teas, tinctures, and poultices. In the early nineteenth century, the emphasis moved to separating active components, beginning with Serturner's separation of morphine from opium in 1803. Other compounds were then isolated, including emetine from *Carapichea ipecacuanha*, strychnine from *Strychnos nux vomica*, quinine from *Cinchona officinalis*, colchicine from *Colchicum autumnale*, atropine from *Atropa belladonna*, papaverine from *Papaver somniferum*, and salicin from *Salix* species (Siddiqui *et al.*, 2014; Süntar, 2020). A few traditional plants with medicinal properties that are commonly used in Indian households have been enlisted in **Table 1**. Some traditional medicinal plants and their applications are discussed below:

### a. *Coriandrum sativum*

*Coriandrum sativum* Linn., also known as coriander, is widely utilized in both culinary and traditional medicine (Mahleyuddin *et al.*, 2022; Prachayasittikul *et al.*, 2018). The plant includes a number of chemical substances, including gallic acid, thymol, and bornyl acetate, which have anticancer, anti-inflammatory, and relaxing benefits (Matsubara *et al.*, 2011; Riella

*et al.*, 2012). Linalool, a major component, has neuroprotective, anxiolytic, anticonvulsant, and analgesic properties (de Lucena *et al.*, 2020). Coriander seeds were traditionally used to treat pain, rheumatoid arthritis, inflammation, mouth ulcers, and eye redness. They also alleviated gastrointestinal problems such as flatulence, diarrhea, indigestion, and nausea by promoting bile and digestive enzyme production (Paniagua-Zambrana *et al.*, 2020). Coriander is recognized in Saudi Arabia, Jordan, and Morocco for its ability to reduce blood glucose levels, has antibacterial capabilities against food-borne diseases, and have aphrodisiac and analgesic effects (Al-Rowais, 2002; Chaudhry & Tariq, 2006). Furthermore, it is used in Turkey and India to treat indigestion, enhance water excretion, and avoid seizures, nervousness, and insomnia. Coriander is used in Morocco to treat diabetes, indigestion, flatulence, sleeplessness, renal diseases, appetite loss, and as a diuretic (Aissaoui *et al.*, 2008; Emamghoreishi & Heidari-Hamedani, 2006).

Tachycardia and bradycardia are examples of arrhythmia, which is defined as an abnormal heart rate or rhythm (Mendel *et al.*, 2021). A study by (Rehman *et al.*, 2016) *Coriandrum sativum* (coriander) seed extract was tested for antiarrhythmic activity, and it was found to reduce pulse rate and normalize ECG patterns as well as cardiac biomarkers such as lactate dehydrogenase (LDH), creatine kinase-MB fraction (CK-MB), aspartate transaminase (AST), and alanine transaminase (ALT). The study discovered that coriander's anti-tachycardia efficacy was equivalent to the beta-blocker propranolol, while its anti-bradycardia impact was less effective than atropine. This function is linked to polyphenolic chemicals in coriander, which interact with beta-adrenergic receptors to modulate heart rate and produce a negative chronotropic impact, perhaps lowering myocytes' action potential and avoiding arrhythmias (Dianat *et al.*, 2014).

**b. *Acorus calamus***

Vacha (*Acorus calamus* Linn.) is a traditional Indian medicinal plant that is used to cure a variety of conditions, including neurological, gastrointestinal, pulmonary, metabolic, renal, and liver issues. Vacha has so far yielded 145 components, including phenylpropanoids, sesquiterpenoids, and monoterpenes. Several extracts and active components of Vacha show remarkable biopotential in metabolic and neurological illnesses, including anticonvulsant, depressive, antihypertensive, anti-inflammatory, immunomodulatory, neuroprotective, cardioprotective, and anti-obesity properties (Sharma *et al.*, 2020). Vacha, which has been used in both Indian Ayurvedic and Chinese medicine, is known for its analgesic, antipyretic, tonic, anti-obesity, and therapeutic effects. It is very helpful against skin ailments, as well as neurological, gastrointestinal, respiratory, and other health issues. Vacha rhizomes and leaves are usually utilized as infusions, powders, pastes, or decoctions (Kingston *et al.*, 2009; Pradhan & Badola, 2008).

Methanolic extract of *Acorus calamus* rhizome (12.5 g/mL) reduced VCAP-1 expression in mouse myeloid leukemia cells and intercellular expression in murine endothelial cells (Tanaka *et al.*, 2001). In an in vitro anti-inflammatory investigation employing the red blood cell membrane stability technique, the maximum dosage of *A. calamus* aqueous rhizome extract (10 mg/mL) exhibited no significant efficacy against hemolysis inhibition and RBC membrane stabilization (Karthiga *et al.*, 2016). In HaCaT cells, aqueous leaf extract of *A. calamus* reduced IL-8 and IL-6 RNA protein levels, as well as IRF3 and NF-κB activation. N-hexane, butanolic, and aqueous fractions of *A. calamus* were investigated for their effects on COX and LOX-mediated eicosanoid synthesis.

The butanolic fraction reduced COX-mediated thromboxane B<sub>2</sub> and LOX product 1, influencing the phospholipase C pathway and protein kinase C in platelets. At a concentration of 300 µg/mL, *A. calamus* essential oil inhibited protein denaturation by 69.56% (Ahmed *et al.*, 2014; Loying *et al.*, 2019).

**Table 1:** Some traditional plants with medicinal properties that are commonly used in Indian households.

Botanical name	Common name	Phytochemicals present	Pharmacological activities	References
<i>Zingiber officinale</i>	Ginger	Phenols, Flavonoids, Steroids, Triterpenoids, Tannins	Analgesic, Anti-arthritic, Antioxidant	(Koh <i>et al.</i> , 2010)
<i>Ocimum sanctum</i>	Holy Basil	Phenols, Terpenoids, Fatty acid, Ursolic acid	Antimicrobial, Anti-cancer, Anti-malarial	(Banik <i>et al.</i> , 2018)
<i>Curcuma longa</i>	Turmeric	Carbohydrates, proteins, alkaloids, saponins	Antioxidant, Antiseptic, Anti-allergic	(Koh <i>et al.</i> , 2010)
<i>Azadirachta indica</i>	Neem	Nimbin, Azadirachtin, Phenols, Flavonoids	Anti-cancer, Anti-fertility, Anti-inflammatory	(Khare, 2007)
<i>Mentha piperita</i>	Mint	Flavonoids, Carbohydrates, Menthol, Carotenes	Antibacterial, Anti-pyretic, Anti-parasitic	(Rita & Animesh, 2011)

### c. *Ficus* sp.

The genus *Ficus*, which belongs to the *Moraceae* family, has roughly 800 species worldwide, including 120 in the Americas, 105 in Africa, and 367 in Asia and Australasia. *Ficus* species are characterized by hood-like stipules and ring scars on twigs, as well as their latex-rich bark, leaves, and branches (Berg & Corner, 2005; Olaoluwa *et al.*, 2022). Their specialized reproductive structure, the Syconium, is also distinctive (Olaoluwa *et al.*, 2022). These plants have therapeutic characteristics and have traditionally been used to treat a variety of human and animal illnesses, such as digestive, respiratory, endocrine, and reproductive issues. This review focuses on nine African *Ficus* species, highlighting their ethnopharmacological, phytochemical, and biological properties (Berg & Hijman, 2021). These species are well-known for their substantial pharmacological activity, which have been widely reported in the literature, offering useful insights into possible pharmaceutical uses.

Anthocyanins are well-known for their antioxidant characteristics, which scavenge free radicals and metals due to their conjugated structure, resulting in stable radical scavenging products. Anthocyanins have been related to a variety of health advantages, including antidiabetic effects through AMPK activation, anti-inflammatory effects via COX-2 downregulation, neuroprotection by improving glutamatergic neurotransmission, and cardiovascular protection by increasing superoxide dismutase activity (Pereira *et al.*, 2017; Takikawa *et al.*, 2010). Various cyanidin and pelargonidin compounds have been extracted from *F. carica* fruits using various extraction techniques. Ceramides and sphingolipids from the *Ficus* genus have biological functions such as anticancer characteristics, nerve function maintenance, and antibacterial actions. Interestingly, elasticamide from *F. elastica* demonstrated anti-proliferative action against human cell lines (Mbosso *et al.*, 2012), whereas lutamide, lutaoside, and benjaminamide from *F. lutea* leaves shown antibacterial efficacy

against diverse pathogens, with inhibition zone sizes ranging from 10 to 17 mm (Poumale Poumale, 2012; Simo *et al.*, 2008). These findings show the wide range of bioactive properties of *Ficus* chemicals.

## 2. Extraction and Pharmacological Validation of traditional plants:

The acquisition of excellent bioactive compounds from medicinal plants is a multi-stage procedure that includes solvent selection and identification approaches. Common solvents range from polar to nonpolar, and extraction techniques include maceration, percolation, and Soxhlet extraction. Chromatography is used for fractionation and purification, whereas spectroscopy is used for chemical identification. Grouping approaches based on expected biological tests can help researchers (Abubakar & Haque, 2020). In an in-silico validation utilizing Biological Spectrum Analysis (PASS), 20 ethnomedicinal herbs were tested for antiviral and anti-inflammatory properties. *Aeginetia indica* L. has the greatest antiviral activity (Pa 0.939), followed by *Bacopa monnieri* (L.) Wettst. with a Pa of 0.880. *Andrographis paniculata* (Burm.f.) Nees had the most anti-inflammatory action (Pa 0.845), followed by *Pisum sativum* L. (Pa 0.749). *Blumea balsamifera* (L.) DC. showed both actions, demonstrating its potential as a multifunctional medicinal plant (Lalbiaknii *et al.*, 2023).

## 5. Conclusion and Future prospects:

The paper underlines the value of ethnopharmacological research in unlocking plants' therapeutic potential and verifying traditional medical practices. Natural products are significant resources for drug development, with around half a million plant species still to be studied for their therapeutic characteristics. Ethnopharmacology is critical in producing herbal medications, conserving cultural history, and encouraging local cultivation for economic and health advantages. Despite worries regarding the safety of herbal medicines, scientific confirmation of traditional

knowledge can help in the identification of new bioactive molecules. By combining traditional wisdom with contemporary research methodologies, ethnopharmacology provides a viable route for discovering novel plant-derived compounds with medicinal potential, benefiting both traditional and modern healthcare systems.

## References

- Abubakar, A. R., & Haque, M. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. *Journal of Pharmacy and Bioallied Sciences* (Vol. 12, Issue 1). [https://doi.org/10.4103/jpbs.JPBS\\_175\\_19](https://doi.org/10.4103/jpbs.JPBS_175_19).
- Ahmed, S., Gul, S., Zia-Ul-Haq, M., & Stanković, M. S. (2014). Pharmacological basis of the use of *Acorus calamus* L. in inflammatory diseases and underlying signal transduction pathways. *Boletin Latinoamericano y Del Caribe de Plantas Medicinales y Aromaticas*, 13(1).
- Aissaoui, A., El-Hilaly, J., Israili, Z. H., & Lyoussi, B. (2008). Acute diuretic effect of continuous intravenous infusion of an aqueous extract of *Coriandrum sativum* L. in anesthetized rats. *Journal of Ethnopharmacology*, 115(1). <https://doi.org/10.1016/j.jep.2007.09.007>.
- Al-Rowais, N. A. (2002). Herbal medicine in the treatment of diabetes mellitus. *Saudi Medical Journal*, 23(11).
- Atanasov, A. G., Waltenberger, B., Pferschy-Wenzig, E. M., Linder, T., Wawrosch, C., Uhrin, P., Temml, V., Wang, L., Schwaiger, S., Heiss, E. H., Rollinger, J. M., Schuster, D., Breuss, J. M., Bochkov, V., Mihovilovic, M. D., Kopp, B., Bauer, R., Dirsch, V. M., & Stuppner, H. (2015). Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnology Advances* (Vol. 33, Issue 8). <https://doi.org/10.1016/j.biotechadv.2015.08.001>.

- Banik, S., Mukherjee, R., Ghosh, P., Karmakar, S., & Chatterjee, S. (2018). Estimation of plant pigments concentration from tulsi (*Ocimum sanctum* Linn.): a six months study. *Journal of Pharmaccognosy and Phytochemistry*, 7(4).
- Berg, C. C., & Corner, E. J. H. (2005). Moraceae: Ficeae. Flora Malesiana - Series 1, *Spermatophyta*, 17(2).
- Berg, C. C., & Hijman, M. E. E. (2021). Flora of Tropical East Africa - Moraceae (1989). In Flora of Tropical East Africa - Moraceae (1989). <https://doi.org/10.1201/9781003071976>.
- Chaudhry, N. M. A., & Tariq, P. (2006). Bactericidal activity of black pepper, bay leaf, aniseed and coriander against oral isolates. *Pakistan Journal of Pharmaceutical Sciences*, 19(3).
- de Lucena, J. D., Gadelha-Filho, C. V. J., da Costa, R. O., de Araújo, D. P., Lima, F. A. V., Neves, K. R. T., & de Barros Viana, G. S. (2020). L-linalool exerts a neuroprotective action on hemiparkinsonian rats. *Naunyn-Schmiedeberg's Archives of Pharmacology*, 393(6). <https://doi.org/10.1007/s00210-019-01793-1>.
- Dianat, M., Amini, N., Badavi, M., & Farbood, Y. (2014). Antidysrhythmic, inotropic and chronotropic effects of ellagic acid and forced exercise in rat. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 5(4).
- Emamghoreishi, M., & Heidari-Hamedani, G. (2006). Sedative-hypnotic activity of extracts and essential oil of Coriander seeds. *Iranian Journal of Medical Sciences*, 31(1).
- Gilani, A. H., & Atta-ur-Rahman. (2005). Trends in ethnopharmacology. *Journal of Ethnopharmacology* (Vol. 100, Issues 1–2). <https://doi.org/10.1016/j.jep.2005.06.001>.
- Karthiga, T., Venkatalakshmi, P., Vadivel, V., & Brindha, P. (2016). In vitro anti-obesity, antioxidant and anti-inflammatory

studies on the selected medicinal plants. International Journal of Toxicological and Pharmacological Research, 8(5).

- Khare, C. P. (2007). Indian Medicinal Plants, An Illustrated Dictionary. Berlin/Heidelberg. Springer-Verlag, 219(508).
- Kingston, C., Jeeva, S., Jeeva, G. M., Kiruba, S., Mishra, B. P., & Kannan, D. (2009). Indigenous knowledge of using medicinal plants in treating skin diseases in Kanyakumari district, Southern India. Indian Journal of Traditional Knowledge, 8(2).
- Koh, H. L., Chua, T. K., & Tan, C. H. (2010). A Guide to Medicinal Plants - An Illustrated, Scientific and Medicinal Approach. In A Guide to Medicinal Plants - An Illustrated, Scientific and Medicinal Approach. <https://doi.org/10.1142/9789812837103>.
- Krupa, J., Sureshkumar, J., Silambarasan, R., Priyadarshini, K., & Ayyanar, M. (2019). Integration of traditional herbal medicines among the indigenous communities in Thiruvarur District of Tamil Nadu, India. Journal of Ayurveda and Integrative Medicine, 10(1). <https://doi.org/10.1016/j.jaim.2017.07.013>.
- Lalbiaknii, P. C., Vanlalruati Ngamlai, E., Ralte, V., Vanlalnunpuia, P. C., & Lalnunmawia, F. (2023). *In-Silico* Validation and Pharmacological Activity of Potent Anti Viral and Anti Inflammatory Ethno Medicinal Plants Used by Traditional Herbalists Within Thorangtlang Wildlife Sanctuary, Mizoram, North-East India. International Journal of Pharmaceutical Sciences and Research, 14(5), 2385. [https://doi.org/10.13040/IJPSR.0975-8232.14\(5\).2385-00](https://doi.org/10.13040/IJPSR.0975-8232.14(5).2385-00).
- Loying, R., Gogoi, R., Sarma, N., Borah, A., Munda, S., Pandey, S. K., & Lal, M. (2019). Chemical Compositions, In-vitro Antioxidant, Anti-microbial, Anti-inflammatory and Cytotoxic Activities of Essential Oil of *Acorus calamus* L. Rhizome from

North-East India. Journal of Essential Oil-Bearing Plants, 22(5).  
<https://doi.org/10.1080/0972060X.2019.1696236>.

- Mahleyuddin, N. N., Moshawih, S., Ming, L. C., Zulkifly, H. H., Kifli, N., Loy, M. J., Sarker, M. M. R., Al-Worafi, Y. M., Goh, B. H., Thuraisingam, S., & Goh, H. P. (2022). *Coriandrum sativum* l.: A review on ethnopharmacology, phytochemistry, and cardiovascular benefits. *Molecules* (Vol. 27, Issue 1). <https://doi.org/10.3390/molecules27010209>.
- Matsubara, E., Fukagawa, M., Okamoto, T., Ohnuki, K., Shimizu, K., & Kondo, R. (2011). (-)-Bornyl acetate induces autonomic relaxation and reduces arousal level after visual displayy terminal work without any influences of task performance in low-dose condition. *Biomedical Research*, 32(2). <https://doi.org/10.2220/biomedres.32.151>.
- Mbosso, E. J. T., Nguedia, J. C. A., Meyer, F., Lenta, B. N., Ngouela, S., Lallemand, B., Mathieu, V., Antwerpen, P. Van, Njunda, A. L., Adiogo, D., Tsamo, E., Looze, Y., Kiss, R., & Wintjens, R. (2012). Ceramide, cerebroside and triterpenoid saponin from the bark of aerial roots of *Ficus elastica* (Moraceae). *Phytochemistry*, 83. <https://doi.org/10.1016/j.phytochem.2012.07.010>.
- Mendel, B., Christianto, C., Setiawan, M., Prakoso, R., & Siagian, S. N. (2021). A Comparative Effectiveness Systematic Review and Meta-analysis of Drugs for the Prophylaxis of Junctional Ectopic Tachycardia. *Current Cardiology Reviews*, 18(1). <https://doi.org/10.2174/1573403x17666210603113430>.
- Mengistu, S., & Kindie, B. (2022). Issue 12 • 1000361 *J Tradit Med Clin Natur*, an open access journal Kindie and Mengistu. *Journal of Traditional Medicine & Clinical Naturopathy*, 11, 12. <https://doi.org/10.4172/2573-4555.1000361>.

- Nur, M., Azam, K., Mannan, A., & Ahmed, N. (n.d.). Medicinal plants Used by The Traditional Medical Practitioners of Barendra and Shamata (Rajshahi & Khulna Division) Region in Bangladesh for treatment of Cardiovascular Disorders.
- Olaoluwa, O., Taiwo, O., Nahar, L., & Sarker, S. D. (2022). Ethnopharmacology, phytochemistry and biological activities of selected African species of the genus *Ficus*. Trends in Phytochemical Research, 6(1). <https://doi.org/10.30495/tpr.2022.1939285.1219>.
- Paniagua-Zambrana, N. Y., Bussmann, R. W., & Romero, C. (2020). *Coriandrum sativum* L. Apiaceae. [https://doi.org/10.1007/978-3-319-77093-2\\_79-1](https://doi.org/10.1007/978-3-319-77093-2_79-1).
- Pereira, S. R., Pereira, R., Figueiredo, I., Freitas, V., Dinis, T. C. P., & Almeida, L. M. (2017). Comparison of anti-inflammatory activities of an anthocyanin-rich fraction from Portuguese blueberries (*Vaccinium corymbosum* L.) and 5-aminoosalicylic acid in a TNBS-induced colitis rat model. PLoS ONE, 12(3). <https://doi.org/10.1371/journal.pone.0174116>.
- Petrovska, B. B. (2012). Historical review of medicinal plants' usage. Pharmacognosy Reviews (Vol. 6, Issue 11). <https://doi.org/10.4103/0973-7847.95849>.
- Poumale Poumale, H. M. (2012). Lutamide, a New Ceramide Isolated from the Leaves of *Ficus lutea*. In A Search for Antibacterial Agents. <https://doi.org/10.5772/32671>.
- Prachayasittikul, V., Prachayasittikul, S., Ruchirawat, S., & Prachayasittikul, V. (2018). Coriander (*Coriandrum sativum*): A promising functional food toward the well-being. Food Research International (Vol. 105). <https://doi.org/10.1016/j.foodres.2017.11.019>.
- Pradhan, B. K., & Badola, H. K. (2008). Ethnomedicinal plant use by Lepcha tribe of Dzongu valley, bordering

Khangchendzonga Biosphere Reserve, in North Sikkim, India. Journal of Ethnobiology and Ethnomedicine, 4. <https://doi.org/10.1186/1746-4269-4-22>.

- Rehman, N., Jahan, N., Khalil-ul-Rahman, Khan, K. M., & Zafar, F. (2016). Anti-arrhythmic potential of *Coriandrum sativum* seeds in salt induced arrhythmic rats. Pakistan Veterinary Journal, 36(4).
- Riella, K. R., Marinho, R. R., Santos, J. S., Pereira-Filho, R. N., Cardoso, J. C., Albuquerque-Junior, R. L. C., & Thomazzi, S. M. (2012). Anti-inflammatory and cicatrizing activities of thymol, a monoterpenoid of the essential oil from *Lippia gracilis*, in rodents. Journal of Ethnopharmacology, 143(2). <https://doi.org/10.1016/j.jep.2012.07.028>.
- Rita, P., & Animesh, D. K. (2011). An Updated Overview on Peppermint (*Mentha Piperita* L.). International Research Journal of Pharmacy, 2(August).
- Sen, S., & Chakraborty, R. (2015). Toward the integration and advancement of herbal medicine: a focus on traditional Indian medicine. Botanics: Targets and Therapy. <https://doi.org/10.2147/btat.s66308>.
- Sharma, V., Sharma, R., Gautam, D. N. S., Kuca, K., Nepovimova, E., & Martins, N. (2020). Role of vacha (*Acorus calamus* Linn.) in neurological and metabolic disorders: Evidence from ethnopharmacology, phytochemistry, pharmacology and clinical study. Journal of Clinical Medicine (Vol. 9, Issue 4). <https://doi.org/10.3390/jcm9041176>.
- Sheng-Ji, P. (2001). Ethnobotanical approaches of traditional medicine studies: Some experiences from Asia. Pharmaceutical Biology, 39(SUPPL.). <https://doi.org/10.1076/phbi.39.7.74.5869>

- Siddiqui, A. A., Iram, F., Siddiqui, S., & Sahu, K. (2014). Role of natural products in drug discovery process. International Journal of Drug Development and Research, 6(2).
- Simo, C. C. F., Kouam, S. F., Poumalle, H. M. P., Simo, I. K., Ngadjui, B. T., Green, I. R., & Krohn, K. (2008). Benjaminamide: A new ceramide and other compounds from the twigs of *Ficus benjamina* (Moraceae). Biochemical Systematics and Ecology, 36(3). <https://doi.org/10.1016/j.bse.2007.08.014>.
- Steenkamp, V., Mathivha, E., Gouws, M. C., & Van Rensburg, C. E. J. (2004). Studies on antibacterial, antioxidant and fibroblast growth stimulation of wound healing remedies from South Africa. Journal of Ethnopharmacology, 95(2-3). <https://doi.org/10.1016/j.jep.2004.08.020>.
- Süntar, I. (2020). Importance of ethnopharmacological studies in drug discovery: role of medicinal plants. Phytochemistry Reviews (Vol. 19, Issue 5). <https://doi.org/10.1007/s11101-019-09629-9>
- Surya, S., Salam, A. D., Tomy, D. V., Carla, B., Kumar, R. A., & Sunil, C. (2014). Diabetes mellitus and medicinal plants-a review. Asian Pacific Journal of Tropical Disease, 4(5). [https://doi.org/10.1016/S2222-1808\(14\)60585-5](https://doi.org/10.1016/S2222-1808(14)60585-5).
- Takikawa, M., Inoue, S., Horio, F., & Tsuda, T. (2010). Dietary anthocyanin-rich bilberry extract ameliorates hyperglycemia and insulin sensitivity via activation of amp-activated protein kinase in diabetic mice. Journal of Nutrition, 140(3). <https://doi.org/10.3945/jn.109.118216>.
- Tanaka, S., Yoichi, S., Ao, L., Matumoto, M., Morimoto, K., Akimoto, N., Honda, G., Tabata, M., Oshima, T., Masuda, T., Asmawi, M. Z. Bin, Ismail, Z., Yusof, S. M., Din, L. B., & Said, I. M. (2001). Potential immunosuppressive and antiinflammatory activities of Malaysian medicinal plants characterized by

reduced cell surface expression of cell adhesion molecules. *Phytotherapy Research*, 15(8). <https://doi.org/10.1002/ptr.778>.

- Vogl, S., Picker, P., Mihaly-Bison, J., Fakhrudin, N., Atanasov, A. G., Heiss, E. H., Wawrosch, C., Reznicek, G., Dirsch, V. M., Saukel, J., & Kopp, B. (2013). Ethnopharmacological in vitro studies on Austria's folk medicine - An unexplored lore in vitro anti-inflammatory activities of 71 Austrian traditional herbal drugs. *Journal of Ethnopharmacology*, 149(3). <https://doi.org/10.1016/j.jep.2013.06.007>.
- Weckerle, C. S., de Boer, H. J., Puri, R. K., van Andel, T., Bussmann, R. W., & Leonti, M. (2018). Recommended standards for conducting and reporting ethnopharmacological field studies. *Journal of Ethnopharmacology*, 210. <https://doi.org/10.1016/j.jep.2017.08.018>.
- World Health Organization (WHO). (2013). WHO Traditional Medicine Strategy 2014-2023. World Health Organization (WHO). <https://doi.org/2013>.

## Ethnopharmacology: Bridging Traditional Knowledge with Modern Medicine

KV Hridhya<sup>1</sup>, P.Srinivasan<sup>1\*</sup>, M.Kulandhaivel<sup>2</sup>,  
M.Rajalakshmi<sup>1</sup>, R Kavya<sup>1</sup>

<sup>1</sup>Department of Microbiology, Rathinam College of Arts and Science, Rathinam Tech Zone Campus, Eeachanari, Coimbatore - 21.

<sup>2</sup>Department of Microbiology, Karpagam Academy of Higher Education, Pollachi Main Road, Eeachanari, Coimbatore - 21.

\*Corresponding Author's e-mail: srini2k8@gmail.com

### Introduction

Ethnopharmacology is an interdisciplinary field that studies the traditional medicinal practices of various cultures to understand the use of natural substances for health and healing. This branch of pharmacology explores the relationship between cultural knowledge and the pharmacological properties of plants, fungi, and other natural resources.

It involves the investigation of ethnobotanical knowledge, which encompasses the identification, collection, and utilization of plants for therapeutic purposes. This field extends beyond plant-based remedies to include animal-derived substances, minerals, and other traditional healing practices.

### The Scope of Ethnopharmacology

- Documentation of Traditional Knowledge:** Recording and preserving indigenous knowledge related to medicinal plants and their applications in healthcare
- Identification of Bioactive Compounds:** Analyzing the chemical constituents of medicinal plants to identify active compounds with pharmacological effects
- Pharmacological Studies:** Investigating the mechanisms of action, efficacy, and safety profiles of traditional remedies

through experimental and clinical research

- **Integration with Modern Medicine:** Exploring the potential integration of traditional medicine into contemporary healthcare systems
- **Conservation and Sustainability:** Addressing issues related to the conservation of medicinal plant species and sustainable use of natural resources

## Importance of Traditional Medicine in Contemporary Healthcare

Traditional medicine plays a significant role in addressing healthcare needs, especially in regions where access to modern healthcare services is limited. Indigenous healing practices have been passed down through generations and offer valuable insights into the treatment of various ailments.

- **Cultural Relevance:** Traditional healing practices are deeply rooted in cultural beliefs and practices, providing holistic approaches to health and well-being
- **Accessibility:** Traditional remedies are often readily available and affordable, making them accessible to communities with limited resources
- **Source of Bioactive Compounds:** Many modern drugs have their origins in natural products derived from traditional medicinal plants, highlighting the potential of ethnopharmacology in drug discovery
- **Complementary Therapies:** Integrative approaches that combine traditional and modern medicine can enhance patient care and treatment outcomes
- **Conservation of Traditional Knowledge:** Ethnopharmacological research contributes to the preservation of indigenous knowledge systems and biodiversity

## Traditional Healing Practices - Historical Perspectives:

Medicinal plants are a gift from God and can cure many diseases in the body. The recognition of plants for medicinal purposes in the West and East dates back to 60,000 years of culture. The use of plants and plant derivatives in the treatment of various ailments has been a practice for many years. Currently, approximately 65% of Indians are addicted to prescription drugs. Traditional medicine is the oldest form of medicine in the world and is used to prevent and treat diseases of the body and mind.

It is also known as complementary, alternative, or ethno medicine, and still plays an important role in many countries today. In China, traditional medicine still plays an important role in healthcare. Western medicine emerged in the 16th century but did not develop until the 19th century. The effectiveness and role of traditional Chinese medicine are based on five thousand years of clinical experience and rich clinical research data. In the 5th and 6th centuries AD, Chinese medicine was introduced to Japan from China. Japanese doctors adapted traditional Chinese medicine very well and adapted it to the treatment of their specific ailments, and gradually transferred to Kampo. Doctors in Japan use the drug Kampo alongside radiation therapy and chemotherapy to treat cancer patients. Another healing method dating back 2,500 years is Unani, an ancient healing system.

The World Health Organization began to attach importance to traditional medicine, and Unani has attracted great attention worldwide, especially in India, since the mid-1970s and has been integrated into healthcare services. Alkaloids, flavonoids, terpenoids, etc. in plants' phytochemical components are useful for medicinal purposes. Ayurveda, the traditional medicinal system practiced in India for nearly 5,000 years, includes a holistic diet and herbal remedies that address the body, mind, and spirit to prevent and cure disease. Antimicrobial resistance is a global problem today as it emerges and spreads worldwide due

to its genetic structure. Ethanol pharmacologists, microbiologists, botanists, and chemists worldwide are searching for phytochemicals and drugs to be developed naturally to treat infectious diseases. Plant materials contain many phytochemicals such as phenolic acids, flavonoids, tannins, lignin, and other small amounts. These drugs have many health effects such as anti-inflammatory, anti-inflammatory, anti-inflammatory, and vasodilator activities. In recent years, many countries have conducted many studies to prove its effectiveness. However, a handful of information is available about the activities of medicinal plants and only a few of the 4,00,000 plant species in the world have been studied. Bioactive compounds generally accumulate as secondary metabolites in all cells, but their content varies depending on the plant, season, and growth stage. The leaves are one of the plants with the most compounds, and one or both of them have some anti-inflammatory properties that cause certain pathogens and are often used for treatment.

In pharmaceuticals, most drugs are derived from natural products. More than a thousand years of knowledge and progress in modern medicine have determined the method of preparation, selection of medicinal products, analysis of medical data, and the best time to obtain different herbs. To increase the effectiveness of the drug and reduce its toxicity, it is necessary to follow a good plan and change the drug.

## Bioactive Compounds from Medicinal Plants

The poor in urban and rural India depend on medicinal plants because it is the only thing they can do. This is the only medical facility in the remote area. It is important to note that herbalists are uncertain about the effectiveness of herbs. The traditional practices of Aboriginal people that affect human health are called ethnomedicine. Ethnomedicine is the mother of all other systems of medicine like Ayurveda, Siddha, Unani, Naturopathy, and even modern medicine.

**Medicinal plants have many properties when used for therapeutic purposes, such as:**

**Synergic medicine:** All properties of plants interact simultaneously, so their uses can complement or damage others or neutralize their possible negative effects.

**Support of official medicine:** This will help reduce the use of antibiotics used when this disease occurs, thus reducing the side effects of synthetic treatment.

**Preventive medicine:** It has been proven that the components of plants are also characterized by their ability to prevent the appearance of some diseases. This will help to reduce the use of chemical remedies which will be used when the disease is already present and reduce the side effects of synthetic treatment.

Plants exhibit a variety of medicinal activities, including anti-inflammatory, anti-inflammatory, anti-cancer and hypolipidemic, cardiovascular, anti-inflammatory, anti-inflammatory and other drugs. The presence of many life-supporting properties in plants has led scientists to study these plants to determine their wound-healing potential.

Many medical laboratories are now focused on identifying bioactive phytochemicals that are valuable for the physical benefits these plants provide to the human body. These products contain a variety of secondary metabolites such as alkaloids, essential oils, flavonoids, tannins, terpenes, saponins, and phenolic compounds. Plants can act as antibiotics against many diseases those are resistant to commercial antibiotics. There is an organizing relationship. Generally speaking, fruit extracts of herbs and spices are a mixture of phytochemicals, containing up to 85% of the main bioactive compounds and other substances found in insects. These bioactive substances may be involved in many types of anti-inflammatory effects, including cell wall

disruption, disruption of the cytoplasmic membrane, leakage of cellular components, changes in fatty acid and phospholipid composition, changes in DNA and RNA synthesis, and protein turnover.

The increased use of antibiotics has caused bacteria to gain protective properties. Therefore, it is necessary to find alternatives to chemotherapy and use herbs that are easily available and have fewer side effects. The use of higher plants and their extracts in the treatment of infectious diseases has been used for a long time in many parts of the world. Botanical remedies can be used in many forms, including raw or boiled powders, liquids, or mixtures such as salves, ointments, and cuts.

The plant has great medicinal value because there are many phytochemical components in the tissues that have certain effects on the body of the human body. Due to the contribution of medicinal plants to health services, many countries have started to research them. The main benefits of using herbal medicines indeed are that they are safer than synthetic products, provide good medical results, and are the cheapest treatments. Current relationships in healthcare show that people are moving away from medical treatment and towards natural remedies such as herbs. Recent studies have shown that extracts from various medicinal plants have broad-spectrum antibacterial effects against pathogenic bacteria.

Antibiotics are effective in the treatment of diseases because they are selectively toxic to pathogenic microorganisms without harming the host. Evaluation of antimicrobial properties of plant extracts and phytochemicals using antimicrobial and anti-inflammatory agents. Antibiotics have become a global problem. The emergence of increased resistance in human pathogenic bacteria is mainly due to the use of ineffective antibiotics often used in the treatment of infectious diseases. Plants were also used in ancient times to flavor food and beverages and for

medicinal purposes, and many successes were achieved in the treatment and prevention of diseases. In the last few years, many studies have been conducted in many countries to prove the effectiveness of herbal products, and thousands of products that are said to be bacteria or antibiotics have been isolated from these plants. A combination of antibiotics is often more effective than the addition of a single antibiotic because some bacteria are not resistant to antibiotics or are killed by the use of antibiotics. Many higher plants contain many bioactive substances such as phenols, flavonoids, quinones, tannins, alkaloids, saponins, sterols, and terpenoids, which are responsible for the protective activity in plants. These phytochemicals contribute to various bioactivities such as antibacterial, allelopathic, antioxidant, and modulatory properties, so these natural products can replace harmful substances in disease control. Therefore, plants used in traditional medicine should be intensively investigated as sources of traditional antimicrobial compounds. Fungal infections can result from interference with fungal protein production, DNA replication, interference with cell metabolism, membrane damage, and fungal cell death that limits fungal growth. The antibiotic activity of secondary metabolites depends on the extraction method and severity, their concentration, and composition.

### **Standardization of herbs and medicinal formulation**

The use of herbal medicines is increasing rapidly worldwide. Among modern medical systems, Indian medicine is popular worldwide for its history, medicinal uses, and healing properties. Traditional medicine is the best way to discover natural bioactive compounds. This demand for safe medicine requires the quality, efficiency, and standards of Indian medicine. General requirements for the design of plants include good ethnobotanical information and they must be free of pesticides, heavy metals, and other chemicals. Formulations based on chemical and activity profiles, safety and security data, animal

model studies, and human efficacy will be encouraged. Clinical evaluation of plants includes macroscopic, microscopic, and pharmacognosy studies. Macroscopic research is the evaluation of the color, smell, taste, shape, size, texture, refraction, surface area, and other characteristics of the plant. It may also be damaged by microbial or chemical substances during processing. Misidentification of herbs, improper contamination, or deliberate adulteration can result in inferior products. Standardization of medicinal plants begins with the collection of plant information for treatment. Multidimensional testing standards for purity and potency of traditional medicine, covering subtle changes such as name, botanical and geographical origin, organoleptic, morphological and anatomical, physical, chemical, and biological. Standardize multiple herbal preparations (more than one herb species) to control and measure quality and safety for effective treatment. This reduces batch-to-batch variation to ensure the safety, effectiveness, quality, and reliability of various herbs. Standardization of formulations should be well-established practice, for herbal formulations it is very important to conduct additional research on many parameters such as pharmacodynamics, pharmacokinetics, dosage, stability, toxicity assessment, and drug evaluation. This will improve the quality and purity of the herbal and formulated medicine.

## **Assessing Pharmacological Properties of Traditional Remedies**

The assessment of pharmacological properties in ethnopharmacology starts with the careful identification and selection of medicinal plants or natural products based on traditional knowledge passed down through generations. Ethnobotanical studies play a crucial role in identifying plants with potential therapeutic benefits, guided by indigenous practices and cultural wisdom. Once potential medicinal plants are identified, various methods are employed to evaluate their pharmacological effects. This includes extracting bioactive

compounds from plant materials using suitable solvents like water, ethanol, or organic solvents. The extracted compounds are then subjected to a series of tests to assess their biological activities.

*In vitro* studies involve using cultured cells or specific enzymes to evaluate the effects of plant extracts or isolated compounds on cellular functions and biochemical processes. These experiments provide insights into potential mechanisms of action and therapeutic targets. *In vivo* studies, conducted on animal models, help to further investigate the pharmacological properties, efficacy, and safety profiles of the identified medicinal plants. These studies contribute valuable data that inform subsequent clinical applications and drug development processes, bridging traditional knowledge with modern pharmacology in ethnopharmacological research.

**Cell Culture Assays:** Cultured cells (e.g., cancer cells, immune cells) are treated with plant extracts or isolated compounds to assess cytotoxicity, anti-inflammatory effects, or other cellular responses. In cell culture assays within ethnopharmacological studies, various types of cultured cells such as cancer cells and immune cells are exposed to plant extracts or isolated compounds to evaluate their effects on cellular behavior and functions. These experiments serve multiple purposes, including assessing cytotoxicity, anti-inflammatory properties, and other cellular responses relevant to health and disease. For instance, researchers may use cancer cell lines to investigate the potential cytotoxic effects of plant compounds, aiming to identify substances that selectively inhibit cancer cell growth or induce cell death (apoptosis). In the context of immune cells, plant extracts can be tested for their ability to modulate immune responses, such as regulating cytokine production or enhancing phagocytosis. Cell culture assays provide valuable insights into the pharmacological actions of natural products derived from traditional remedies, helping researchers understand their

mechanisms of action at the cellular level. These studies contribute essential data for further exploration and development of potential therapeutic interventions in ethnopharmacology.

**Enzyme Inhibition Assays:** Plant extracts are tested for their ability to inhibit specific enzymes relevant to disease processes (e.g., microbial enzymes, metabolic enzymes). Enzyme inhibition assays are critical in ethnopharmacological studies to evaluate the potential of plant extracts in modulating specific enzymatic activities associated with disease processes. In these assays, researchers assess the ability of plant extracts to inhibit the activity of target enzymes, which could include microbial enzymes involved in pathogenic processes or metabolic enzymes implicated in disease pathways. For example, plant extracts may be tested for their inhibitory effects against key enzymes involved in microbial virulence or biofilm formation, aiming to identify natural compounds with antimicrobial properties. Similarly, metabolic enzyme inhibition assays can help identify plant-derived compounds that regulate metabolic pathways linked to conditions like diabetes or cardiovascular diseases. Enzyme inhibition studies provide crucial information on the pharmacological actions of natural products, guiding the selection and development of potential therapeutic agents from traditional remedies. Understanding enzyme interactions with plant-derived compounds offers insights into novel treatment strategies for various health disorders within the realm of ethnopharmacology.

In ethnopharmacology, *in vivo* studies play a crucial role in evaluating the safety, efficacy, and potential therapeutic benefits of traditional remedies derived from medicinal plants and natural products. These studies involve administering plant extracts or isolated compounds to living organisms, typically animal models such as mice, rats, or other species, to assess their pharmacological effects and physiological responses.

*In vivo*, investigations aim to replicate human biological processes within controlled experimental settings. Researchers examine parameters such as toxicity, pharmacokinetics (absorption, distribution, metabolism, and excretion), and pharmacodynamics (mechanisms of action and therapeutic effects) of the tested compounds. By conducting *in vivo* studies, researchers can better understand how traditional remedies interact with living systems, identify potential health benefits, and assess any associated risks or adverse effects. This knowledge contributes to the development of evidence-based practices in ethnopharmacology and informs further research for integrating traditional medicine into modern healthcare approaches.

**Animal Models in Ethnopharmacology:** *In vivo* studies involve administering plant extracts or isolated compounds to animal models like mice or rats to assess their pharmacological effects, toxicity, and potential therapeutic benefits. These studies aim to mimic human physiological responses and provide valuable insights into the safety and efficacy of traditional remedies. Researchers evaluate parameters such as physiological changes, organ function, and behavioral effects to understand how these natural compounds interact with living organisms, guiding further investigations and potential clinical applications in ethnopharmacology.

### Pharmacokinetic Studies in Ethnopharmacology:

Pharmacokinetic studies focus on understanding the absorption, distribution, metabolism, and excretion (ADME) of bioactive compounds derived from medicinal plants in animal models. These studies investigate how natural substances behave within living organisms, including their bioavailability and systemic circulation.

By elucidating the ADME profile, researchers gain insights into the pharmacological behavior and potential therapeutic

applications of plant-derived compounds, informing dosage regimens and optimizing treatment strategies in ethnopharmacology research.

**Mechanistic Studies in Ethnopharmacology:** Mechanistic studies utilize molecular and biochemical techniques such as Western blotting and gene expression analysis to elucidate the underlying mechanisms of action of traditional remedies at a cellular and molecular level. These studies investigate how plant-derived compounds interact with specific targets, signaling pathways, or biomolecules involved in disease processes. Understanding the mechanisms of action helps validate traditional medicinal practices, identify new drug targets, and optimize therapeutic interventions derived from natural products in ethnopharmacology.

### ***In vitro and In vivo Studies on Efficacy and Safety***

*In vitro* studies assess traditional remedies' efficacy and safety using cellular models, dose-response analyses, and cytotoxicity evaluations. These studies inform the development of therapeutic interventions by identifying effective concentrations and understanding selectivity. Dose-response relationships are crucial for determining optimal efficacy while minimizing toxicity in ethnopharmacology. Investigating cellular and molecular interactions elucidates mechanisms of action against disease processes like inflammation. *In vivo*, studies in animal models evaluate therapeutic efficacy by assessing disease effects and physiological parameters, guiding clinical development. Safety assessments in animal models ensure the safety profile of traditional remedies, informing risk-benefit evaluations for clinical applications in ethnopharmacology.

## Integrative Approaches Combining Traditional and Modern Therapies

Integrative medicine aims to leverage the strengths of both traditional and modern healthcare systems for comprehensive patient care. This includes:

- **Collaborative Care:** Engaging healthcare providers from diverse backgrounds (e.g., herbalists, and physicians) to develop personalized treatment plans
- **Evidence-Based Practice:** Integrating scientific research and clinical data to inform decision-making and optimize therapeutic outcomes
- **Patient-Centered Care:** Empowering patients to make informed choices about their health by incorporating traditional remedies alongside conventional treatments

In conclusion, pharmacological studies in ethnopharmacology employ diverse methods to assess the pharmacological properties of traditional remedies. From in vitro experiments to clinical trials, these studies contribute valuable insights into the therapeutic potential, safety, and clinical applications of natural products derived from traditional healing practices. Integrative approaches that combine traditional and modern therapies offer promising avenues for enhancing patient care and advancing holistic healthcare practices.

### References:

- Abdullahi, A. (2011). Trends and challenges of traditional medicine in Africa. *African Journal of Traditional, Complementary and Alternative Medicines*, 8(5S), 115-123.
- Al-Snafi, A. E. (2015). A review on the pharmacology and phytochemistry of medicinal plants used to treat bacterial infections in humans. *Asian Journal of Pharmaceutical Sciences*, 5(4), 214-220.

- Bhat, K. P. L., & Negi, P. S. (2012). Phytochemicals and antimicrobial activity of Himalayan medicinal plants. *Journal of Ethnopharmacology*, 103(2), 259-263.
- Rajesh Kumari, C., & Kumar, A. (2016). Standardization of herbal drugs: A review. *International Journal of Pharmaceutical Sciences and Research*, 7(3), 1054-1063.
- Suntar, I. P., Akkol, E. K., & Keles, H. (2012). Traditional wound healing plants used in the South-Eastern Anatolia region (Turkey) and their ethno-botanical properties. *Journal of Ethnopharmacology*, 139(2), 523-530.

## 3

## Bioactive Phytochemicals: Recent Developments in Extraction, Isolation, Characterization and Applications

Ramachandran A M\*, Paul Abiaa J,  
Arivishnu G. Joel J S Vijay and Vishmitha M

Department of Microbiology,

Dr. N. G. P. Arts and Science College, Coimbatore -641 048

\*Corresponding Author: Dr. Ramachandran A M

Email: ramachandran@drngpasc.ac.in, luxyram40@gmail.com

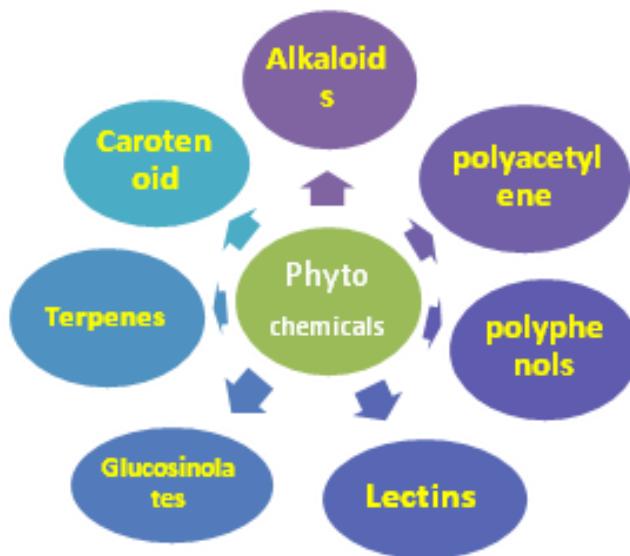
### Introduction:

Phytochemicals are plant secondary metabolites which possess outnumber of bioactive properties include antimicrobial, anti-inflammatory, anti protozoal, antifungal, anti cancerous, anti diarrheal, anti helminthic and anti viral (1). Their role in the clinical treatment have been progressively increased and it usages can help reduce the cost of the medication by providing alternative to more expensive treatments. Moreover, the use of phytochemicals as medicine may have low risk of adverse effects (2). A lot of sources produce phytochemicals, including nuts, cereals, barks, flowers, root and leaves of various plants. A large number of phytochemicals have been identified which are enlisted as follows like carotenoids, isoprenoids, saponins, phytosterols, fibers and unique polysaccharides (3).

Recent multidimensional research has augmented the value of phytochemical importance in the nutraceutical arena. The successful extractions of phytochemicals from various sources of plant play a significant role in the application part. The selection of suitable extraction method is a critical one because the inappropriate method may reduce the quantity and the standard of phytochemical compounds. There are number of techniques to extract and purify the compounds derived from plants. Besides, the selection of the suitable solvent also influences the quality of phytochemical recovery.

The solvents can be classified in to two categories such as green solvents (water, ethanol, glycerol, fatty acids or oils, acetic acid, carbon dioxide, etc.) and other solvents encompass acetone, chloroform, butanol, methanol, ethyl acetate, benzene, hexane, cyclohexane, etc. (4). However, certain conventional methods to extract the phytochemicals are maceration, percolation, decoction, reflux extraction and Soxhlet extraction and the recent methods are pressurized liquid extraction (PLE), high hydrostatic pressure extraction (HHP). Microwave assisted extraction (MAE), ultrasound assisted extraction (UAE), pulsed electric field extraction (PEF), vibro-cavitation extraction, extraction under vacuum -oscillating boiling conditions, extraction in mills, Enzyme assisted extraction (EAE), and supercritical fluid extraction (SFE) (5).

Because of their different shapes and structures, phytochemicals have not been categorized precisely (Fig.1) (6).



**Figure 1.** Classification of phytochemical compounds

Generally many solvents have been employed to extract phytocompounds and also release the interference of water simultaneously. It has been chosen based on the polarity of the solute of interest. Multiple solvents can be used in order to regulate the impurities and the identical compounds in the desired yield. The degree of polarity from low to high polar, of a few solvents is as follows: Hexane < Chloroform < Ethylacetate < Acetone < Methanol < Water (7).

**Table1.** List of phytochemical extraction methods from the plants

S. No.	Methods	Procedure	Applications	Reference
1.	Supercritical Fluid Extraction (SFE)	Macerate the plant sample and introduce the CO <sub>2</sub> as solvent in the SFE system under the specific temperature (31° C or room temperature) and pressure (100 -450 bar). After extraction, the extract is moved to the fraction chamber and depressurized due to which CO <sub>2</sub> loses solvating ability leads to the material as precipitate	<ul style="list-style-type: none"> <li>❖ Applied to extract natural products such as coffee, tea, spices, essential oil from black pepper.</li> <li>❖ It is used to determine fat content from vegetables eg. sun flower, cotton seed oil etc.</li> </ul>	Quit'ero, E., Grossio, <i>et al.</i> , 2022. (8)
2.	Enzyme Assisted Extraction (EAE)	The procedure involved adding 25 mg of enzyme formulation to 20 mL of selected buffer with 1 g of air-dried sample. The sample was then stirred for 2 hours at 50°C, and 1 mL samples were taken every hour. The final extract was obtained through centrifugation and freeze-drying. Buffers used were disodium phosphate-citric acid at pH 5 and Tris-HCl at pH 8, based on the enzyme type.	<ul style="list-style-type: none"> <li>❖ Enzymes can help extract different phenolic compounds like flavonoids and anthocyanidins.</li> <li>❖ Factors such as enzyme activity, treatment time, substrate ratio, and particle size are crucial for the best results during enzymatic treatment.</li> </ul>	Pontillo, A.R.N.; <i>et al.</i> , 2021.(9)  Streimikyte, P <i>et al.</i> , 2022 (10).

3.	Ultrasound Assisted Extraction (UAE)	<p>The UAE unit used was a closed rectangular ultrasonic processor with a maximum power of 400 W at a frequency of 20 KHz was used for the extraction process. The extracted sample was then filtered and concentrated using a vacuum rotary evaporator before being dried in a freeze drier at -55°C and 0.15 mmHg. Finally, the dried samples were stored in darkness at 18°C for further analysis.</p>	<ul style="list-style-type: none"> <li>❖ UAE is a highly efficient technique that offers advantages such as shorter operation time, easier operation, reduced solvent consumption and temperature, saved energy, and increased yield.</li> <li>❖ It works through cavitation phenomena and improved mass transfer to enhance extraction yields.</li> </ul>	Sharayei, P., et al., 2018 (11)
4.	Pressurized Liquid Extraction (PLE)	<p>PLE, involved continuously flowing solvent through a fixed substrate bed using a specific extraction unit. 5.0 grams of sample formed the fixed bed in a 50 ml jacketed stainless steel column. The sample was ground in a domestic blender for homogenization and to aid in extraction. Temperatures of 40, 60, 80, and 100 °C were tested using different solvents such as pure ethanol, water-ethanol, and acidified water. The pressure and solvent mass flow rate were maintained at <math>10.0 \pm 0.5</math> MPa and 5.0 g/min, respectively. The extraction time was determined through preliminary tests. Extracts were stored at -18 °C in glass flasks for further analyses.</p>	<ul style="list-style-type: none"> <li>❖ PLE to efficiently extract phenolic compounds from plants using solvents and/or water.</li> <li>❖ It highlights the challenge of extracting individual compounds. For eg. From grape marc, a complex mixture of bioactive compounds, and recommends the use of PLE as an efficient technique to improve extraction and maximize compound recovery from plant matrices.</li> </ul>	Pereira. D.T.V., et al., 2018 (12)

5.	Microwave Assisted Extraction (MAE)	<p>We used a special microwave system with a rotor for 6 sample vessels to extract compounds. We mixed the samples with different amounts of solvent and collected the extracts at different time intervals. Then we evaporated the extracts using a rotary evaporator. We studied how the extraction yield varied with different microwave powers and solvent ratios. We conducted thirteen experimental runs with five different solvents. The extraction yield was measured as the percentage of compounds extracted from the sample.</p>	<ul style="list-style-type: none"> <li>❖ MAE is a method that can extract phenolics from various plant materials, such as citrus peels, peanut skins, grape seeds, tomatoes, blueberries, spent filter coffee, chokeberries, and walnut leaves.</li> <li>❖ MAE has shorter extraction times and requires less solvent because it uses heat and mass gradients together, resulting in faster extraction and higher yields.</li> </ul>	Kaderides K., <i>et al.</i> , 2019 (13)
6.	High Hydrostatic Pressure Extraction (HHP)	<p>Protein sample source and solvent mixture was placed in a sterile polythene bag and vacuum sealed. Then, apply the pressure up to 600 MPa. In which water can be used as a transmission medium. The pressure rose at a rate of about 300 MPa/min. After the mixture is placed in the pressure chamber the pressure can be gradually increased at 150, 300, 450 and 600 MPa for 5 min and the temperature can be set initially at 25oC and then increased by 3o C for every 100 MPa. After the procedure followed, the extract is vacuum concentrated, freeze dried and filtered through Whatman No.4 paper and further analysis can be performed at 4 C.</p>	<ul style="list-style-type: none"> <li>❖ Anti oxidant content yield analysis.</li> <li>❖ It is economical.</li> <li>❖ Eco friendly.</li> <li>❖ It is an efficient extraction method in the food and cosmetic industry.</li> </ul>	Wu SJ <i>et al.</i> , 2017 (14)

7.	Pulsed Electric Field Extraction (PEF)	<p>PEF unit consist a set of devices which includes a high voltage pulse generator, chamber, monitor, control system, charger (to convert AC in DC), capacitor and a battery. The treatment chamber has two electrodes and facilitates to place the samples. PEF procedure has followed in to two categories such as batch and continuous extraction. For batch extraction method need pretreatment of the sample with solvent and placed in to chamber between two electrodes. Then the PEF generator can be connected. The treated sample can be removed from the chamber and stirred at various velocities by using magnetic agitator. In continuous PEF method the oscilloscope can read the output (up to 40 kV pulse voltage) and the frequency range is 40-3000 Hz. During the PEF continuous extraction procedure the solvent mixture is pumped in to the chamber by a peristaltic pump. Simultaneously, the temperature can be maintained at 25°C by thermostat. After the completion of extraction procedure the extract can be used for further analysis.</p>	<ul style="list-style-type: none"> <li>❖ PEF method of extraction is widely used in food industries to extract anti oxidants, Phenolic compounds and nutritional elements like vitamins, minerals etc.,</li> <li>❖ This method is also used to preserve the microbes and the enzymes for a specific period of time.</li> </ul>	Ranjha <i>et al.</i> , 2021 (15).
----	--	--	--	-----------------------------------

## Applications and Characterization of Bioactive Phytochemicals

### (i) Pharmacology Activity & antioxidants

The plant-based antioxidants found in herbal remedies are getting more and more important. Not only in the field of nutrition but also in the field of preventative medicine. Because of its high

polyphenol content, researchers have focused their attention on the Lamiaceae family of plants. In addition to the essential oil components, rosemary's potent antioxidant properties were mostly attributed to its essential diterpenes, carnosol (Fig 2.) and carnosol acid. However, due to their pharmacological properties, the essential oils and their active constituents are now of great interest. Typically, rosemary leaves are used to flavor food and as a source of antioxidant chemicals that are utilized to preserve food. The clinical literature identified a number of extraction techniques for the selective extraction of rosemary leaves. This covers mechanical urgent techniques, alkaline pH water, solvent extraction using vegetable or animal fats, and natural solvents (e.g. hexane, ethyl ether, chloroform, ethanol, methanol, dioxane, and ethylene dichloride). These days, dried rosemary leaves are typically used to make rosemary extracts. Partial deodorization or extract decolorizations are frequently included in the extraction process of all the current technologies. In general, depending on the raw material utilized, different authors have reported yields of rosemary extract ranging from 2% to 26%. (16).

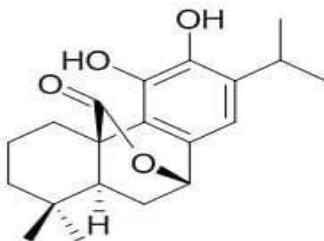


Figure 2: Carnosol Acid

## (ii) Camphor

Waxy and colorless, camphor has a potent scent. It is categorized as both a cyclic ketone and a terpenoid. Additionally, it can be found in certain other closely related plant. For example, rosemary leaves (*R. officinalis*) include 0.05 to 0.5% camphor, whereas camphorweed (*Heterotheca*) has about 5%. (17)

### (iii) Rosemarinic Acid

Regions found that the content of rosmarinic acid in rosemary leaf extract ranged from 0.27 to 2.49%. The derivatives of rosemary, such rosmarinic acid, are its most significant components. These substances have anti-oxidant properties. One of the phenolic rings in rosmarinic acid is derived from phenylalanine through the action of caffeic acid, while the other is obtained from tyrosine through the action of dihydroxyphenyl-lactic acid. When phenylalanine and tyrosine are fed exogenously, *Coleus blumei* cell culture can produce rosmarinic acid on a somewhat big scale. The skin and digestive tract both effectively absorb rosmarinic acid. It suppresses the complement system and causes human polymorphonuclear leucocytes to produce less leukotriene B4 and more prostaglandin E2. The findings indicate that rosemary and its components, particularly caffeic acid derivatives like rosmarinic acid, may be used therapeutically to treat or prevent a variety of conditions, including cancer, hepatotoxicity, bronchial asthma, spasmogenic disorders, peptic ulcers, inflammatory diseases, atherosclerosis, ischemic heart disease, cataracts, and impaired sperm motility (18).

### (iv) Skin Cancer

Human melanoma A375 cells were used to investigate the effects of the *Rosmarinus officinalis* hydro alcoholic extract the extract reduced human melanoma cell growth in a dose-dependent manner. Excessive exposure to sunlight is linked to mutations in melanocytes, which can lead to the formation of melanomas. The *R. officinalis* hydroalcoholic extract's cytotoxic and cytostatic properties prevented cell cycle growth in vitro. It has been demonstrated that carnosic acid plays a significant preventive function against melanoma. By phosphorylating signaling molecules (Akt, FAK, and Sr) and suppressing the expression of cell migration markers (MMP-9, TIMP-1, uPA, and VCAM-1), this secondary metabolite suppressed the adhesion and proliferation of B16F10 melanoma cells in a dose-dependent manner (19).

### (v) *In vitro* wound healing potential

Wound healing involves the formation and remodeling of new tissues, with cell migration and proliferation at the wound edge being essential for closing the wound and repairing the injured tissue. Many plants in folk medicine are used to accelerate this process and prevent infection. The study demonstrated that the essential oil of *R. officinalis* did not show acute toxicity in rats. Rats treated with the essential oils of *R. officinalis* showed a significantly higher healing percentage compared to the other groups ( $4.99 \pm 2.3$ ,  $4.22 \pm 0.19$ ,  $36.48 \pm 1.12$ ,  $29.99 \pm 0.8$  and  $84.82 \pm 6.41\%$  for the untreated, placebo, *R. officinalis*, *Populus alba*, and Madecassol® group respectively). The treated groups had smaller wound surface areas at day 25, indicating a protective effect during the wound healing process (20).

## References

- Brewer MS. 2011. Natural antioxidants: sources, compounds, mechanisms of action, and potential applications. Comprehensive Reviews in Food Science and Food Safety 10(4):221 – 247.
- Débora Tamires Vitor Pereira, Adriana Gadioli Tarone, Cinthia Baú Betim Cazarin, Gerardo Fernández Barbero, Julian Martínez, 2018. Pressurized liquid extraction of bioactive compounds from grape marc, Journal of Food Engineering. doi: 10.1016/j.jfoodeng.2018.07.019
- Eze, M.N., Odoh, H.E., Apeh, M.C., 2023. Assessment of phytochemicals and antioxidant potential of the stem bark of *Albizia chevalieri*. Biosci. J. 11 (1), 43–56.
- Gupta, C., Prakash, D., 2014. Phytonutrients as therapeutic agents. J. Complement. Integr. Med. 11 (3), 151–169. <https://doi.org/10.1515/jcim-2013-0021>.
- K. Tamta, 2022, International journal of health science, A review on Pharmacological Phytochemical and medicinal properties of *Rosemarinus officinalis* . 6 (S6) 3491- 3500.
- Khaleel, A., Shehadil, I., Al-Shamisi, M., 2010. Nanostructured chromium-iron mixed oxides: physicochemical properties and

- catalytic activity. *Colloids Surf. A* 355, 75–82.
- Koche, D., Shirsat, R., Kawale, M.A.H.E.S.H., 2016. An overer view of major classes of phytochemicals: their types and role in disease prevention. *Hislopia. J.* 9 (1/2), 09762124.
  - Kyriakos Kaderides, Lygeri Papaoikonomou, Melania Serafim, Athanasia M. Goula, 2019. Microwave-assisted extraction of phenolics from pomegranate peels: Optimization, kinetics, and comparison with ultrasounds extraction, *Chemical Engineering and Processing - Process Intensification*, Volume137, Pages 1-11, ISSN 0255-2701. <https://doi.org/10.1016/j.cep.2019.01.006>.
  - Lucas Malvezzi de Macedo, 2020, Multidisciplinary digital publishing institute, Rosemary (*Rosemarinus officinalis* L., *syn* *salvia rosmarinus* spenn). And its Topical Applications: A Review, 9(5), 651.
  - Pontillo, A.R.N., Papakosta-Tsigkri, L, Lymeropoulou, T, Mamma D, Kekos, D, Detsi, A. 2021. Conventional and Enzyme-Assisted Extraction of Rosemary Leaves (*Rosmarinus officinalis* L.): Toward a Greener Approach to High Added-Value Extracts. *Appl. Sci.* , 11, 3724. <https://doi.org/10.3390/app11083724>
  - Quit'ero, E., Grosso, C., Ferraz, R., Delerue-Matos, C., Soares, C., 2022. A critical comparison of the advanced extraction techniques applied to obtain health-promoting compounds from seaweeds. *Mar. Drugs* 20 (11), 677.
  - Quitério, E.; Grosso, C.; Ferraz, R.; Delerue-Matos, C.; Soares, C. 2022. A Critical Comparison of the Advanced Extraction Techniques Applied to Obtain Health-Promoting Compounds from Seaweeds. *Marine Drug.* 20, 677.
  - Ranjha, M.M.A.N.; Kanwal,R.; Shafique, B.; Arshad, R.N.; Irfan,S.; Kieliszek, M.; Kowalczewski, P.Ł.;Irfan, M.; Khalid, M.Z.; Roobab, U.; (2021). A Critical Review on Pulsed Electric Field: A Novel Technology for the Extraction of Phytoconstituents. *Molecules*, 26, 4893. <https://doi.org/10.3390/molecules26164893>
  - Sharayei, P., Azarpazhooh, E., Zomorodi, S., Ramaswamy, H.S., 2018. Ultrasound assisted extraction of bioactive compounds

from pomegranate (*Punica granatum* L.) peel, LWT - Food Science and Technology doi: <https://doi.org/10.1016/j.lwt.2018.11.031>.

- Sharma, B.R.; Kumar, V.; Gat, Y.; Kumar, N.; Parashar, A.; Pinakin, D.J. 2018. Microbial maceration: A sustainable approach for phytochemical extraction. *3 Biotech*, 8, 401.
- Shikov, A.N, Mikhailovskaya, I.Y., Narkevich, I.A., Flisyuk, E.V., Pozharitskaya, O.N. 2022. Methods of extraction of medicinal plants. In Evidence-Based Validation of Herbal Medicine; Elsevier: Amsterdam, The Netherlands,; pp. 771–796.
- Streimikyte, P, Viskelis, P, Viskelis, J. 2022. Enzymes-Assisted Extraction of Plants for Sustainable and Functional Applications. *Int. J. Mol. Sci.*, 23, 2359.
- Wong, P.Y.Y.; Kitts, D.D. 2006. Studies on the dual antioxidant and antibacterial properties of parsley (*Petroselinum crispum*) and cilantro (*Coriandrum sativum*) extracts. *Food Chem.*, 97, 505–515.
- Wu SJ, Chen YW, Wang CY, Shyu YT (2017). Anti-inflammatory properties of high pressure-assisted extracts of *Grifola frondosa* in lipopolysaccharide-activated RAW 264.7 macrophages. *Int J Food Sci Technol* 52:671–678.
- Zhang, Huangxian, Ting Huang, Xiaoning Liao, Yaohong Zhou, Shangxing Chen, Jing Chen, and Wanming Xiong. 2022. "Extraction of Camphor Tree Essential Oil by Steam Distillation and Supercritical CO<sub>2</sub> Extraction" *Molecules* 27, no. 17: 5385. <https://doi.org/10.3390/molecules27175385>

## Isolation, Characterization and Extraction of Bioactive Compounds from Plant Extracts

Sarumathi, S,\*Senthil Kumar, V., Abdhul, K.,  
Karthik, S., & Poonkothai, M

PG & Research Department of Biotechnology  
Nandha Arts and Science College,  
Erode - 638 052, Tamil Nadu, India

\*Corresponding author: senthilkumarsen73@gmail.com

### Abstract

The chemical diversity found in natural products made from medicinal plants, whether they are standardised extracts or pure chemicals, opens up a world of possibilities for creating novel therapeutic targets. The need for chemical diversity in screening programmes and the hunt for medicinal drugs derived from natural sources have led to an increase in interest in edible plants throughout the world. Herbal and botanical products meant for medicinal purposes contain a wide variety of bioactive compounds.

The primary analytical techniques covered in this paper are the extraction, isolation, and characterization of active ingredients in botanical and herbal remedies. Common problems and major roadblocks in the process of extracting, isolating, and characterising the active ingredients in botanical and herbal remedies are discussed. As the most important step in analysing the components of botanical and herbal remedies is extraction, the benefits and drawbacks of different extraction techniques are discussed. Examining the bioactive compounds present in plant extracts is done using chromatographic techniques, Fourier Transform Infrared (FT-IR), and standard phytochemical screening tests.

## 1. Introduction:

Plants are rich in beneficial phytochemicals that may complement the human body's needs by functioning as natural antioxidants (Boots *et al.*, 2008). Plant compounds defend cells against environmental threats, including pollution, stress, drought, UV exposure, and pathogenic assault (Mathai, 2000). The study of different plants and their byproducts has grown significantly in search of beneficial bioactive chemicals (Kumar *et al.*, 2015). Depending on their function in plant metabolism, phytochemicals are categorized as major or secondary components in the current year. Common sugars, proteins, amino acids, chlorophyll, and the purines and pyrimidines of nucleic acids are examples of primary components. The remaining plant compounds, known as secondary ingredients, include lignans, alkaloids, terpenes, flavonoids, curcuminoids, plant steroids, saponins, phenolics, flavonoids, and glucosides (Lampe and Messina, 1998). About 20% of all plants have been the subject of pharmacological research, which has improved healthcare for example, curing cancer and other dangerous diseases (Naczk & Shahidi, 2006). Phytochemicals, also known as phytonutrients, are naturally occurring bioactive substances that are abundant in whole grain products, fruits, vegetables, legumes, nuts & seeds, tea, and dark chocolate (Zhao *et al.*, 2019). Although phytochemicals are categorized according to their biological functions, a single component may have many biological roles, such as acting as an antioxidant and an antibacterial agent (Ngozi *et al.*, 2014). The most well-known phytochemicals are called polyphenolics, and they are made up of flavonoids and phenolic acids, which are the building blocks of proanthocyanidins and hydrolyzable and condensed tannins, also known as polymeric tannins (Puupponen-Pimia *et al.*, 2005). Polyphenols, carotenoids, flavonoids, coumarins, indoles, isoflavones, lignans, organosulfur compounds, catechins, phenolic acids, isothiocyanates, saponins, procyanidins, phenylpropanoids, anthraquinones, and ginsenosides are the most prevalent phytochemicals found in food (Xiao & Bai, 2019). They protect plants from illness and harm while also enhancing their colour,

fragrance, and flavour (Gibson *et al.*, 1998). The outer layers of the different plant tissues frequently contain the majority of phytochemicals, especially the color molecules. These compounds are known as plants' secondary metabolites and have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation, and modulation of hormone metabolism and anticancer properties (Rao, 2003).

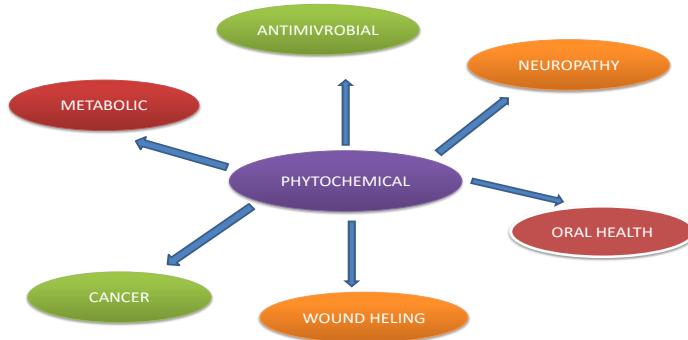
New natural bioactive substances with anti-aging and lifespan-extending properties have been the focus of recent studies (Sutphim *et al.*, 2012). Consuming phytochemicals is linked to a lower risk of developing several chronic illnesses because of their capacity to scavenge free radicals and act as antioxidants (Zhang *et al.*, 2015). Additionally, recent studies have demonstrated their potential contribution to enhanced vascular blood flow and endothelial function (Du *et al.*, 2016).

### 1.1. Phytochemicals and their Functions:

S. No.	Compounds	Sources	Functions
1	Carotenoids	Pulp of Mango, leaves of spinach, Papaya, citrus fruits, Guava.	Regulates gene transcription, strengthens immunity, and enhances vision (Crupi <i>et al.</i> , 2023)
2	Polyphenols	Grapefruit, oats, chili, pepper, and watermelon	Benefits that guard against allergies, inflammation, and cardiovascular illnesses.
3	Isoprenoids	Lemon, lime, orange, bay leaves, sweet basil and cucumber	Anti-stress qualities and a part in Alzheimer's disease neuroprotection
4	Saponins	Sunflower, almond, garden pea and walnut.	Diarrhoea, skin inflammation, and antifungal properties.

5	Phytosterols	Banana, pepper, avocado, soybean, onion, potato and coffee.	Utilised to treat asthma, allergies, and has anti-cancer properties.
6	Dietary fiber (Pectin, cellulose)	Apple, orange, carrot, rice, and wheat.	Reduces the quantity of harmful cholesterol and cancer risk.
7	Polysaccharides (amylose and starch)	Corn, rice, oats, peas, and beans.	Improves intestinal health and reduces the chance of diabetes, cancer, and obesity.

For the most part, this work concentrated on analytical techniques., such as extraction procedures and the analysis and identification of bioactive compounds found in plant extracts using a variety of chromatographic and detection methods.



## 2. Materials and Methods:

### 2.1. Phytochemical Extractions:

Extraction is an important initial step in medicinal plant analysis because it is required to remove the desired chemical components from the plant materials before further separation and characterization. The fundamental operation includes processes

such as pre-washing, drying of plant materials or freeze drying, grinding to generate a homogeneous sample, and often optimizing the kinetics of analytic extraction as well as increasing sample surface contact with the solvent system. Proper steps must be followed to ensure that possible active ingredients are not lost, altered, or destroyed during the extraction of plant materials (Fabricant & Farnsworth, 2001).

Various solvent solutions are available for extracting bioactive compounds from natural sources. Hydrophilic chemicals are extracted using polar solvents such as methanol, ethanol, or ethyl-acetate. More lipophilic chemicals are extracted using dichloromethane or a 1:1 combination of dichloromethane and methanol. In other circumstances, chlorophyll is removed using hexane extraction (Cos *et al.*, 2006). Plant extracts can also be made by macerating or percolating dried powdered plant material or fresh green plants in water and/or organic solvent systems. It is crucial to separate the bioactive components that are extracted from the plants from co-strives. The extracted chemicals are further fractionated according to their molecular size, polarity, or acidity. The different methods of extraction of phytochemicals are given below.

### **2.1.1. Solvent Extraction Method:**

The dried powder of different plant components was put in a glass thimble and extracted with different solvents. The process, which lowers the temperature of the corresponding solvents by a small amount, is repeated ten times for every extract. To ascertain whether phytoconstituents are present, the resultant solvent extract is filtered, concentrated in a concentrator, and examined.

### **2.1.2. Cold Extraction Method:**

During the extraction process, the plant components are ground into a powder, weighed, and dried at 15-25 °C in a controlled setting. After adding the powder and solvents to a beaker, the mixture is allowed to sit at room temperature for half

an hour. For seven days, the contents are shaken once every twenty-four hours. The extract is dried at room temperature in a watch glass dish after being filtered with Whatman filter paper under vacuum. Both before and after the powder dries, its weight is noted (Harborne, 1998).

### **2.1.3. Serial Exhaustive Extraction:**

This extraction method ensures a broad polarity range of chemicals being extracted and prepares crude extracts by solvents of increasing polarity, from a non-polar solvent (hexane) to a polar solvent (methanol) (Velavan, 2015).

## **2.2. Identification and Characterization:**

The separation of plant extracts continues to be a significant problem for the identification and characterization of bioactive compounds since they often exist as a mixture of several types of bioactive compounds or phytochemicals with distinct polarity. It is standard procedure to utilize a variety of separation methods, including Thin Layer Chromatography, Partition Chromatography, Ion-Exchange Chromatography, Affinity Chromatography, Size-Exclusion Chromatography (Sec).

The structure and biological activity of the pure chemicals are subsequently ascertained. In addition, non-chromatographic methods including FTIR, phytochemical screening test, can be utilized to identify bioactive chemicals

### **2.2.1. Chromatographic Techniques:**

The process of separating molecules according to their size, shape, and charge is called chromatography (Heftmann, 1992). Analytes in chromatography pass through a solid phase that serves as a sieve material while they are in a solvent. The molecules separate as they pass through the molecular sieve.

### 2.2.1.1. Thin Layer Chromatography (TLC):

TLC is a technique used to isolate non-volatile mixtures. Additional tests include examining the plate under a UV light source or spraying phytochemical screening reagents, which alter colour based on the phytochemicals present in a plant extract. This has also been applied to verify the identification and purity of separated chemicals. A helpful method for identifying bioactive compounds with antibacterial activity in plant extract is bioautography. The localization and target-directed isolation of active ingredients in a mixture are made easier by TLC bioautographic techniques, which combine chromatographic separation and *in situ* activity assessment. The growth inhibition of microorganisms has been a traditional bioautographic approach for identifying the antimicrobial components in extracts chromatographed on a TLC layer. This approach is thought to be the most effective test for finding antimicrobial substances (Shahverdi *et al.*, 2007).

Three methods are used by bio-autography to pin-point antibacterial activity on a chromatogram. They are:

1. Direct bio-autography, in which the microorganism grows directly on the thin-layer chromatographic (TLC) plate;
2. Contact bio-autography, in which the antimicrobial compounds are transferred directly from the TLC plate to an inoculated agar plate; and
3. Agar overlay bio-autography, in which the TLC plate is directly covered with a seeded agar medium (Hamburger & Cordell, 1987).

The position of the bioactive molecule with antimicrobial activity in the TLC fingerprint will be seen concerning  $R_f$  values using the inhibition zones created on TLC plates by one of the mentioned bioautographic techniques (Homans & Fuchs, 1970).

### 2.2.1.2. Partition Chromatography:

In partition chromatography, the molecules to be separated will interact between two immiscible liquid phases according to their relative solubility. This process is also referred to as liquid chromatography.

### 2.2.1.3. Ion-Exchange Chromatography:

Ions and polar compounds can be separated using ion exchange chromatography, also known as ion chromatography, according to their affinity for ion exchangers. Thus, the reversible exchange of ions between the target ions in the sample solution and the ions on ion exchangers provides the basis for the concept of separation (Ingle *et al.*, 2017).

### 2.2.1.4. Affinity chromatography:

A highly specific macromolecular binding interaction between a bio molecule and another substance is the basis for the affinity chromatography technique, which is used to separate a bio molecule from a mixture.

### 2.2.1.5. Size-Exclusion Chromatography:

Size-exclusion chromatography (SEC) often referred to as gel filtering or molecular sieve chromatography is a technique that divides molecules in solution based on their molecular weight or size. Its use is usually directed towards large molecules or macromolecular complexes, like industrial polymers and proteins.

## 2.3. Methods of Detection:

### 2.3.1. FT-IR Spectroscopy:

It is an useful method for identifying functional groups in plant extracts. It aids in the identification and structural determination of the molecule. There are several techniques to prepare samples for FT-IR analysis. For liquid samples, the simplest method is to insert one drop of material between two plates of sodium chloride. The drop produces a thin layer between the plates. Solid samples can be milled using potassium bromide (KBr) and compressed into thin pellets for analysis. Otherwise, solid samples can be dissolved in a solvent, such as methylene chloride, and a few drops of the solution are put on a single High Attenuated Total Reflectance (HATR) plate, with spectra recorded in terms of % transmittance. The peaks at specified wave numbers were allocated by the bonding and functional groups.

### 2.3.2. Nuclear Magnetic Resonance (NMR) Spectroscopy:

NMR reveals the chemical, biological, and physical characteristics of materials. Although two-dimensional NMR methods might be utilized to get the complex structure of the molecules, one-dimensional approaches are commonly employed. Stable condition Solids' molecular structure may be ascertained via NMR spectroscopy. To determine the different forms of carbon that are present in the chemical, NMR is utilized. H-NMR is used to identify the different forms of hydrogen that are present in the chemical and to determine the bonding between the hydrogen atoms.

### 2.4. Possible Health Advantages of Phytochemicals and Some of the Foods

Phytochemical	Foods	Benefits
Beta and lycopene carotenoids	Cooked tomatoes, carrots, sweet potatoes, orange squash, and green vegetables like broccoli	Could boost immunity, reduce the risk of cardiovascular disease, and stop the growth of cancer cells.
Phenols	Whole grains, coffee, tea, walnuts, citrus fruits, soybeans, berries, and apples	Possibly reduce DNA damage, stop the growth of tumours, and fight inflammation
Anthocyanins	Berries	Possibly advantageous for reducing blood pressure
Sulforaphane, one of the isothiocyanates	Broccoli, cauliflower, and kale are examples of cruciferous vegetables.	Might provide defence against cancer and heart disease
Zeaxanthin and lutein	Leafy greens with dark colours, like chard and spinach	potentially enhance eye health

### 2.4.1. Benefits of Phytochemicals:

- The immune system's operation
- Prevent harm to cells and DNA that could result in cancer.
- Diminish inflammatory response
- Certain cancer cells' development rate can be slowed
- Control hormones
- Safeguard your vision and eye health.
- Boost bone and skin health
- Sustain a healthy weight

### 3. Conclusion:

Phytochemicals are bioactive plant components that can be either nutritious or non-nutritional and can be found in fruits, vegetables, grains, and other plant foods. In addition to providing basic nourishment, they also provide health benefits including reducing the risk of serious chronic illnesses. Carotenoids, polyphenols, isoprenoids, phytosterols, saponins, dietary fibres, and polysaccharides are the major phytochemicals found in plants. The need to extract plant bioactive components is increasing, which drives ongoing research into practical extraction techniques. Conventional techniques can be used to extract these compounds. More comparison research is needed to better understand appropriate extraction methods. Subsequent research endeavours ought to concentrate on contrasting extraction methodologies that operate on analogous concepts. These investigations will offer a more precise and insightful comparison of the methods and solvents employed in the extraction of different phytochemicals.

### References

- Boots, A. W., Haenen, G. R., & Bast, A. (2008). Health effects of quercetin: from antioxidant to nutraceutical. *European Journal of Pharmacology*, 585(2-3), 325-337.
- Cos, P., Vlietinck, A. J., Berghe, D. V., & Maes, L. (2006). Anti-infective potential of natural products: How to develop a stronger *in vitro* 'proof-of-concept'. *Journal of Ethnopharmacology*, 106(3), 290-302.

- Crupi, P., Faienza, M. F., Naeem, M. Y., Corbo, F., Clodoveo, M. L., & Muraglia, M. (2023). Overview of the potential beneficial effects of carotenoids on consumer health and well-being. *Antioxidants*, 12(5), 1069.
- Du, G., Sun, L., Zhao, R., Du, L., Song, J., Zhang, L., He, G., Zhang, Y., & Zhang, J. (2016). Polyphenols: Potential source of drugs for the treatment of ischaemic heart disease. *Pharmacology & Therapeutics*, 162, 23-34.
- Fabricant, D. S., & Farnsworth, N. R. (2001). The value of plants used in traditional medicine for drug discovery. *Environmental Health Perspectives*, 109(11), 69-75.
- Gibson, E. L., Wardle, J., & Watts, C. J. (1998). Fruit and vegetable consumption, nutritional knowledge and beliefs in mothers and children. *Appetite*, 31(2), 205-228.
- Hamburger, M. O., & Cordell, G. A. (1987). A direct bioautographic TLC assay for compounds possessing antibacterial activity. *Journal of Natural Products*, 50(1), 19-22.
- Harborne, A. J. (1998). *Phytochemical Methods A Guide to Modern Techniques of Plant Analysis*. Springer Science & Business Media, (ISBN: 978-0-412-57260-9).
- Heftmann, F. (1992). Chromatography: Fundamentals and Application of Chromatographic and Electrophoretic Methods. Elsevier, Amsterdam. pp. 952.
- Homans, A. L., & Fuchs, A. (1970). Direct bioautography on thin-layer chromatograms as a method for detecting fungi toxic substances. *Journal of Chromatography A*, 51(2), 327-329.
- Ingle, K. P., Deshmukh, A. G., Padole, D. A., Dudhare, M. S., Moharil, M. P., & Khelurkar, V. C. (2017). Phytochemicals: Extraction methods, identification, and detection of bioactive compounds from plant extracts. *Journal of Pharmacognosy and Phytochemistry*, 6(1), 32-36.
- Kumar, J., Park, K. C., Awasthi, A., & Prasad, B. (2015). Silymarin extends lifespan and reduces proteotoxicity in *C. Elegans* Alzheimer's model. *CNS & Neurological Disorders-Drug Targets*, 14(2), 295-302.
- Lampe, J., & Messina, M. (1998). Are phytoestrogens nature's cure for what ails us? A look at the research. Interview by Nancy I. Hahn. *Journal of the American Dietetic Association*, 98(9), 974-977.

- Mathai, K. (2000). Nutrition in the Adult Years. *Krause's Food, Nutrition, & Diet Therapy, 10<sup>th</sup> Edition*, LK Mahan and S. Escott-Stump, 271, 274-275.
- Naczk, M., & Shahidi, F. (2006). Phenolics in cereals, fruits, and vegetables: occurrence, extraction and analysis. *Journal of Pharmaceutical and Biomedical Analysis*, 41(5), 1523-1542.
- Ngozi, E. A., Chinene, M. O., & Florence, I. A. (2014). Phytochemical, proximate, and anti-nutrient compositions of four leafy vegetables used in South Eastern Nigeria. *African Journal of Biotechnology*, 13(50), 4541-4546.
- Puupponen-Pimiä, R., Nohynek, L., Alakomi, H. L., & Oksman-Caldentey, K. M. (2005). Bioactive berry compounds - novel tools against human pathogens. *Applied Microbiology and Biotechnology*, 67(1), 8-18.
- Rao, B. N. (2003). Bioactive phytochemicals in Indian foods and their potential in health promotion and disease prevention. *Asia Pacific Journal of Clinical Nutrition*, 12(1), 9-22.
- Shahverdi, A. R., Abdolpour, F., Monsef-Esfahani, H. R., & Farsam, H. (2007). A TLC bioautographic assay for the detection of nitrofurantoin resistance reversal compound. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*. 850(1-2), 528-530.
- Sutphin, G. L., Bishop, E., Yanos, M. E., Moller, R. M., & Kaeberlein, M. (2012). Caffeine extends life span, improves healthspan, and delays age-associated pathology in *Caenorhabditis elegans*. *Longevity & Healthspan*, 1, 9.
- Velavan, S. (2015). Phytochemical Techniques- A Review. *World Journal of Science and Research*, 1(2), 80-91.
- Xiao, J., & Bai, W. (2019). Bioactive phytochemicals. *Critical Reviews in Food Science and Nutrition*, 59(6), 827-829.
- Zhang, Y. J., Gan, R. Y., Li, S., Zhou, Y., Li, A. N., Xu, D. P., & Li, H. B. (2015). Antioxidant phytochemicals for the prevention and treatment of chronic diseases. *Molecules*, 20(12), 21138-21156.
- Zhao, C., Yang, C., Wai, S. T. C., Zhang, Y., Portillo, M., Paoli, P., Wu, Y., San Cheang, W., Liu, B., Carpéné, C., Xiao, J., & Cao, H. (2019). Regulation of glucose metabolism by bioactive phytochemicals for the management of type 2 diabetes mellitus. *Critical Reviews in Food Science and Nutrition*, 59(6), 830-847.

## Pharmacognosy and Phytochemistry of Flavonoids

S.Ambika,

Assistant Professor,

Department of Biotechnology,

PSG College of Arts & Science, Coimbatore

### Abstract

Flavonoids are secondary metabolites, made of phenolic substances that provide the flavor and color to fruits and vegetables, with six major subclasses: flavones, flavonols, flavanones, catechins, anthocyanidins, and isoflavones. These compounds have significant antioxidant, anticancer, antimicrobial, and anti-inflammatory activity. Over 9,000 different flavonoids have been reported across the kingdoms, the structural and functional diversity has allowed the scientific community to explore their interaction with different targets at the subcellular level. A considerable amount of research is required to understand the mechanism of bioactivity. This review provides a reference for basic and applied research on flavonoid compounds.

**Keywords:** flavonoids; classification; biological activity; application

### Introduction

Flavonoids are natural compounds found in plants with variable phenolic structures. In 1930, a new substance was isolated from oranges and it was believed to be a member of vitamins and was designated as vitamin P, Laterit was identified as a flavonoid (rutin).<sup>(1)</sup> Flavonoids, known for their wide distribution across the plant kingdom are a diverse group of plant secondary metabolites obtained from primary metabolic precursors and are generated via various biosynthetic pathways namely, shikimate, phenylpropanoid, and flavonoids pathway. They are characterized by 15 carbon atoms arranged in three rings (A, B, and C) with a

heterocyclic ring (ring C) linking rings A and B, their polyphenolic structure.(1-3). Flavonoids are polyphenolic compounds based on a C15 (C<sub>6</sub>C<sub>3</sub>C<sub>6</sub>) structure (Fig-1). They contain a chroman ring (C-ring) with a second aromatic ring (B-ring) at the C-2, C-3, or C-4 position.

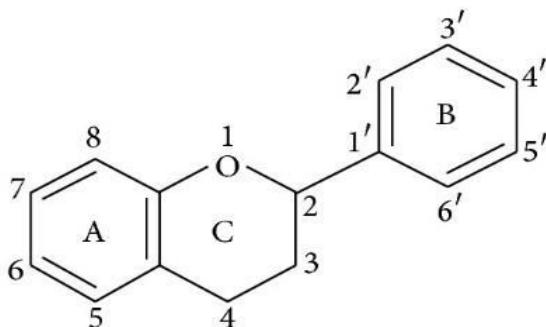


Figure: 1 – Structure of Flavonoid

Flavonoids play essential roles in plants, serving as UV protectants, pigments for flower coloration, and signaling molecules in response to environmental stresses. Additionally, as plant defence mechanisms against pathogens and herbivores. In human nutrition, flavonoids are recognized for their potential health benefits, and antioxidant properties, including anti-inflammatory, anti-cancer, and cardiovascular protective effects. Fruits, vegetables, tea, and red wine are a few dietary sources of flavonoids. The bioactivity and bioavailability of flavonoids depend on chemical structure, food matrix, and metabolic processes in the body. Despite their potential health benefits, optimizing their therapeutic application and understanding the mechanisms of action remains a challenge. (2,3)

In the present review, an attempt is made to discuss the current trends of research and development on flavonoids, working mechanisms, functions, and applications as potential drugs for chronic diseases, and future research directions.

## Classification of Flavonoids

Flavonoids can be subdivided depending on the carbon of the C ring on which the B ring is attached and the degree of unsaturation and oxidation of the C ring. Isoflavones in which the B ring is linked in position 3 of the C ring. Neoflavonoids in which the B ring is linked in position 4 and the B ring is linked in position 2 can be further subdivided based on the structural features of the C ring, as follows: (Fig-2) flavones, flavonols, flavanones, flavanonols, flavanols or catechins, anthocyanins, and chalcones (4,5).

### Flavones

Flavones consist of 4H-chromen-4-one bearing a phenyl substituent at position 2. Flavones mostly occur as 7-O-glycosides, which are found in celery, red pepper, parsley, chamomile, ginkgo, and mint. Apigenin and luteolin are two common flavones. Apigenin can regulate antioxidant enzyme activity and scavenge free radicals. Flavones are one of the largest classes of flavonoids.

### Flavonols

Flavonols, also called 3-hydroxy flavone, have substitutions in their A- and B-rings, which are connected by a three-carbon chain. Flavonols possess hydroxyl groups at positions 5 and 7 in the A-ring. Flavonol bioactivity includes antioxidant, antibacterial, cardioprotective, anticancer, and antiviral activities.

### Flavanones

Flavanones also known as (dihydro-flavones) possess a saturated C-ring. The saturated double bond between positions 2 and 3 in the C-ring. Flavanones are mainly distributed in citrus fruits, contain hydroxyl groups at positions 5 and 7 in the A-ring, and possess hydroxyl/methoxy substituents at the C3 or C4 positions of the B-ring. Naringenin and hesperetin, are the main

dietary flavanones. Naringin can increase the activity of antioxidant enzymes.

## Isoflavonoids

Isoflavones have a B-ring at the C3 position of the heterocyclic C-ring of the diphenylpropane (C6-C3-C6) backbone, which represents their only chemical structural difference from other flavonoids. Isoflavonoids play essential roles in nodule induction and microbial signaling in legumes and are characteristic metabolites of leguminous plants.

## Flavanols

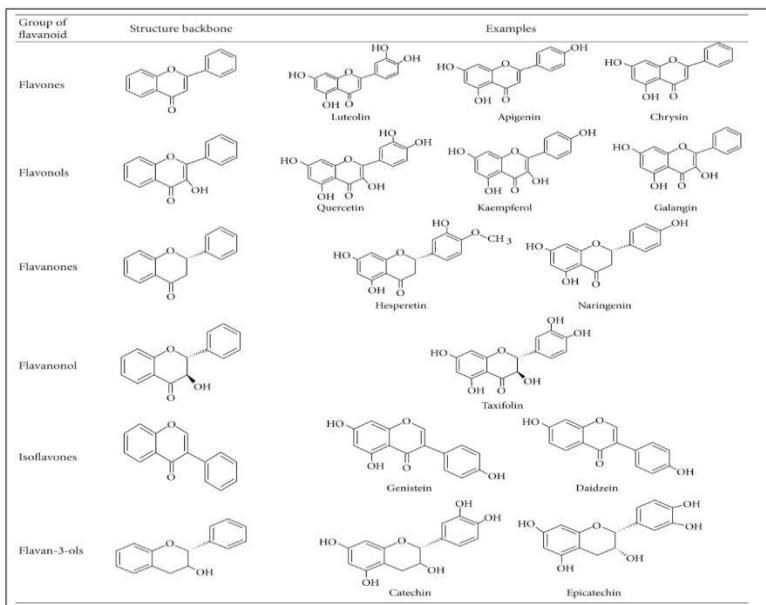
Flavanols, also called flavan-3-ols or catechins, are characterized by a hydroxyl group at position 3 in the C-ring. Flavanols lack a double bond between positions 2 and 3 in the C-ring. Several flavanols, including catechin, gallocatechin 3-gallate, gallocatechin, epicatechin, epicatechin 3-gallate, catechin 3-gallate, and epicatechin 3-gallate, are widely distributed in many fruits

## Anthocyanins

Anthocyanins are glycosylated polyphenolic compounds, soluble vacuolar pigments that possess a range of colors, from orange, red, and purple to blue, depending on the pH of the micro-environment of the flowers, seeds, fruits, and vegetative tissues.

## Chalcones

Chalcones (1,3-diaryl-2-propen-1-ones) are natural open-chain flavonoids, carrying up to three modified or unmodified C5-, C10-, and C15-prenyl moieties on both their A and B-rings. These bioactive products are widely distributed in the Fabaceae, Moraceae, Zingiberaceae, and Cannabaceae families. They exhibit a wide spectrum of pharmacological effects, including antioxidant, antibacterial, anthelmintic, antiulcer, antiviral, antiprotozoal, and anticancer effects.

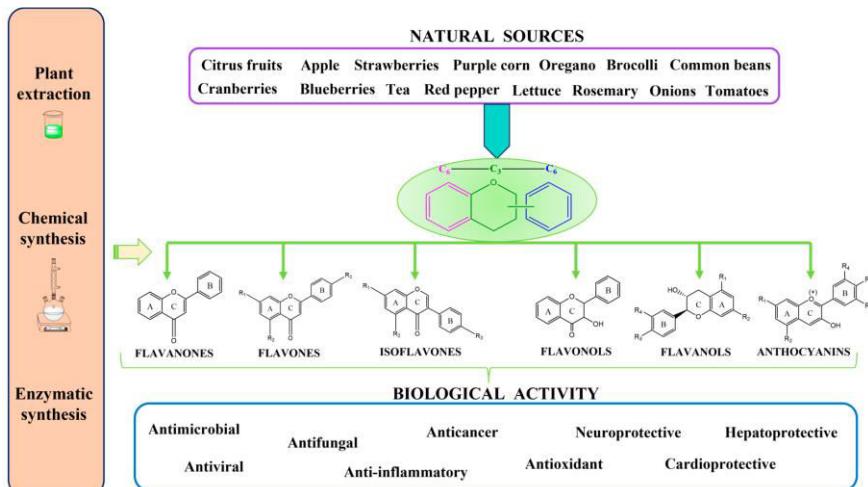


**Figure 2. Classification of Flavonoids**

## Extraction of Flavonoids

Flavonoids are found in fruits, vegetables, seeds, flowers, and other plant parts, as well as in beer, wine, and tea(Fig:3). There are two types of extraction methods for flavonoids: conventional and unconventional (6,7). Various conventional techniques have been proposed, including maceration, boiling, soaking, percolation, hydro-distillation, reflux, and soxhlet. Though these techniques are simple they require large amounts of organic solvents with high purity, with long extraction times, lower extraction yields, low selectivity, and thermal degradation of target compounds. This solvent extraction process is associated with environmental concerns and costs, compared to other techniques. Advanced techniques and strategies are frequently being developed to overcome the limitations of conventional methods for the extraction of these compounds. These techniques include microwave-assisted extraction (MAE), solid phase extraction (SPE), ultrasound-assisted extraction (UAE),

supercritical fluid extraction (SFE), enzyme-assisted extraction, pressurized liquid extraction (PLE or accelerated solvent extraction - ASE), extraction assisted by a pulsed electric field (PEF), and a combination of different techniques. These current techniques are very effective in the extraction of flavonoids from different sources. More significantly, they require less organic solvents and replace them with alternative "green" solvents however attaining high yields in a short period.



**Figure-3** Sources of Flavonoids and Extraction Process Source:  
Liga *et al.*, (2023)

## Spectral Characteristics of Flavonoids

Spectral studies on flavonoids have revealed that most flavones and flavonols display two major absorption bands: Band-I (320–385 nm) represents the B ring absorption, while Band-II (250–285 nm) corresponds to the A ring absorption. A shift in absorption on functional groups attached to the flavonoid skeleton from 367 nm in kaempferol (3,5,7,4'-hydroxyl groups) to 371 nm in quercetin (3,5,7,3',4'-hydroxyl groups) and 374 nm in myricetin (3,5,7,3',4',5'-hydroxyl groups). The lack of a 3-hydroxyl group in flavones differentiates them from flavonols. As determined by their UV spectral characteristics Flavanones have a saturated

heterocyclic C ring, with no conjugation between the A and B rings. Flavanones exhibit a very strong Band-II absorption maximum between 270 and 295 nm, namely, 288 nm (naringenin) and 285 nm (taxifolin), and only a shoulder for Band I at 326 and 327 nm. Band II appears as one peak (270 nm) in compounds with a mono substituted Bring, but as two peaks or one peak (258 nm) with a shoulder (272 nm) when a di-, tri-, or o-substituted B ring is present. As anthocyanins show distinctive Band I peaks in the 450–560 nm region due to the hydroxyl cinnamoyl system of the Bring and Band II peaks in the 240–280 nm region due to the benzoyl system of the A ring, the color of the anthocyanins varies with the number and position of the hydroxyl groups (10).

## Pharmacological Properties of Flavonoids

**Antioxidant Activity:** Flavonoids possess many biochemical groups to act as antioxidants, depending upon the arrangement of functional groups in the nuclear structure. The configuration, substitution, and total number of hydroxyl groups significantly influence several mechanisms of antioxidant activity such as radical scavenging and metal ion chelation ability.

Occurrence, position, structure, and total number of sugar moieties in flavonoids (flavonoid glycosides) play an important role in antioxidant activity. Aglycones are more potent antioxidants than their corresponding glycosides. The most significant is the B ring hydroxyl configuration which is the determinant of scavenging ROS and RNS because it donates hydrogen and an electron to hydroxyl, peroxy, and peroxy nitrite radicals, stabilizing them and making them relatively stable flavonoid radical (11).

Mechanisms of antioxidant action can include (1) suppression of ROS formation either by inhibition of enzymes or by chelating trace elements involved in free radical generation; (2) scavenging of ROS; and (3) upregulation or protection of antioxidant defences.

**Hepatoprotective Activity:** Flavonoids like catechin, apigenin, quercetin, naringenin, rutin, and venoruton are reported for their

hepatoprotective activities. Silymarin can stimulate the enzymatic activity of DNA-dependent RNA polymerase 1 and subsequent biosynthesis of RNA and protein, resulting in DNA biosynthesis and cell proliferation leading to the regeneration of liver (11).

**Antibacterial Activity:** Flavonoids are known to be synthesized by plants in response to microbial infection; several flavonoids including apigenin, galangin, flavone and flavonol glycosides, isoflavones, flavanones, and chalcones have been shown to possess potent antibacterial activity. The mechanism behind this is attributed to alteration of membrane fluidity in hydrophilic and hydrophobic regions reducing the fluidity of the outer and inner layers of membranes. Also, the B ring of the flavonoids may intercalate or form hydrogen bonds with the nucleic acid bases leading to inhibition of DNA and RNA synthesis in bacteria. Naringenin and sophoraflavanone G have intensive antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and *Streptococci*. (12)

**Anti-Inflammatory Activity:** Flavonoids such as hesperidin, apigenin, luteolin, and quercetin are reported to possess anti-inflammatory and analgesic effects. Flavonoids inhibit the kinases by competitively binding with ATP at catalytic sites on the enzymes, especially tyrosine and serine-threonine protein kinases. These enzymes are involved in signal transduction and cell activation processes involving cells of the immune system. It has been reported that flavonoids can inhibit the expression of isoforms of inducible nitric oxide synthase, cyclooxygenase, and lipooxygenase, which are responsible for the production of a great amount of nitric oxide, prostaglandins, leukotrienes, and block the synthesis of inflammatory mediators such as IL-1 $\beta$ , TNF- $\alpha$ , NO, and COX-2, suppress VEGF and ICAM-1 expression, along with the activation of STAT3, NFkB, NLRP3 inflammasome, and MAP kinases pathways (14).

**Anticancer Activity:** Flavonoids have a dual function regarding ROS homeostasis—they act as antioxidants under normal conditions and are potent pro-oxidants in cancer cells triggering the apoptotic

pathways and downregulating pro-inflammatory signaling pathways. A few molecular mechanisms of flavonoids are by down-regulation of mutant p53 protein, cell cycle arrest, inhibition of heat shock proteins, tyrosine kinase, expression of Ras proteins, and estrogen receptor binding capacity. It has been experimentally proved that increased signal transduction in human breast cancer cells is markedly reduced by quercetin acting as an antiproliferative agent. Tyrosine kinase expression is thought to be involved in oncogenesis via an ability to override normal regulatory growth control. Flavonoids inhibiting tyrosine kinase activity have possible antitumor activity without the cytotoxic side effects seen with conventional chemotherapy. Quercetin was the first tyrosine kinase-inhibiting compound tested in a human phase I trial (13,15,16).

**Antiviral Activity:** The flavonoids act at different stages of viral infection, such as viral entrance, replication, and translation of proteins. Flavan-3-ol was more effective than flavones and flavonones in selective inhibition of HIV-1, HIV-2, and similar immunodeficiency virus infections. (17) Catechins are also known to inhibit DNA polymerases of HIV-1. Flavonoids such as demethylatedgardenin A and robinetin are known to inhibit HIV-1 proteinase, various combinations of flavones and flavonols have been shown to exhibit synergism. Kaempferol and luteolin show synergistic effects against herpes simplex virus (HSV). Synergism has also been reported between flavonoids and other antiviral agents.

## Future Prospects

Many reports validating the potential therapeutic role and efficacy of flavonoids in cardiovascular diseases, osteoarthritis, Parkinson's disease, colitis, cancer pain, arthritis, and neuropathic pain are available, yet the mechanisms of action of flavonoids are to be fully elucidated. Indeed, flavonoids are multi-target molecules, and increasing attention has been given to these molecules due to their anti-inflammatory and analgesic properties. Diminishing the activity of varied pathways seems to present

fewer side effects than abolishing the activity of one target, since, the targets also have endogenous physiological roles (18).

Regardless of their broad pharmacological properties, flavonoids show poor water solubility, inadequate permeability, and constrained bioavailability, potentially requiring high doses to show efficacy in humans. Generally, most flavonoids undergo sulfation, methylation, and glucuronidation in the digestive system and liver and conjugated metabolites can be found in plasma after flavonoid ingestion. Nevertheless, some metabolites are still active. In conclusion, given the pharmacological activities of flavonoids, the development of protective delivery formulations, improving intestinal absorption utilizing absorption enhancers, and novel delivery systems; improving metabolic stability; and changing the site of absorption from the large intestine to the small intestine does provide an arena for the researchers. Thus, flavonoid pharmacology, therapeutics, and pharmaceutical development remain a hot topic in inflammatory diseases and pain treatment.

## Conclusion

The therapeutic effects of flavonoids have been proved in the majority of pre-clinical studies. Alternate approaches should be used in clinical trials to enhance the absorption and bioavailability of flavonoids. Further, the conjugates of flavonoids with other important drugs may enhance the potency of those compounds. Conclusively, more research work is needed to produce flavonoids through metabolic engineering and modified biosynthetic pathways to meet the requirements, and pharmacokinetic/pharmacodynamic parameters and toxicological studies, have to be explored. Flavonoid structures will always inspire research for the design and synthesis of new effective drugs for different types of diseases.

## References

- Buer, C.S., Imin, N. and Djordjevic, M.A. (2010), Flavonoids: New Roles for Old Molecules. *Journal of Integrative Plant Biology*, 52: 98-111.
- Chaves JO, de Souza MC, daSilva LC, Lachos-Perez D, Torres-Mayanga PC, Machado APdF, Forster-Carneiro T, Vázquez-Espinosa M, González-de-Peredo AV, Barbero GF and Rostagno MA (2020) Extraction of Flavonoids From Natural Sources Using Modern Techniques. *Front. Chem.* 8:507887.
- Chaves, J. O., De Souza, M. C., Da Silva, L. C., César, P., Machado, A. P., Velasco, A., Barbero, G. F., & Rostagno, M. A. (2020). Extraction of Flavonoids from Natural Sources Using Modern Techniques. *Frontiers in Chemistry*, 8, 507887.
- Chen, S.; Wang, X.; Cheng, Y.; Gao, H.; Chen, X. A Review of Classification, Biosynthesis, Biological Activities and Potential Applications of Flavonoids. *Molecules* 2023, 28, 4982. <https://doi.org/10.3390/molecules28134982>.
- H.Tsuchiya and M. Iinuma, "Reduction of membrane fluidity by antibacterial sophoraflavanone G isolated from *Sophoraexigua*," *Phytomedicine*, vol. 7, no. 2, pp. 161–165, 2000.
- Kopustinskiene, D. M., Jakstas, V., Savickas, A., & Bernatoniene, J. (2020). Flavonoids as Anticancer Agents. *Nutrients*, 12(2), 457. <https://doi.org/10.3390/nu12020457>.
- Liga, S.; Paul, C.; Péter, F. Flavonoids: Overview of Biosynthesis, Biological Activity, and Current Extraction Techniques. *Plants*, 2023, 12, 2732. <https://doi.org/10.3390/plants12142732>.
- Liu W, Feng Y, Yu S, Fan Z, Li X, Li J, Yin H. The Flavonoid Biosynthesis Network in Plants. *International Journal of Molecular Sciences*. 2021; 22(23):12824.
- M. J. Tunon, M. V. Garcia-Mediavilla, S. Sanchez-Campos, and J. Gonzalez-Gallego, "Potential of flavonoids as anti-inflammatory agents: modulation of pro-inflammatory gene expression and signal transduction pathways," *Current Drug Metabolism*, vol. 10, no. 3, pp. 256–271, 2009.

- Martyna Krysa, Monika Szymańska-Chargot, Artur Zdunek, FT-IR and FT-Raman fingerprints of flavonoids – A review, *Food Chemistry* 393 (2022) 133430. <https://doi.org/10.1016/j.foodchem.2022.133430>.
- Middleton EJ. Effect of plant flavonoids on immune and inflammatory cell function. *Advances in Experimental Medicine and Biology*. 1998; 439:175–182.
- Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: an overview. *Journal of nutritional science*, 5, e47.
- Singhal, R. L. Y. A. Yeh, N. Prajda, E. Olah, G. W. Sledge, and G. Weber, "Quercetin down-regulates signal transduction in human breast carcinoma cells," *Biochemical and Biophysical Research Communications*, vol. 208, no. 1, pp. 425–431, 1995.
- Sara Luisa Rodríguez De Luna, R. E. Ramírez-Garza, Sergio O. Serna Saldívar, "Environmentally Friendly Methods for Flavonoid Extraction from Plant Material: Impact of Their Operating Conditions on Yield and Antioxidant Properties", *The Scientific World Journal*, vol. 2020, Article ID 6792069, 38 p, 2020. <https://doi.org/10.1155/2020/6792069>.
- Shashank Kumar, Abhay K. Pandey, "Chemistry and Biological Activities of Flavonoids: An Overview", *The Scientific World Journal*, vol. 2013, Article ID 162750, 16 pages, 2013. <https://doi.org/10.1155/2013/162750>.
- T. P. T. Cushnie and A. J. Lamb, "Antimicrobial activity of flavonoids," *International Journal of Antimicrobial Agents*, vol. 26, no. 5, pp. 343–356, 2005.
- Tariq H, Asif S, Andleeb A, Hano C, Abbasi BH. Flavonoid Production: Current Trends in Plant Metabolic Engineering and De Novo Microbial Production. *Metabolites*. 2023; 13(1):124.
- Ullah, A., Munir, S., Badshah, S. L., Khan, N., Ghani, L., Poulsen, B. G., Emwas, A. H., & Jaremko, M. (2020). Important Flavonoids and their Role as a Therapeutic Agent. *Molecules* (Basel, Switzerland), 25(22), 5243.

## Bioactive Phytochemicals: Isolation, Characterization and Synthesis

Senthilkumar P\*, Anagha A. and Jishiga Jigeesh

Department of Biotechnology,

Hindusthan College of Arts & Science, Coimbatore - 641 028

\* Corresponding Author: senthilkumar.p@hicas.ac.in

### Abstract

A large number of diverse bioactive compounds are produced by plants. Fruits and vegetables are the main sources of natural antioxidants; over 8000 distinct phenolic compounds have been identified. Researchers employ a number of techniques and methodologies to extract, quantify, and identify bioactive chemicals from a wide variety of fruits and vegetables. Phytochemicals help in the prevention of growth of viruses and bacteria in the body and also helps in reducing inflammatory diseases. Due to their positive impact on human health and enormous health benefits for consumers, phytochemicals are of tremendous interest and have great antioxidant potential. The main stages of isolation, characterization and Synthesis of a novel bioactive phytochemical include the isolation, extraction, purification and characterization of the phytochemicals. The isolation and extraction involve many methods like Soxhlet method, maceration, decoction, Microwave assisted, Ultrasound assisted, then these compounds are purified with the help of chromatographic methods which are then later characterized with the help of the spectrophotometric methods like UV-visible spectrophotometry, Nuclear Magnetic Resonance (NMR), Mass spectrometry. The synthesis of the extracted bioactive compounds is done by using cell suspension culture technique. Soft drinks, functional foods, and numerous other food products with high nutritional value and economic significance are made with phytochemicals. Around 5000 years old, Ayurveda primarily

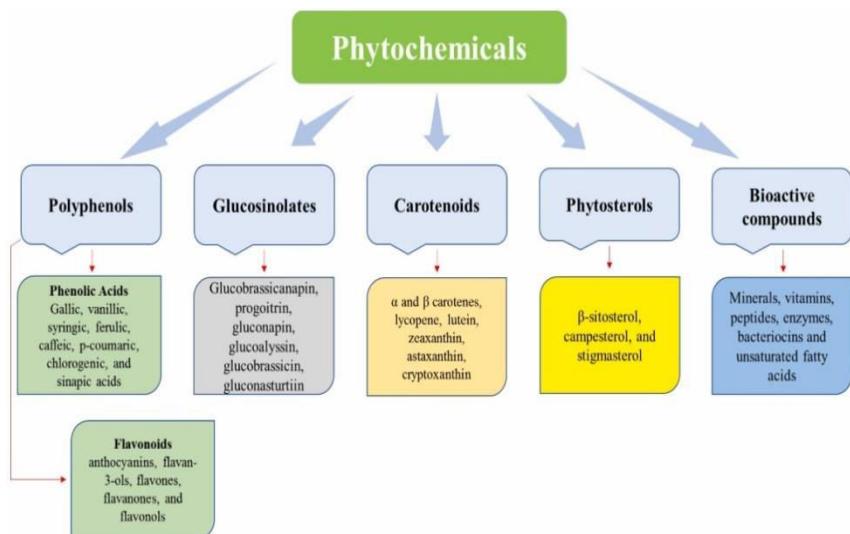
employs phytochemicals in its formulations and preparations. In view of the above, the present chapter highlights the bioactive phytochemical compounds synthesis, isolation and characterization.

## Introduction

Global interest in edible plants has increased due to the growing need for chemical diversity in screening programs and the search for therapeutic medicines from natural sources. Medicinal herbal and botanical preparations contain a variety of bioactive components (1). In many African countries, traditional medicine (TM) practitioners, livestock owners, indigenous people, and rural residents can all benefit from the usage of medicinal plants to cure a variety of illnesses and maladies. If used effectively, the traditional knowledge of medicinal plants can shed light on the critical role that these plants play in the creation of new drugs(2). Plants used for their medicinal purposes contain a wide variety of bioactive compounds, which are the phytochemicals of the whole plants are referred in general. Such high value metabolites have demonstrated exceptional therapeutic potentials in several therapies. They have been used to develop several herbal treatments, natural drugs and standardized extracts have been used for the treatment of many diseases around the world. Bioactive compounds are ideal molecular candidates for enhancement of human health, since the phytochemicals can be used as standard extracts or pure substances. In recent years, the world has witnessed an increase demand for this chemical diversity in screening programs.

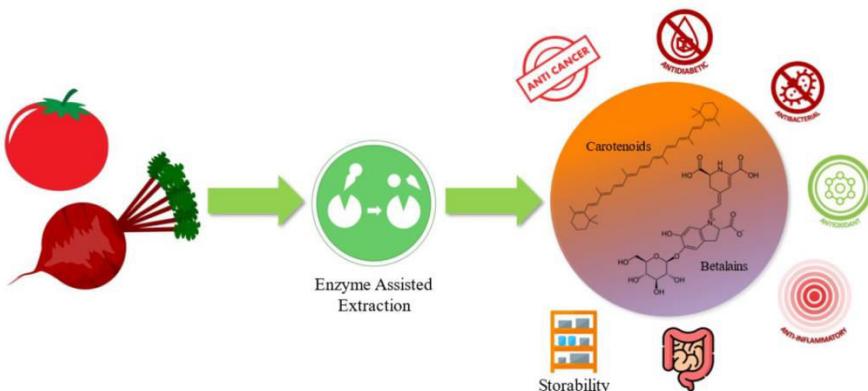
Phytochemicals are natural ingredients active crops. The search for the therapeutic drugs from natural products occupies a prominent place of interest and has focussed primarily on plants. The growing demand for new biomolecules, extraction of bioactive composites from plants and evaluation, both quantitative and qualitative, remain significantly important. The discovery and identification of bioactive chemicals, plant extraction becomes a key research concern. The selection of an extraction strategy is

crucial to drug discovery because extraction is the fundamental first step in medicinal plant research, pharmacognosy, and photochemistry to obtain the targeted molecule. The separation and analysis of the extracted compound of interest is done and then it is characterized (3). The separation of plant extracts bioactive chemicals is also a familiar practice, which would have been instrumental in many ways. Chromatographic techniques like HPLC, TLC, OPLC, GC-MS may be performed to analyse plant extract and detection processes like FTIR, NMR, and MS are employed to identify these bioactive compounds. The properties of these compounds such as its antioxidant capability, antibacterial property are then determined in relation to the simplicity, specificity, and rapidity of the process (4). Finding bioactive substances in natural sources is becoming more and more of a focus for research. The common phytochemicals isolated are mentioned in figure 1.



**Figure 1.** Common type of phytochemical compounds present in almost all types of plants (15)

Consuming phenolics found in food sources may in part lower the chance of developing some chronic illnesses, including hypertension, type 2 diabetes, and improper cholesterol metabolism. Important secondary metabolites found in plants are phenolic chemicals, based on their structural skeletons, they are typically categorised as derivatives of phenolic acids, flavonoids, tannins, lignans, and stilbenes. Numerous biological characteristics, including anti inflammatory, anti diabetic, anticancer, antibacterial, and cytoprotective effects, are displayed by these substances. Among these flavonoids in particular can reduce lipid peroxidation and phenolic have received a lot of attention (5). Lignans have shown promise in hormone-related therapies, particularly in reducing the risk of breast cancer. Additionally, stilbenes like resveratrol are being studied for their neuroprotective effects and potential in managing neurodegenerative diseases. Human health is significantly impacted by the equilibrium between the production and removal of reactive oxygen species (ROS) and free radicals. A disruption in the antioxidant stress, human disorders like aging, atherosclerosis, diabetes mellitus, and rheumatoid arthritis are directly linked to oxidative stress. According to stress reports, oxidative stress can harm islets and reduce insulin gene expression and secretion. Further, alpha glucosidase plays a crucial role in the breakdown of carbohydrates and the consequent uptake of glucose. Therefore, it is imperative to find potent antioxidant components and alpha-glucosidase inhibitors for the treatment of type2 diabetes (5). Because of the unparalleled abundance of chemical variety, natural products derived from the medicinal plants, whether as pure compound or as standardized extracts, offer countless chances for novel therapeutic leads. Growing need for a wider range of chemicals in screening programs, the search for natural compounds that can be used to make therapeutic medications and the increased interest in culinary plants has expanded globally. Medicinally herbal and botanical preparations contain a variety of bioactive components.



**Figure 2.** Some benefits of the bioactive phytochemicals from plants. (16)

This study centres on analytical techniques, encompassing the processes of extracting, isolating, and characterizing active compounds found in botanical and herbal medicines. There is a discussion of the prevalent issues and significant difficulties in the extraction, separation and characterization of active components in botanical and herbal products. Given that extraction is the most crucial stage in the examination of the ingredients found in botanical and herbal remedies, the advantages and disadvantages of various extraction methods are explored. The examination of the bioactive substances found in the plant extracts using the standard phytochemical screening tests and chromatographic methods like TLC and HPLC (6). One of the main techniques which is cost effective and also easier to operate is Thin Layer Chromatography. In many years, ordinary chemistry laboratories have employed thin layer chromatography as a straightforward affordable, and user friendly planar chromatographic technology for the everyday separation of chemical and biological substances. The analyte spots on the TLC plate are often visible using chemical and optical techniques. It is also often used to identify contaminants in a chemical (7). Alkaloids and other bioactive chemicals can be extracted from natural products extracts using the preparative HPLC approach, then the alkaloids were extracted

by maceration and ultrasonication. These analyses were preceded thorough sample preparation. Analyte extraction before chromatographic determination is a major issue in natural products analysis that arises from the complexity of the matrices.

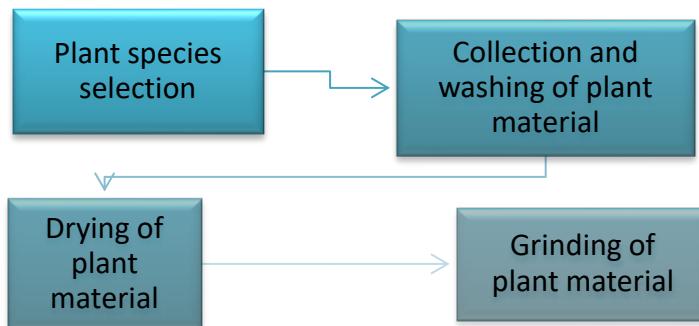
Sample treatment is an important stage in the identification of bioactive chemicals in natural goods and can occasionally impede the advancement of analytical techniques (8). Frequently occurring heterocyclic fragments, polyether units are important synthons for the production of pharmacologically significant substances and are found in many natural products of major biological significance. Similar to molecules with alkyl branched hydrocarbon chains that are stereo chemically specified are widely found in nature developing novel synthetic techniques to generate them in high yields and with high degrees of stereo control is currently a difficult task in organic synthesis (9). The bioactive components are very much essential, these are extracted from plants and they are required for the human beings in various fields specially in preparing medicine and formulation of various drugs. There are uses of various instruments to characterize the bioactive compounds from plants includes HPLC, HPTLC, TLC. There are even uses of Nuclear Magnetic Resonance, Mass spectrometry, Infrared spectroscopy and UV-visible spectroscopy.

## Materials and methods

Phytochemicals are the active constituents derived naturally from plants. Find a novel biomolecules, it is necessary to do quantitative and qualitative assessment of the extracted bioactive composites from plants. Plant extraction and separation are still important issues in the identification and characterization of bioactive chemicals. The major stages included in the isolation, characterization and synthesis of bioactive phytochemicals are: Isolation of plant based bioactive compounds, Extraction of bioactive compounds, Purification of the extracted bioactive phytochemicals, and Characterization of the bioactive phytochemicals.

## Isolation of plant based bioactive compounds

The bioactive compounds present in plants could be isolated by various methods. There are many steps involved in the isolation of bioactive phytochemicals from the plants, which is given in the figure 3.



**Figure 3.** Basic steps involved in isolation of bioactive compounds from plants

### Plant species selection

Plant species and plant materials are to be selected at randomly using previous literature. The random screening method, ethnobotanical bioprospecting method and chemotaxonomy method can be used.

### Collection and washing of plant material

One of the most important parts of isolation of plant material is the part of plant from which it is extracted. Depending on where the metabolites are gathered and kept, aerial portions such as flowering tops, leaves, stems, barks, seed and fruits and the subterranean parts like bulbs, roots are collected and preserved.

### Drying of plant material

After being collected, the specimens are properly ventilated and dried at room temperature on trays. This is very important to stop

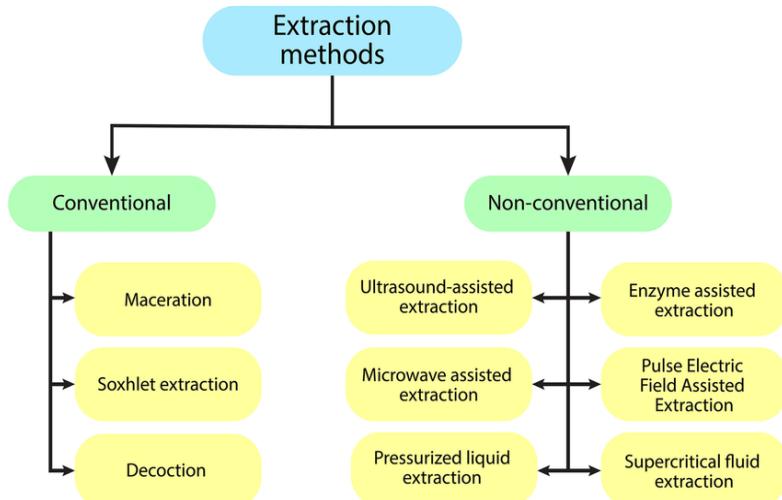
the microbial growth and thus less and subsequent loss of plant components (10).

## Grinding of the plant material

The dried plant specimens are then ground into a fine powder. This powder could be used fresh or could be stored at 4°C and can be used later for the extraction. It is more favourable to use the fresh specimen.

## Extraction of bioactive phytochemicals

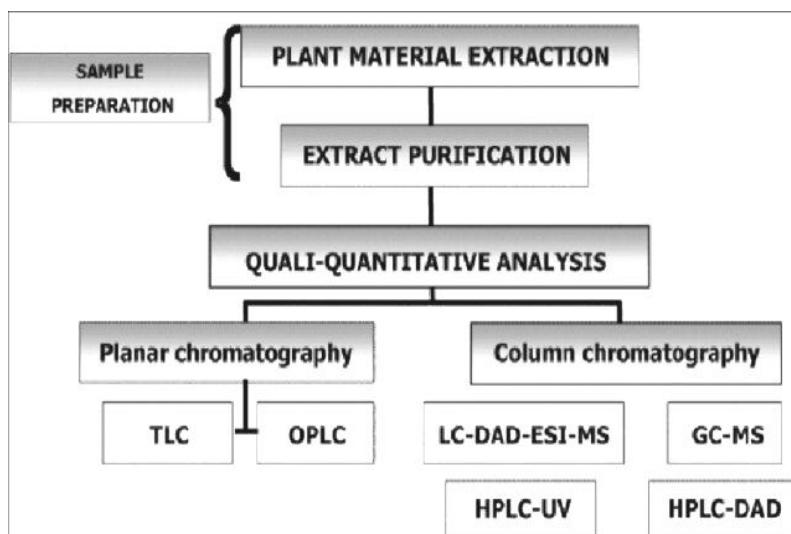
The extraction process typically includes the following processes: solvent extraction, distillation, compression and sublimation. There are many types of solvents used for extraction, which includes water, ethanol, acetone, methanol, benzene, petroleum ether, chloroform. There are many methods followed for extraction of phytochemicals which is given in the figure 4.



**Figure 4.** Different types of extraction methods used for phytochemical isolation. (11)

## Purification of the extracted bioactive phytochemicals

The targeted bioactive phytochemical's physical characteristics like their solubility, molecular weight, stability, dipole moment are essential for a successful isolation strategy (6). Plant metabolites are isolated via chromatographic techniques like TLC, Column chromatography, Gas chromatography, HPLC, and HPTLC (Figure 5).



**Figure 5.** Various Chromatographic techniques employed for purification of bioactive phytochemicals (12)

## Characterization of the bioactive phytochemicals

Variety of spectroscopic methods, including UV-visible, infrared (IR), Nuclear Magnetic Resonance (NMR), and Mass spectroscopy are used to determine the structure of certain compounds (1).

## UV-visible spectroscopy

It is possible to identify certain groups of substances in both pure and biological mixtures and conduct qualitative analyses using this technique. The UV-visible spectroscopy can identify phenolic compounds, such as anthocyanins tannins, polymer dyes and phenol complexes with iron in them.

## Nuclear Magnetic Resonance

The main focus of NMR is on the magnetic characteristics of certain atomic nuclei, such as the hydrogen atom nucleus, the proton, the carbon atom, and an isotope of carbon. It provides a clear image of the locations of the magnetic nuclei inside the molecule. It'll also show which atoms are found in the nearby groupings.

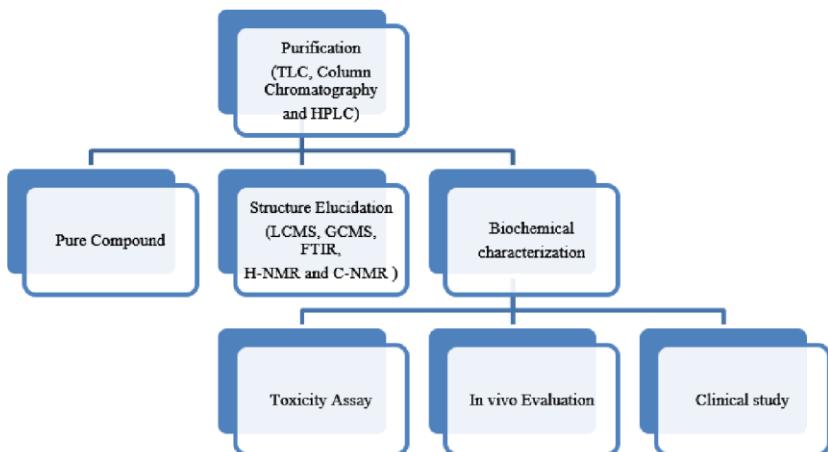
## Mass spectrometry

In the Mass spectroscopy, organic molecules are blasted with electrons or a laser, which transforms them into very energetic charged ions. The ratio between anions' mass and charge is measured. This ratio m/c and the location of the molecule's fragments are known; a molecule's molecular mass could be calculated.

## Infrared spectroscopy

When infrared light travels through an organic compound, some of the frequencies will be absorbed, other frequencies will pass through the sample unbated. The vibrational alterations thus taking place within a molecule upon exposure to infrared are associated with infrared absorption. The different types of bonds like C-C, C=C, C≡C, C-O, C=O, O-H, and N-H are observed.

**Bioactive profiling:** It reveals the different pharmacological properties of the isolated phytochemicals. In this, we analyse the properties such as antioxidant property, antimicrobial property, anticancer and antiinflammatory activities.



**Figure 6.** Methods used for characterization and profiling of the bioactive compounds (13)

## Results and Discussion

The bioactive phytochemicals are isolated by various conventional and non-conventional methods which are then purified and characterized for its biological, chemical and physical properties. According to the WHO organization, majority of the people are still depending on traditional medicines. There are many medicines produced from the bioactive compounds of various plants. There are many compounds found in plants which are used in traditional medicine that can be utilized to treat both infectious and chronic illnesses (14).

The bioactive phytochemicals are extracted from the plant sample usually by the methos like Maceration, Soxhlet extraction, Decoction, ultrasound- assisted extraction, and enzyme assisted extraction. The solvents used for the extraction process are ethyl alcohol, water, benzene, methanol, chloroform and other organic solvents. The better solvents were found to be water and ethyl alcohol for the extraction of phytochemicals from the medicinal

plants. This was due to higher affinity of the phytochemicals towards water and ethanol.

The extract is then purified by using the chromatographic techniques like HPLC, HPTLC, TLC, and GC once they are purified, they are sent for the characterization. The extracted phytochemicals were screened based on their physical, chemical and moreover, the most important, biological properties by using various spectroscopic techniques like UV-visible spectroscopy, NMR spectroscopy, FTIR spectroscopy, Mass spectrometry. The biological properties were assessed with the help of the various biological and biochemical assays to understand how effective the biologically active compound is. As the bioactive phytochemicals are purified and characterized, are then synthesized in bulk, usually by using cell suspension culture.

## Conclusion

In this chapter, the detailed protocol of isolation, purification, characterization and synthesis are discussed. The bioactive phytochemicals are those compounds extracted from the plants that have a very high biological activity and are very useful in treating many infections and diseases. The isolation of phytochemical, their extraction by numerous conventional and non-conventional methods, purification by chromatographic and other analytical techniques and the characterization of the compound by using a wide range of spectroscopic techniques, immune-assays, biological assays and biochemical assays that will determine the properties of the extracted phytochemicals and will let us know how much effective the compound is. In the preliminary screening, we can identify the required bioactive phytochemical and start synthesizing it in a larger scale using cell suspension culture.

## References

- Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G., & Lightfoot, D. A. (2017). Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*, 6(4), 42. Chicago
- Atlabachew, M., Chandravanshi, B. S., & Redi-Abshire, M. (2017). Preparative HPLC for large scale isolation, and salting-out assisted liquid-liquid extraction-based method for HPLC-DAD determination of khat (*Catha edulis* Forsk) alkaloids. *Chemistry Central Journal*, 11, 1-10.
- Dawurung, C. J., Nguyen, M. T., Pengon, J., Dokladda, K., Bunyong, R., Rattanajak, R., & Pyne, S. G. (2021). Isolation of bioactive compounds from medicinal plants used in traditional medicine: Rautandiol B, a potential lead compound against *Plasmodium falciparum*. *BMC Complementary Medicine and Therapies*, 21, 1-12.
- Diaz, D. D., Betancort, J. M., & Martin, V. S. (2007). The Nicholas reaction: a powerful tool for the stereoselective synthesis of bioactive compounds. *Synlett*, 2007(03), 0343-0359.
- Duraipandiyan, V., & Ayyanar, M. S. Ignacimuthu.(2006). Antimicrobial activity of some ethnomedicinal plants used by paliyar tribe from Tamilnadu, India. *BMC Complementary and alternative medicine*, 6, 35.
- Kumar, S., Jyotirmayee, K., & Sarangi, M. (2013). Thin layer chromatography: a tool of biotechnology for isolation of bioactive compounds from medicinal plants. *International Journal of Pharmaceutical Sciences Review and Research*, 18(1), 126-132.
- Lombardelli, C., Benucci, I., Mazzocchi, C., & Esti, M. (2022). Green enzymatic recovery of functional bioactive compounds from unsold vegetables: storability and potential health benefits. *Applied Sciences*, 12(23), 12249.
- Pai, S., Hebbar, A., & Selvaraj, S. (2022, March 2). A critical look at challenges and future scopes of bioactive compounds and their incorporations in the food, energy, and pharmaceutical

sector. Environmental Science and Pollution Research International.

- Pradhan, D., Tripathy, G., Mohapatra, R., & Pradhan, S. (2015). Ethnopharmacological Approach in Extraction, Isolation and Characterization of Bioactive Compounds.
- Roopashree, K. M., & Naik, D. (2019). Advanced method of secondary metabolite extraction and quality analysis. *Journal of Pharmacognosy and Phytochemistry*, 8(3), 1829-1842.
- Sarker, S. D., Latif, Z., & Gray, A. (2006). An introduction to natural products isolation. *Methods Mol Biol*, 864, 1-25.
- Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., & Latha, L. Y. (2011). Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African journal of traditional, complementary and alternative medicines*, 8(1).
- Siddiqui, S. A., Khan, S., Mehdizadeh, M., Bahmid, N. A., Adli, D. N., Walker, T. R., & Câmara, J. S. (2023). Phytochemicals and bioactive constituents in food packaging: A systematic review. *Heliyon*.
- Sruthi, D., Dhanalakshmi, M., Rao, H. Y., Parthasarathy, R., & Jayabaskaran, C. (2023). Extraction, isolation, and characterization of phytochemicals, the bioactive compounds of plants. In *Recent Frontiers of Phytochemicals* (pp. 1-8). Elsevier.
- Thakur, R., Mehta, N., Singh, A., Joshi, K., Gautam, A., & Bithel, N. (2022). Techniques for the Isolation of Plant-Based Bioactive Compounds. In *Isolation, Characterization, and Therapeutic Applications of Natural Bioactive Compounds* (pp. 280-296). IGI Global.
- Zhao, T., Sun, M., Kong, L., Xue, Q., Wang, Y., Wang, Y., & Cheng, G. (2021). Bioactivity-guided isolation of phytochemicals from *Vaccinium dulanianum* Wight and their antioxidant and enzyme inhibitory activities. *Molecules*, 26(7), 2075.

## Mechanisms of Action of Phytochemical in Disease Prevention and Treatment

**Dr. R. Ravi<sup>1</sup>, Dr. M. Murugan<sup>2</sup> and Dr. B. Shanmugapriya<sup>3</sup>**

<sup>1</sup>Assistant Professor & Head, Department of Biotechnology,  
Jairam Arts & Science College, Salem - 636 008

<sup>2</sup>Assistant Professor & Head, Department of Biotechnology,  
Sowdeshwari College, Salem - 636 010

<sup>3</sup>Assistant Professor, Department of Biotechnology,  
Vivekanandha Arts and Science College for Women,  
Sangari, Salem - 637 303

### Abstract

Secondary metabolites, synthesized by diverse organisms like plants, fungi, and bacteria, serve specialized roles like defense and attraction. Alkaloids, derived from amino acids, hold historical medicinal uses, while terpenoids, from isoprene units, aid in plant defense and have commercial applications. Phenolic compounds, with phenol rings, contribute to plant physiology and human health, especially through antioxidant properties. Polyketides possess significant pharmaceutical importance. These bioactive compounds impact human health positively, exhibiting antioxidant, anti-inflammatory, immuno-modulatory, antimicrobial, antiviral, and anticancer effects. Understanding their biosynthesis, functions, and ecological roles is vital for medical, agricultural, and industrial applications. Phytochemicals, abundant in plant-based diets, offer therapeutic potential through antioxidant, anti-inflammatory, immunomodulatory, antimicrobial, and anticancer effects, targeting specific receptors and enzymes crucial in disease pathways. Comprehending their mechanisms and clinical efficacy is essential for natural drug development and human health promotion. Further research into their applications will enhance disease prevention and therapy.

## Introduction to Secondary Metabolites

Secondary metabolites are organic compounds produced by plants, fungi, bacteria, and other organisms. They are distinct from primary metabolites, which are essential for growth, development, and basic cellular functions (Dixon & Strack 2003). Unlike primary metabolites, secondary metabolites are not directly involved in the organism's primary metabolic processes but often serve specialized functions such as defense against predators, attraction of pollinators, competition with other organisms, and adaptation to environmental stressors (Wink, 2010).

These molecules exhibit remarkable structural diversity, with tens of thousands of different compounds identified to date (Pichersky & Lewinsohn 2011). They can be broadly classified into several major groups based on their chemical structures and biosynthetic origins. These groups include alkaloids, terpenoids, phenolic compounds, polyketides, and others (Wink, 2010). Each group encompasses numerous subclasses and individual compounds, each with unique properties and biological activities.

Alkaloids are nitrogen-containing compounds derived from amino acids and are widely distributed across the plant kingdom. They exhibit diverse pharmacological effects and have been utilized for centuries by humans for medicinal, recreational, and ritualistic purposes (Roberts & Wink 1998). Examples of alkaloids include morphine, caffeine, nicotine, and quinine.

Terpenoids, also known as isoprenoids, constitute another major class of secondary metabolites. These compounds are derived from the isoprene unit and are characterized by their structural diversity and wide range of biological activities (Tholl, 2015). Terpenoids play crucial roles in plant defense, communication, and adaptation to environmental stressors. They also have significant commercial value as flavors, fragrances, pharmaceuticals, and industrial chemicals.

Phenolic compounds represent a diverse group of secondary metabolites characterized by their phenol rings and various functional groups. These compounds are ubiquitous in plants and contribute to numerous physiological processes, including UV protection, defense against pathogens and herbivores, and regulation of growth and development (Dixon & Paiva, 1995). Phenolic compounds have attracted considerable attention due to their antioxidant properties and potential health benefits. Examples include flavonoids, tannins, lignans, and phenolic acids.

Polyketides are a structurally diverse class of secondary metabolites synthesized by bacteria, fungi, and plants through the polyketide biosynthetic pathway. These compounds exhibit a wide range of biological activities, including antimicrobial, antitumor, and immunosuppressive effects (Hertweck, 2009). Polyketides have significant pharmaceutical importance and have served as lead compounds for the development of numerous drugs, including antibiotics, anticancer agents, and immunosuppressants.

Secondary metabolite biosynthesis, governed by intricate enzymatic pathways, utilizes primary metabolism precursors (Bohlmann & Keeling, 2008). Regulatory mechanisms respond to developmental, environmental, and physiological cues, governing gene expression and metabolite production. Phytochemicals, alkaloids, terpenoids, phenolics, and polyketides, synthesized by various organisms, exhibit bioactive properties beneficial to human health. For instance, flavonoids and polyphenols, abundant in plants, possess antioxidant abilities, countering oxidative stress (Pandey & Rizvi, 2009; Calder *et al.*, 2020). Secondary metabolites play vital roles in defense, communication, stress adaptation, and physiological regulation, offering potential for medicinal, agricultural, and industrial applications (Demain & Fang, 2000). They provide natural alternatives in supplements and functional foods, contributing to wellness (Cirmi *et al.*, 2016).

Therefore, secondary metabolites represent a valuable resource for disease prevention in humans, offering a wide range

of bioactive compounds with diverse therapeutic potentials. Research into the biosynthesis, function, and applications of these compounds is essential for unlocking their full potential in promoting human health and well-being.

## Prevention Actions of Secondary Metabolites

### Antioxidant Activity:

Phytochemicals, found abundantly in various plant-based foods, exhibit diverse modes of action in antioxidant activity, playing a crucial role in promoting health and preventing diseases. Firstly, these bioactive compounds serve as potent free radical scavengers, effectively neutralizing reactive oxygen species (ROS) by donating electrons. This scavenging action helps prevent oxidative damage to cellular components such as proteins, lipids, and DNA, thus mitigating the risk of oxidative stress-related diseases (Halliwell, 2007). In addition to their direct antioxidant effects, certain phytochemicals possess metal-chelating properties, enabling them to bind to transition metal ions like iron and copper. By sequestering these metal ions, phytochemicals can inhibit their ability to catalyze ROS generation via Fenton chemistry, thereby reducing oxidative stress within cells (Ninfali *et al.*, 2005).

Moreover, phytochemicals have been demonstrated to modulate the activity of endogenous antioxidant enzymes. These compounds can upregulate the expression and activity of enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase. By enhancing the cellular antioxidant defense system, phytochemicals bolster the detoxification of ROS and maintain redox homeostasis, thereby conferring protection against oxidative damage (Valko *et al.*, 2007).

Furthermore, phytochemicals exert their antioxidant effects through the modulation of various signaling pathways involved in oxidative stress responses and inflammation. These compounds can target key molecular signaling cascades, such as nuclear

factor- $\kappa$ B (NF- $\kappa$ B) and mitogen-activated protein kinases (MAPKs), which regulate the expression of genes involved in antioxidant defense and inflammatory processes. By modulating these pathways, phytochemicals exert anti-inflammatory and antioxidant effects at the molecular level, contributing to their overall health-promoting properties (Lee, Bode, & Dong, 2011).

The phytochemicals exhibit multiple modes of action in antioxidant activity, including free radical scavenging, metal chelation, induction of antioxidant enzymes, and modulation of signaling pathways. Through these mechanisms, phytochemicals help mitigate oxidative stress, a critical factor in the development of various chronic diseases, including cancer, cardiovascular disorders, and neurodegenerative conditions. Incorporating phytochemical-rich foods into the diet can thus provide an effective strategy for enhancing antioxidant defenses and promoting overall health and well-being.

### **Anti-inflammatory Effects:**

Phytochemicals, compounds found in plants, exert anti-inflammatory effects through diverse mechanisms, contributing to their potential in promoting health and mitigating inflammatory diseases. Firstly, these bioactive compounds possess direct anti-inflammatory properties by inhibiting the activity of enzymes involved in the production of inflammatory mediators, such as cyclooxygenase (COX) and lipoxygenase (LOX). By blocking the synthesis of prostaglandins and leukotrienes, phytochemicals attenuate the inflammatory response and alleviate associated symptoms (Gupta, Patchva & Aggarwal 2013).

Additionally, phytochemicals exhibit antioxidant activity, which plays a crucial role in combating inflammation. By scavenging free radicals and reducing oxidative stress, these compounds mitigate the activation of inflammatory signaling pathways and the production of pro-inflammatory cytokines. Moreover, certain phytochemicals can upregulate the expression of endogenous antioxidant enzymes, further enhancing the

cellular defense against oxidative damage-induced inflammation (Halliwell, 2007).

Furthermore, phytochemicals modulate immune responses by regulating the activity of immune cells and the production of cytokines. For example, some compounds can inhibit the activation of nuclear factor-kappa B (NF- $\kappa$ B), a key transcription factor involved in the expression of inflammatory genes. By suppressing NF- $\kappa$ B activation, phytochemicals reduce the secretion of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF- $\alpha$ ) and interleukin-6 (IL-6), thereby attenuating inflammation (Pan & Lai 2002).

Moreover, phytochemicals exert anti-inflammatory effects by interfering with cellular signaling pathways involved in inflammatory processes. These compounds can target various kinases and transcription factors, including MAPKs and signal transducer and activator of transcription proteins (STATs), which regulate the expression of inflammatory genes. By modulating these signaling pathways, phytochemicals inhibit the production of inflammatory mediators and suppress inflammatory responses (Surh *et al.*, 2001).

The phytochemicals exert anti-inflammatory effects through multiple mechanisms, including inhibition of inflammatory enzyme activity, antioxidant activity, modulation of immune responses, and interference with inflammatory signaling pathways. These compounds hold promise as natural agents for managing inflammatory conditions and promoting overall health and well-being.

### **Immunomodulatory Properties:**

Phytochemicals, bioactive compounds found in plant-based foods, exhibit immunomodulatory properties through various mechanisms, contributing to their potential in promoting immune health and modulating immune responses. Firstly, these compounds can directly influence immune cell function by

regulating the proliferation, differentiation, and activity of immune cells such as T cells, B cells, macrophages, and natural killer (NK) cells. For example, certain phytochemicals have been shown to enhance T cell activation and proliferation, leading to improved immune surveillance and response to pathogens (Yin *et al.*, 2018).

Moreover, phytochemicals exert their immunomodulatory effects by regulating the production and secretion of cytokines, which are key mediators of immune responses. These compounds can modulate the expression of pro-inflammatory and anti-inflammatory cytokines, helping to balance immune responses and maintain immune homeostasis. For instance, some phytochemicals have been found to suppress the production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF- $\alpha$ ) and interleukin-6 (IL-6), while enhancing the secretion of anti-inflammatory cytokines such as interleukin-10 (IL-10) (Li *et al.*, 2016).

Additionally, phytochemicals exhibit antioxidant activity, which can indirectly modulate immune function by reducing oxidative stress and inflammation. Oxidative stress has been implicated in immune dysfunction and inflammatory diseases, and phytochemicals with antioxidant properties help alleviate oxidative damage, thereby supporting optimal immune function. Furthermore, certain phytochemicals can upregulate the expression of endogenous antioxidant enzymes, further enhancing cellular antioxidant defenses and promoting immune health (Sies, 2017).

Furthermore, phytochemicals may interact with pattern recognition receptors (PRRs) on immune cells, such as toll-like receptors (TLRs), influencing immune cell activation and cytokine production in response to pathogen-associated molecular patterns (PAMPs). By modulating PRR signaling pathways, phytochemicals can regulate innate immune responses and contribute to the host defense against infections (Yang *et al.*, 2016).

The phytochemicals possess immunomodulatory properties through various mechanisms, including direct effects on immune cell function, regulation of cytokine production, antioxidant activity, and modulation of PRR signaling pathways. These compounds play a crucial role in supporting immune health and may have therapeutic potential in managing immune-related disorders.

### **Anti-microbial and Anti-viral Activity:**

Phytochemicals, bioactive compounds found in plants, exhibit remarkable anti-microbial, anti-viral, and anti-fungal properties, offering promising avenues for natural antimicrobial therapies. Firstly, these compounds can directly inhibit the growth and replication of microorganisms by disrupting their cellular structures or metabolic processes. For instance, certain phytochemicals such as alkaloids, flavonoids, and tannins have been shown to interfere with microbial cell membrane integrity, leading to leakage of cellular contents and eventual cell death (Cowan, 1999).

Moreover, phytochemicals possess antimicrobial activity by targeting specific microbial enzymes or proteins essential for their survival and virulence. For example, polyphenolic compounds like resveratrol and catechins can inhibit the activity of microbial proteases and DNA gyrase, disrupting essential cellular functions and inhibiting microbial growth (Gibson *et al.*, 1999). Additionally, phytochemicals may interfere with microbial adhesion and biofilm formation, preventing the colonization of surfaces and tissues by pathogenic microorganisms (Nostro *et al.*, 2007).

Furthermore, phytochemicals exhibit anti-viral properties by interfering with viral entry, replication, and assembly. These compounds can target viral envelope proteins, viral enzymes, or host cell receptors involved in viral infection. For instance, flavonoids like quercetin and epigallocatechin gallate (EGCG) have been shown to inhibit viral attachment and entry into host

cells by blocking viral glycoprotein interactions with cellular receptors (Cushnie & Lamb 2005).

Additionally, phytochemicals can modulate host immune responses to combat viral infections. These compounds can enhance innate immune defenses by stimulating the production of antiviral cytokines and promoting the activity of natural killer cells and macrophages. Furthermore, certain phytochemicals possess immunomodulatory properties that help regulate excessive inflammation and cytokine storms associated with severe viral infections (Saikia *et al.*, 2017).

Moreover, phytochemicals exhibit anti-fungal properties by disrupting fungal cell membrane integrity, inhibiting fungal cell wall synthesis, or interfering with fungal nucleic acid synthesis. Compounds such as alkaloids, terpenoids, and phenolic compounds have been shown to possess potent antifungal activity against a wide range of pathogenic fungi, including *Candida* species and dermatophytes (Nascimento *et al.*, 2000). The phytochemicals demonstrate diverse mechanisms of action in exerting antimicrobial, antiviral, and antifungal properties, including direct inhibition of microbial growth and virulence, interference with viral entry and replication, modulation of host immune responses, and disruption of fungal cellular processes. These bioactive compounds hold great potential for the development of natural antimicrobial agents and may offer effective alternatives to conventional antibiotics and antiviral drugs.

### **Anti-cancer Effects:**

Phytochemicals, bioactive compounds derived from plants, possess multifaceted mechanisms of action in exerting anti-cancer effects, offering promising avenues for cancer prevention and therapy. Firstly, these compounds can inhibit cancer cell proliferation by inducing cell cycle arrest at various checkpoints. For instance, polyphenols such as resveratrol and curcumin have been shown to block the cell cycle progression of cancer cells by

regulating the expression of cyclins, cyclin-dependent kinases (CDKs), and cell cycle inhibitors, leading to the suppression of tumor growth (Aggarwal *et al.*, 2003).

Moreover, phytochemicals exhibit anti-cancer properties by promoting apoptosis, or programmed cell death, in cancer cells. These compounds can activate intrinsic and extrinsic apoptotic pathways, leading to the activation of caspases and the cleavage of cellular substrates, ultimately resulting in cell death. For example, flavonoids like quercetin and genistein have been demonstrated to induce apoptosis in various cancer cell lines by modulating Bcl-2 family proteins and death receptor signaling pathways (Shanmugam *et al.*, 2015).

Additionally, phytochemicals possess anti-cancer effects by inhibiting angiogenesis, the process of new blood vessel formation that is essential for tumor growth and metastasis. Compounds such as epigallocatechin gallate (EGCG) and lycopene can suppress the expression and activity of angiogenic factors like vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs), thereby inhibiting tumor angiogenesis and neovascularization (Siddiqui *et al.*, 2009).

Furthermore, phytochemicals exhibit anti-cancer properties by modulating signaling pathways involved in cell survival, proliferation, and metastasis. These compounds can target key oncogenic signaling cascades, including the PI3K/Akt/mTOR pathway, the MAPK pathway, and the NF-κB pathway, leading to the inhibition of cancer cell growth and the suppression of tumor progression (Surh *et al.*, 2008).

Moreover, phytochemicals possess anti-inflammatory effects, which contribute to their anti-cancer properties. Chronic inflammation is closely linked to cancer development and progression, and phytochemicals with anti-inflammatory activity can inhibit inflammatory signaling pathways and the production of pro-inflammatory cytokines, thereby reducing the risk of cancer

initiation and progression (Pan *et al.*, 2009). The phytochemicals exert anti-cancer effects through multiple mechanisms, including inhibition of cancer cell proliferation, promotion of apoptosis, suppression of angiogenesis, modulation of signaling pathways, and anti-inflammatory activity. These bioactive compounds hold great promise as natural agents for cancer prevention and therapy, and further research into their mechanisms of action and clinical efficacy is warranted.

## Modulation of Cellular Signaling Pathways

Phytochemicals, bioactive compounds derived from plants, play a crucial role in modulating cellular signaling pathways, influencing various physiological processes and contributing to their potential health benefits. Firstly, these compounds can target key signaling molecules and receptors involved in cell proliferation, differentiation, and survival. For example, polyphenols such as epigallocatechin gallate (EGCG) and curcumin have been shown to inhibit the activity of receptor tyrosine kinases (RTKs) and downstream signaling pathways like the PI3K/Akt and MAPK pathways, leading to the suppression of cancer cell growth and proliferation (Surh *et al.*, 2013).

Moreover, phytochemicals exhibit anti-inflammatory effects by modulating signaling pathways involved in immune responses and inflammatory processes. Compounds like resveratrol and quercetin can inhibit the activation of nuclear factor-kappa B (NF- $\kappa$ B) and activator protein-1 (AP-1), transcription factors that regulate the expression of pro-inflammatory genes. By suppressing NF- $\kappa$ B and AP-1 activation, phytochemicals reduce the production of pro-inflammatory cytokines and chemokines, thereby alleviating inflammation (Pan *et al.*, 2010).

Additionally, phytochemicals can influence signaling pathways involved in oxidative stress responses and cellular redox balance. These compounds can activate antioxidant response pathways, such as the Nrf2-Keap1 pathway, leading to the upregulation of antioxidant enzyme expression and enhanced

cellular defense against oxidative damage. For instance, sulforaphane, a phytochemical found in cruciferous vegetables, has been shown to activate Nrf2 and induce the expression of phase II detoxification enzymes, protecting cells from oxidative stress-induced damage (Kensler *et al.*, 2007).

Furthermore, phytochemicals can modulate signaling pathways involved in apoptosis, or programmed cell death. Compounds like resveratrol and genistein can activate intrinsic apoptotic pathways by regulating the expression of Bcl-2 family proteins and promoting the release of cytochrome c from mitochondria. Moreover, some phytochemicals can induce extrinsic apoptotic pathways by activating death receptors like Fas and TRAIL receptors, leading to caspase activation and apoptotic cell death (Maioli *et al.*, 2010).

Moreover, phytochemicals possess neuroprotective effects by modulating signaling pathways involved in neuronal survival and synaptic plasticity. Compounds such as flavonoids and polyphenols can activate signaling pathways like the PI3K/Akt and BDNF/TrkB pathways, which promote neuronal survival, growth, and synaptic function. By enhancing neurotrophic signaling, phytochemicals support brain health and may protect against neurodegenerative diseases (Spencer, 2009).

The phytochemicals exert their effects through modulation of cellular signaling pathways, influencing various physiological processes such as cell proliferation, inflammation, oxidative stress responses, apoptosis, and neuronal function. By targeting key signaling molecules and pathways, these bioactive compounds offer potential therapeutic benefits for a wide range of health conditions, including cancer, inflammation, oxidative stress-related disorders, and neurodegenerative diseases.

## Major Classes of Phytochemicals Include:

Polyphenols, carotenoids, alkaloids, terpenoids, organosulfur compounds, phytosterols, capsaicinoids, glucosinolates, indoles, curcuminoids, tannins, and phenethylamines constitute a diverse array of phytochemicals with varied health benefits. They include flavonoids, phenolic acids, stilbenes, lignans,  $\alpha$ -carotene,  $\beta$ -carotene, lycopene, lutein, zeaxanthin, caffeine, nicotine, morphine, quinine, monoterpenes, diterpenes, triterpenes, saponins, allicin, isothiocyanates,  $\beta$ -sitosterol, campesterol, stigmasterol, capsaicin, dihydrocapsaicin, ellagic acid, EGCG, indole-3-carbinol, curcumin, phenylethylamine, and soyasaponins.

## Drug Targets of Polyphenols

**Enzymes:** Cyclooxygenase (COX), Lipoxygenase (LOX), Cell Cycle Regulators: Cyclins, Cyclin-dependent Kinases (CDKs), Apoptotic Pathways: Intrinsic and Extrinsic Apoptotic Pathways, Angiogenic Factors: Vascular Endothelial Growth Factor (VEGF), Matrix Metalloproteinases (MMPs), Neurotrophic Signaling Pathways: PI3K/Akt, BDNF/TrkB and Inflammatory Signaling Pathways: Nuclear Factor-kappa B (NF- $\kappa$ B), Activator Protein-1 (AP-1).

Polyphenols have been extensively studied for their pharmacological effects on various disease processes due to their ability to interact with multiple cellular targets. For instance, they inhibit enzymes like COX and LOX, which are involved in the production of pro-inflammatory mediators, thus displaying anti-inflammatory effects (Pan *et al.*, 2010). Moreover, polyphenols modulate cell cycle regulators such as cyclins and CDKs, leading to cell cycle arrest and inhibition of cancer cell proliferation (Aggarwal *et al.*, 2003). Additionally, these compounds target apoptotic pathways, inducing programmed cell death in cancer cells through intrinsic and extrinsic pathways (Maioli *et al.*, 2010). Furthermore, polyphenols inhibit angiogenic factors like VEGF and MMPs, thereby suppressing tumor angiogenesis and

metastasis (Siddiqui *et al.*, 2009). They also activate neurotrophic signaling pathways such as PI3K/Akt and BDNF/TrkB, promoting neuronal survival and synaptic plasticity, which may have implications in neuroprotection (Spencer, 2009). Lastly, polyphenols interfere with inflammatory signaling pathways like NF-κB and AP-1, reducing the expression of pro-inflammatory cytokines and chemokines, thus exhibiting anti-inflammatory effects (Pan *et al.*, 2010).

## Drug Targets of Carotenoids

**Retinoic Acid Receptors (RARs) and Retinoid X Receptors (RXRs) & Nuclear Receptor ROR $\alpha$ :** Carotenoids are known for their role as precursors of vitamin A and their antioxidant properties. However, recent studies have also identified specific drug targets for carotenoids. For instance, carotenoids can act as ligands for retinoic acid receptors (RARs) and retinoid X receptors (RXRs), which are nuclear receptors involved in gene transcription and cellular differentiation (Tanoury *et al.*, 2013). Additionally, carotenoids have been shown to interact with the nuclear receptor ROR $\alpha$  (retinoic acid receptor-related orphan receptor alpha), which plays a role in immune regulation and inflammatory responses (Jetten *et al.*, 2019). These interactions with nuclear receptors suggest that carotenoids may have potential therapeutic applications beyond their traditional roles as antioxidants and vitamin A precursors.

## Drug Targets of Different Types of Alkaloids

**Nicotinic Acetylcholine Receptors, Dopamine Receptors, Opioid Receptors, Serotonin Receptors & Muscarinic Acetylcholine Receptors:** Alkaloids are a diverse group of nitrogen-containing compounds found in various plant species, many of which have pharmacological effects on the human body. Different types of alkaloids target specific receptors in the central nervous system and peripheral tissues, leading to various physiological responses. For example, nicotine, an alkaloid found in tobacco, acts as an agonist at nicotinic acetylcholine receptors,

leading to the release of neurotransmitters such as dopamine and norepinephrine (Dani & De Biasi, 2001). Similarly, alkaloids like morphine and codeine target opioid receptors in the brain and spinal cord, exerting analgesic effects (Inturrisi, 2002). Additionally, alkaloids such as scopolamine and atropine bind to muscarinic acetylcholine receptors, leading to anticholinergic effects such as pupil dilation and decreased gastrointestinal motility (Hansen *et al.*, 2008).

## Drug Targets of Different Types of Terpenoids (Isoprenoids)

HMG-CoA Reductase, Farnesyl Pyrophosphate Synthase, Squalene Synthase, and Geranylgeranyl Pyrophosphate Synthase & Lanosterol Synthase: Terpenoids, also known as isoprenoids, comprise a large and diverse group of compounds derived from the mevalonic acid pathway. Several types of terpenoids target enzymes involved in cholesterol biosynthesis and other cellular processes, making them potential drug targets for various diseases. For example, statins, which are terpenoid-based drugs, inhibit HMG-CoA reductase, a key enzyme in cholesterol biosynthesis, leading to reduced cholesterol levels in the blood (Endo, 1992). Moreover, bisphosphonates, another class of terpenoid-based drugs, target enzymes such as farnesyl pyrophosphate synthase and geranylgeranyl pyrophosphate synthase, which are involved in the mevalonate pathway and play a role in bone metabolism and cancer progression (Russell, 2011). Understanding the drug targets of terpenoids is essential for developing therapeutically relevant compounds and elucidating their mechanisms of action.

## Drug Targets of Different Types of Organosulfur Compounds

Histone Deacetylases (HDACs), Cytochrome P450 Enzymes, Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2), Glutathione S-Transferases (GSTs) & Hydrogen Sulfide (H<sub>2</sub>S)-Releasing Compounds: Organosulfur compounds are a diverse group of compounds containing sulfur atoms bonded to carbon atoms. They have been extensively studied for their pharmacological

properties and therapeutic potential. Several types of organosulfur compounds target specific molecular pathways and enzymes in the body, making them potential drug targets for various diseases. For example, certain organosulfur compounds, such as diallyl sulfide and diallyl disulfide, are known to inhibit histone deacetylases (HDACs), enzymes involved in the regulation of gene expression and chromatin structure (Herman-Antosiewicz *et al.*, 2007). Additionally, organosulfur compounds can modulate the activity of cytochrome P450 enzymes, which play a crucial role in drug metabolism and detoxification processes in the liver (Igoli *et al.*, 2020). Furthermore, some organosulfur compounds activate nuclear factor erythroid 2-related factor 2 (Nrf2), a transcription factor that regulates the expression of antioxidant and detoxification enzymes, providing cellular protection against oxidative stress (Wu *et al.*, 2019). Moreover, certain organosulfur compounds, such as allyl sulfides, are substrates for glutathione S-transferases (GSTs), enzymes involved in the detoxification of xenobiotics and reactive metabolites (Galvano *et al.*, 2007). Lastly, hydrogen sulfide (H<sub>2</sub>S)-releasing compounds, which contain organosulfur moieties, have emerged as novel therapeutic agents with diverse biological activities, including vasodilation, anti-inflammatory, and cytoprotective effects (Módis *et al.*, 2019).

## Drug Targets of Different Types of Phytosterols

ATP-binding Cassette Transporters (ABC Transporters), Niemann-Pick C1-like 1 (NPC1L1) Protein, Liver X Receptors (LXRs), Peroxisome Proliferator-Activated Receptors (PPARs) & Sterol Regulatory Element-Binding Proteins (SREBPs).

Phytosterols, plant-derived sterols structurally similar to cholesterol, have gained attention for their potential health benefits, particularly in reducing cholesterol levels and preventing cardiovascular diseases. These compounds exert their effects through various molecular targets in the body. For example, phytosterols are known to interact with ATP-binding cassette transporters (ABC transporters), inhibiting cholesterol absorption

in the intestine and promoting its excretion (Zhang *et al.*, 2020). Moreover, phytosterols competitively inhibit the Niemann-Pick C1-like 1 (NPC1L1) protein, a key mediator of intestinal cholesterol absorption, leading to reduced cholesterol uptake from the diet (Plat *et al.*, 2020). Additionally, phytosterols can modulate the activity of nuclear receptors such as liver X receptors (LXRs) and peroxisome proliferator-activated receptors (PPARs), which play crucial roles in lipid metabolism and cholesterol homeostasis (Zhou *et al.*, 2019). Furthermore, phytosterols regulate the expression of sterol regulatory element-binding proteins (SREBPs), transcription factors that control the synthesis of cholesterol and fatty acids, thereby influencing lipid biosynthesis and cholesterol levels (Srivastava *et al.*, 2019).

## Conclusion

Phytochemicals exhibit diverse health benefits by acting as antioxidants, anti-inflammatories, immunomodulators, and antimicrobials. They protect against oxidative stress-related diseases, reduce inflammation, enhance immune responses, and combat infections. Moreover, they show promise in cancer prevention and therapy by modulating cellular signaling pathways and physiological processes. Incorporating phytochemical-rich foods into the diet promotes overall health and well-being by targeting multiple molecular pathways and cellular processes, offering potential avenues for disease prevention and management.

## Reference

- Aggarwal, B. B., Shishodia, S., Takada, Y., Banerjee, S., Newman, R. A., Bueso-Ramos, C. E., & Price Jr, J. E. (2003). Curcumin suppresses the paclitaxel-induced nuclear factor- $\kappa$ B pathway in breast cancer cells and inhibits lung metastasis of human breast cancer in nude mice. *Clinical Cancer Research*, 9(1), 325-332.

- Bohlmann, J., & Keeling, C. I. (2008). Terpenoid biomaterials. *Plant Journal*, 54(4), 656-669.
- Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, 12(4), 564-582.
- Cushnie, T. P., & Lamb, A. J. (2005). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents*, 26(5), 343-356.
- Dani, J. A., & De Biasi, M. (2001). Cellular mechanisms of nicotine addiction. *Pharmacology, Biochemistry, and Behavior*, 70(4), 439-446.
- Dixon, R. A., & Paiva, N. L. (1995). Stress-induced phenylpropanoid metabolism. *Plant Cell*, 7(7), 1085.
- Dixon, R. A., & Strack, D. (2003). Phytochemistry meets genome analysis, and beyond. *Phytochemistry*, 62(6), 815-816.
- Endo, A. (1992). The discovery and development of HMG-CoA reductase inhibitors. *Journal of Lipid Research*, 33(11), 1569-1582.
- Galvano, F., La Fauci, L., Lazzarino, G., Fogliano, V., Ritieni, A., Ciappellano, S., Battistini, N. C., Tavazzi, B., & Galvano, G. (2007). Cyanidins: metabolism and biological properties. *Journal of Nutritional Biochemistry*, 18(11), 759-770.
- Gibson, M. R., Nurhayati, R. W., & Bittencourt, A. J. (1999). The antimicrobial activities of catechins and their interactions with orthophosphate ions. *Journal of Applied Microbiology*, 87(4), 432-440.
- Gupta, S. C., Patchva, S., & Aggarwal, B. B. (2013). Therapeutic roles of curcumin: lessons learned from clinical trials. *The AAPS Journal*, 15(1), 195-218.
- Halliwell, B. (2007). Dietary polyphenols: Good, bad, or indifferent for your health? *Cardiovascular Research*, 73(2), 341-347.
- Hansen, P. E., Christensen, S. B., Jensen, S. R., & Nielsen, K. (2008). Acetylcholinesterase and butyryl cholinesterase inhibitory compounds from *Corydalis cava* Schweigg. & Kort. *Journal of Ethnopharmacology*, 116(2), 308-312.
- Herman-Antosiewicz, A., Johnson, D. E., Singh, S. V. (2007).

Sulforaphane causes autophagy to inhibit release of cytochrome C and apoptosis in human prostate cancer cells. *Cancer Research*, 67(23), 11173-11180.

- Hertweck, C. (2009). The biosynthetic logic of polyketide diversity. *Angewandte Chemie International Edition*, 48(26), 4688-4716.
- Igoli, J. O., Igoli, N. P., Onyiriuka, S. O., & Adeyemi, O. O. (2020). Pharmacology of selected medicinal plants in the treatment of neuropsychiatric disorders: A review. *Archives of Basic and Applied Medicine*, 8(1), 71-78.
- Inturrisi, C. E. (2002). Clinical pharmacology of opioids for pain. *Clinical Journal of Pain*, 18 (4 Suppl), S3-S13.
- Jetten, A. M., Takeda, Y., Slominski, A., Kang, H. S. (2019). Retinoic acid-related orphan receptor  $\gamma$  (ROR $\gamma$ ): connecting sterol metabolism to regulation of the immune system and autoimmune disease. *Current Opinion in Toxicology*, 13, 30-35.
- Kensler, T. W., Wakabayashi, N., & Biswal, S. (2007). Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. *Annual Review of Pharmacology and Toxicology*, 47, 89-116.
- Lee, K. W., Bode, A. M., & Dong, Z. (2011). Molecular targets of phytochemicals for cancer prevention. *Nature Reviews Cancer*, 11(3), 211-218.
- Li, Y., Yao, J., Han, C., Yang, J., Chaudhry, M. T., Wang, S., & Liu, H. (2016). Quercetin, inflammation and immunity. *Nutrients*, 8(3), 167.
- Maioli, E., Torricelli, C., Valacchi, G., & Rottler, F. (2010). Protective effects of N-acetyl-L-cysteine and resveratrol on apoptosis and oxidative stress in a neuroinflammatory model of Parkinson's disease. *Journal of Neuroimmune Pharmacology*, 5(3), 580-593.
- Módis, K., Coletta, C., Erdélyi, K., Papapetropoulos, A., & Szabo, C. (2019). Intramitochondrial hydrogen sulfide production by 3-mercaptopyruvate sulfurtransferase maintains mitochondrial electron flow and supports cellular bioenergetics. *FASEB Journal*, 33(1), 1288-1298.

- Ninfali, P., Aluigi, G., & Bacchiocca, M. (2005). Polyphenols and antioxidant capacity of vegetables under fresh and frozen conditions. *Journal of Agricultural and Food Chemistry*, 53(20), 8618-8623.
- Nostro, A., Cellini, L., Di Bartolomeo, S., Cannatelli, M. A., Di Campli, E., Procopio, F., & Bisignano, G. (2007). Effects of combining extracts (from propolis or *Zingiber officinale*) with clarithromycin on Helicobacter pylori. *Phytotherapy Research*, 21(5), 415-418.
- Pan, M. H., & Lai, C. S. (2002). Ho, C. T. Anti-inflammatory activity of natural dietary flavonoids. *Food and Function*, 3(8), 886-896.
- Pan, M. H., Lai, C. S., & Ho, C. T. (2010). Anti-inflammatory activity of natural dietary flavonoids. *Food & Function*, 1(1), 15-31.
- Pan, M. H., Lai, C. S., Wu, J. C., & Ho, C. T. (2009). Epigenetic and disease targets by polyphenols. *Current Pharmaceutical Design*, 15(22), 3043-3059.
- Pichersky, E., & Lewinsohn, E. (2011). Convergent evolution in plant specialized metabolism. *Annual review of plant biology*, 62, 549-566.
- Plat, J., Mensink, R. P., & Baumgartner, S. (2020). Plant sterols and stanols in the treatment of dyslipidemia: new insights into targets and mechanisms related to cardiovascular risk. *Current Pharmaceutical Design*, 26(12), 1322-1329.
- Roberts, M. F., & Wink, M. (1998). Alkaloids: biochemistry, ecology, and medicinal applications. Springer Science & Business Media.
- Russell, R. G. (2011). Bisphosphonates: mode of action and pharmacology. *Pediatrics*, 127(3), 742-746.
- Saikia, P., Roy, P., Borah, A. J., & Baruah, G. (2017). Role of natural products in modulating histone deacetylases in cancer. *Annals of Pharmacology and Pharmaceutics*, 2(9), 1052.
- Shanmugam, M. K., Warrier, S., Kumar, A. P., Sethi, G., & Arfuso, F. (2015). Potential role of natural compounds as anti-angiogenic agents in cancer. *Current Vascular Pharmacology*,

13(5), 698-719.

- Siddiqui, I. A., Adhami, V. M., Bharali, D. J., Hafeez, B. B., Asim, M., & Mukhtar, H. (2009). Introducing nanochemoprevention as a novel approach for cancer control: proof of principle with green tea polyphenol epigallocatechin-3-gallate. *Cancer Research*, 69(5), 1712-1716.
- Sies, H. (2017). Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress: Oxidative eustress. *Redox Biology*, 11, 613-619.
- Spencer, J. P. (2009). Flavonoids and brain health: multiple effects underpinned by common mechanisms. *Genes & Nutrition*, 4(4), 243-250.
- Srivastava, R. A. K., Bhaswant, M., & Nair, A. (2019). Mechanism of cholesterol-lowering action of phytosterols: review of literature. *Journal of AOAC International*, 102(2), 507-512.
- Surh, Y. J., Chun, K. S., Cha, H. H., Han, S. S., Keum, Y. S., Park, K. K., & Lee, S. S. (2001). Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: down-regulation of COX-2 and iNOS through suppression of NF- $\kappa$ B activation. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 480, 243-268.
- Surh, Y. J., Kundu, J. K., & Na, H. K. (2013). Nrf2 as a master redox switch in turning on the cellular signaling involved in the induction of cytoprotective genes by some chemopreventive phytochemicals. *Planta Medica*, 79(13), 1541-1547.
- Surh, Y. J., Kundu, J. K., Na, H. K., & Lee, J. S. (2008). Redox-sensitive transcription factors as prime targets for chemoprevention with anti-inflammatory and antioxidative phytochemicals. *Journal of Nutrition*, 138(1), 185-192.
- Tanoury, Z., Piskunov, A., Rochette-Egly, C. (2013). Vitamin A and retinoid signaling: Genomic and nongenomic effects. *Journal of Lipid Research*, 54(7), 1761-1775.

- Tholl, D. (2015). Biosynthesis and biological functions of terpenoids in plants. In Advances in biochemical engineering/biotechnology (Vol. 148, pp. 63-106). Springer.
- Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T., Mazur, M., & Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry & Cell Biology*, 39(1), 44-84.
- Wink, M. (2010). Introduction: biochemistry, physiology and ecological functions of secondary metabolites. In Annual Plant Reviews Volume 40: Biochemistry of Plant Secondary Metabolism (pp. 1-19). John Wiley & Sons.
- Wu, K. C., McDonald, P. R., Liu, J., Klaassen, C. D. (2019). Screening of natural compounds as activators of the Keap1-Nrf2 pathway. *Planta Medica*, 85(5), 352-361.
- Yang, Y., Kim, B., Park, Y., & Lee, J. H. (2016). A systematic review on the anticancer activity of *Sasa borealis* extract in human cancer cells. *Journal of Cancer Prevention*, 21(4), 203-209.
- Yin, D., Jiang, N., Zhang, Y., & Wang, D. (2018). Anti-inflammatory effects of phytochemicals from fruits, vegetables, and food legumes: A review. *Critical Reviews in Food Science and Nutrition*, 58(8), 1260-1270.
- Zhang, Y., Lin, X., Lu, X., & Wang, R. (2020). Phytosterols and the ATP-binding cassette transporters ABCG5 and ABCG8: implications in cardiovascular diseases. *Critical Reviews in Food Science and Nutrition*, 60(17), 2925-2933.
- Zhou, J., Zeng, Y., Wang, H., & Zhang, X. (2019). Liver X receptors and phytosterols: implications for cardiovascular diseases. *Journal of Agricultural and Food Chemistry*, 67(8), 2199-2205.

## Mechanism of Action of Phytochemicals in Disease Prevention and Management

Gowri S, Vidhya shree M, KanageswaranV,  
Rajalakshmi M, Akilan A

Department of Biochemistry,

Dr.N.G.P Arts and Science College, Coimbatore - 641048

### Introduction

Millions of individuals worldwide suffer from chronic illnesses like cancer, diabetes, and cardiovascular disease (CVD), which also cause disability and death. Research has indicated that fruits, vegetables, and grains can prevent the onset of certain chronic illnesses. Phytochemicals, which are bioactive non-nutrient molecules found in fruits, vegetables, grains, and other plants, are primarily responsible for their protective role. Tannins, flavones, triterpenoids, steroids, saponins, and alkaloids are some of the recognised phytochemicals. (Zhang *et al.*, 2015). Consuming phytochemicals like carotenoids, polyphenols, isoprenoids, phytosterols, saponins, dietary fibres, polysaccharides, etc. has been linked in recent studies to health benefits like preventing chronic diseases (Kumar *et al.*, 2023).

This chapter, which focuses on the possible mechanisms of action in the prevention and treatment of chronic diseases, summarises, discusses, and provides an extensive and deep understanding of phytochemicals in human health and diseases.

### Major Phytochemicals

Plants create bioactive molecules known as phytochemicals to defend themselves. There are many different sources of phytochemicals, including whole grains, fruits, vegetables, nuts, and herbs. To date, over a thousand phytochemicals have been

identified. Carotenoids, polyphenols, isoprenoids, phytosterols, saponins, dietary fibres, and certain polysaccharides are a few of the important phytochemicals. In addition to having potent antioxidant properties, these phytochemicals have antiviral, antibacterial, anthelmintic, antiallergic, and antidiarrheal properties (Sharma *et al.*, 2018).

## Cardiac Diseases

The capacity of bioactive compounds obtained from various plant sections to demonstrate cardioprotective properties has been widely exploited. The bulk of known potential phytochemicals that may have significant cardioprotective effects include alkaloids, iridoids, phenolics, flavonoids, terpenoids, glycosidic derivatives, and saponins. By reducing blood lipids, obesity factors, hyperglycemia and type 2 diabetes impacts, oxidative stress factors, inflammation modulation, and platelet aggregation inhibition, these phytochemicals safeguard heart health (Bachheti *et al.*, 2022).

Many bioactive substances, mostly derived from terrestrial plants, have been shown to lower the risk of cardiovascular diseases and support cardioprotection. These include isoflavones, diosgenin, resveratrol, quercetin, catechin, sulforaphane, tocotrienols, and carotenoids. The antioxidative, antihypercholesterolemic, antiangiogenic, anti-ischemic, inhibition of platelet aggregation, and anti-inflammatory properties of the different phytochemicals may be responsible for their cardioprotective benefits (Vasantha *et al.*, 2012). Broccoli sprouts, or *Brassica oleracea* species, are abundant in glucosinolates. *B. oleracea* exhibits antithrombotic, anti-inflammatory, and antioxidant properties through its interactions with various molecular signalling pathways like NF- $\kappa$ B, nuclear factor-2 (Nrf2), c-Jun N-terminal kinase (JNK), MAPK, Akt/PKB, AMPK/SIRT-1/peroxisome proliferator-activated receptor- $\alpha$  (PPAR $\alpha$ )/uncoupling protein-2 (UCP2) and helps improving cardiac health (Pagliaro *et al.*, 2015).

Terpenoids, which naturally suppress NF-B through p65 translocation, DNA binding, and IB phosphorylation are able to regulate the effects of cardiovascular disease. Natural anti-inflammatory artemisinin is present in *Artemisia annua* and works by preventing NF-B activation (Bachheti *et al.*, 2022). Quinones show cardioprotective properties through preserving mitochondrial function and prevention of oxidative injury in the rat cardiac myocytes. Thymoquinone is also a quinone found in the plant *Nigella sativa* seeds, *Nepeta distans*, *Thymus vulgaris*, *Calocedrus decurrens*, *Eupatorium ayapana*, *Origanum* and *Satureja* species (Diego *et al.*, 2019).

Natural polyphenolic chemical resveratrol is found in *Polygonum cuspidatum*, grapes, peanuts, and berries. The pathway of silent mating type information regulation-1 (SIRT-1) and 5'-adenosine monophosphate-activated protein kinase (AMPK) is the primary molecular mechanism responsible for the biological effects of resveratrol and helps in prevention of age-related disorders, cancer, and cardiovascular and cerebro-vascular illnesses (Yun *et al.*, 2014).

Curcumin (diferuloylmethane) is a naturally occurring phenolic compound isolated as a yellow pigment from spice turmeric (*Curcuma Longa*). A standard dose of curcumin protected against cerebral ischemic insult, as well as aging-related cerebro-vascular dysfunction via AMPK/UCP2 pathway and protects neurons against ischemic injury through Akt/Nrf2 pathway (Pu *et al.*, 2013).

Rich in flavonoids, triterpenes, and tannins, as well as additional phytoconstituents like arjunetin, polyphenols,  $\beta$ -sitosterol, freidelin, and arjunic acid, the *Terminalia arjuna* plant has the potential to be a safe and effective cardiotonic with potential cardioprotective effects. (Cespedes *et al.*, 2008). *T. cordifolia*'s leaves, fruits, roots, and stem contain tinosporin, tinosporic acid, tinosporol, giloin, giloinin, gilosterol, columbin, chasmanthin, palmarin, steroids, glycosides, sesquiterpenoids,

diterpenoid lactones, and berberine, all of which have been shown to have cardioprotective properties. In MI models, these substances decreased the infarct's size and the amount of lipid peroxide (Sharma *et al.*, 2011). By promoting blood flow and preventing hypoxia in the heart muscles, *Ginkgo biloba*'s numerous phytoconstituents including flavones glycosides, flavonol, ascorbic acid, diterpen lactones, catechin, sesquiterpenes, iron-based superoxide dismutase, fatty acids, resins, essential oils, tannins, carotenoids, quercetin, and myricetin help to improve cardiac health (Badore *et al.*, 2017; DeFeudis, 1998).

## Inflammatory disorders

Inflammation is the immune system's required response to outside stimuli like damage and infection. Prolonged production of inflammatory mediators, either locally or systemically, is associated with prolonged inflammation, even though inflammation is an essential component of the host defensive response. Additionally, there is a strong correlation between the pathophysiology of certain inflammatory illnesses and inflammatory cytokines as well as non-cytokine mediators including ROS and NO. Uncontrolled inflammation is often the cause of chronic illnesses such as vascular disease, dementia, autoimmune disorders, cancer, diabetes, and arthritis (Chen *et al.*, 2017).

Phytochemicals that exhibit anti-inflammatory processes that reduce chronic inflammation may be a therapeutic option for a range of inflammatory disorders. These phytochemicals function by altering several important inflammatory signalling pathways, including NF-B, Nrf-2, MAPKs, and STAT signalling. Here, we discuss the characteristics of anti-inflammatory phytochemicals in a variety of chronic inflammatory diseases (Shin *et al.*, 2020).

The alkaloid colchicine is found in *Colchicum autumnale L.* (Colchicaceae), also referred to as autumn crocus or meadow saffron. For gout sufferer's intolerant to NSAIDs, this phytochemical offers an alternative treatment. Colchicine binds to

tubulin and inhibits microtubule polymerization, lowering urate crystal inflammation and suppressing leukocyte and other inflammatory cell proliferation (Leung *et al.*, 2015).

Escin comes from the horse chestnut triterpenoid saponin *Aesculus hippocastanum L.*, which has been demonstrated to have anti-inflammatory, anti-nociceptive, anti-edematous, and vasoprotective properties. In traditional Chinese medicine, escin is used to treat cerebral edema and chronic venous insufficiency. According to current research, escin works by lowering vascular permeability to lessen edema in inflammatory tissues (Gallelli, 2019).

A synthetic material derived from the lignan of the Chinese medicinal herb *Schisandra chinensis* Fructus (Turcz.) Baill, also known as Schisandra C. The Chinese Medical Association approved this anti-inflammatory drug for liver issues. Among the methods of action include cytochrome P-450 stimulants, free radical-scavenging HSP70 stimulants, and protein kinase C inhibitors (Bao and Liu, 2008).

Bromelain is the name for the group of enzymes found in pineapple juice and the fruit's stem. Bromelain activates inflammatory pathways to produce chemicals that lessen pain and inflammation. As a result, it is usually advised for osteoarthritis, hay fever, ulcerative colitis, and debridement. TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 are not released when inflammation causes activated immune cells to produce an overabundance of cytokines (Varilla *et al.*, 2021).

Propolis contains apigenin, ferulic acid, galangin, quercetin, naringenin, and caffeic acid phenethyl ester as its main anti-inflammatory constituents. The flavonoid components of propolis are effective in reducing the activity of LOX and COX-2, the inducible isoform of COX, which in turn reduces the generation of nitric oxide, prostaglandins, and leukotrienes. Moreover, propolis and its flavonoids suppress the activities of myeloperoxidase, NADPH-oxidase, ornithine decarboxylase, tyrosine-protein kinase, and hyaluronidase (Orsolić, 2022).

## Neurodegenerative diseases

The physiologically active substances called phytochemicals that are present in plants have attracted a lot of interest lately because they may be able to treat a wide range of illnesses in humans, including neurological conditions. The progressive loss of the nervous system's structure and function is a hallmark of neurodegenerative disorders, including Alzheimer's, Parkinson's, and Huntington's disease. Since there are presently no effective treatments for these disorders, the main goal of care is to manage symptoms rather than stop the disease from getting worse. This has sparked a fervent hunt for cutting-edge therapeutic strategies, with phytochemicals emerging as intriguing contenders (Sarker & Nahar, 2007).

Owing to a comparatively high concentration of oxygen free radicals and insufficient levels of antioxidative defences, the brain is thought to be especially susceptible to oxidative stress. Antioxidant-rich phenols, flavonoids, vitamins, and terpenoids are just a few of the many compounds found in plants that scavenge free radicals. The human body can scavenge free radicals thanks to a variety of plants, citrus fruits, and leafy vegetables that include ascorbic acid, vitamin E, carotenoids, flavanols, and phenolics (Madsen & Bertelsen, 1995; Cai & Sun, 2003).

Elevated oxidative/nitrosative stress, mitochondrial dysfunction, protein misfolding/aggregation, loss of synapses, and reduced neuronal survival are the principal physiological signs of degenerative disorders. A significant quantity of energy is required to protect neurons and immune cells exposed to harmful proteins from the accumulating oxygen and nitrogen species that cause stress in the surrounding environment (Hartman, 2006).

*Dioscorea nipponica* has a substance called diosgenin, which is widely used as a traditional medicinal herb to treat neurological illnesses, diabetes, and inflammation. It has been observed that diosgenin decreases oxidative damage in neural tissues and increases the activity of antioxidant enzymes. It has the potential to lessen the build-up of neurotoxic amyloid- $\beta$  plaques (Woo et al., 2014).

By scavenging free radicals and preventing apoptosis, rosmarinic acid, a polyphenolic molecule included in a variety of herbs including sage, basil, and rosemary, has demonstrated promise in the treatment of neurodegenerative illnesses. *M. officinalis* includes rosmarinic, ursolic, and oleanolic acids that modulate blood levels of corticosterone and gamma-aminobutyric acid (GABA) transaminase, thereby increasing the number of cells and promoting the development of neuroblasts in the dentate gyrus (Yoo et al., 2011).

The phenolic components curcumin, demethoxycurcumin, and bisdemethoxycurcumin are found in the turmeric plant (*Curcuma longa*), which is a member of the *Zingiberaceae* family of ginger plants. Through the induction of BDNF and the application of antiapoptotic, antineuroinflammatory, antioxidant, and neuritogenesis-inducing activities, curcumin has been demonstrated to provide neuroprotection and mediate the recovery of learning and memory deficits in a variety of neurodegenerative models (Akbitik et al., 2014).

*Zingiber officinale*, also known as ginger, is a member of the *Zingiberaceae* family. It has been used for millennia as a spice in food preparation and in traditional Indian, Chinese, Arabic, Tibetan, Unani, and Siddha medicinal traditions. It contains the phenolic phytochemical component 6-shogaol. Strong action against AD, memory improvement, and antioxidant system stimulation are all provided by 6-shogaol. In LPS-treated astrocytes, 6-shogaol has a neuroprotective impact by reducing Bax and increasing Bcl-2, Bcl-xL, and BDNF (Shim et al., 2012).

Huperzine A is a powerful reversible acetylcholinesterase (AChE) inhibitor that is present in *Huperzia serrata*. It is a sesquiterpene alkaloid molecule. By blocking AChE, changing the processing of A $\beta$  peptides, lowering oxidative stress, and encouraging the development of antiapoptotic protein and NGF, huperzine A has a neuroprotective impact against AD (Zhu et al., 2004).

## Cancer

Cancer is one of the leading causes of death across the world. Although conventional cancer treatments such as chemotherapy and radiotherapy have effectively decreased cancer progression, they come with many dose-limiting side-effects. Phytochemicals that naturally occur in spices, fruits, vegetables, grains, legumes, and other common foods are surprisingly effective complements to conventional cancer treatments. These biologically active compounds demonstrate anticancer effects via cell signalling pathway interference in cancerous cells. In addition, phytochemicals protect non-cancerous cells from chemotherapy-induced side-effects (Jain et al., 2021).

Red tomatoes (*Solanum lycopersicum*; Solanaceae) are rich in lycopene, a naturally occurring substance that gives fruits and vegetables their red colour. In men who consumed a higher level of lycopene, there was a marginally significant reduction in the probability of prostate cancer diagnosis, according to Chen et al.'s meta-analysis of six cohort and eleven nested case-control studies (Chen et al., 2013). Epigallocatechin is a major catechin found in green tea (*Camellia sinensis*). Numerous studies using cell lines and animal models have established anticancer activity of Epigallocatechin (Wang et al., 2002).

Tetracyclic triterpenoids called cucurbitacins are present in traditional Chinese medicinal plants that are part of the cucurbitaceae family. Curcubitacin B (CuB), the most active form of the eight different forms of cucurbitacins, has demonstrated promise in a number of cancer models. CuB's several anti-cancer mechanisms are listed in numerous investigations have demonstrated that CuB suppresses STAT3 signalling in a range of cancer types (Cai et al., 2015).

Soy-derived isoflavones, such genistein, have been shown to have strong anti-tumor actions against gastric, breast, prostate, leukaemia, lymphoma, and non-small cell lung cancer. (Sarkar et al., 2002). An alkaloid phytochemical called piperlongumine or

piplartine is taken from the roots of long pepper plants. Many cancers, including multiple myeloma, melanoma, pancreatic cancer, colon cancer, oral squamous cell carcinoma, non-small-cell lung cancer, gastric cancer, biliary cancer, and prostate cancer, have been reported to respond well to piperlongumine treatment. (Wang et al., 2015)

## Conclusion

In conclusion, many chronic diseases are caused by persistent inflammation and overproduction of oxidants. Antioxidant phytochemicals are therefore among the most promising therapeutic agents for long-term illnesses. They have a wide range of biological activities and health advantages, including the capacity to scavenge free radicals and act as antioxidants. They also have anti-inflammatory, anticancer, and anti-aging properties, as well as preventive properties against disorders of the heart, diabetes, obesity, and the nervous system. It is necessary to identify and isolate more antioxidant phytochemicals from foods and medicinal plants, as well as to do additional research on their bioactivities and mechanisms of action. Furthermore, the possible negative effects of antioxidant phytochemicals on humans should be taken into consideration.

## References

- Akbik D., M. Ghadiri, W. Chrzanowski, and R. Rohanizadeh, "Curcumin as a wound healing agent," *Life Sciences*, 2014.
- Asai, N. Iwata, A. Yoshikawa (2007) "Berberine alters the processing of Alzheimer's amyloid precursor protein to decrease A $\beta$  secretion," *Biochemical and Biophysical Research Communications*, 2007, Volume 352, Issue 2, 12 January 2007, Pages 498-502
- Bachheti RK, Worku LA, Gonfa YH, Zebeaman M, Deepti, Pandey DP, Bachheti A. Prevention and Treatment of Cardiovascular Diseases with Plant Phytochemicals: A Review. *Evid Based Complement Alternat Med.* 2022 Jul 4;2022:5741198.
- Badore NS, Das PK, Pillai S, Thakur A. Role of Ginkgo biloba

extract, against isoproterenol induced cardiac toxicity in rats. Indian J Pharm Educ. 2017;51(4): S691–S699.

- Bao, X. Q., and Liu, G. T. (2008). Bicyclol: A novel antihepatitis drug with hepatic heat shock protein 27/70-inducing activity and cytoprotective effects in mice. Cell. Stress Chaperones 13, 347–355.
- Cai Y., Fang X., He C., Li P., Xiao F., Wang Y., Chen M. Cucurbitacins: A Systematic Review of the Phytochemistry and Anticancer Activity. Am. J. Chin. Med. 2015;43 :1331–1350.
- Cai YZ and Sun M (2003) Antioxidant activity of betalins from plants of the *Amaranthacea*. Journal of Agriculture and Food Chemistry., 51: 2288-2294.
- Céspedes CL, El-Hafidi M, Pavon N, Alarcon J. Antioxidant and cardioprotective activities of phenolic extracts from fruits of Chilean blackberry *Aristotelia chilensis* (Elaeocarpaceae), maqui. Food Chem. 2008;107(2):820–829.
- Chen J., Song Y., Zhang L. (2013). Lycopene/tomato consumption and the risk of prostate cancer: a systematic review and meta-analysis of prospective studies. J. Nutr. Sci. Vitaminol (Tokyo) 59 (3), 213–223.
- Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, Li Y, Wang X, Zhao L. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget. 2017 Dec 14;9(6):7204-7218.
- DeFeudis FV. *Ginkgo biloba Extract (EGB 761): From Chemistry to the Clinic*. Wesbaden: Ullstein Medical; 1998.
- Diego A., María F., Yolanda E., Eduardo F., Marcelo A., Iván P. Natural bioactive compounds as protectors of mitochondrial dysfunction in cardiovascular diseases and aging. Molecules . 2019;24:p. 4259.
- Ercisli S. Chemical composition of fruits in some rose (*Rosa* spp.) species. Food Chem. 2007;104:1379–1384. doi: 10.1016/j.foodchem.2007.01.053.
- Fang W Y. Deng, Y. Li et al., "Blood brain barrier permeability and therapeutic time window of Ginkgolide B in ischemia-reperfusion injury," European Journal of Pharmaceutical Sciences, 2010.

- Gallelli L. Escin: a review of its anti-edematous, anti-inflammatory, and venotonic properties. *Drug Des Devel Ther.* 2019 Sep 27;13:3425-3437.
- Hartman RE, Shah A, Fagan AM, Schwetye KE, Parsadanian M, Schulman RN, Finn MB, Holtzman DM. Pomegranate juice decreases amyloid load and improves behavior in a mouse model of Alzheimer's disease. *Neurobiol Dis.* 2006 Dec;24(3):506-15
- Jain A, Madu CO, Lu Y. Phytochemicals in Chemoprevention: A Cost-Effective Complementary Approach. *J Cancer.* 2021 Apr 30;12(12):3686-3700.
- Kumar A, P N, Kumar M, Jose A, Tomer V, Oz E, Proestos C, Zeng M, Elobeid T, K S, Oz F. Major Phytochemicals: Recent Advances in Health Benefits and Extraction Method. *Molecules.* 2023 Jan 16;28(2):887.
- Leung YY, Yao Hui LL, Kraus VB. Colchicine--Update on mechanisms of action and therapeutic uses. *Semin Arthritis Rheum.* 2015 Dec; 45(3):341-50.
- Madsen HL and Bertelsen G (1995) Spices as antioxidants. *Trends Food Science and Technology,* 6: 271-277.
- Pagliaro B, Santolamazza C, Simonelli F, Rubattu S. Phytochemical Compounds and Protection from Cardiovascular Diseases: A State of the Art. *Biomed Res Int.* 2015;2015:918069.
- Pu Y., Zhang H., Wang P., et al. Dietary curcumin ameliorates aging-related cerebrovascular dysfunction through the ampk/uncoupling protein 2 pathway. *Cellular Physiology and Biochemistry.* 2013;32(5):1167-1177. doi: 10.1159/000354516.
- Ranjan A, Ramachandran S, Gupta N, Kaushik I, Wright S, Srivastava S, Das H, Srivastava S, Prasad S, Srivastava SK. Role of Phytochemicals in Cancer Prevention. *Int J Mol Sci.* 2019 Oct 9;20(20):4981..
- Sarkar F.H., Li Y. Mechanisms of cancer chemoprevention by soy isoflavone genistein. *Cancer Metastasis Rev.* 2002;21:265-280.
- Sharma AK, Kishore K, Sharma D, et al. Cardioprotective

activity of alcoholic extract of *Tinospora cordifolia* (Willd.) miers in calcium chloride-induced cardiac arrhythmia in rats. *J Biomed Res.* 2011;25(4):280.

- Sharma B.R., Kumar V., Gat Y., Kumar N., Parashar A., Pinakin D.J. Microbial maceration: A sustainable approach for phytochemical extraction. *3 Biotech.* 2018; 8:401.
- Shim, S. Kim, Y.-B. Kwon, and J. Kwon, "Protection by [6]-shogaol against lipopolysaccharide-induced toxicity in murine astrocytes is related to production of brain-derived neurotrophic factor," *Food and Chemical Toxicology*, 2012.
- Shin SA, Joo BJ, Lee JS, Ryu G, Han M, Kim WY, Park HH, Lee JH, Lee CS. Phytochemicals as Anti-Inflammatory Agents in Animal Models of Prevalent Inflammatory Diseases. *Molecules.* 2020 Dec 15;25(24):5932.
- Varilla C, Marcone M, Paiva L, Baptista J. Bromelain, a Group of Pineapple Proteolytic Complex Enzymes (*Ananas comosus*) and Their Possible Therapeutic and Clinical Effects. A Summary. *Foods.* 2021 Sep 23;10(10):2249.
- Vasanthi HR, ShriShriMal N, Das DK. Retraction Notice: Phytochemicals from plants to combat cardiovascular disease. *Curr Med Chem.* 2012;19(14):2242-51.
- Wang F., Mao Y., You Q., Hua D., Cai D. Piperlongumine induces apoptosis and autophagy in human lung cancer cells through inhibition of PI3K/Akt/mTOR pathway. *Int. J. Immunopathol. Pharmacol.* 2015;28:362–373.
- Wang Y. C., Bachrach U. (2002). The specific anti-cancer activity of green tea (-)-epigallocatechin-3-gallate (EGCG). *Amino Acids* 22 (2), 131–143.
- Yoo DY, Choi JH, Kim W, Yoo KY, Lee CH, Yoon YS, Won MH, Hwang IK. Effects of *Melissa officinalis* L. (lemon balm) extract on neurogenesis associated with serum corticosterone and GABA in the mouse dentate gyrus. *Neurochem Res.* 2011 Feb;36(2):250-7
- Yun H., Park S., Kim M.-J., et al. AMP-activated protein kinase mediates the antioxidant effects of resveratrol through regulation of the transcription factor FoxO1. *FEBS Journal.*

2014;281(19):4421–4438.

- Zhang YJ, Gan RY, Li S, Zhou Y, Li AN, Xu DP, Li HB. Antioxidant Phytochemicals for the Prevention and Treatment of Chronic Diseases. *Molecules*. 2015, Nov 27;20(12):21138-56.
- Zhang, X. Tian, Y. Luo, and X. Meng, "Ginkgolide B attenuates ethanol-induced neurotoxicity through regulating NADPH oxidases," *Toxicology*, 2011.
- Zhu X Z, X.-Y. Li, and J. Liu (2004) "Recent pharmacological studies on natural products in China," *European Journal of Pharmacology*, 500,1-3, 1: 221-230.

## Mechanism of Action of Phytochemicals in Disease (Cancer) Prevention and Treatment

Karthika B<sup>1</sup>, Pradeepan K<sup>1</sup>, Sharumathy U<sup>1</sup>,  
Arunavarsini K<sup>2</sup> & Mahenthiran R<sup>3</sup>

<sup>1</sup>M.Sc. Microbiology;

<sup>2</sup>PhD Research Scholar & <sup>3</sup>Assistant Professor  
Department of Microbiology

Dr. N.G.P. Arts and Science College, Coimbatore - 641 048

### Abstract

Phytochemicals are the abundant bioactive natural chemical compounds, which are produced by the plant system. And these phytochemicals are absorbed by the human body in the form of diet consisting of fruits and vegetables. Major functional classes of phytochemical are like carotenoids, alkaloids, polyphenols, phytosterols, glucosinolates, limonoids, flavonoids, terpenoids, saponins, fibres, polysaccharides, etc., and they have various properties including anti-oxidant, anti-inflammatory, anti-microbial, anti-cancerous, neuroprotective effect, cardioprotective effect and many other beneficial properties. Each major functional class of phytochemicals constitutes wide range of chemicals with varying potency depending upon their nature. Phytochemicals helps to resist the growth and function of pathogens such as bacteria, fungi, viruses, etc, and some phytochemicals are synthesised in order to prevent the consumption of plants by insects and animals. Phytochemicals also protect the cell components such as genetic material, proteins and membrane by neutralising the harmful free radicals as a part of antioxidant property. Intake of sufficient amount of diet rich in phytochemicals with antioxidant property helps in balancing the reactive species production within the body, as according to reports majority of the disease are associated with the free radicals

or reactive species inside the cell which oxidize the biological components. Emerging research on phytochemicals helps the medicinal field to get rid of consequences arises by various diseases by finding out appropriate medication for treatment and prevention of various deadliest diseases.

**Keywords:** *Phytochemical, Medicinal uses, Disease Treatment & Prevention*

## Objective of the Review

- Phytochemical compounds with potential medicinal properties.
- Role of phytochemicals in Treatment and Prevention
- Cancer treatment and prevention

## Introduction

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans as medicinal ingredients and nutrients. They protect plants from disease and damage, and also contribute to the plant's colour, aroma and flavour. In general, the plant chemicals that protect plants from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals. Recently, it has been clearly shown that they also have roles in the protection of human health, when their dietary intake is significant. Till date over 4,500 phytochemicals have been reported and are classified on the basis of their protective functions, and physical and chemical characteristics, amongst these all about 350 phytochemicals have been studied in detail till now. Wide-ranging dietary phytochemicals are found in fruits, vegetables, legumes, whole grains, nuts, seeds, fungi, herbs and spices. Broccoli, cabbage, carrots, onions, garlic, whole wheat bread, tomatoes, grapes, cherries, strawberries, raspberries, beans and soy foods are also the common sources of phytochemicals. Phytochemicals accumulate in different parts of the plants, such as in the root, stem, leaf, flower, fruit and seed. Many phytochemicals,

particularly the pigment molecules like anthocyanines and flavonoids, are often concentrated in the outer layers of the various plant parts like leaves and fruits of vegetables. However, the levels of these phytochemicals vary from plant to plant depending upon the variety, climate, and growing conditions. These compounds have biological properties such as antioxidant activity, anti-microbial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property. The exact classification of phytochemicals has not been given so far, because of their diverse forms and structures. Classically, the phytochemicals have been classified as primary or secondary metabolites, depending on their role in plant metabolism. Primary metabolites include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlorophyll etc. Secondary metabolites are the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumins, saponins, phenolics and glucosides (Fig. 1).

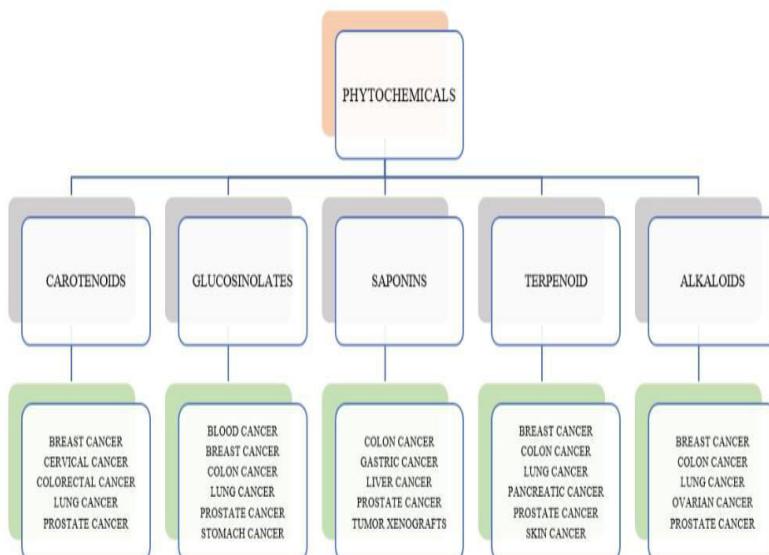


Figure 1. Phytochemicals Classification

The second leading cause of mortality worldwide is Cancer. Cancer occurs by a series of successive mutations in genes thereby changing the cell functions. The genes that are mutated are the oncogenes. The multiple genetic defects including mutations, translocations, and amplifications of oncogenes and are reduced to homozygosity for putative tumor suppressor genes arises the cancer cells. Chemical compounds have a major role in causing gene mutations and cancer cells. In general, cancer disrupts cellular relations and results in the dysfunction of vital genes leading to abnormal proliferation of cells. Proto-oncogenes are responsible for cell division and growth under normal condition, but become oncogenes during genetic mutation, which are most dangerous for cell existence. The lack of tumor suppressor genes triggers uncontrolled cells division. OH-induced mutations in purines, pyrimidines, and chromatin proteins affect genome stability and dynamics of gene expression and are widely accepted as a cause for cancer initiation. Cancer cells undergo sequential changes that enable the "non-tumor" cells to acquire a wide range of forward mutations including ones that are essential for oncogenicity, followed by revertant mutations in the "para-tumor" cells to avoid growth retardation by excessive mutation load. Early identification of cancer is essential for proper treatment. Cancer may present in a variety of ways. Common causes of cancer are Tobacco, Alcohol, Areca nut, Pollution, Lifestyle factors, Occupational exposure, Radiation, Biological agents. Some of the cancer treatments include Clinical examination, Radiologic assessment, Radiotherapy, Chemotherapy, Palliative care. The major types of cancer are carcinoma, sarcoma, melanoma, lymphoma, and leukaemia.

## Review of Literature

This study states the different types of phytochemicals and their medicinal properties against various types of cancer. Various types of phytochemicals are used in the cancer treatment and prevention.

## 1. Carotenoids

Carotenoids are a diverse group of natural pigments and are present in many fruits and vegetables. The data surrounding carotenoids and their potential roles in carcinogenesis have been rapidly growing over the past two decades. These are a group of phytochemicals that are responsible for different colours of the foods such as red, yellow and orange. Carotenoids give the characteristic colour to pumpkins, carrots, parsnips, corn, tomatoes, canaries, flamingos, salmon, lobster, shrimp, and daffodils. Fruits and vegetables mediate their beneficial effects via several mechanisms that include metabolism, immune modulation and hormonal induction. They are recognized as playing an important role in the prevention of human diseases and maintaining good health. In addition to being potent antioxidants some carotenoids also contribute to dietary vitamin. There is scientific evidence in support of the beneficial role of phytochemicals such as carotenoids and  $\beta$ -carotene in the prevention of several chronic diseases like cancer. Unlike some other carotenoids, lycopene does not have pro-vitamin A properties. Because of the unsaturated nature of lycopene, it is considered to be a potent antioxidant and a singlet oxygen quencher. According to their structure most carotenoids exhibit absorption maxima at around 450 nm. Filtering of blue light has been proposed as a mechanism protecting the macula lutea against photooxidative damage. There is increasing evidence from human studies that carotenoids protect the skin against photo oxidative damage.

Approximately two of every five people will develop cancer in their lifetime. Dietary modifications are one of the most promising lifestyle changes that can adjust the risk of developing cancer by nearly 30% - 40%. Oxidative stress is an important contributor to the risk of chronic diseases. Dietary guidelines recommend increased consumption of fruits and vegetables to combat the incidence of human diseases such as cancer, cardiovascular disease, osteoporosis and diabetes. Fruits and vegetables are good

sources of antioxidant phytochemicals that mitigate the damaging effect of oxidative stress. However, in recent years oxidative stress, induced by reactive oxygen species (ROS) that are generated by normal metabolic activity as well as lifestyle factors such as smoking, exercise and diet, have been implicated in the causation and progression of several chronic diseases. Antioxidants that can mitigate the damaging effects of ROS have been the focus of recent research.

Carotenoid ability in inhibiting or in enhancing apoptosis depends on several factors: carotenoid concentration, concerted action of multiple micronutrients, cell type, and redox status. This review summarizes the available evidence for a modulatory action of carotenoids on apoptosis and focuses on the main molecular pathways involved in this process. Neoplastic changes of the cells are often associated with changes in the expression of growth factors and growth factor receptors. The apoptotic process is characterized by particular morphological and ultrastructural features, which can be evidenced by several assays, including terminal deoxynucleotidyl transferase nick end labelling (TUNEL), FITC-conjugated annexin V method, acridine orange assay, and DNA fragmentation.

## 2. Glucosinolates

Glucosinolates are amino acid-derived plant secondary metabolites whose role in plant defense has been well characterized. Glucosinolates (thioglucoside-N-hydroxysulphates) constitute a homogeneous class of naturally occurring thiosaccharidic compounds mainly found in the botanical order *Brassicales*. The enzyme myrosinase hydrolyzes glucosinolates to form isothiocyanates, which are chemical protectors. Glucosinolate metabolites exert the cancer-preventive activity through different mechanisms, including induction of the Nrf2 transcription factor, inhibition of expression of tumor necrosis factor- $\alpha$  (TNF $\alpha$ ) and interleukin-1 $\beta$  (IL-1 $\beta$ ), induction of apoptosis through inhibiting phase I enzymes and inducing phase II enzymes, interruption of

caspase pathways, STAT1/STAT2, inhibition of sulfotransferases. They are effective in cancer treatment by inhibiting angiogenesis, upregulating natural killers, increasing expression of p53, p21, caspase 3 and 9, and modulating NF- $\kappa$ B. Some isothiocyanates can increase the risk of tumors. So, further studies are needed to obtain an accurate and effective dose for each glucosinolates to treat different types of tumors. Antagonistic activity of BITC, PEITC and SFN against cancer was related with the direct/indirect interaction with Nrf2 and NF- $\kappa$ B protein. Most of research on glucosinolates today focuses on treatment or effective medicine against cancer. So, here the anticancerous activities of glucosinolates are discussed based on reference articles.

Cancer is a type of regenerative disease which can affect any part of body by abnormal and uncontrolled cell growth. According to many reports there are no effective medicine found to cure certain cancers completely, as cancers are one of the leading causes of mortality world-wide, which can be controlled or prevented by a proper food diet which have essential phytochemical to reduce the risk factors associated with formation of cancer cells. Glucosinolate's medicinal value is not only limited to pathogenic diseases, among hundreds of glucosinolates, PEITC, BITC, SFN and AITC were most strongly associated with potential chemo preventive effects targeting multiple biological pathways by modulating cellular events leading to apoptotic cell death. However, the mechanisms facilitated by glucosinolates in inducing apoptotic pathways are not fully understood. In summary, glucosinolates and its derivatives isothiocyanates were found to be a potent source of chemo preventive therapy. The mechanism of anti-carcinogenic properties of glucosinolates includes the modification of apoptotic pathway leading to cell death. Mechanism of action of different classes of glucosinolates differ from each other. The phytochemical compound isothiocyanates which are effective against colon cancer, lung cancer, prostate cancer, breast cancer has mechanisms such as apoptosis by condensation of nuclear heterochromatin, changes in cell membrane by shrinkage and changes in the orientation of intra

cytoplasmic organelles and controlled proliferation and cancer cell death. Phenethyl isothiocyanate (PEITC) which are effective against prostate cancer, lung cancer, colon cancer, blood cancer causes biotransformation of phase-2 detoxification enzyme, mitochondrial dysfunction and oxidation stress, alteration of caspase activity, anti-angiogenesis, and inhibition of histone deacetylation on a panel of cancer cell lines and cell cycle arrest occurs in G2/M phase of cell cycle. Sulforaphane that are effective against lung cancer, colon cancer, stomach cancer alters the cell cycle progression stomach cancer and induces apoptosis thereby increasing the expression of p53 and Bax and negatively affected the expression of Bcl-2 and also inhibition of histone deacetylase activity. The molecular mechanisms of action of most of the glucosinolates are still under research.

### 3. Saponins

Historically, saponins were primarily derived from vegetables and herbs. Saponins from herbs include soapwort, ginseng, ginsenosides, gypenosides, soapberry rhizomes from the *Liliaceae*, *Dioscoreaceae*, *Agavaceae*, *Primulaceae*, *Sapotaceae* and *Caryophyllaceae* families. Furthermore, different types of saponins can be isolated within the same plant species. Saponins are one of the broadest classes of high-molecular weight, natural compounds. Saponins are a group of naturally occurring plant glycosides, characterized by their strong foam-forming properties in aqueous solution. The glucose parts were oligosaccharides arranged in linear or branched pattern. Saponins are found in about more than 100 plant families and also marine organisms. About 150 kinds of natural saponins have been found to possess significant anti-cancer properties. Saponins are usually found in roots, tubers, leaves, blooms or seeds. Saponins inhibit the tumor cell growth by cell cycle arrest and apoptosis with IC<sub>50</sub> values of up to 0.2 µM, antioxidant activity, cellular invasion inhibition, induction of apoptosis and autophagy. There are more than 11 distinguished classes of saponins including dammaranes, tirucallanes, lupanes, hopanes, oleananes, taraxasteranes, ursanes, cycloartanes,

lanostanes, cucurbitanes and steroids. Most of research on saponins today focus on treatment or effective medicine against cancer. So, here the anti-cancerous activity of saponins is discussed based on reference articles.

Cancer is a type of regenerative disease which can affect any part of body by abnormal and uncontrolled cell growth. According to many reports there are no effective medicine found to cure certain cancers completely, as cancers are one of the leading causes of mortality world-wide, which can be controlled or prevented by a proper food diet which have essential phytochemical to reduce the risk factors associated with formation of cancer cells. Saponin's medicinal value is not only limited to pathogenic diseases, it also has reported on anti-cancerous activity. The mechanism of anti-carcinogenic properties of saponins include immune-modulatory effects, direct cytotoxicity, bile acid binding and normalisation of carcinogen-induced cell proliferation. The phytochemical compound Cycloartanes which are effective against colon cancer, tumor xenografts have mechanisms such as down-regulated expression of the HCC tumor marker alpha-fetoprotein, suppression of HepG2 cell growth by apoptosis and modulating independent ERK-NF-kappa-Bsignalling pathway. Diosgenin which are effective against prostate cancer inhibit metastasis by P13K signalling pathway. Dioscin effective against gastric cancer causes cell cycle arrest occurs in G2/M phase of cell cycle and also induces apoptosis. Mechanism of action of different classes of saponins differs from each other. The molecular mechanisms of action of most of the saponins are still under research.

#### 4. Terpenoids

Terpenoids are represented as one of the most abundant phytochemicals with wide applications and also reported as major component present in essential oils. Most of the terpenoids are present in the form of volatile oil in higher group of medicinal plants like *Myrtaceae*, *Cannabaceae*, *Celastraceae*, *Umbelliferae*, *Pinaceae*, *Magnoliaceae*, *Oleaceae*, *Taxaceae*, etc.

Due to the diversity of structure with atleast 40000 compounds each type of terpenoids has unique property. Terpenoids are classified into various types based on presence of number of carbon and isoprene units, they include monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), triterpenes (C30), and tetraterpenes (C40). Monoterpenes and sesquiterpenes are the two terpenoid which are mainly found in medicinal plant derived essential oils while compared with other types. Terpenoids possess enormous medicinal value in treatment and prevention of several diseases, including cancer, and also have antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, anti-inflammatory, and immunomodulatory properties. Most of research on terpenoids today focus on treatment or effective medicine against cancer. So, here the anti-cancerous activity of terpenoids is discussed based on reference articles.

Cancer is a type of regenerative disease which can affect any part of body by abnormal and uncontrolled cell growth. According to many reports there are no effective medicine found to cure certain cancers completely, as cancers are one of the leading causes of mortality world-wide, which can be controlled or prevented by a proper food diet which have essential phytochemical to reduce the risk factors associated with formation of cancer cells. Terpenoid's medicinal value is not only limited to pathogenic diseases, it also have reported on anti-cancerous activity. They play an important role on cancer prevention by inhibiting the protein, enzymes or signalling pathways which activates the cancer cells, or by activation of DNA repair mechanism and also induce the production or stimulating the anti oxidation action and protective enzymes against cancer cells. Mechanism of action of different classes of terpenoids differ from each other. The molecular mechanisms of action of most of the terpenoids are still under research.

## 5. Alkaloids

Alkaloids are the naturally occurring organic phytochemical compound which contains atleast one nitrogen atom, it gained the name Alkaloids due to its alkaline nature and most of the Alkaloids are bitter in taste. Alkaloids are divided into three major types - true alkaloids, protoalkaloids, and pseudoalkaloids. Alkaloids are present in most of the plant groups, but plant groups rich in Alkaloids are *Amaryllidaceae*, *Papaveraceae*, *Solanaceae*, *Ranunculaceae*, *Fabaceae*, *Apocynaceae*, *Rubiaceae*, *Berberidaceae*, and *Boraginaceae* and *Rutaceae*. Alkaloids have wide pharmacological applications such as anaesthetics, anti-inflammatory, cardioprotective, anti-cancerous, anti-hyperglycaemic, anti-hypertensive, neuropharmacological, antimicrobial and anti-asthmatic. Numerous Alkaloids have shown effective in anti-cancerous activity both *in vitro* and *in vivo*. So, here the activity of alkaloids against cancer cells are discussed based on reference articles.

Cancer is a multifactorial disease which results in alteration in physiological and biochemical functions in human body which depends upon stage and type of cancer occurred. Cancer is one of the fatal diseases in the world with no fully effective medicine. Researches on plant derived phytochemicals are undergoing to find fully effective medicine against cancer without side effects. According to many reports cancer can be controlled or prevented by a proper food diet which have essential phytochemical to reduce the risk factors associated with formation of cancer cells.

Studies on alkaloids have proven that they are capable of modifying cell cycle, key signalling pathways involved in proliferation of cancer cells and metastasis, which in term suppress the cancerous activity. Alkaloids such as vinblastine, vincristine, vinorelbine and vindesine have already been successfully developed as anticancer drugs. Most of the compounds in Alkaloid groups play a role in inhibiting mitosis and disrupting the microtubule polymerization. Microtubules are

important in intracellular transport, cell division and maintaining cellular shape. Alkaloid molecules like vincristine, vinblastine binds to tubulin protein (which are the building block of microtubules) and prevents its polymerization to form microtubules, which disrupts the mitotic spindle formation, which is necessary for segregation of chromosome during cell division, hence, multiplication of cancerous cells are inhibited. Alkaloids also induce cell cycle arrest and triggers apoptosis in rapidly multiplying cancer cells, this process occurs during M and S phases. These are the basic mechanisms involved in preventing and treating the cancer disease.

## Conclusion

Whatever the final assessment of the overall contribution of diets rich in fruits and vegetables to cancer prevention turns out to be, there is no doubt that the phytochemicals they contain do exert a range of fascinating and potentially-important biological effects on human cells. In conclusion, the complicated mechanism of action exhibited by different phytochemicals offers a promising result in prevention and treatment of various diseases. By possessing various properties such as anti-oxidant, anti-inflammatory, anti-cancer, and other biological activities phytochemicals offers a wide range of therapeutic benefits across various health conditions. Intake of diet rich in phytochemicals such as fruits, vegetables and other plant-based sources helps us in improving the health benefits and also reduce the chance of disease occurrence. Notably in cancer prevention and treatment their role is tremendous, as they exhibit potential to disrupt cancer initiation, proliferation and metastasis, which inhibits the growth of cancer cells. Further research must be undergone to know the full potential and specific mechanism of individual phytochemicals in chronic diseases and other health care conditions and diving deep into the mechanism of action of phytochemicals also leads to drug discovery for untreatable diseases without any side effects.

## Reference

- Adams LS, Phung S, Yee N, Seeram NP, Li L, Chen S. Blueberry phytochemicals inhibit growth and metastatic potential of MDA-MB-231 breast cancer cells through modulation of the phosphatidylinositol 3-kinase pathway. *Cancer Res.* 2010;70(9):3594–605.
- Asvinidevi Arumugam, Ahmad Faizal Abdull Razis. (2018). Apoptosis as a Mechanism of the Cancer Chemopreventive Activity of Glucosinolates: a Review. *Asian Pacific Journal of Cancer Prevention.* 19(6). 1439-1448.
- Bachran, Christopher,Bachran, Silke, Sutherland, Mark, Bachran, Diana; Fuchs, Hendrik. (2008). Saponins in Tumor Therapy. *Mini Reviews in Medicinal Chemistry.* 8(6). 575-584.
- Dhan Prakash, Charu Gupta. (2012). Glucosinolates: The Phytochemicals of Nutraceutical Importance. *Journal of Complementary and Integrative Medicine.* 9(1).
- Finley JW, Kong AN, Hintze KJ, Jeffery EH, Ji LL, Lei XG. Antioxidants in foods: state of the science important to the food industry. *J Agric Food Chem.* 2011;59(13):6837–46.
- Jaeger R., Cuny E. Terpenoids with special pharmacological significance: A review. *Nat. Prod. Commun.* 2016; 11:1934578X1601100946.
- Jiang Y., Chen L., Taylor R.N., Li C., Zhou X. Physiological and pathological implications of retinoid action in the endometrium. *J. Endocrinol.* 2018; 236: R169–R188.
- John D. Hayes, Michael O. Kelleher, Ian M. Eggleston. (2008). The cancer chemopreventive actions of phytochemicals derived from glucosinolates. *Eur J Nutr.* 47(2). 73-88.
- Kim J, Cha YN, Surh YJ. A protective role of nuclear factor-erythroid 2-related factor-2 (Nrf2) in inflammatory disorders. *Mutat Res.* 2010;690(1-2):12-23.
- Mohammad Bagher Majnooni, Sajad Fakhri, Syed Mustafa Ghanadian, Gholamreza Bahrami, Kamran Mansouri, Amin Iranpanah, Mohammad Hosein Farzaei, Mahdi Mojarrab. (2023). Inhibiting Angiogenesis by Anti-Cancer Saponins: From Phytochemistry to Cellular Signaling Pathways. *Metabolites.* 13(3). 323.

- Moreno, M.Carvajal, C.López-Berenguer, C.García-Viguera. (2006). Chemical and biological characterisation of nutraceutical compounds of *broccoli*. Journal of Pharmaceutical and Biomedical analysis. 41(5). 1508-1522.
- Neda Orouji, Siamak Kazemi Asl, Zahra Taghipour, Solomon Habtemariam, Seyed Mohammad Nabavi, Roja Rahimi. (2023). Glucosinolates in cancer prevention and treatment: experimental and clinical evidence. Medical Oncology. 40 (12). 344.
- Olusola Olalekan Elekofehinti, Opeyemi Iwaloye, Femi Olawale, Esther Opeyemi Ariyo. (2021). Saponins in Cancer Treatment: Current Progress and Future Prospects. Pathophysiology. 28(2). 250-272.
- Rao, M.-K. Sung. (1995). Saponins as Anticarcinogens. The Journal of Nutrition. 125(3). 717S-724S.
- Rowles J.L., 3rd, Erdman J.W., Jr. Carotenoids and their role in cancer prevention. Biochimica et Biophysica Acta. Mol. Cell Biol. Lipids. 2020; 1865:158613.
- Seyed Hossein Hassanpour, Mohammadamin Dehghani. (2017). Review of cancer from perspective of molecular. Journal of Cancer Research and Practice. 4(4). 127-129.
- Sharma B.R., Kumar V., Gat Y., Kumar N., Parashar A., Pinakin D.J. Microbial maceration: A sustainable approach for phytochemical extraction. 3 Biotech. 2018; 8:401.
- Shuli Man, Wenyuan Gao, Yanjun Zhang, Luqi Huang, Changxiao Liu.(2010).Chemical study and medical application of saponins as anti-cancer agents. Fitoterapia. 81(7). 703-714.
- Surh YJ, Kundu JK, Na HK. Nrf2 as a master redox switch in turning on the cellular signaling involved in the induction of cytoprotective genes by some chemopreventive phytochemicals. Planta Med. 2008;74(13):1526–39.
- Thinh Nguyen, Jon Stewart, Michel Lopez, Irina Ioannou, Florent Allais. (2020). Glucosinolates: Natural Occurrence, Biosynthesis, Accessibility, Isolation, Structures, and Biological Activities. Molecules. 25. 4537.

## Exploring the Therapeutic Potential of Phytochemicals in Tuberculosis Treatment

**Dr. Saritha E<sup>\*1</sup>, Saranya M<sup>2</sup>, Hemasri M S<sup>2</sup>, Roshini S A<sup>2</sup>**

<sup>\*1</sup>Assistant Professor, Department of Biotechnology

PSG College of Arts & Science, Coimbatore - 641014.

<sup>2</sup>B.Sc., Biotechnology, Department of Biotechnology

PSG College of Arts & Science, Coimbatore - 641014.

### Abstract

The fight against tuberculosis (TB) demands innovative therapeutic approaches beyond traditional antibiotics. Phytocompounds, derived from plants, offer a promising avenue due to their diverse pharmacological properties and lower risk of resistance development. This review delves into the therapeutic potential of key phytocompounds in TB treatment, elucidating their mechanisms of action, efficacy, and synergistic effects. Phytochemicals such as curcumin, allicin, and resveratrol exhibit notable antimycobacterial activity by inhibiting mycobacterial growth and disrupting cell wall formation. These compounds, sourced from botanical reservoirs, present a holistic strategy for combating TB, particularly in regions with logistical challenges for conventional pharmaceutical interventions. Extensive clinical trials are imperative to ascertain the safety, efficacy, and optimal dosing regimens of these compounds. Furthermore, exploring synergistic interactions between phytocompounds and conventional TB drugs could unveil potent combination therapies with enhanced efficacy and reduced emergence of drug resistance.

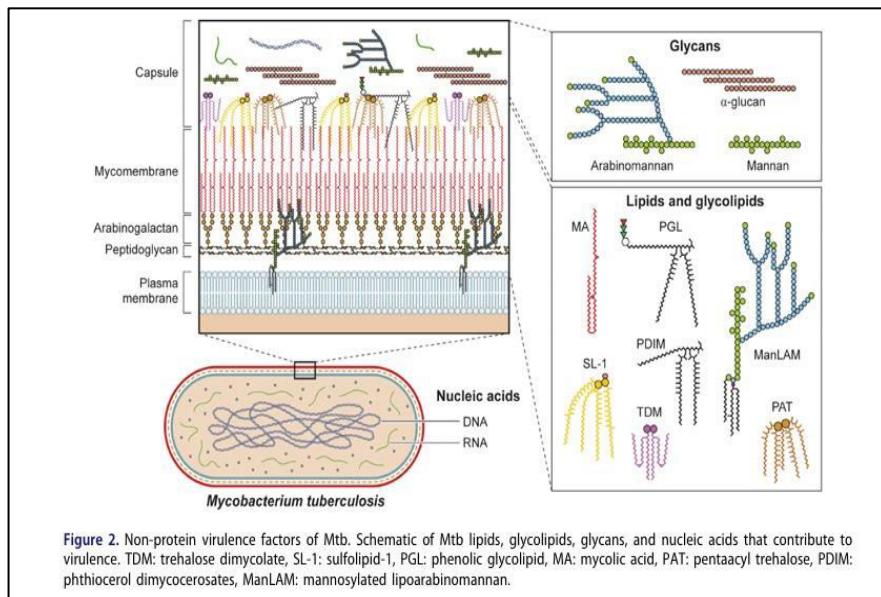
**Keywords:** *Mycobacterium, Bioactive Compounds, Anti TB Phytochemicals, Traditional Medicine*

## Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is the second-deadliest bacterial disease globally (Ansari *et al.*, 2023). The World Health Organization (WHO) reported 10.6 million new TB cases in 2021, with an incidence rate of 134 per 100,000 people, including 5.6 million men, 3.3 million women, and 1.1 million children. TB was the 13<sup>th</sup> leading cause of death worldwide in 2021, with fatalities among HIV-negative individuals rising to 1.4 million, up from 1.28 million in 2020. The TB mortality rate was 17 per 100,000, marking the second year of increased deaths since 2005 (Shan *et al.*, 2024). The COVID-19 pandemic caused an 18% decline in TB case notifications, with studies showing a significant drop in diagnoses across 19 countries. While lockdowns may have temporarily reduced TB transmission, concerns persist about long-term impacts on prevention efforts (Nalunjogi *et al.*, 2023).

The *M. tuberculosis* complex (MTC), including *M. bovis* and, *M. africanum*, causes TB. Current treatments for active TB, such as isoniazid, rifampicin, and pyrazinamide, often lead to severe side effects like hepatotoxicity, gastrointestinal disturbances, neurotoxicity, and hypersensitivity. The emergence of multi-drug resistant (MDR) and extensively drug-resistant (XDR) TB necessitates second-line drugs with serious side effects, including nephrotoxicity and ototoxicity. This highlights the urgent need for safer, more effective treatments. Increasingly, plant-based natural products are being explored for their chemical diversity and potential as phyto-drugs. These phytonutrients are less toxic and have fewer side effects than conventional treatments. WHO estimates that 80% of the population in developing countries relies on traditional medicines, underscoring the importance of medicinal plant extracts and phytochemicals in treating TB (Singh *et al.*, 2020).

## Cell Wall of *M. tuberculosis*



**Figure 2.** Non-protein virulence factors of Mtb. Schematic of Mtb lipids, glycolipids, glycans, and nucleic acids that contribute to virulence. TDM: trehalose dimycolate, SL-1: sulfolipid-1, PGL: phenolic glycolipid, MA: mycolic acid, PAT: pentaacyl trehalose, PDIM: phthiocerol dimycocerosates, ManLAM: mannosylated lipoarabinomannan.

### Fig.1: Cell wall of *Mycobacterium tuberculosis*

(Source: Rahlwes *et al.*, 2023)

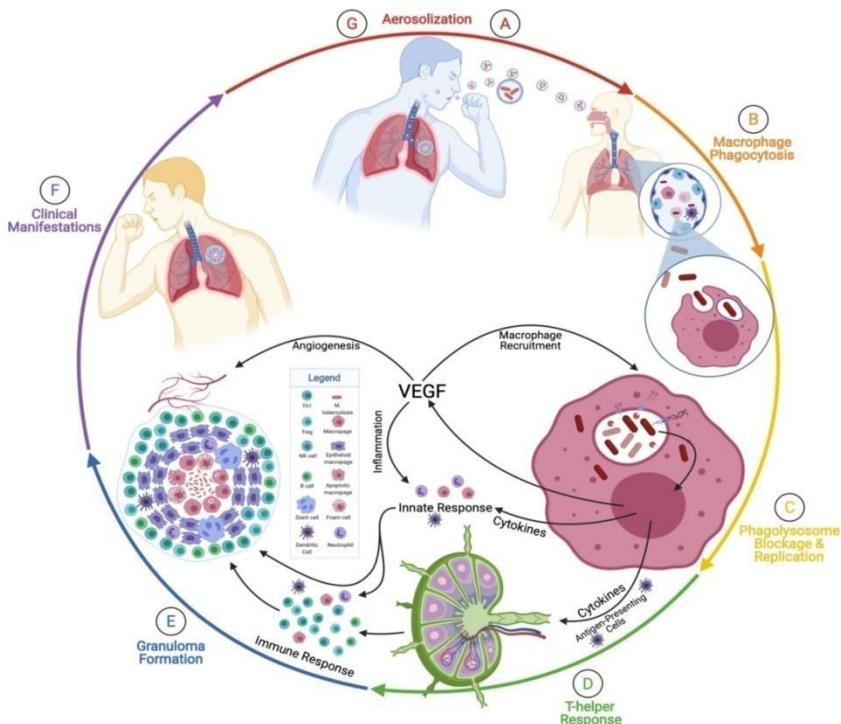
The cell wall of *M. tuberculosis* is composed of layers of peptidoglycan, arabinogalactan, and mycolic acid, which provide structural integrity and antibiotic resistance. The outermost layer contains lipids, glycolipids, and mycosides, contributing to the bacterium's resilience and virulence. Beneath the cell wall lies the plasma membrane, which houses proteins such as lipoarabinomannan (LAM), crucial for the bacterium's survival within macrophages and serving as a diagnostic antigen for TB. Additionally, cytoplasmic (soluble) antigens, including antigen 5, 6, and 60, are utilized in the serodiagnosis of tuberculosis (Sastry and Bhat, 2023).

## Mode of Transmission

Airborne transmission is the primary mode, involving inhalation of aerosols containing *M. tuberculosis*. These aerosols, produced during coughing, sneezing, or speaking, contain tiny dry droplet nuclei ( $<5\text{ }\mu\text{m}$ ) that can linger in the air, facilitating inhalation. Each cough may contain up to 3,000 infectious nuclei. Direct skin contact transmission is rare, with inoculation being uncommon. Ingestion of sputum, especially in infants, or consumption of unpasteurized infected milk can also transmit the disease (Sastry and Bhat, 2023).

## Pathogenesis

1. Inhalation of droplet nuclei containing tubercle bacilli occurs, with most being expelled by the body's mucosal cell ciliary action. Only a small fraction reaches the alveoli.
2. Tubercle bacilli adhere to macrophages through their surface lipoarabinomannan (LAM), binding to complement and mannose receptors on macrophage surfaces, leading to internalization.
3. Macrophages phagocytose the bacilli, facilitated by complement-mediated opsonization.
4. Bacilli survive within macrophages by inhibiting phagosome-lysosome fusion via LAM, which prevents an increase in intracellular  $\text{Ca}^{2+}$  and phosphatidylinositol 3-phosphate.
5. Successful prevention of phagolysosome fusion allows bacilli to replicate within macrophages. Eventually, macrophages rupture, releasing bacilli to infect other phagocytes, perpetuating the infection cycle (Sastry and Bhat, 2023).



**Figure 2: Pathogenesis of Tuberculosis (Source: Maison, 2022)**

## Classification of Tuberculosis

### Pulmonary Tuberculosis (PTB):

Pulmonary Tuberculosis (PTB) accounts for 60-90% of all TB cases and can be further divided into primary, affecting children, and post-primary (secondary), affecting adults (Sastry and Bhat, 2023).

### Extrapulmonary Tuberculosis (EPTB):

Extrapulmonary Tuberculosis (EPTB) occurs when tubercle bacilli spread through the bloodstream. It comprises 10-40% of all TB cases, with a higher prevalence among HIV-positive individuals. EPTB is categorized based on the organs affected: Tuberculous

lymphadenitis is the most common form, representing 35% of all EPTB cases, often affecting cervical and supraclavicular lymph nodes. Pleural tuberculosis, accounting for 20% of EPTB cases, manifests as pleural effusion, with tuberculous empyema being a complication. Genital tuberculosis in females mainly involves the fallopian tubes and endometrium, leading to infertility. Skeletal tuberculosis, notably affecting weight-bearing joints such as the spine and hips, can cause vertebral collapse and abscess formation. Tuberculosis of the central nervous system includes tuberculous meningitis and tuberculoma. Gastrointestinal tuberculosis primarily affects the terminal ileum and cecum.

Tuberculous skin lesions include Scrofuloderma and Lupus vulgaris. Miliary or disseminated tuberculosis is characterized by tiny granulomatous lesions, often seen in HIV-infected individuals. Post-TB aspergillosis may develop, presenting as simple aspergilloma or chronic cavitary aspergillosis. HIV-associated tuberculosis is common among HIV-infected individuals, with extrapulmonary TB being more prevalent than pulmonary TB, with presentations including lymphatic, disseminated, pleural, and meningitis involvement (Sastry and Bhat, 2023).

## Phytochemicals

The Ayurvedic medical system have made an extensive use of ethnomedicinal tools, including the use of plants like Amalaki (*Emblica officinalis* (Gaertn.)), Guduchi (*Tinospora cordifolia* (willd.)), Sariva (*Hemidesmus indicus* (R.Br.)), Kustha (*Saussurea lappa* (Falc.)), turmeric (*Curcuma longa* (Mal.) and Green tea (*Camellia sinensis* (Linn.)). These sources are high in flavonoids, polyphenols, tannins and catechins, has been shown to reduce the risk of TB (Nalam *et al.*, 2023).

## List of Phytocompounds in Treating TB

**Epigallocatechin gallate (EGCG):** Epigallocatechin gallate (EGCG) from *Camellia sinensis* (green tea) has demonstrated promising anti-tuberculosis activity by targeting the pantothenate synthetase of

MDR-TB and XDR-TB, making it effective in treating MDR and XDR *M. tuberculosis* and *M. smegmatis* (Ansari *et al.*, 2023).

**Eugenol (4-allyl-2-methoxyphenol):** Clove essential oil, containing the extract eugenol (4-allyl-2-methoxyphenol), exhibits antimicrobial properties by disrupting the cell membrane of the Mtb strain H37Rv (Ghallab *et al.*, 2024).

**Curcumin:** Curcumin from the rhizome of *Curcuma longa* (turmeric) exhibits antibacterial, antioxidant, and anti-inflammatory properties against *M. tuberculosis* (Gautam *et al.*, 2007)

**Resveratrol:** Resveratrol from *Vitis vinifera* (common grape vine) increases resistance to *M. tuberculosis* infection by preventing the inhibition of Sirt1 through the downregulation of TAK1, (Ansari *et al.*, 2023).

**Artemisinin(AN):** Extracts from *Artemisia annua* and *Artemisia afra* demonstrate potent bactericidal activity against *M. tuberculosis*, with artemisinin (AN), an antimalarial and antitubercular agent, inhibiting Mtb survival under hypoxic conditions by blocking the DosRST system essential for Mtb's non-replicating persistence, and also exhibiting bactericidal effects during aerated growth, potentially through lipid peroxidation, while also being effective against *M. abscessus* (Martini *et al.*, 2020).

**Nimbidin:** Nimbidin, a crude bitter principle extracted from neem seed oil, has demonstrated several biological activities; *in vitro*, it can completely inhibit the growth of *M. tuberculosis* and has also been found to be bactericidal (Mandal *et al.*, 2011)

**Quercetin:** Quercetin from *Citrus spp.* inhibits subunit B of DNA gyrase, beta-ketoacyl ACP synthase III of the mycolic acid pathway, uridine 5'-diphosphategalactopyranosemutase, glutamine synthetase, isocitratlyase of the glyoxylate shunt, suppresses hyaluronan-dependent growth of mycobacterium, and inhibits sulfotransferase to block sulfur metabolism of mycobacterium, making it effective against *M. tuberculosis* (Ansari *et al.*, 2023).

**Sesquiterpene zingiberene (ZGB):** Extracts from *Couroupitaguianensis* and *Tithonia diversifolia*, specifically the sesquiterpene zingiberene (ZGB) from TD leaves and the phthalate ester bis-2 (ethylhexyl) phthalate (BEHP) from CG leaves, demonstrate potent inhibitory effects against *M. tuberculosis* H37Ra, indicating their potential as anti-TB drug molecules (Priyadarshini *et al.*, 2022).

**Cryptolepine hydrochloride:** Cryptolepine hydrochloride from *Cryptolepis anguinolenta* exhibits anti-mycobacterial activity, making it effective against *M. fortuitum* (Ansari *et al.*, 2023).

**Bisbenzylisoquinoline alkaloids:** Bisbenzylisoquinoline alkaloids from *Tiliacoratri andra* exhibit anti-mycobacterial activity, making them effective against clinical isolates of MDR-TB (Ansari *et al.*, 2023).

**Curcumin:** Curcumin from *Curcuma longa* (turmeric) enhances the clearance of *M. tuberculosis* in primary human alveolar macrophages and differentiated THP-1 human monocytes, and induces apoptosis in *M. tuberculosis*-infected THP-1 cells through enhanced expression of bax and cytochrome-c and reduced activation of NF-κB, making it effective against *M. tuberculosis* and *M. abscessus* (Ansari *et al.*, 2023).

**Diallyl sulfide 2-methylpropenyl:** Active organosulfur compounds from *Allium sativum*, such as Diallyl sulfide and 2-methylpropenyl, act against isoniazid-resistant isolates of *M. tuberculosis* (Ansari *et al.*, 2023).

**Allicin:** Allicin from *Allium sativum* selectively kills intracellular *M. tuberculosis* in macrophages and restrains the entry of *M. tuberculosis* by inhibiting the ICAM-1 receptor. It exhibits activity against *M. smegmatis*, *M. phlei*, and *M. tuberculosis* (Ansari *et al.*, 2023).

**Diallyl trisulfide:** Diallyl trisulfide from *Allium sativum* exhibits potent antimycobacterial activity against the H37Rv strain of *M. tuberculosis*, with a MIC of 2.5 µg/mL, while diallyl tetra sulfide

demonstrates significant inhibitory activity against the same strain of *M. tuberculosis*. (Ansari *et al.*, 2023).

**Tropane alkaloids:** Tropane alkaloids such as scopolamine, hyoscyamine, and atropine, found in the leaf extract of *Datura metel* L., are not commonly associated with direct anti-TB activity but may have pharmacological effects that indirectly contribute to combating tuberculosis (Gautam *et al.*, 2007).

**Catechins, flavonoids, tannins, proanthocyanidins:** The root extract of *Acacia catechu* (L.) Willd contains catechins, flavonoids, tannins, and proanthocyanidins, with tannins and flavonoids particularly showing potential antimicrobial activity against tuberculosis (Gautam *et al.*, 2007).

**Andrographolides:** The leaf extract of *Andrographis paniculata* Nees., containing andrographolides, exhibits antimicrobial properties against tuberculosis (Gautam *et al.*, 2007).

**Bis 2-(ethyl hexyl) phthalate (BEHP):** The leaves of *Couroupita guianensis* (Aubl.) contain bis 2-(ethyl hexyl) phthalate (BEHP), which exhibits sensitivity against the MTB H37Ra strain at concentrations of ≤60 µg/mL (Gautam *et al.*, 2007).

**Zingiberene (ZGB):** The leaves of *Tithonia diversifolia* (Hemsl.) A. Gray contains zingiberene (ZGB), which exhibits antimycobacterial activity against the *M. tuberculosis* strain H37Ra (Gautam *et al.*, 2007).

**Tannins, Flavonoids, Vitamin C, Gallic acid, Ellagic acid:** *Emblica officinalis* (Gaertn.) (syn. *Phyllanthus emblica* L.), commonly known as amla, contains tannins, flavonoids, vitamin C, gallic acid, and ellagic acid, which together exhibit antimicrobial, anti-inflammatory, and antioxidant properties against *M. tuberculosis* (Gautam *et al.*, 2007).

**Indole alkaloids, Suaddimins-A, Suaddimins-B, Suaddimins C, and Dragamine Voacangine:** Indole alkaloids from *Alstoniascholaris*, Suaddimins-A, Suaddimins-B, and Suaddimins C from *Melodinus*

*suaveolens*, and Dragamine Voacangine from *Tabernaemontana elegans* exhibit anti-mycobacterial activity against *M. tuberculosis* (Ansari *et al.*, 2023).

## Conclusion

Ayurveda emerges as a promising avenue for tuberculosis (TB) management and prevention, particularly due to its potential to integrate with existing drugs, mitigating side effects such as liver toxicity a common issue with allopathy drugs. With phytochemicals showing fewer or no side effects compared to allopathy medications notorious for hepatotoxicity, Ayurveda offers a safer and more holistic approach. Its affordability further underscores its societal value, especially in regions where costly anti-TB drugs pose significant financial barriers to treatment access. Embracing Ayurveda for TB care not only enhances efficacy but also ensures equitable healthcare, benefiting communities affected by this global health challenge. Additionally, several phytochemicals have synergistic effects with antibiotics routinely used to treat TB, improving their efficacy and decreasing the risk of resistance development. Interestingly, phytocompounds have been shown to reduce isoniazid- and ethambutol-induced hepatotoxicity by reversing serum levels of AST, ALP, ALT, bilirubin, MDA, urea, creatinine, and albumin to their normal range, leading to attenuation of inflammation and hepatic necrosis. Consequently, phytochemicals represent a promising field of research for the development of new TB medicines.

## References

- Ansari, M. A., Shoaib, S., Alomary, M. N., Ather, H., Ansari, S. M. A., Hani, U., Jamous, Y. F., Alyahya, S. A., Alharbi, J. N., Imran, M. A., Wahab, S., Ahmad, W., & Islam, N. (2023). Deciphering the emerging role of phytocompounds: Implications in the management of drug-resistant tuberculosis and ATDs-induced hepatic damage. *Journal of Infection and Public Health*, 16(9), 1443–1459. <https://doi.org/10.1016/j.jiph.2023.07.016>.

- Gandhi, Y., Grewal, J., Jain, V., Rawat, H., Mishra, S. K., Kumar, V., Kumar, R., Shakya, S. K., Sharma, P., Dhanjal, D. S., Prasad, S. B., Charde, V., Arya, J., Narasimhaji, C., Singh, A., Singh, R., Srikanth, N., & Acharya, R. (2023). *Emblica officinalis*: A promising herb confining versatile applications. *South African Journal of Botany*, 159, 519–531. <https://doi.org/10.1016/j.sajb.2023.06.041>.
- Gautam, R., Saklani, A., & Jachak, S. M. (2007). Indian medicinal plants as a source of antimycobacterial agents. *Journal of Ethnopharmacology*, 110(2), 200–234. <https://doi.org/10.1016/j.jep.2006.12.031>.
- Ghallab, Y. E., Aainouss, A., Messaoudi, M. D. E., & Derfoufi, S. (2024). Synthesis, Biological activity and In Silico Study of Alkyl Eugenol Derivatives as *Mycobacterium Tuberculosis* Inhibitors. *Chemical Physics Impact*, 8, 100508. <https://doi.org/10.1016/chphi.2024.100508>.
- Kerr, P. G. (2013). Plants and tuberculosis. In *Elsevier eBooks* (pp. 45–64). <https://doi.org/10.1016/b978-0-12-398539-2.00005-7>.
- Maison, D. P. (2022). Tuberculosis pathophysiology and anti-VEGF intervention. *Journal of Clinical Tuberculosis and Other Mycobacterial Diseases*, 27, 100300. <https://doi.org/10.1016/j.jctube.2022.100300>.
- Mangwani, N., Singh, P. K., & Kumar, V. (2020). Medicinal plants: Adjunct treatment to tuberculosis chemotherapy to prevent hepatic damage. *Journal of Ayurveda and Integrative Medicine*, 11(4), 522–528. <https://doi.org/10.1016/j.jaim.2019.02.004>.
- Martini, M. C., Zhang, T., Williams, J. T., Abramovitch, R. B., Weathers, P. J., & Shell, S. S. (2020). *Artemisia annua* and *Artemisia afra* extracts exhibit strong bactericidal activity against *Mycobacterium tuberculosis*. *Journal of Ethnopharmacology*, 262, 113191. <https://doi.org/10.1016/j.jep.2020.113191>.

- Mobed, A., Darvishi, M., Kohansal, F., Dehfooli, F. M., Alipourfard, I., Tahavvori, A., & Ghazi, F. (2024). Biosensors; nanomaterial-based methods in diagnosing of *Mycobacterium tuberculosis*. *Journal of Clinical Tuberculosis and Other Mycobacterial Diseases*, 34, 100412. <https://doi.org/10.1016/j.jctube.2023.100412>.
- Nalam, S. M., Chintamaneni, P. K., Pal, R. S., Chaitanya, M., Singh, S. K., Saranya, P., Arora, S., Sharma, S., Pandey, P., Mazumder, A., Babu, R., Amoateng, P., & Singh, A. (2023). From nature's bounty to drug discovery: Leveraging phytochemicals and molecular approaches to combat multi-drug-resistant (MDR) tuberculosis. *Indian Journal of Tuberculosis/Indian Journal of Tuberculosis*. <https://doi.org/10.1016/j.ijtb.2023.08.007>.
- Nalunjogi, J., Mucchino-Toscano, S., Sibomana, J. P., Centis, R., D'Ambrosio, L., Alffenaar, J., Denholm, J., Blanc, F., Borisov, S., Danila, E., Duarte, R., García-García, J., Goletti, D., Ong, C. W., Rendon, A., Thomas, T. A., Tiberi, S., Van Den Boom, M., Sotgiu, G., & Migliori, G. B. (2023). Impact of COVID-19 on diagnosis of tuberculosis, multidrug-resistant tuberculosis, and on mortality in 11 countries in Europe, Northern America, and Australia. A Global Tuberculosis Network study. *International Journal of Infectious Diseases*, 130, S25-S29. <https://doi.org/10.1016/j.ijid.2023.02.025>.
- Pawase, P. A., Goswami, C., Shams, R., Pandey, V. K., Tripathi, A., Rustagi, S., & G, D. (2024). A conceptual review on classification, extraction, bioactive potential and role of phytochemicals in human health. *Future Foods*, 9, 100313. <https://doi.org/10.1016/j.fufo.2024.100313>.
- Priyadarshini, N., Veeramani, A., Chinnathambi, P., Palanichamy, A., Al-Dosary, M. A., Ali, M. A., Lee, J., & Paulraj, B. (2022). Antimycobacterial effect of plant derived phthalate against *Mycobacterium tuberculosis* H37Ra. *Physiological and Molecular Plant Pathology*, 117, 101761. <https://doi.org/10.1016/j.pmpp.2021.101761>

- Rahlwes, K. C., Dias, B. R., Campos, P. C., Alvarez-Arguedas, S., & Shiloh, M. U. (2023). Pathogenicity and virulence of *Mycobacterium tuberculosis*. *Virulence*, 14(1). <https://doi.org/10.1080/21505594.2022.2150449>.
- Repele, F., Alonzi, T., Navarra, A., Farroni, C., Salmi, A., Cuzzi, G., Delogu, G., Gualano, G., Puro, V., De Carli, G., Girardi, E., Palmieri, F., Martineau, A. R., & Goletti, D. (2024). Detection of *Mycobacterium tuberculosis* DNA in CD34+ peripheral blood mononuclear cells of adults with tuberculosis infection and disease. *International Journal of Infectious Diseases*, 141, 106999. <https://doi.org/10.1016/j.ijid.2024.106999>.
- Sastry, A. S., & Bhat, S. (2023). Essentials of medical microbiology. Jaypee Brothers Medical Publishers Pvt Limited.
- Shan, L., Wang, Z., Wu, L., Qian, K., Peng, G., Wei, M., Tang, B., & Jun, X. (2024). Statistical and network analyses reveal mechanisms for the enhancement of macrophage immunity by manganese in *Mycobacterium tuberculosis* infection. *Biochemistry and Biophysics Reports*, 37, 101602. <https://doi.org/10.1016/j.bbrep.2023.101602>.
- Tawde, K., Gacche, R., & Pund, M. (2012). Evaluation of selected Indian traditional folk medicinal plants against *Mycobacterium tuberculosis* with antioxidant and cytotoxicity study. *Asian Pacific Journal of Tropical Disease*, 2, S685-S691. [https://doi.org/10.1016/s2222-1808\(12\)60244-8](https://doi.org/10.1016/s2222-1808(12)60244-8).
- Venkatappa, T., Shen, D., Ayala, A., Li, R., Sorri, Y., Punnoose, R., & Katz, D. (2023). Association of *Mycobacterium tuberculosis* infection test results with risk factors for tuberculosis transmission. *Journal of Clinical Tuberculosis and Other Mycobacterial Diseases*, 33, 100386. <https://doi.org/10.1016/j.jctube.2023.100386>.

## Global Markets and Commercial Opportunities in Medicinal Phytochemicals

Supriya L<sup>1</sup>, Sakthikokilambal.S<sup>2</sup>, Maneeshaa K<sup>3</sup>,  
Durga Devi L<sup>4</sup> & Mahenthiran.R<sup>5</sup>

<sup>1-3</sup> I.M.Sc., Microbiology, Department of Microbiology,  
Dr.N.G.P. Arts and Science College, Coimbatore, Tamilnadu.

<sup>4</sup> Ph.D. Scholar, Department of Microbiology,  
Dr.N.G.P. Arts and Science College, Coimbatore, Tamilnadu

<sup>5</sup> Assistant professor, Department of Microbiology,  
Dr.N.G.P. Arts and Science College, Coimbatore, Tamilnadu

### Abstract

Phytochemicals are bioactive compounds inherent in plant-derived food products such as fruits, vegetables, nuts, and grains. Plants use Phytochemicals as a natural defence mechanism. Phytochemicals safeguard cells through the process of neutralising free radicals that harm the cellular components such as the membrane, proteins, and genetic material. Given their favourable impact on human health, phytochemicals offer viable solutions to prevalent health issues like cardiovascular diseases, cancer, and various neurodegenerative conditions like Alzheimer's and Parkinson's disease. Categorically, phytochemicals are organised based on their chemical characteristics into polyphenols, carotenoids, anthocyanidins, catechins, flavonoids, glucosinolates, polysaccharides, lectins, terpenes, alkaloids, polyacetylenes, allium compounds, chlorophyll, capsaicinoids, and betalains. Noteworthy attributes of phytochemicals encompass antioxidant, anticarcinogenic, anti-inflammatory, and neuroprotective properties, contributing significantly to overall health and well-being. Additional advantages of phytochemicals include the prevention of cellular damage, mitigation of DNA damage,

reduction of inflammation, and stimulation of the immune system. Their anti-aging attributes have led to their utilisation in skincare products and cosmetics. The global markets for phytochemicals are harnessed across different industries due to their nutritional value and additional components like antioxidants, antiseptics, and antimicrobials. The increasing demand for phytochemicals in the global market is driven by their health benefits as dietary supplements and medicinal properties. The application of medicinal phytochemicals is observed in numerous regions worldwide such as America, Asia, Africa, and others.

**Keywords:** *Phytochemicals, flavonoids, alkaloids, antioxidant, health benefits, Global markets.*

## Objective of the Study

- To identify and study new phytochemical compounds with potential medicinal properties.
- Establish standards for the extraction, purification, and dosage of phytochemical-based medicines to ensure consistency and quality.
- For their anti-inflammatory and anti oxidant qualities as well as for the prevention and treatment of a number of illness, including diabetes, cancer, and cardiovascular disorders.
- Educate healthcare professionals and the public about the importance of phytochemicals in health promotion and disease prevention.
- To be aware of the market for natural remedies instead of manufactured ones and the financial potential that comes with growing and extracting phytochemicals.

## Introduction

Phytochemicals are produced by all plants, including fruits, vegetables, cereals and beans. Traditional species of plants have medicinal properties due to their phytochemical components, which have specific pharmacological effects on

human anatomy. As a component of the immune system, they aid in defending the plant against bacteria, fungus, viruses and parasites. The phytochemical components can be broadly classified into two groups based on the metabolism activity in the plant; primary constituents, which are primarily, composed of sugars, amino acids, proteins and chlorophyll, and secondary constituents, which are made up of alkaloids, flavanoids, saponins, tannins and phenolic compounds. Although some phytochemicals might be thought as vitamins as well, they are not as vital to health as vitamins and minerals are. Rather, they shield your cells against harm brought on by toxins in the environment and the body's normal chemical (metabolic) processes. It can be protected against infections and disease without experiencing over reactions or persistent inflammation, immune system in balance and is neither underactive nor overactive. A healthy immune system can be attained and maintained with the use of phytochemicals. Additionally, phytochemical have antibacterial properties. They lessen the possibility that germs and viruses will proliferate within the body. According to preliminary studies phytochemical assist in ensuring that your immune system mounts a suitable defence in the event of an illness. Additionally, they can lessen persistent inflammation brought on by inflammatory illnesses. Carotenoids, polyphenol, isoprenoids, phytosterols, saponins, dietary fibres, and certain polysaccharides are a few of the important phytochemicals have antiviral, antibacterial, antidiarrheal, and antihelmintic and anti allergic properties. In addition, they support immunity promote gap junction communication, control gene transcription, and guard against prostate and lung malignancies. The features of functional foods have been expanded by the new emphasis on translational research. Following their extraction from a variety of sources, phytochemical are widely used in the creation of nutraceuticals and functional foods. The affinity of phytochemicals for solvents and their heat tolerance vary. The quality of the recovered phytochemical and its use in the creation of food and nutraceuticals are also impacted by the solvent choice.

## Review of Literature

As phytochemistry has developed over the last few decades and become its own field, humans have always associated plants with civilization. An estimated 5,000 years old Sumerian clay slab holds the earliest known record of medicinal herbs being utilised to make medications. Twelve recipes for preparing medications from over two hundred and fifty different plants some of which have significant alkaloids were presented. The market for phytochemical is highly competitive due to the rise of new companies and the growing demand for dietary supplements. The market is seeing potential due to the presence of small scale business that prioritises innovation over product sales. The major industry participants are expanding their production capacity by building and purchasing manufacturing facilities. Persistence Research has released a new research study on the global phytochemicals that assesses the potential and state of the industry while offering updates and insights into related market segments for the 2023-2033 forecast periods. The research provides comprehensive insights into the markets expected growth during the 2023-2033 forecast periods. Manufacturers, suppliers, distributors, and investors in the phytochemicals industry might find the report useful.

### Medicinal Phytochemical

Medicinal phytochemicals are natural compounds found in plants that have been studied for their potential health benefits and therapeutic properties. These bioactive substances are not considered essential nutrients like vitamins and minerals, but they are believed to play a role in promoting health and preventing diseases. There are thousands of different phytochemicals found in various plant-based foods, and each may have unique effects on the body. Some well-known examples of medicinal phytochemicals include:

1. **Polyphenols:** Polyphenols are a large group of naturally occurring compounds found abundantly in fruits,

vegetables, tea, coffee, and red wine. These compounds are known for their potent antioxidant properties, which help neutralize harmful free radicals in the body. The consumption of polyphenols is linked to a lower risk of several chronic diseases, including heart disease and cancer. Polyphenols are found in fruits, vegetables, tea, coffee, and red wine, polyphenols have antioxidant properties and are associated with reduced risk of chronic diseases such as heart disease and cancer.

2. **Flavonoids:** A subgroup of polyphenols, flavonoids are found in fruits, vegetables, cocoa, and legumes. They have antioxidant and anti-inflammatory effects and may help in reducing the risk of cardiovascular disease and improving cognitive function. Moreover, they have been studied for their potential benefits in enhancing cognitive function and memory, making them valuable in the prevention of neurodegenerative diseases such as Alzheimer's.
3. **Carotenoids:** Found in colorful fruits and vegetables such as carrots, tomatoes, and spinach, carotenoids have antioxidant properties and are important for eye health and immune function. Carotenoids, such as beta-carotene, lycopene, and lutein, have strong antioxidant properties, supporting overall immune function and reducing the risk of chronic diseases. Their role in skin health is also significant, as they help protect against UV damage and promote a healthy complexion.
4. **Alkaloids:** These nitrogen-containing compounds are found in plants such as coffee, tea, and certain medicinal herbs. Alkaloids have diverse effects on the body, including pain relief, mood enhancement, and stimulation of the central nervous system. Alkaloids are also used in medicine for their therapeutic properties, including their roles as analgesics, stimulants, and antimalarials.
5. **Terpenoids:** Found in essential oils and aromatic plants, terpenoids have various biological activities including anti-inflammatory, antimicrobial, and anticancer properties.

## Sources of Phytochemicals

Phytochemicals	Source Plant	Health Benefits
$\alpha$ -linolenic acid (ALA)	Flax seeds	Cancer preventive, reduce risk of coronary heart disease
Allicin	Garlic, onion	Antibacterial, anticancer, antifungal, anti-inflammatory, chemopreventive, hepato-protective, hypolipidemic, hypotensive, and neuroprotective
Anthocyanins	Blackberry, cherry, orange, purple corn, raspberry, red grapes	Anti-allergic, anti-inflammatory, antioxidants, and pigments
Apigenin	Apple, artichoke, basil, celery, cherry, grapes, nuts, parsley	Anti-inflammatory, antioxidant, Antispasmodic, chemo-preventive, induce apoptosis, and inhibits breast and ovarian cancers
Caffeic acid	Artichoke, pear, basil, oregano	Anti-inflammatory, anti-fatigue and anti-stress properties
Carotene	Carrots, Leafy greens and red, orange and yellow vegetables, pumpkin	Anti-carcinogenic, enhances release of immunogenic cytokines IL-1 and TNF-alpha, provide cornea protection against UV light, stimulate DNA repair enzymes
Catechins	Tea	Antioxidant, CNS stimulant, and Diuretic
Curcumin	Turmeric	Anti-hypertensive, anti-inflammatory, antioxidant, and cancer preventive
Diosgenin	Fenugreek seeds	Hypolipidemic
Ellagic acid	Cranberry, grapes, pecans, pomegranates, raspberry, strawberry, walnuts	Anticancer, and antioxidant
Ferulic acid	Oats, rice, orange, pineapple, peanut	Protect against cancer, bone degeneration, menopausal symptoms (hot flushes)
Gallic acid	Tea, mango, strawberries, soy	Cytotoxic and antioxidative activities, Anti-leukemic, antioxidant, anticancer, anti-neoplastic, anti-inflammatory, anti-diabetic
Genistein	Alpha-alfa sprouts, red clover,	acts as a phytoestrogens, antioxidant,

**Figure 1. Source of phytochemicals**

## Extraction

Extraction of medicinal phytochemicals refers to the process of isolating bioactive compounds from plants for various purposes, including pharmaceuticals, nutraceuticals, and cosmetics. These methods vary in complexity, efficiency, and suitability for different types of phytochemicals and plant materials. Here are some of the commonly employed extraction methods:

### 1. Solvent Extraction:

- **Maceration:** Simple soaking of plant material in a solvent to extract phytochemicals.
- **Percolation:** Continuous flow of solvent through a packed bed of plant material.
- **Soxhlet Extraction:** Continuous extraction using a reflux-condensation cycle.

### 2. Solid-Liquid Extraction:

- **Ultrasound-Assisted Extraction (UAE)**
- **Microwave-Assisted Extraction (MAE)**
- **Pulsed Electric Field Extraction (PEF)**

### 3. Supercritical Fluid Extraction (SFE):

### 4. Fractionation Techniques:

- **Liquid-Liquid Extraction (LLE)**
- **Solid-Phase Extraction (SPE)**
- **Thin-Layer Chromatography (TLC)**
- **Column Chromatography**

Both maceration and percolation are versatile and widely used in herbal medicine, pharmaceuticals, and research laboratories due to their simplicity, scalability, and effectiveness in extracting a broad

spectrum of phytochemicals. Additionally, these methods can be easily adapted for use with various solvents and plant materials, making them suitable for diverse applications.

### Extraction of Curcumin from Turmeric Process;

1. **Extraction-** Powdered turmeric rhizomes are soaked with solvent placed in Soxhlet extractor.
2. **Filtration-** After extraction, filter the solvent-turmeric mixture to remove solid residues using the rotary evaporator, leaving behind a concentrated extract.
3. **Purification-** It is done using column chromatography or recrystallization to obtain high purity curcumin.
4. **Drying-** Filter out the precipitated curcumin and dry it under vacuum or at a low temperature.

### Extraction; Supercritical fluid extraction (SFE)

Powdered turmeric is subjected to supercritical  $\text{CO}_2$  which dissolves the curcumin by penetrating into turmeric matrix at a constant temperature  $75^\circ\text{C}$  and pressure 425bar. The mixture is then depressurized to separate  $\text{CO}_2$  and curcumin mixture and collect the curcumin.

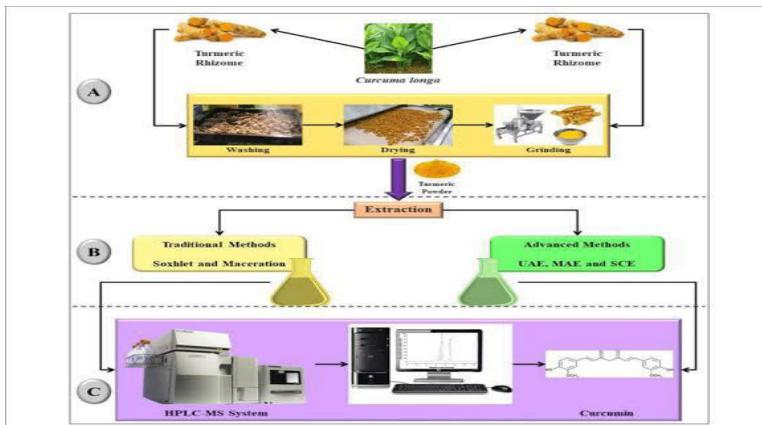


Figure 2: Image references from researchgate.com

## Current Global Market Size and Forecast of Medicinal Phytochemical

The global market for medicinal phytochemicals, which include herbal and plant-based extracts used for therapeutic purposes, is experiencing substantial growth. The market is projected to grow significantly in the coming years, driven by increasing consumer preference for natural and holistic health solutions. The global phytochemical market is set to record a market value of US\$ 7,436 million in 2023, pushing the market size to US\$ 14,821 million in 2033. The overall market is expected to witness healthy growth at 7.1% CAGR. Surging application across diverse end-use sectors such as food & beverages, nutraceuticals, and pharmaceutical continue to augment growth in the phytochemical market. While the food and beverages application segment may hold the leading share, sales in the animal nutrition segment are to record a 13.4% CAGR through 2033. There has been an unexpected increase in natural plant ingredients in ayurvedic manufacturing due to the recent surge in the consumption of traditional medicines, or ayurvedic. Phytochemicals are products derived from organic plants and used in manufacturing organic food and supplement manufacturing.

The forecast outlook for the global medicinal phytochemicals market indicates robust growth driven by increasing consumer demand for natural and plant-based products, advancements in extraction technologies, and expanding applications across various industries.

### Phytochemical Products Demands on Consumers Analysis from (2017-2022) Vs Market Estimation (2023-2033);

Consumer's preference for natural products as it is the safer alternative to synthetic drugs, with fewer side effects and derived from natural sources without artificial additives. Longevity and quality of life that supports healthy aging. The demand for the medicinal phytochemicals among consumers is driven by a combination of health and wellness trends, cultural

influences lifestyle changes, technological advancements and enhanced market accessibility all contribute to the growing consumer interest in medicinal phytochemicals. Currently, the U.S. holds 57.7% of total market share in North America and the market value accounts for US\$ 815.5 Mn as on 2022 and grows at a CAGR of 8.4% therefore it is largest. The consumption for phytochemicals is growing in the U.S. due to the growing aging population which is leading to an upsurge in the consumption of phytochemicals and plant extracts. Moreover, nutraceuticals of preventing chronic diseases improving health, and delaying the aging process have added the best driver for phytochemicals in the U.S. Carotenoids accounts for a market share of 27.4% of total phytochemicals. As on 2022, carotenoids valued at US\$ 1,713.9 Mn and grow at a CAGR of 10.6%. Carotenoids are mainly ruling the health and wellness industry. Researches have shown that dietary carotenoids play a vital role in reducing the cardiovascular diseases and the risk of cancer, and it is also used in the treatment of cataract. Moreover, it is also used in the cosmetics industry as an anti-aging agent as it contains anti-oxidant properties. Phytochemicals are used in the food and beverage Industry, cosmetics & personal care products, pharmaceuticals, nutraceuticals, animal feed and others. Among all these application industries, phytochemicals are majorly used in the food and beverage industry, it accounts for 34.6% of the total market share. Phytochemicals are used in the food and beverage Industry, cosmetics & personal care products, pharmaceuticals, nutraceuticals, animal feed and others. Among all these application industries, phytochemicals are majorly used in the food and beverage industry, it accounts for 34.6% of the total market share.

## **Commercial Opportunities and Companies In medicinal Phytochemicals**

The commercial opportunities for the medicinal phytochemicals are vast and diverse involving multiple industries following the below;

## 1. Pharmaceutical Industry

- Involved in the drug development and in phytopharmaceuticals which are the standardised plant extracts that are used in medicinal products. Many companies are involved in developing new medication from plants for various diseases including cancer, cardiovascular disease and neurodegenerative disorders. Examples of the phytochemical product
- Paclitaxel(taxol)- chemotherapy drug from pacific yew tree for cancers
- Aspirin-from Willow bark for pain relief and as anti inflammatory

## 2. Nutraceuticals and Dietary Supplements

- There is a growing market for the foods and beverages with phytochemicals used for their health benefits. The dietary supplements containing phytochemicals like curcumin, resveratrol, gingseng is increasing consumers seek to boost their health and wellness through natural means.
- Curcumin (from Turmeric): Curcumin supplements, which are marketed for their anti-inflammatory and antioxidant properties and used in dietary supplements, skincare products, and functional foods.
- Resveratrol: Resveratrol supplements, known for their potential anti-aging and cardio protective effects. Extracted from Red Grapes and Japanese Knotweed and Widely used in the nutraceutical and cosmetic industries, found in supplements, skincare products, and anti-aging creams.

## 3. Cosmetics and Personal Care;

- Phytochemicals are increasingly being used in cosmetics and personal care products for their antioxidant, anti inflammatory and anti aging properties. They are used in preparation of skincare products, hair care products and oral care products.

- Aloevera; For soothing and moisturising properties they are used in skincare products.
- Chamomile extract; used in creams and lotion for its calming and anti inflammatory effects.
- They are also widely used in preparing products in the veterinary medicine sectors, agricultural sectors for pesticides and fungicide production as well as for crop nutritional enhancements and growth (quercetin). Leveraging these opportunities requires a combination of innovation, research, strategic partnership and effective marketing to meet the growing consumer demand for natural and plant based health solutions. The market for natural health products continues to grow, driven by consumer interest in holistic wellness and natural alternatives to traditional medicine.

## Companies and Their Challenges

- **Kothari Phytochemicals International**, established in 1974, is a leading manufacturer and exporter of Bulk drugs and Phytochemicals.
- **Acumen life science**-A healthcare and the food & beverage industry and the cosmetics industry
- **Cifal herbal**-Production of citrus products
- **Kaiwal biotech** -A manufacturer and supplier of phytochemicals and herbal extract.
- **Natura**-Production of essential oils , food, beverage and cosmetic products.
- **Sunpure Extracts pvtltd,**
- **Nestle health science**
- **DSM nutritional products**
- **BASF**
- **Sabinsacorporation**
- **Synthite Industries Ltd (India),**
- **Vidya Herbs (India).**

A multifaceted business with interests in a wide range of industries, including natural specialties, phytochemicals, herbal extracts, nutraceuticals, ayurveda, and spices.

## Challenges

Companies involved face a range of challenges from regulatory hurdles and quality control issues to supply chain disruptions, high research cost and intense competitions. Addressing these challenges requires a strategic approach that encompasses regulatory compliance, quality assurance, supply chain management, innovation, and effective marketing. Companies that can navigate these hurdles successfully stand to capitalise on the growing demand for natural health products and realize significant business opportunities.

- **Regulatory Hurdles:** Compliance with complex regulations governing production, labelling, and marketing.
- **Quality control and standardisation:** Risk of adulteration and quality variation.
- **Supply Chain Risks:** Vulnerability to crop failures, seasonal variations, and environmental factors affecting raw material supply.
- **Research and development cost:** Significant investments needed for scientific validation, safety efficacy analysis and R&D.
- **Competition and Market Saturation:** Intense competition leading to price pressure and reduced profit margins.

## Conclusion

Phytochemicals are the non-nutritive parts which are produced by plants against pathogens. Ethiopia and other developing nations in Asia and Africa continue to rely heavily on medicinal plants in their daily lives. Medicinal plants improve the health and safety of the local population in addition to supplementing or replacing contemporary medical therapies,

which are frequently insufficiently available. Traditional medicine, according to the World Health Organisation (WHO), is the entirety of knowledge and methods that can be formally explained or applied in the prevention and treatment of physical, mental, or social imbalances, with a sole reliance on firsthand experience and observation that is verbally or in writing passed down from generation to generation.

Phytochemicals are used in medical field not only because of their antimicrobial properties (including antibacterial, anti-fungal, antiviral) it has other properties like controlling many metabolic disorders such as obesity, diabetes mellitus, cardiovascular disease (CVD), non-alcoholic fatty liver disease. It can also be used against cancer as it has anticancerous properties. With an expanding population comes an increase in the utilisation of native plants in commercial medicine. Demand rose as a result of plant extracts' antibacterial qualities. To overcome this situation, plant tissue culture has shown to be a dependable substitute for extracting bioactive chemicals from plants. Medicinal plants can produce more phytochemicals when grown in artificial environments. Biotechnologist's favour the solution of in vitro plant production. Through the regulated manipulation of ambient conditions, growth regulators, and techniques that can improve both production and overall yield of phytochemicals, in vitro technology makes it possible to cultivate plants consistently. It is possible to prevent plants from having an unstable chemical composition by cultivating them on media that has been prepared strictly to meet the nutritional needs of the plant under controlled environmental circumstances, such as temperature, length and intensity of light, and light. Therefore, phytochemicals has health benefits which can be cultivated in an effective manner apart from harming the nature plants. The future of phytochemical products is promising with numerous advancements on the technological innovations; personalised medicine, sustainable practices and integration with mainstream healthcare are set to driven growth and development in this sector. Enhancing consumer education and awareness, companies can harness the full potential of

phytochemicals to meet the growing demand for natural and effective heal solution.

## References

- Bhagwan K Sakhale, Namrata A Giri, BB Borse. 2023. "Role of Phytochemicals in Human Health." In: Novel Processing Methods for Plant-Based Health Foods. Academic Press. pags. 187-205.
- Cora J Dillard, J Bruce German. First published: 28 July 2000, "Phytochemicals: Nutraceuticals and Human Health." Journal of the Science of Food and Agriculture, vol. 80, issue 12, pp. 1744-1756.
- Gbemisola O Onipede, Abosede O Fawole, Olalekan J Odukoya, John O Onuh. 2024. "Production, Stability, and Industrial Commercialization of Bioactive Phytochemicals." In: Plant Food Phytochemicals and Bioactive Compounds in Nutrition and Health. Elsevier. pags. 79-98.
- Harry L Brielmann, William N Setzer, Peter B Kaufman, Ara Kirakosyan, Leland J Cseke. 2006. "Phytochemicals: The Chemical Components of Plants." Natural Products from Plants, vol. 2, pp. 1-49.
- Hock Eng Khoo, Azrina Azlan, Kin Weng Kong, Amin Ismail. 2016. "Phytochemicals and Medicinal Properties of Indigenous Tropical Fruits with Potential for Commercial Development." Evidence-Based Complementary and Alternative Medicine, vol. 2016, pp. 7591951.
- Kumar, V Krishnan. 2017. "Phytochemistry and Functional Food: The Needs of a Healthy Life." J Phytochem Biochem, vol. 1, no. 1, pp. 103.
- Michael Wink. 2022. "Current Understanding of Modes of Action of Multicomponent Bioactive Phytochemicals: Potential for Nutraceuticals and Antimicrobials." Annual Review of Food Science and Technology, vol. 13, pp. 337-359.
- Monika Thakur, Karuna Singh, Renu Khedkar. 2020. "Functional and Preservative Properties of Phytochemicals." In: Phytochemicals: Extraction Process, Safety Assessment,

Toxicological Evaluations, and Regulatory Issues. Springer, Singapore. pags. 341-361.

- Rajesh Chandra Misra, Ramesha Thimmappa, Mercedes Bonfill. 2024. "Advances in Discoveries of Plant Phytochemicals." Frontiers in Plant Science, vol. 15, art. 1414150.
- Raman Manoharlal, GVS Saiprasad, Shradha Devi Dwivedi, Manju Rawat Singh, Deependra Singh. 2023. "Commercial Aspect and Market Potential of Phytoactive Products." In: Phytopharmaceuticals and Herbal Drugs. CRC Press. pags. 281-301.

## Vegetation Computation of Perundurai, Erode District, Tamil Nadu and Phytochemical Screening of Aqueous Leaf Extract of *Costus Pictus* D.Don.

<sup>1</sup>M. Bhuvaneswari, <sup>2\*</sup>C. Chitra Vadivu,

<sup>3</sup>K. Keerthishree, <sup>4</sup>S. Sakthi and <sup>5</sup>A. Marthupandian

<sup>1</sup>Ph.D., Scholar, <sup>2,5</sup>Assistant Professor & <sup>3</sup>M. Sc., Students

<sup>1,2,3,4</sup>PG& Research Department of Botany,

Vellalar College for Women (Autonomous),

Thindal, Erode, Tamil Nadu.

<sup>5</sup>Ethno pharmacology and Algal Biotechnology Laboratory,

Department of Botany,

School of Life Sciences, Periyar University, Salem, Tamil Nadu.

\*Corresponding Author e-mail id: [chitravadivuchinnu@gmail.com](mailto:chitravadivuchinnu@gmail.com)

### Abstract

Nature has abundant source of medicinal plants, which are more capable of containing phytochemicals. Those phytochemicals were used to cure different diseases and disorders. To assess the vegetation status, this is a preliminary endeavor of collecting plant species from the Perundurai, Erode District and totally 145 plant species identified. From the survey 129 genera, 57 families classified into dicots were 130 species which is 122 genera carries 47 families. The monocot 15 species concerned with 15 genera carries 10 different families (Table - 2). The highest species distribution were herb (62 species), followed by shrub (52 species) followed by tree (29 species) and climber (2 species). The above survey one of the potential medicinal plant *Costus Pictus* D.Don. aqueous leaf extract qualitative phytochemical analysis resulted protein, amino acids, carbohydrates, flavonoids, phenols, glycosides, terpenoids, saponins and quinones were present. The preliminary analyses may useful for novel drug discovery.

**Key words:** *Floristic Survey, Aqueous leaf extract, Qualitative phytochemical analysis*

## Introduction

Medicinal plants are part of our culture and are still valued in India, one of the world's largest biodiversity rich countries. Ayurveda, Siddha, Unani, and homoeopathy are India's primary traditional medical systems. In India, 75% of the significant medicinal plant species exist in nearly wild conditions (Johnson *et al.*, 2015). Tropical areas biodiversity loss is mostly caused by anthropogenic habitat degradation and destruction, which is now accredited as global issues (Sukumaran *et al.*, 2008). Urbanization has drastically changed the floristic diversity of the study area (Saranya *et al.*, 2023). The optimal process for identifying conservation areas necessitates a thorough understanding of species diversity and ecological distribution (Pitchairamu *et al.*, 2008).

India is home to two mega diversity centres and has an enormous amount of biological diversity. The safeguarding and sustainable use of these vast bio resources, however, is significant issues and 10% of the world's plant species, or over 48,000 species, are thought to exist in India. The nation is also home to 320 species of wild relatives of domesticated crops and 167 significant cultivated plant species (Jayanthi Palanisamy and Rajendran Arumugam, 2014). One of the medicinally potent plants was chosen for the study from the floristic survey Namely *Costus pictus* D.Don. used for anti - diabetic, obesity, antiulcerolytic and antimicrobial activities.

## Materials and methods

### Study Site

The frequent visit of survey was conducted in Perundurai, Erode District which lies on 11.2746° N, 77.5827° E. The study site

has more potential medicinal plants which are used to cure several diseases.

## Floristic Diversity

Survey was conducted during November 2023 - April 2024 and identified plant species were arranged alphabetical order with in the form of Binomial Name, Family, Common Name, Tamil Name, Useful Parts and Medicinal Uses. The plant species were identified by using 'Flora of the Presidency of Madras (Gamble,1915-1935).

## Collection of plant

From the above survey medicinally important plant species *Costus pictus D.Don.* was chosen for the present study.

## Plant extraction preparation for Phytochemical Analysis

The collected *Costus pictus D.Don.* was rinsed properly, cut into pieces and stored under room temperature. The dried plant material was made into powder and used to prepare extract through Soxhlet apparatus. The extraction was used for phytochemical analysis and remaining extracts stored at 4°C.

## Qualitative Phytochemical Analysis

The qualitative phytochemical screening for Protein (Biuret Test), Carbohydrate (Barfoed's Test), Amino acids (Ninhydrin Test), Alkaloids (Wagner's Test), Flavonoids (Ammonium Hydroxide Test), Phenols (Lead Acetate Test), Tannin (Ferric Chloride Test), Glycoside (Borntrager's Test), Terpenoids (Liebermann test), Saponin (Foam test), Coumarins (NaOH test), Quinone (Sulphuric acid test), Anthroquinone (Borntrager's test), Fixed Oil and Fat (Stain test) and for Gums and mucilage (Absolute alcohol test) tests were carried out.

## Results and Discussion

We identified 145 plant species based on the findings of the floristic survey of plants for medicinal purposes (Plate -1). Among these 129 genera, 57 families categorized, further dicots were 130 species belonging to 122 genera belong to 47 families. The monocot 15 species belonging to 15 genera carries 10 different families (Table - 2& Fig - 1). The most of the plant species were herb (62 species), followed by shrub (52 species), tree (29 species) and climber (2 species) (Table -1& Fig - 2). The above classified plants were again considered into large quantity such as *Fabaceae* (23 species), *Solanaceae* (10 species), *Apocynaceae* (8 species), *Malvaceae* (6 species), *Amaranthaceae* and *Poaceae* (5 species), *Lamiaceae*, *Boraginaceae*, *Euphorbiaceae*, *Nyctaginaceae* and *Oleaceae* (4 Species and the remaining families contains lesser species count (Table -2) similarly, Jayanthi Palanisamy and Rajendran Arumugam (2014) documented that Madukkarai hills floristic diversity resulted a total of 300 plant species from 206 genera and 72 families have been identified as well as 241 species were dicots, including 169 genera and 65 families, and 59 species from monocots, comprising 35 genera and 7 families. Kumar *et al.*, 2023 recorded that floristic diversity of Nandha Gopalasamy hill temple exhibits 54 plant species from that 22 herbs, 2 climbers 12 shrubs and 17 trees. The preliminary phytochemical screening of aqueous leaf extract of *Costus pictus* leaves revealed the presence of Protein, Carbohydrates, Amino acids, Alkaloids, Flavonoids, Phenols, Tannins, Glycosides, Terpenoids, Saponin and the absence of Tannin, Anthraquinone, Fixed oil, fat, Gums and mucilage (Table - 4).

## Enumeration

The plant species were arranged in the format of tamil name, botanical Name, family, habit, Cotyledon (Table - 1) and medicinal uses (Table - 3). The below table describes about 145 plant species identified from the Perundurai, Erode District.

**Table -1: Floristic Diversity of Habit and cotyledons of species in Perundurai, Erode District**

S.N o	Botanical name	Tamil Name	Families	Life forms	Cotyle dons
1	<i>Abelmoschus esculentus</i> (L.) Moench	Vendakkai	Malvaceae	Herb	Dicot
2	<i>Abrus precatorius</i> L.	Kundumani	Fabaceae	Climbe r	Dicot
3	<i>Abutilon indicum</i> (L.) Sweet.	Thuthikeerai	Malvaceae	Shrub	Dicot
4	<i>Achras sapota</i> L.	Sapota	Sapotaceae	Tree	Dicot
5	<i>Achyranthus aspera</i> L.	Nayuruvi	Amaranthaceae	Herb	Dicot
6	<i>Adenanthera pavonina</i> L.	Aanai Kundumani	Fabaceae	Shrub	Dicot
7	<i>Aegle marmelos</i> (L.) Correa.	Vilvamaram	Rutaceae	Tree	Dicot
8	<i>Aerva lanata</i> (L.) Juss. Ex Schult.	Siru peelai	Amaranthaceae	Shrub	Dicot
9	<i>Albizia amara</i> (Roxb) Boivin.	Unja maram	Fabaceae	Tree	Dicot
10	<i>Allamanda cathartica</i> Linn.	Manjalpatti	Apocynaceae	Shrub	Dicot
11	<i>Allium cepa</i> L.	Vengayam	Liliaceae	Herb	Monocot
12	<i>Aloe vera</i> (L.) Webb.	Sotrukatalai	Asphodelaceae	Herb	Monoc ot
13	<i>Amaranthus tricolor</i> L.	Nerunjil	Zygophyllaceae	Herb	Dicot
14	<i>Amaranthus viridis</i> L.	Mullaikeerai	Amaranthaceae	Shrub	Dicot
15	<i>Annona squamosa</i> L.	Seetha maran	Annonaceae	Shrub	Dicot
16	<i>Areca catechu</i> L.	Pakku maram	Arecaceae	Tree	Monoc ot
17	<i>Asystasia gangetica</i> (L.) T. Anderson.	Mithikeerai	Acanthaceae	Herb	Dicot

18	<i>Azadirachta indica</i> A. Juss.	Veppamaram	Meliaceae	Tree	Dicot
19	<i>Barleria acuminata</i> Nees.	Vellai kurunj	Acanthaceae	Shrub	Dicot
19	<i>Bauhinia tomentosa</i> L.	Thiruvath	Fabaceae	Tree	Dicot
20	<i>Boerhavia diffusa</i> L.	Mukaratai Sarai	Nyctaginaceae	Herb	Dicot
21	<i>Boerhavia verticillata</i> Poir.	Mukaratai Keerai	Nyctaginaceae	Herb	Dicot
22	<i>Bougainvillaea glabra</i> Choisy.	Kakitha poo	Nyctaginaceae	Shrub	Dicot
23	<i>Caesalpinia pulcherrima</i> (L.) Sw.	Mayil Kondrai	Fabaceae	Shrub	Dicot
24	<i>Cajanus cajan</i> (L.) Millsp.	Thuvarai	Fabaceae	Shrub	Dicot
25	<i>Calotropis gigantea</i> (L.) W. T. Aiton.	Erukku	Apocynaceae	Shrub	Dicot
26	<i>Canna indica</i> L.	Kal Vazhai	Cannaceae	Shrub	Monocot
27	<i>Capsicum annuum</i> L.	Kudamilagai	Solanaceae	Shrub	Dicot
28	<i>Cardiospermum halicacabum</i> L.	Mudakkaruthan	Sapindaceae	Herb	Dicot
29	<i>Carica papaya</i> L.	Pappali	Caricaceae	Shrub	Dicot
30	<i>Carissa carandas</i> L.	Kalakkai	Apocynaceae	Shrub	Dicot
31	<i>Cassia auriculata</i> Linn.	Avaram poo	Fabaceae	Shrub	Dicot
32	<i>Cassia roxburghii</i> DC.	Senkondrai	Fabaceae	Tree	Dicot
33	<i>Celosia argentea</i> L.	Pannaikeerai	Amaranthaceae	Herb	Dicot
34	<i>Centella asiatica</i> (L.) Urban.	Vallarai	Apiaceae	Herb	Dicot
35	<i>Chloris barbata</i> Sw.	Kodaipullu	Poaceae	Herb	Monocot
36	<i>Cissus quadrangularis</i> L.	Pirandai	Vitaceae	Herb	Dicot

37	<i>Cleome viscosa</i> L.	Naikaduku	Capparidaceae	Herb	Dicot
38	<i>Clitoria ternatea</i> L.	Sangu poo	Fabaceae	Herb	Dicot
39	<i>Coccinia indica</i> (L.) Voigt.	Kovai	Cucurbitaceae	Herb	Dicot
40	<i>Cocculus hirsutus</i> (L.) Diels.	Sirukattukodi	Menispermaceae	Shrub	Dicot
41	<i>Cocos nucifera</i> L.	Thennamaram	Arecaceae	Tree	Monocot
42	<i>Coldenia procumbens</i> L.	Seruppadi	Boraginaceae	Herb	Dicot
43	<i>Colocasia esculenta</i> (L.) Schott.	Seppankizhaingu	Araceae	Herb	Monocot
44	<i>Commelina benghalensis</i> L.	Aduthinnathalai	Commelinaceae	Herb	Monocot
45	<i>Cordia sebestena</i> L.	Achirunaruvi zhi	Boraginaceae	Shrub	Dicot
46	<i>Coriandrum sativum</i> L.	Kottumalli	Apiaceae	Herb	Dicot
47	<i>Costus pictus</i> D. Don.	Insulin chedi	Costaceae	Herb	Monocot
48	<i>Crotalaria juncea</i> L.	Sanal	Fabaceae	Herb	Dicot
49	<i>Cuminum cyminum</i> L.	Seeragam	Apiaceae	Herb	Dicot
50	<i>Cymbopogon nardus</i> L. Rendle.	Kamachi pul	Poaceae	Shrub	Monocot
51	<i>Cyperus rotundus</i> L.	Korai kilangu	Cyperaceae	Herb	Monocot
52	<i>Datura metel</i> L.	Karu oomathai	Solanaceae	Shrub	Dicot
53	<i>Dichrostachys cinerea</i> (L.) Wight.&Arn.	Vidathalai	Fabaceae	Shrub	Dicot
54	<i>Dolichos biflorus</i> L.	Kollu	Fabaceae	Herb	Dicot
55	<i>Emblica officinalis</i> L.	Nellikai	Phyllanthaceae	Tree	Dicot

56	<i>Euphorbia heterophylla var. cyathifolia</i> L.	Palperukki	Euphorbiaceae	Herb	Dicot
57	<i>Euphorbia hirta</i> L.	Ammanpachai risi	Euphorbiaceae	Herb	Dicot
58	<i>Evolvulus alsinoides</i> (L.) L.	Vishnukiranti hi	Convolvulaceae	Herb	Dicot
59	<i>Feronia elephantum</i> Corr.	Vilamaram	Rutaceae	Tree	Dicot
60	<i>Ficus benghalensis</i> L.	Aalamaram	Moraceae	Tree	Dicot
61	<i>Ficus religiosa</i> L.	Arasamaram	Moraceae	Tree	Dicot
62	<i>Gomphrena globosa</i> L.	Vadamalli	Amaranthaceae	Herb	Dicot
63	<i>Gymnema sylvestre</i> R. Br.	Sirukurinchai	Apocynaceae	Shrub	Dicot
64	<i>Heliotropium indicum</i> L.	Thelkodukku	Boraginaceae	Herb	Dicot
65	<i>Hemidesmus indicus</i> (L.) R. Br.	Nannari	Apocynaceae	Shrub	Dicot
66	<i>Hibiscus cannabinus</i> (Kenaf)	Pulichai keerai	Malvaceae	Herb	Dicot
67	<i>Hibiscus rosa-sinensis</i> L.	Semparuthi	Malvaceae	Shrub	Dicot
68	<i>Inigofera enneaphylla</i> L.	Seepu Nerunjil	Fabaceae	Herb	Dicot
69	<i>Ipomoea alba</i> L.	Chathiragandhi	Convolvulaceae	Herb	Dicot
70	<i>Ixora coccinea</i> L.	Viruchi	Rubiaceae	Shrub	Dicot
71	<i>Jasminum angustifolium</i> (L.) Willd.	Kattumalligai	Oleaceae	Shrub	Dicot
72	<i>Jasminum grandiflorum</i> L.	Sathimalligai	Oleaceae	Shrub	Dicot
73	<i>Jasminum sambac</i> (L.) Aiton.	Kodimalligai	Oleaceae	Shrub	Dicot
74	<i>Jatropha curcas</i> L.	Kattamanaku	Euphorbiaceae	Shrub	Dicot

75	<i>Justicia tranquebariensis Roxb.</i>	Thavasi murungai	Acanthaceae	Herb	Dicot
76	<i>Lawsonia inermis L.</i>	Maruthani	Lythraceae	Shrub	Dicot
77	<i>Leucas aspara (Wild.) Link.</i>	Thumbai	Lamiaceae	Shrub	Dicot
78	<i>Ludwigia peruviana (L.) Hara</i>	Poonaikodi	Onagraceae	Shrub	Dicot
79	<i>Luffa acutangula (L.) Roxb.</i>	Peerkangai	Cucurbitaceae	Tree	Dicot
80	<i>Mangifera indica L.</i>	Mamaram	Anacardiaceae	Tree	Dicot
81	<i>Martynia annua L.</i>	Thetkoduki	Martyniaceae	Herb	Dicot
82	<i>Mentha spicata L.</i>	Pudina	Lamiaceae	Herb	Dicot
83	<i>Millingtonia hortensis L. f.</i>	Kattumalli	Bignoniaceae	Tree	Dicot
84	<i>Mimosa pudica L.</i>	Thottasurunk	Fabaceae	Shrub	Dicot
85	<i>Mirabilis jalapa L.</i>	Anthimantha ai	Nyctaginaceae	Herb	Dicot
86	<i>Mollugo nudicaulis Lam.</i>	Parpadagam	Molluginaceae	Herb	Dicot
87	<i>Momordica charantia L.</i>	Pakarkai	Cucurbitaceae	Herb	Dicot
88	<i>Moringa oleifera Lam.</i>	Murungai	Moraginaceae	Tree	Dicot
89	<i>Murraya koenigii (L.) Spreng.</i>	Karuveppilai	Rutaceae	Tree	Dicot
90	<i>Musa paradisiaca L.</i>	Vazhai	Musaceae	Herb	Monocot
91	<i>Nerium oleander L.</i>	Chevarali	Apocynaceae	Shrub	Dicot
92	<i>Nyctanthes arbor-tristis L.</i>	Pavazhamalli	Oleaceae	Shrub	Dicot
93	<i>Ocimum basilicum L.</i>	Thiruneetru pachilai	Lamiaceae	Herb	Dicot
94	<i>Ocimum</i>	Tulsi	Lamiaceae	Shrub	Dicot

	<i>tenuiflorum</i> L.				
95	<i>Oldenlandia umbellata</i> L.	Chayaver	Rubiaceae	Shrub	Dicot
95	<i>Opuntia humifusa</i> Mill.	Chapathikalli	Cactaceae	Shrub	Dicot
96	<i>Oryza sativa</i> L.	Nel	Poaceae	Herb	Monocot
97	<i>Pavonia zeylanica</i> (L.) Cav.	Chitramutti	Malvaceae	Herb	Dicot
98	<i>Pedalium murex</i> L.	Yanainerunjil	Pedaliaceae	Herb	Dicot
99	<i>Phylla nodiflora</i> (L.) Greene.	Poduthalai	Verbenaceae	Herb	Dicot
100	<i>Phyllanthus niruri</i> L.	Keezhanelli	Phyllanthaceae	Herb	Dicot
101	<i>Physalis minima</i> L.	Sodakkuthakli	Solanaceae	Herb	Dicot
102	<i>Piper betle</i> L.	Vetrilai	Piperaceae	Climber	Dicot
103	<i>Pithecellobium dulce</i> (Roxb.) Benth.	Kodukkapuli	Fabaceae	Tree	Dicot
104	<i>Polyalthia longifolia</i> (Sonn.) Thwaites.	Nettilingam	Annonaceae	Tree	Dicot
105	<i>Polygala rosmarinifolia</i> W.&A.	Periyangal	Polygalaceae	Herb	Dicot
106	<i>Pongamia pinnata</i> (L) Pierre.	Punga maran	Fabaceae	Herb	Dicot
107	<i>Portulaca oleracea</i> L.	Tharaikeerai	Portulacaceae	Herb	Dicot
108	<i>Priva cordifolia</i> (L.) R. S. Rao & Kamathy.	Adai otti	Verbenaceae	Herb	Dicot
109	<i>Psidium guajava</i> L.	Koyya	Myrtaceae	Tree	Dicot
110	<i>Punica granatum</i> L.	Madhulai	Lythraceae	Shrub	Dicot

111	<i>Rhynchosia aurea</i> DC.	Kattu Kol	Fabaceae	Herb	Dicot
112	<i>Ricinus communis</i> L.	Amanakku	Euphorbiaceae	Shrub	Dicot
113	<i>Saccharum officinarum</i> L.	Karumbu	Poaceae	Herb	Monocot
114	<i>Santalum album</i> L.	Santhanam	Santalaceae	Tree	Dicot
115	<i>Saraca indica</i> L.	Ashoka maram	Fabaceae	Tree	Dicot
116	<i>Sesbania grandiflora</i> (L.) Pers.	Sevagathi	Fabaceae	Shrub	Dicot
117	<i>Solanum insanum</i> L.	Kathirikai	Solanaceae	Shrub	Dicot
118	<i>Solanumlycopersicum</i> L.	Thakkali	Solanaceae	Shrub	Dicot
119	<i>Solanum nigrum</i> L.	Manathakkali	Solanaceae	Shrub	Dicot
120	<i>Solanum pubescens</i> L.	Chundal	Solanaceae	Shrub	Dicot
121	<i>Solanum torvum</i> Sw.	Sundaikkai	Solanaceae	Shrub	Dicot
122	<i>Solanum trilobatum</i> L.	Thuthuvalai	Solanaceae	Shrub	Dicot
123	<i>Sphaeranthus indicus</i> L.	Kottaikarandi	Rhamnaceae	Tree	Dicot
124	<i>Swietenia macrophylla</i> King.	Mahogany	Meliaceae	Tree	Dicot
125	<i>Tabebuia rosea</i> (Bertol.) DC.	Vasantharani	Bignoniaceae	Tree	Dicot
126	<i>Tamarindus indica</i> L.	Puli	Fabaceae	Tree	Dicot
127	<i>Tecomella stans</i> (L.) Juss. exKunth.	Nagasampagam	Bignoniaceae	Shrub	Dicot
128	<i>Tephrosia purpurea</i> (L.) Pers.	Kolunchi	Fabaceae	Herb	Dicot
129	<i>Terminalia</i>	Nattu	Combretaceae	Tree	Dicot

	<i>cattappa</i> L.	vathumai			
130	<i>Thespesia populnea</i> (L.) Soland ex Correa.	Poovarasu	Malvaceae	Shrub	Dicot
131	<i>Thevetia peruviana</i> (Pers.) K. Schum	Thirvachipoo	Apocynaceae	Herb	Dicot
132	<i>Tinospora cordifolia</i> (Willd.)Miers.	Seenthil kodi	Menispermacea	Shrub	Dicot
133	<i>Trianthema decandra</i> L.	Vellaisharunai	Aizoaceae	Herb	Dicot
134	<i>Trichodesma indicum</i> (L.)R. Br.	Kavizhthumbai	Boraginaceae	Herb	Dicot
135	<i>Tridax procumbens</i> L.	Vettakaya poondu	Asteraceae	Herb	Dicot
136	<i>Typha angustifolia</i> L.	Sambu	Typhaceae	Herb	Dicot
137	<i>Vetiveria zizanoides</i> (L.) Nash.	Vettiver	Poaceae	Herb	Monocot
138	<i>Vigna radiata</i> (L.) Wilczek.	Pachapayaru	Fabaceae	Herb	Dicot
139	<i>Vigna unguiculata</i> (L.) Walp.	Karamani	Fabaceae	Herb	Dicot
140	<i>Withania somnifera</i> (L.) Dunal	Amukkura	Solanaceae	Shrub	Dicot
141	<i>Wrightia tinctoria</i> (Roxb.) R. Br.	Vetpalai arisi	Apocynaceae	Tree	Dicot
142	<i>Zizyphus jujuba</i> Mill.	Illandai	Rhamnaceae	Tree	Dicot
143	<i>Zizyphus mauritiana</i> Lam.	Illandhai	Rhamnaceae	Shrub	Dicot

**Table – 2: Family wise and cotyledon of species distribution of Perundurai, Erode District**

S. No	Families	Cotyledons	No. of species
1.	<i>Fabaceae</i>	Dicot	23
2.	<i>Solanaceae</i>	Dicot	10
3.	<i>Apocynaceae</i>	Dicot	8
4.	<i>Malvaceae</i>	Dicot	6
5.	<i>Amaranthaceae</i>	Dicot	5
6.	<i>Poaceae</i>	Monocot	5
7.	<i>Boraginaceae</i>	Dicot	4
8.	<i>Euphorbiaceae</i>	Dicot	4
9.	<i>Lamiaceae</i>	Dicot	4
10.	<i>Nyctaginaceae</i>	Dicot	4
11.	<i>Oleaceae</i>	Dicot	4
12.	<i>Acanthaceae</i>	Dicot	3
13.	<i>Apiaceae</i>	Dicot	3
14.	<i>Bignoniaceae</i>	Dicot	3
15.	<i>Cucurbitaceae</i>	Dicot	3
16.	<i>Rhamnaceae</i>	Dicot	3
17.	<i>Rutaceae</i>	Dicot	3
18.	<i>Annonaceae</i>	Dicot	2
19.	<i>Arecaceae</i>	Monocot	2
20.	<i>Convolvulaceae</i>	Dicot	2
21.	<i>Lythraceae</i>	Dicot	2

22.	<i>Meliaceae</i>	Dicot	2
23.	<i>Menispermaceae</i>	Dicot	2
24.	<i>Moraceae</i>	Dicot	2
25.	<i>Phyllanthaceae</i>	Dicot	2
26.	<i>Rubiaceae</i>	Dicot	2
27.	<i>Verbenaceae</i>	Dicot	2
28.	<i>Aizoaceae</i>	Dicot	1
29.	<i>Anacardiaceae</i>	Dicot	1
30.	<i>Araceae</i>	Monocot	1
31.	<i>Asphodelaceae</i>	Monocot	1
32.	<i>Asteraceae</i>	Dicot	1
33.	<i>Cactaceae</i>	Dicot	1
34.	<i>Caricaceae</i>	Dicot	1
35.	<i>Cannaceae</i>	Monocot	1
36.	<i>Capparidaceae</i>	Dicot	1
37.	<i>Combretaceae</i>	Dicot	1
38.	<i>Commelinaceae</i>	Monocot	1
39.	<i>Costaceae</i>	Monocot	1
40.	<i>Cyperaceae</i>	Monocot	1
41.	<i>Liliaceae</i>	Monocot	1
42.	<i>Martyniaceae</i>	Dicot	1
43.	<i>Molluginaceae</i>	Dicot	1
44.	<i>Moraginaceae</i>	Dicot	1
45.	<i>Musaceae</i>	Monocot	1

46.	<i>Myrtaceae</i>	Dicot	1
47.	<i>Onagraceae</i>	Dicot	1
48.	<i>Pedaliaceae</i>	Dicot	1
49.	<i>Piperaceae</i>	Dicot	1
50.	<i>Polygalaceae</i>	Dicot	1
51.	<i>Portulacaceae</i>	Dicot	1
52.	<i>Santalaceae</i>	Dicot	1
53.	<i>Sapindaceae</i>	Dicot	1
54.	<i>Sapotaceae</i>	Dicot	1
55.	<i>Typhaceae</i>	Dicot	1
56.	<i>Vitaceae</i>	Dicot	1
57.	<i>Zygophyllaceae</i>	Dicot	1

**Table - 3: Floristic diversity and medicinal uses of species in Perundurai, Erode District**

S. No	Botanical Name	Tamil Name	Families	Useful parts	Medicinal Uses
1	<i>Abelmoschus esculentus</i> (L.) Moench	Vendakkai	<i>Malvaceae</i>	Fruits	Ulcers, gastroprotective, cerebro vascular diseases and hemorrhoids
2	<i>Abrus precatorius</i> L.	Kunduma ni	<i>Fabaceae</i>	Leaves	Eye complaint,cough urine problems
3	<i>Abutilon indicum</i> (L.) Sweet.	Thuthikere rai	<i>Malvaceae</i>	Leaves	Facial paralysis and joint disorders and aphrodisiac
4	<i>Ahras sapota</i> L.	Sapota	<i>Sapotaceae</i>	Fruits	Anti-spasmodic agent, skin infections and anti-microbial
5	<i>Achyranthus aspera</i> L.	Nayuruvi	<i>Amaranthaceae</i>	Leaves	Boils, asthma, bleeding, bronchitis, debility, dropsy, cold, colic,

					cough, dog bite, snake bite, scorpion bite and dysentery
6	<i>Adenanthera pavonina</i> L.	Aanai Kunduman ni	<i>Fabaceae</i>	Leaves	Boils, inflammation, blood disorders, arthritis, cholera, paralysis, epilepsy, convulsion, spasm and indigestion
7	<i>Aegle marmelos</i> (L) Correa.	Vilvamaram	<i>Rutaceae</i>	Fruits	Anti -diabetics, respiratory problem, inflammation, dysentery and diarrhea.
8	<i>Aerva lanata</i> (L.) Juss. Ex Schult.	Sirupeelai	<i>Amaranthaceae</i>	Leaves and flowers	Headache, diuretic, analgesic and urolithiasis
9	<i>Albizia amara</i> (Roxb) Boivin.	Unja maram	<i>Fabaceae</i>	Leaves	Boils and ulcers, skin diseases and poisonous bites, astringent piles, diarrhea and gonorrhoea
10	<i>Allamanda cathartica</i> Linn.	Manjalpatti	<i>Apocynaceae</i>	Leaves and flowers	Tumors, jaundice, splenomegaly and malaria
11	<i>Allium cepa</i> L.	Vengayam	<i>Liliaceae</i>	Bulbs and leaves	Antimicrobial, anticancer, antidiabetic, antioxidant, antiplatelet, antihypertensive, antidepressant effects and antiparasitic effects
12	<i>Aloe vera</i> (L.) Webb.	Sotrukatalai	<i>Asphodelaceae</i>	Mucilage substances	Antioxidant and antibacterial, dental plaque, accelerating wound healing, preventing wrinkles, and managing blood sugar
13	<i>Amaranthus tricolor</i> L.	Nerunjil	<i>Zygophylaceae</i>	Leaves, stem and flowers	Antiradical and antibacterial activity
14	<i>Amaranthus viridis</i> L.	Mullaikereerai	<i>Amaranthaceae</i>	Stem and leaves	Anti-inflammatory, antipyretic, antimicrobial and antioxidant
15	<i>Annona squamosa</i> L.	Seetha maram	<i>Annonaceae</i>	Fruits	Anti-bacterial and antioxidant activity
16	<i>Areca catechu</i>	Pakku	<i>Arecaceae</i>	Seeds	Diarrhea, helminth

	L.	maram	e		infections, urinary disorders, obesity, anemia, leprosy and leucoderma
17	<i>Asystasia gangetica</i> (L.) T. Anderson.	Mithikeerai	<i>Acanthaceae</i>	Leaves	Rheumatism, stomachache, anthelmintic, heart pains, asthma, astringent, diaphoretic and women infertility
18	<i>Azadirachta indica</i> A. Juss.	Veppamar am	<i>Meliaceae</i>	Leaves, flowers, seeds, fruits, root and bark	Antifungal, antibacterial arthritis and exfoliate
19	<i>Barleria acuminata</i> Nees.	Vellai kurunji	<i>Acanthaceae</i>	Leaves	Boils, glandular swellings, dropsey, toothache and rheumatism
20	<i>Bauhinia tomentosa</i> L.	Thiruvathi	<i>Fabaceae</i>	Root, bark, leaves and flowers	Liver inflammation, abscess, tumors, wounds, and hyperlipidemia
21	<i>Boerhavia diffusa</i> L.	Mukaratai Sarai	<i>Nyctaginaceae</i>	Leaves	Anti - inflammatory, liver-kidney disorders, skin problems
22	<i>Boerhavia verticillata</i> Poir.	Mukaratai Keerai	<i>Nyctaginaceae</i>	Leaves	Dyspepsia, jaundice, enlargement of the spleen and abdominal pain
23	<i>Bougainvillae a glabra</i> Choisy.	Kakitha poo	<i>Nyctaginaceae</i>	Flowers	Reduce heartburn, treat sore throat, leucorrhea, blood vessels and hepatitis
24	<i>Caesalpinia pulcherrima</i> (L.) Sw.	Mayil Kondrai	<i>Fabaceae</i>	Leaves and flower	Tonic, stimulant and emmenagogue, Abortifacient
25	<i>Cajanus cajan</i> (L.) Millsp.	Thuvarai	<i>Fabaceae</i>	Seeds	Toothache, dizziness, diabetes, stomachache, female ailments and chronic infections.
26	<i>Calotropis gigantea</i> (L.) W. T. Aiton.	Erukku	<i>Apocynaceae</i>	Latex and flowers	Skin, digestive, respiratory, circulatory and neurological disorders

					elephantiasis, nausea, vomiting and diarrhea
27	<i>Canna indica</i> L.	Kal Vazhai	<i>Cannaceae</i>	Seeds	Menstrual pains, gonorrhea and amenorrhoea
28	<i>Capsicum annuum</i> L.	Kudamila gai	<i>Solanaceae</i>	Fruits	Osteoarthritis, shingles, rheumatoid arthritis, post-herpetic neuralgia, trigeminal neuralgia, diabetic and Neuropathy
29	<i>Cardiospermum halicacabum</i> L.	Mudakkar uthan	<i>Sapindaceae</i>	Leaves	Rheumatism, abdominal pain, orchitis, dropsy,lumbago, skin diseases, cough, nervous disorders, and hyperthermia
30	<i>Carica papaya</i> L.	Pappali	<i>Caicaceae</i>	Leaves and fruits	Wound healing, anti-cancer, hypolipidemic and hypoglycemic properties
31	<i>Carissa carandas</i> L.	Kalakkai	<i>Apocynaceae</i>	Seeds	Indigestion, fresh and infected wounds, skin diseases, urinary disorders
32	<i>Cassia auriculata</i> Linn.	Avaram poo	<i>Fabaceae</i>	Leaves and flowers	Anti-diabetics, pink eye, joint and muscle pain and constipation
33	<i>Cassia roxburghii</i> DC.	Senkondrai	<i>Fabaceae</i>	Leaves and flowers	Anti - diabetics, antimicrobial and liver disorders
34	<i>Celosia argentea</i> L.	Pannaikee rai	<i>Amaranthaceae</i>	Leaf	Bloody stool, haemorrhoid bleeding, uterine bleeding,leucorrhoea, dysentery and diarrhoea
35	<i>Centella asiatica</i> (L.) Urban.	Vallarai	<i>Apiaceae</i>	Leaves	Heal wounds, improve mental clarity, treat for leprosy and psoriasis.
36	<i>Chloris barbata</i> Sw.	Kodaipullu	<i>Poaceae</i>	Leaves	Anti-diabetic, analgesic, antibacterial, anti-hyperlipidemic and rheumatism
37	<i>Cissus quadrangularis</i> L.	Pirandai	<i>Vitaceae</i>	Stem	Anti - diabetics, obesity, high cholesterol, bone fractures, allergies, cancer,

					stomach upset, painful menstrual periods, asthma, malaria and wound healing
38	<i>Cleome viscosa</i> L.	Naikaduk u	<i>Capparid aceae</i>	Leaves and seed	Rheumatic arthritis, hypertension, malaria, neurasthenia and wound healing
39	<i>Clitoria ternatea</i> L.	Sangu poo	<i>Fabaceae</i>	Flowers	Memory enhancer, nootropic, antistress, anxiolytic, antidepressant, anticonvulsant, tranquilizing and sedative agent
40	<i>Coccinia indica</i> (L.) Voigt.	Kovai	<i>Cucurbit aceae</i>	Fruits	Anti-inflammatory, antioxidant, antidiabetic, high cholesterol to high blood pressure and obesity
41	<i>Cocculus hirsutus</i> (L.) Diels.	Sirukattuk odi	<i>Menisper maceae</i>	Leaves	Leprosy, skin diseases and dyspepsia
42	<i>Cocos nucifera</i> L.	Thennamaram	<i>Arecacea e</i>	Fruits	Antibacterial, antifungal, antiviral, antiparasitic, antidermatophytic, antioxidant, hypoglycemic, hepatoprotective, immunostimulant.
43	<i>Coldenia procumbens</i> L.	Seruppadi	<i>Boragina ceae</i>	Leaves	Rheumatic swellings, immature abscesses, leucorrhoea, menorrhagia, anti-diabetic and anti-arthritis
44	<i>Colocasia esculenta</i> (L.) Schott.	Seppankiz hangu	<i>Araceae</i>	Rhizome	Nervine tonic, antioxidant, anti-inflammatory, anti-lipid peroxidative activity and hepatoprotective
45	<i>Commelina benghalensis</i> L.	Aduthinna thalai	<i>Commeli naceae</i>	Leaves	Leprosy, sore throat, ophthalmia, burns, depressant, demulcent, emollient and laxative

46	<i>Cordia sebestena L.</i>	Achirunar uvizhi	<i>Boraginaceae</i>	Leaves	Hepatoprotective activity, hypoglycemic, hypolipidemic and potent antioxidant, anti-inflammatory and analgesic activity
47	<i>Coriandrum sativum L.</i>	Kottumalli	<i>Apiaceae</i>	Leaves and seeds	Antioxidant, antidiabetic, antimutagenic, anthelmintic, anticonvulsant, anxiolytic and hepatoprotective
48	<i>Costus pictus D. Don.</i>	Insulin chedi	<i>Costaceae</i>	Leaves	Anti - diabetics and urolithiasis
49	<i>Crotalaria juncea L.</i>	Sanal	<i>Fabaceae</i>	Leaves	Fever, soriasis, emmenagogue, colic and astringent , blood purifier
50	<i>Cuminum cyminum L.</i>	Seeragam	<i>Apiaceae</i>	Seeds	Hypolipidemia, anti - cancer and anti - diabetics
51	<i>Cymbopogon nardus L. Rendle.</i>	Kamachi pul	<i>Poaceae</i>	Leaves	Antiviral, antibacterial and antifungal activities
52	<i>Cyperus rotundus L.</i>	Korai kilangu	<i>Cyperaceae</i>	Leaves	Diarrhea,diabetes, pyresis, inflammation, malaria, stomach and bowel disorders
53	<i>Datura metel L.</i>	Karu oomathai	<i>Solanaceae</i>	Leaves and fruits	Fever with catarrh, insanity, heart diseases, and diarrhea
54	<i>Dichrostachys cinerea (L.) Wight.&amp;Arn .</i>	Vidathalai	<i>Fabaceae</i>	Leaves	Rheumatism, anti - diabetics, coughs, asthma,kidney disorders, gonorrhea, syphilis, malaria, tuberculosis, headache, and scabies.
55	<i>Dolichos biflorus L.</i>	Kollu	<i>Fabaceae</i>	Seeds	Piles, constipation, wounds, urinary calculi, cough, edema and asthma
56	<i>Emblica officinalis L.</i>	Nellikai	<i>Phyllanthaceae</i>	Fruits	Diarrhea, jaundice and anti- inflammatory
57	<i>Euphorbia heterophylla</i>	Palperukk i	<i>Euphorbiaceae</i>	Leaves, root and	Gonorrhea, wounds, anti - asthma, bronchitis,

	<i>var. cyathifolia L.</i>			flowers	cancer, scorpion bites, earache,toothache and diarrhea
58	<i>Euphorbia hirta L.</i>	Ammanpa charisi	<i>Euphorbi aceae</i>	Stem and Leaves	Female disorders, dysentery, jaundice,pimples, gonorrhea,digestive problems and tumors
59	<i>Evolvulus alsinoides (L.) L.</i>	Vishnukir anthi	<i>Convolvu laceae</i>	Leaves	Cough, cold, anti - inflammatory and fever
60	<i>Feronia elephantum Corr.</i>	Vilamara m	<i>Rutaceae</i>	Fruits	Astringent, carminative, vomiting,dysentery and indigestion
61	<i>Ficus benghalensis L.</i>	Aalamara m	<i>Moraceae</i>	Leaves and fruits	Anti- diabetics, diarrhea, dysentery,wounds, burns, and rheumatism
62	<i>Ficus religiosa L.</i>	Arasamara m	<i>Moraceae</i>	Fruits	Asthma, blood sugar level, diarrhea, brain disorder, gastrointestinal troubles, anti-inflammatory activity, infectious activity and sexual concerns
63	<i>Gomphrena globosa L.</i>	Vadamalli	<i>Amarant haceae</i>	Flowers	High blood pressure and used for oliguria and empacho
64	<i>Gymnema sylvestre R. Br.</i>	Sirukurinc han	<i>Apocyna ceae</i>	Leaves	Anti - diabetic, anti- microbial and anti - obesity
65	<i>Heliotropium indicum L.</i>	Thelkoduk ku	<i>Boragina ceae</i>	Leaves	Wound healing, antidote, bone fracture, febrifuge, cures eye infection,menstrual disorder, nerve disorder, kidney problem and antiseptic purpose
66	<i>Hemidesmus indicus (L.) R. Br.</i>	Nannari	<i>Apocyna ceae</i>	Leaves	Rheumatism, leprosy, impotence, urinary tract and skin infections.
67	<i>Hibiscus cannabinus (Kenaf)</i>	Pulichai keerai	<i>Malvacea e</i>	Leaves	Dysentery and bilious, blood and throat disorders
68	<i>Hibiscus rosa-sinensis L.</i>	Semparut hi	<i>Malvacea e</i>	Flowers, leaves and root	Hypertension, cholesterol production and anti - cancer
69	<i>Inigofera enneaphylla</i>	Seepu Nerunjil	<i>Fabaceae</i>	Leaves	Antiscorbutic , diuretic, burns and epilepsy

	L.				
70	<i>Ipomoea alba</i> L.	Chathirag andhi	<i>Convolvulaceae</i>	Leaves	Snakebites, purgative, filariasis, laxative, febrifuge, abdominal pains, burns, tumors and irregular menstrual cycle
71	<i>Ixora coccinea</i> L.	Viruchi	<i>Rubiaceae</i>	Flowers	Antioxidative, antibacterial, antinociceptive, antimutagenic, antineoplastic and chemopreventive effects
72	<i>Jasminum angustifolium</i> (L.) Willd.	Kattumalli gai	<i>Oleaceae</i>	Flowers	Analgesic, antidepressant, antiseptic, expectorant, aphrodisiac, sedative, stomachic, diuretic, depurative, astringent, anthelmintic and anti-inflammatory
73	<i>Jasminum grandiflorum</i> L.	Sathimalli gai	<i>Oleaceae</i>	Flowers	Pains, toothache, stomach ache, ulcers, and sexual impotency
74	<i>Jasminum sambac</i> (L.) Aiton.	Kodimalli gai	<i>Oleaceae</i>	Flowers	Healing agent for painful periods, skin infections, and backaches. anti-bacterial, anti-fungal and anti-inflammatory properties
75	<i>Jatropa curcas</i> L.	Kattamana kku	<i>Euphorbiaceae</i>	Seeds	Ailments related to skin, cancer, digestive, respiratory and infectious diseases
76	<i>Justicia tranquebariensis</i> Roxb.	Thavasi murungai	<i>Acanthaceae</i>	Leaves and flowers	Anti- inflammatory, anti-allergic, anti-tumoral, anti-viral and analgesic activities
77	<i>Lawsonia inermis</i> L.	Maruthani	<i>Lythraceae</i>	Leaves, flowers and stem	Jaundice, spleen enlargement, calculus care, leprosy, antimicrobial, antiamoebiasis, astringent, antihemorrhagic,

					hypotensive and sedative effect
78	<i>Leucas aspara</i> (Wild.) Link.	Thumbai	<i>Lamiaceae</i>	Leaves	Antifungal, antioxidant, antimicrobial, antinociceptive and cytotoxic activity
79	<i>Ludwigia peruviana</i> (L.) Hara	Poonaikodi	<i>Onagraceae</i>	Leaves	Poultice in wound dressings and as remedy for dysentery
80	<i>Luffa acutangula</i> (L.) Roxb.	Peerkangi	<i>Cucurbitaceae</i>	Fruits	Colds, nasal swelling, sinus problems, arthritis pain, muscle pain, and chest pain
81	<i>Mangifera indica</i> L.	Mamaram	<i>Anacardiaceae</i>	Fruits	Heat stroke, asthma and as an astringent. Fumes from the burning leaves are inhaled for relief from hiccups and affections of the throat.
82	<i>Martynia annua</i> L.	Thetkodukki	<i>Martyniaceae</i>	Leaves and flowers	Epilepsy, dysentery, cardiac problems, worm infestation, constipation, haemorrhage and antibacterial infection
83	<i>Mentha spicata</i> L.	Pudina	<i>Lamiaceae</i>	Leaves	Gastrointestinal, respiratory, bad breath, carminative, anti-spasmodic, diuretic and sedative agents.
84	<i>Millingtonia hortensis</i> L. f.	Kattumalli	<i>Bignoniaceae</i>	Flowers	Asthma, sinusitis, chologogue and tonic
85	<i>Mimosa pudica</i> L.	Thottasurunki	<i>Fabaceae</i>	Leaves	Alopecia, diarrhea, constipation, leprosy, dysentery, insomnia, tumor, blood disorders and various urogenital infections
86	<i>Mirabilis jalapa</i> L.	Anthimantharai	<i>Nyctaginaceae</i>	Leaves and flowers	Virus inhibitory activity and anti tumour activity
87	<i>Mollugo</i>	Parpadaga	<i>Mollugin</i>	Leaves	Curing whooping cough

	<i>nudicaulis</i> Lam.	m	<i>aceae</i>		and jaundice.
88	<i>Momordica charantia</i> L.	Pakarkai	<i>Cucurbit aceae</i>	Fruits	Antidiabetic, abortifacient, anthelmintic, contraceptive, dysmenorrhea, eczema, emmenagogue, antimalarial and pneumonia
89	<i>Moringa oleifera</i> Lam.	Murungai	<i>Moragin aceae</i>	Leaves and flower	Asthma, diabetics and breast-feeding
90	<i>Murraya koenigii</i> (L.) Spreng.	Karuveppi lai	<i>Rutaceae</i>	Leaves and flower	Piles, inflammation, itching, fresh cuts, dysentery, bruises, purgative and edema
100	<i>Musa paradisiaca</i> L.	Vazhai	<i>Musaceae</i>	Leaves, flower and stem	Ulcers, dysentery, and bronchitis, anti - diabetics, astringent, dysentery and diarrhea
101	<i>Nerium oleander</i> L.	Chevarali	<i>Apocynaceae</i>	Leaves and flowers	Ulcers, haemorrhoids, leprosy, to treat ringworm, herpes, and abscesses
102	<i>Nyctanthes arbor-tristis</i> L.	Pavazham alli	<i>Oleaceae</i>	Flowers	Anti-helminthic and anti-pyretic besides its use as a laxative, rheumatism, skin ailments
103	<i>Ocimum basilicum</i> L.	Thiruneetr u pachilai	<i>Lamiacea e</i>	Leaves and seeds	Headaches, coughs, diarrhea, constipation, warts, worms, and kidney malfunctions
104	<i>Ocimum tenuiflorum</i> L.	Tulsi	<i>Lamiacea e</i>	Leaves	Cough, asthma, diarrhea, fever, dysentery, arthritis, eye diseases, indigestion and gastric ailments
106	<i>Oldenlandia umbellata</i> L.	Chayaver	<i>Rubiacea e</i>	Leaves	Bronchitis, asthma, tuberculosis, constipation, and leprosy
107	<i>Opuntia humifusa</i> Mill	Chapathik alli	<i>Cactaceae</i>	Mucilage Substance	Anti - diabetics, high cholesterol, obesity and

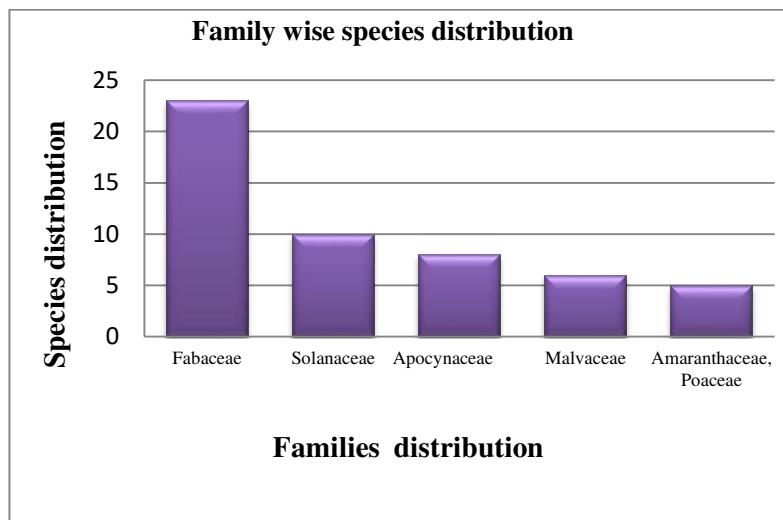
				s	anti - inflammatory
108	<i>Oryza sativa L.</i>	Nel	<i>Poaceae</i>	Seeds	Skin to treat boils, sores, swellings, blemishes, stomach upsets, heartburn, and indigestion
109	<i>Pavonia zeylanica (L.) Cav.</i>	Chitramutti	<i>Malvaceae</i>	Leaves	Chronic rheumatoid arthritis, skin diseases and Chronic menstrual disorders
110	<i>Pedalium murex L.</i>	Yanainerunjil	<i>Pedaliaceae</i>	Leaves and flower	Kidney stone formation, incontinence of urine, gonorrhoea, antibilious agent, dysuria and control white discharge
111	<i>Phylla nodiflora (L.) Greene.</i>	Poduthalai	<i>Verbenaceae</i>	Leaves	Constipation and knee pain
112	<i>Phyllanthus niruri L.</i>	Keezhaneli	<i>Phyllanthaceae</i>	Root, leaves and fruit	Antibacterial, anti-hyperglycemia, anti-viral, diuretic, hepatoprotector, and immunomodulator
113	<i>Physalis minima L.</i>	Sodakkuthakali	<i>Solanaceae</i>	Leaves and fruits	Diuretic, laxative and antiinflammatory activities
114	<i>Piper betle L.</i>	Vetrilai	<i>Piperaceae</i>	Leaves	Cold, cough, bronchial asthma, rheumatism, stomachalgia, bad breath, boils, abscesses, conjunctivitis, constipation and swelling of gums
115	<i>Pithecellobium dulce (Roxb.) Benth.</i>	Kodukkupuli	<i>Fabaceae</i>	Fruits	Chronic diarrhea, dysentery, constipation and tuberculosis
116	<i>Polyalthia longifolia (Sonn.) Thwaites.</i>	Nettilingam	<i>Annonaceae</i>	Leaves	Dermatological ailments and antimicrobial
117	<i>Polygala rosmarinifolia W.&amp;A.</i>	Periyanangai	<i>Polygalaceae</i>	Leaves	Anti-inflammatory, hepatoprotective, antioxidant,

					antihyperglycemic and antihyperlipidemic
118	<i>Pongamia pinnata</i> (L.) Pierre.	Punga maram	<i>Fabaceae</i>	Leaves and flowers	Tumors, piles, skin diseases, ulcers, gonorrhea, cleaning gums, teeth, ulcers and is used in vaginal and skin Diseases
119	<i>Portulaca oleracea</i> L.	Tharaikere ai	<i>Portulaceae</i>	Leaves	Purgative, cardiac tonic, emollient, muscle relaxant and anti-inflammatory
120	<i>Priva cordifolia</i> (L.) R. S. Rao & Kamathy.	Adai otti	<i>Verbenaceae</i>	Leaves	Anti-migraine and common headache
121	<i>Psidium guajava</i> L.	Koyya	<i>Myrtaceae</i>	Leaves, flowers and fruits	Diarrhea, dysentery, stomach aches and indigestion
122	<i>Punica granatum</i> L.	Madhulai	<i>Lythraceae</i>	Fruits	Ulcers, diarrhea, and male infertility
123	<i>Rhynchosia aurea</i> DC.	Kattu Kol	<i>Fabaceae</i>	Root, stem and leaves	Anti-diabetic, abortifacient, anti-bacterial, wound healing, hepatoprotective, rheumatism and skin infections
124	<i>Ricinus communis</i> L.	Amanakk u	<i>Euphorbiaceae</i>	Seed	Abdominal disorders, arthritis, backache, muscle aches, bilharziasis, chronic backache and sciatica, chronic Headache and constipation
125	<i>Saccharum officinarum</i> L.	Karumbu	<i>Poaceae</i>	Stem	Antidote, antiseptic, antivinous, bactericide, cardiotonic, demulcent, diuretic, intoxicant, laxative, pectoral, piscicide, refrigerant, and

					Stomachic
126	<i>Santalum album</i> L.	Santhana m	<i>Santalaceae</i>	Stem	Antipyretic, antiseptic, antiscabetic, diuretic , bronchitis, cystitis, dysuria, and diseases of the urinary tract
127	<i>Saraca indica</i> L.	Ashoka maram	<i>Fabaceae</i>	Bark and leaves	Uterine tonic and has been indicated in menstrual irregularities
128	<i>Sesbania grandiflora</i> (L.) Pers.	Sevagathi	<i>Fabaceae</i>	Leaves, flowers	Cardioprotective, antibacterial, anxiolytic, hepatoprotective and antiulcer
129	<i>Solanum insanum</i> L.	Kathirikai	<i>Solanaceae</i>	Fruits	Sore throat, stomach-ache, head- ache, painful menstruation, liver pain, malaria, hypertension and stomach problem
130	<i>Solanum lycopersicum</i> L.	Thakkali	<i>Solanaceae</i>	Fruits	Burns, scalds, sunburn, rheumatism and headaches
131	<i>Solanum nigrum</i> L.	Manathak kali	<i>Solanaceae</i>	Leaves and fruits	Antibacterial, cough and indigestion, antiproliferative, antioxidant, antiviral, anti-inflammatory,
132	<i>Solanum pubescens</i> L.	Chundal	<i>Solanaceae</i>	Fruits	Hemorrhoids, inflammation, cancer, whooping cough, diarrhea, headache, menstrual pain and tuberculosis
133	<i>Solanum torvum</i> Sw.	Sundaikkai	<i>Solanaceae</i>	Fruits	Anti - diabetics, hypertension, tooth decay, and reproductive problems
134	<i>Solanum trilobatum</i> L.	Thuthuvalai	<i>Solanaceae</i>	Leaves and fruits	Chest congestion, sinusitis, bronchial asthma and tuberculosis
135	<i>Sphaeranthus indicus</i> L.	Kottaikaran dai	<i>Rhamnaceae</i>	Leaves and fruits	Gastric disorders, skin diseases, anthelmintic, glandular swelling, nervous depression, analgesic and antibiotics

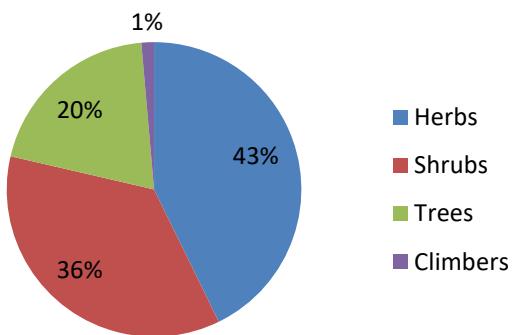
136	<i>Swietenia macrophylla</i> King.	Mahogany	<i>Meliaceae</i>	Fruits	Leishmaniasis and abortion, hypertension, anti - diabetics and malaria
137	<i>Tabebuia rosea</i> (Bertol.) DC.	Vasantha rani	<i>Bignonia ceae</i>	Fruits	Fevers, pain, cause sweating and anti - inflammatory
138	<i>Tamarindus indica L.</i>	Puli	<i>Fabaceae</i>	Leaves and fruit	Laxative effects and fight against certain fungi and bacteria
139	<i>Tecoma stans</i> (L.) Juss. exKunth.	Nagasamp agam	<i>Bignonia ceae</i>	Leaf and flower	Anti-cancer, anti - diabetics, arthritis antioxidant, wound healing, antispasmodic, antiproliferative, anti-inflammatory and antimicrobial
140	<i>Tephrosia purpurea</i> (L.) Pers.	Kolunchi	<i>Fabaceae</i>	Leaves and flower	Liver disorders, kidney disorders, intestinal worms and lung diseases
141	<i>Terminalia cattappa</i> L.	Nattu vathumai	<i>Combretaceae</i>	Seed	Scabies, leprosy wounds and skin diseases
142	<i>Thespesia populnea</i> (L.) Soland ex Correa.	Poovarasu	<i>Malvaceae</i>	Leaves and seed	Astringent, antibacterial, anti-inflammatory, antinociceptive and hepatoprotective
143	<i>Thevetia peruviana</i> (Pers.)J.K. Schum	Thirvachip oo	<i>Apocynaceae</i>	Flower and fruit	Wounds, infected area, ring worms and Tumours
144	<i>Tinospora cordifolia</i> (Willd.)Miers.	Seenthil kodi	<i>Menispermaceae</i>	Leaves	Anti-diabetics, high cholesterol, gout, lymphoma and other cancers, rheumatoid and to boost the immune system
145	<i>Trianthema decandra</i> L.	Vellaishar unnai	<i>Aizoaceae</i>	Leaves	Anti-inflammatory, Analgesic, Wound healing, hepatoprotective , anti-ulcer, antibacterial and Anti-cancer
146	<i>Trichodesma indicum</i> (L.)R. Br.	Kavizhthu mbai	<i>Boraginaceae</i>	Leaves and flowers	Arthritis, fever, skin disease, arthralgia and dysentery
147	<i>Tridax procumbens</i> L.	Vettakaya poondu	<i>Asteraceae</i>	Leaves and flowers	Wounds, skin diseases and to stop blood clotting in folk medicine,

					anticoagulant,
148	<i>Typha angustifolia</i> L.	Sambu	<i>Typhaceae</i>	Flowers	Wound healing and antimicrobial activities
149	<i>Vetiveria zizanoides</i> (L.) Nash.	Vettiver	<i>Poaceae</i>	Root	Relieving stress, lice, repelling insects It is also used for arthritis, stings, insomnia and burns
150	<i>Vigna radiata</i> (L.) Wilczek.	Pachapaya ru	<i>Fabaceae</i>	Seeds	Detoxification activities alleviate heat stroke and reduce swelling in the summer
151	<i>Vigna unguiculata</i> (L.) Walp.	Karamani	<i>Fabaceae</i>	Seeds	Astringent, antipyretic, diuretic and cardiovascular diseases
152	<i>Withania somnifera</i> (L.) Dunal	Amukkura	<i>Solanaceae</i>	Leaves	CNS disorders, Alzheimer disease, Parkinson disease, cerebral -ischemia, epilepsy and stress
153	<i>Wrightia tinctoria</i> (Roxb.) R. Br.	Vetpalai arisi	<i>Apocynaceae</i>	Leaves	Analgesic, anti-inflammatory, anthelmintic, antiulcer, antidiysentric, antidiabetic, anticancer and antipyretic
154	<i>Zizyphus jujuba</i> Mill.	Illandai	<i>Rhamnaceae</i>	Fruits	Asthma, cough, laryngitis, gastrointestinal problems constipation, colitis and liver diseases,cardiovascular and genitourinary system diseases
155	<i>Zizyphus mauritiana</i> Lam.	Illandhai	<i>Rhamnaceae</i>	Fruits	Heartburn biliousness, biliousness, astringency, scabies, diuretic and nausea



**Fig - 1:** Floristic diversity of Perundurai family wise species distribution

### Life forms distribution



**Fig - 2:** Floristic diversity of Perundurai life forms distribution

**Plate - 1****List of few medicinally important plants in Vellakoil, Tiruppur District, Tamil Nadu**

*Phyllanthus niruri* L.



*Costus pictus* D. Don



*Azadirachta indica* L.



*Opuntia humifusa* Mill.



*Hibiscus rosa-sinensis* L.

*Mangifera indica**Cleome viscosa L.**Azadirachta indica*  
A.Juss.*Pithecellobium dulce (Roxb.) Benth.*

**Table 4: The qualitative phytochemical analysis of aqueous leaf extract of *Costus pictus* D.Don.**

S. No	Phytochemicals	Results obtained
1.	Protein	+
2.	Carbohydrate	+
3.	Amino acids	+
4.	Alkaloids	+
5.	Flavonoids	+

6.	Phenols	+
7.	Tannin	-
8.	Glycosides	+
9.	Terpenoids	+
10.	Saponin	+
11.	Quinone	+
12.	Anthraquinone	-
13.	Fixed oil & fat	-
14.	Gums & mucilage	-

## Conclusion

Biodiversity is the collection of resources that support the livelihoods of families, communities, and future generations. Since the dawn of time, humans have been closely linked to the plant kingdom to ensure their existence. The floristic diversity explores the medicinal value of different plants to protect the surroundings and utilization for future generations.

## References

- Anbarasu, Boominathan, Samuel Thomas, Althaf Ahmed Kabeer. (2019). Floristic diversity in the Kallar Corridor Western Ghats, Coimbatore. International Journal of Botanical studies. 4(2): 41-48.
- Gamble, J.S. (1915-36) Fischer CEC. Flora of the Presidency of Madras. Vol. 1-3, Adlard and Son Ltd., London.Gamble, J.S. (1915-36) Fischer CEC. Flora of the Presidency of Madras. Vol. 1-3, Adlard and Son Ltd., London.

- Johnson, M., Maharajan, M., & Janakiraman, N. (2015). Floristic diversity and medicinal importance of South vagaikulam region Tirunelveli, Tamil Nadu, South India. *Journal of Medicinal Herbs and Ethnomedicine* 1: 125.
- Kumar, A. M., Rathika, D., Rakkimuthu, R., & Sathishkumar, P. (2023). Floristic diversity and ethnobotanical studies of Nandha gopalasamy hill temple sacred grove of Western Ghats, Pollachi Taluk, Coimbatore. *Agricultural Science Digest-A Research Journal*. 43(1): 57-62.
- Kuralarasi, R., Sundarapandi, G., Sundar, M., Lingakumar, K., & Ganesan, V. (2017). Floristic survey of Arunachalapuram village, Virudhunagar District, Tamil Nadu. *International Journal of Botanical studies*. 2(4):49- 53.
- Packialakshmi, M., Divya, M. P., Baranidharan, K., Parthiban, K. T., Geetha, S., Ganesan, K. N., & Ravi, R. (2023). Floristic composition and structural analysis of flora in Nilgiris biosphere reserve, Western Ghats of Southern India. *Journal of Tropical Forest Science*, 35(3), 270-282.
- Palanisamy, J., & Arumugam, R. (2014). Phytodiversity in the Madukkarai Hills of South Western Ghats. *Check List*, 10(4): 883-892.
- Pitchairamu, C., Muthuchelian, K., & Siva, N. (2008). Floristic inventory and quantitative vegetation analysis of tropical dry deciduous forest in piramalai forest, Eastern Ghats, Tamil Nadu, India. *Ethnobotanical Leaflets*, (1): 25.
- Saranya, N., Anirudhan, B., Kavimalar, S., Vinoth Kumar, V., & Sri Santhya, V. (2023). Campus survey on floristic biodiversity at Nehru Arts and Science College, Thirumalayampalayam, Coimbatore, Tamil Nadu,

India. International Journal of Research in Engineering and Science, 11(4): 580-586.

- Sukumaran, S., Jeeva, S., Raj, A. D. S., & Kannan, D. (2008). Floristic diversity, conservation status and economic value of miniature sacred groves in Kanyakumari district, Tamil Nadu, Southern Peninsular India. Turkish Journal of Botany, 32(3): 185-199.

## Phytochemical Screening of Marine Seaweeds Collected from Gulf of Mannar and Its Evaluation of Antimicrobial Potential Using Solvent Extraction Method

P.Sagadevan<sup>1</sup>, S. Theyaneshvar<sup>1</sup>, M.Raghunath<sup>1</sup>,  
M. Nagarajan<sup>1</sup> and P. Janarthanan<sup>2</sup>

<sup>1</sup>KSG College of Arts and Science, Coimbatore, Tamilnadu India

<sup>2</sup>Laboratory Technician, Aviagen India, Tamilnadu

Corresponding author email id: sagadevan.biotech@gmail.com

### Abstract

Phytochemicals with nutraceutical properties present in food are of enormous significance due to their beneficial effects on human health since they offer protection against numerous diseases or disorders such as cancer, coronary heart disease, diabetes, high blood pressure, inflammation, microbial, viral and parasitic infections, ulcers, osteoporosis and associate disorders. The result of this study shows the presence of some phytochemicals such as alkaloids, steroids and tannins in extracts of seaweeds. Alkaloids are already known to have antifungal and antimicrobial. Compounds with antioxidant, antifungal, antimicrobial activities have been detected in algae. Reactive oxygen species such as hydroxyl, superoxide and peroxy radicals are found in human cells result in extensive oxidative damage which can lead to aged related degenerative conditions, cancer and various human diseases. In spite of their wide applications in food and feed industries, they have gained importance as medicinal source because of high healing, antimicrobial and antioxidant properties.

**Keywords:** *Phytochemical, antimicrobial Activity, Seaweeds, Alkaloids, Steroids, Antioxidant.*

## Introduction

Biodiversity plays vital roles in maintaining human and animal health. A wide variety of plants, animals and fungi are used as medicine, essential vitamins, painkillers etc. Natural products have been recognized and used as medicines by ancient cultures all around the world. Many animals are also known to self-medicate using plants and other materials available to them. More than 60% of the world population relies almost entirely on the plant medicine for primary health care. About 119 pure chemicals are extracted from less than 90 species of higher plants and used as medicines throughout the world, for example, caffeine, methyl salicylate and quinine.

A lot of plant species are used in today's studies and have been exhaustively studied for their potential value as source of drugs. It is possible that some plant species may be a source of drugs against high blood pressure, AIDS or heart troubles. In China, Japan, India and Germany, there is a great deal of interest in and support for the search for new drugs from higher plants. Medicinal and aromatic plants (MAPs) are gaining popularity globally as a source of raw material for pharmaceuticals and traditional health care system. More than 85% of herbal medicines used in traditional health care systems are derived from medicinal plants [Farnsworth, 1988; Phondani *et al.*, 2014] and ensure the livelihoods of millions of people, especially in the Indian Himalayan region (Phondani *et al.*, 2011).

The edible red seaweeds such as *Palmaria palmata* and *Gracilaria cervicornis* are having the highest protein content which possesses many potential biological activities and seaweeds are good nutrition value such as carbohydrates, minerals, proteins, phenols, and good biofertilizer Flavonoids, the largest group of phenolic compounds are known to contain a broad spectrum of chemical and biological activities including antioxidant and free radical scavenging properties. Flavonoids include flavonols, flavones, catechins, proanthocyanidins,

anthocyanidins and isoflavonoids (Ndhlala *et al.*, 2007). Seaweeds are considered to be the main source of bioactive compounds with a wide range of biological activities, such as antibiotics, antioxidant and anti-inflammatory. Some macroalgae have bioactive components which affected the germination of some pathogenic bacteria (Kolanjinathan *et al.*, 2009). Hornsey and Hide (1985) found that many species of marine algal crude extracts have inhibition activity against pathogenic bacteria. Seaweeds are macro phytic algae, a primitive type of plants. This work aims to evaluate the antimicrobial activity of some seaweeds extracts from Red sea coast against some collected clinical multidrug resistant bacterial isolates in order to find alternative drugs and promising source of pharmaceutical agents. Most of the bioactive substances isolated from marine algae are chemically classified as brominated, aromatics, nitrogenheterocyclic, nitrosulphuric-heterocyclic, sterols, dibutanoids, proteins, peptides and sulphated polysaccharides.

The crude extract thus obtained is subjected to broad based biological screening for antifungal, antiviral, antibacterial, antimalarial, antifilarial, hypoglycaemic and antifertility activity. On other hand, the algae are also used as food stuff, animal fodder, fertilizer, industrial material such as agar and minor medicines. The green algae *Calorpha peltada* contains 1-4diacetoxy butadiene and fatty esters which possess antibacterial, anti-ichthyotoxic and antihypertensive properties.

## Materials and Methods

### Collection of Sample:

The Seaweeds *Ulva lactuca*, *Egregia menziesii*, *Chaetomorpha crassa*, *Thalassia testudinum*, *Halimeda opuntia*, *Sargassum swartzii*, were collected from Gulf of Mannar and it was authenticated from Botanical Survey of India (BSI No BSI/SRC/5/23/2019/Tech/303, 304, 305) Coimbatore, Tamil Nadu, India. The Geographical location of Gulf of Mannar is 8° 47' to 9° 15' N latitude and 78° 12' to 79° 14' E longitude. The preferred sample was collected by

hand, washed with seawater at the sampling site to remove the adhered sediments and impurities and then packed in polythene bags and brought to the laboratory for further analysis.



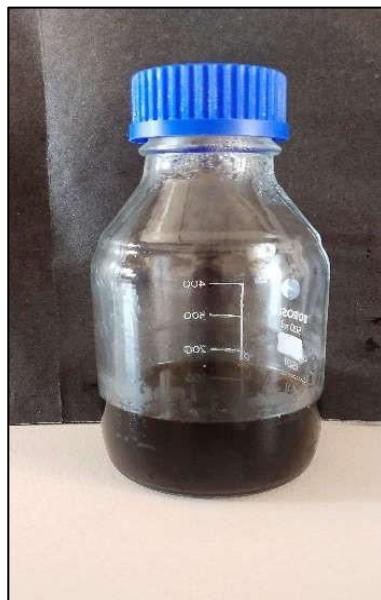
**Figure 1:** Collection of Seaweeds from Gulf of Mannar  
(Bay of Bengal)

#### **Preparation of Seaweeds:**

Seaweed was washed with the running tap water and distilled water to remove the salt on the surface. After draining the water, Seaweeds were spread out on the blotting paper to remove leftover water. The cleaned seaweeds were unleashed for shade dry. Then the dried samples were crushed using blender.

## Extract Preparation:

The crushed samples was weighted for 15grams and dissolved in solvents of 60ml. Each Seaweed samples was dissolved in all the 3 solvents. The different solvent used was aqueous, ethanol and ethyl acetate. It was allowed for boiling 15 to 20 minutes till the crude extract gets ready. So a sample had three different extracts. The extracted solvent was then used for preliminary phytochemical screening.



**Figure 2:** Solvent Extract

## Preliminary Phytochemical Screening (Edeoga *et al.*, 2005)

Phytochemicals are naturally occurring and biologically active compound that have potential disease inhibiting capabilities. Seaweeds are endowed with various Phytochemicals screening of alkaloids, flavonoids, terpenoids, tannins, steroids, phenols, saponins and reducing sugar.

## Solvent Extraction by Soxhlet Apparatus (Jensen *et al.*, 2007)

Solvent Extraction, also known as liquid-liquid extraction and partitioning, is a method to separate compounds or metal complexes based on their relative solubilities in two different immiscible liquids. Solvent Extraction method was carried with Soxhlet apparatus with three solvents namely aqueous, ethanol and ethyl acetate. The extraction solvent inside the boiling flask is evaporated and re-condensed in the distillation column. Then it falls down onto the solid material requiring extraction. The chamber containing the solid material is connected to the boiling flask below by a siphoning mechanism, which allows the chamber to fill to a point, at which it will empty its contents and start to fill again and the extracted compounds will accumulate in the boiling flask below. Chemists use it to remove a material from a solid. It is useful when removing material that does not dissolve well in the solvent. The soxhlet extractor applies the solvent repeatedly to the solid until enough of the material dissolves.



**Figure 3:** Solvent Extraction by Soxhlet Apparatus

## Thin layer Chromatography (Satheesh kumar *et al.*, 2013)

Thin layer chromatography technique used to separate nonvolatile mixtures. It is performed on a sheet of glass, plastic or aluminum foil coated with adsorbent material, silica gel. After the sample had applied on the plate, a solvent or solvent mixture (mobile phase) is drawn up the plate via capillary action.

### Dot plot Assay

Dot plot assay was carried out with TLC plates, which is coated with thin layer of silica gel. It acts as adsorbent material. Since 3 concentrations (150 $\mu$ l, 300 $\mu$ l, 450 $\mu$ l) so TLC plate was cut into length and breadth of 9cm and 3cm respectively. TLC plate was divided into three equal segments.

### Results and Discussion

**Table 1:** Phytochemical Screening Result

Test	<i>Egregiamen ziesii</i>			<i>Chaetomorpha crassa</i>		
	Aqueous	Ethanol	Ethyl Acetate	Aqueous	Ethanol	Ethyl Acetate
Alkaloids	--	--	++	--	--	++
Flavonoids	--	--	--	--	--	--
Terpenoids	--	--	++	--	++	++
Tannins	++	--	--	--	--	++
Steroids	--	++	++	--	++	++
Phenols	--	--	--	--	--	--
Saponins	++	--	--	++	--	--
Reducing Sugar	++	--	--	--	--	--

## Antibacterial Activity

**Table 2:** Antibacterial Screening Result

S.No	Organism	Activity of Seaweed Extracts Diameter in cm			
		25µl	50µl	75µl	100µl
1	<i>Escherichia coli</i>	-	-	-	0.5
2	<i>Bacillus subtilis</i>	-	-	0.5	0.7
3	<i>Pseudomonas aeruginosa</i>	-	-	-	-
4	<i>Serratia marcescens</i>	-	-	-	-

## Antifungal Activity

**Table 3:** Antifungal Screening

S. No	Samples	Organism	Activity of Seaweeds Diameter in cm			
			25µl	50µl	75µl	100µl
1	Aqueous	<i>Aspergillus flavus</i>	--	--	--	--
2	Aqueous	<i>Fusarium sp.,</i>	--	--	--	--
3	Ethanol	<i>Aspergillus flavus</i>	0.1	0.13	0.2	0.4
4	Ethanol	<i>Fusarium sp.,</i>	--	--	--	--
5	Ethyl acetate	<i>Aspergillus flavus</i>	0.2	0.25	0.4	0.45
6	Ethyl acetate	<i>Fusarium sp.,</i>	0.1	0.12	0.19	0.2

Phytoconstituents present in some seaweeds collected from Gulf of Mannar, Tamil Nadu, India. Phytochemical screening was carried out using standard methods. The present study screened the phytochemical properties of six seaweeds. Phytochemical screening is done to evaluate the presence and absence of secondary metabolites. Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases.

Phytochemicals are primary and secondary compounds (Abirami *et al.*, 2017). The presence or absence of phytoconstituents depends upon the solvent medium used for the extractions and physiology properties of seaweeds. It concluded that the seaweeds may be used as a broad-spectrum agent after extensive investigations and the similar type of results were observed in (Fatma *et al.*, 2016). We employed solvent extraction with ethanol, ethyl acetate and aqueous with samples *Egregia menziesii*, *Halimeda opuntia*, *Sargassum swartzii* (Jensen *et al.*, 2007) Soxhlet apparatus method followed by antimicrobial and antioxidant was examined.

Microorganisms which cause many infectious diseases for animal and mankind, in ancient study and also according to literature survey, seaweeds have played a vital in antibacterial activities *E.coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Serratia marcescens* was investigated for antibacterial activity.

The result shows values on 100 $\mu$ l with 0.7cm zone of inhibition was observed in the aqueous solvent with *Bacillus subtilis*, 50 $\mu$ l with 1cm zone of inhibition was observed in the ethanol solvent with *E.coli* and 50 $\mu$ l with 1.2cm zone of inhibition was observed in Ethyl acetate with *E.coli*.

Antifungal activities *A. flavus* and *Fusarium sp.*, was evaluated with Yeast Extract Peptone Dextrose Agar medium, the result shows promising values,no zone was observed in aqueous solvent, in 100 $\mu$ l 0.4cm zone was observed in ethanol solvent with *A. flavus*, in 100 $\mu$ l 0.45cm zone was observed in ethyl acetate solvent with *A. flavus*.

Antioxidant properties which have multiple functions in biological system, such as defense against oxidative damage caused by the action of reactive oxygen species. FRAP, DPPH assay, H<sub>2</sub>O<sub>2</sub> scavenging assay and superoxide radical scavenging assay were determined in ethanol, ethyl acetate and aqueous extracted seaweeds. The result shows that they contained higher amount of scavenging property, similar to this (Wang *et al.*, 2011)

and (Yan *et al.*, 1999) which reveals that seaweeds contain large number of polyphenols and DPPH radical scavenging activity. Dot plot assay helps to know the antioxidant property visually. We observed the presence of secondary metabolites, shows that seaweeds contain higher number of therapeutic agents and antioxidant property in them.

## Conclusion

The result of this study shows the presence of some phytochemicals such as alkaloids, steroids and tannins in extracts of seaweeds. Alkaloids are already known to have antifungal and antimicrobial. Compounds with antioxidant, antifungal, antimicrobial activities have been detected in algae.

Reactive oxygen species such as hydroxyl, superoxide and peroxyl radicals are found in human cells result in extensive oxidative damage which can lead to aged related degenerative conditions, cancer and various human diseases. In spite of their wide applications in food and feed industries, they have gained importance as medicinal source because of high healing, antimicrobial and antioxidant properties.

In future these types of marine macro algae will definitely play a vital role as a remedy for challenging diseases like diabetes, cancer, HIV and it will also act as a supply of food, nutrition, health, fertilizers and soil conditioners, animal feed, fish feed, biomass for fuel, cosmetics, Integrated aquaculture, treats waste water to reduce nitrogen and phosphorus containing compounds, removal of toxic metals, extraction of industrial gums and chemicals. All these are used on the odd occasion nowadays but in future there will great demand for the marine seaweeds.

## Reference

- Abirami P, Yamuna P, Sharmila M, Vijaya Shalini P. Qualitative phytochemical analysis of *Gompherensa globsa* Linn and *Gompherensa decumbens* Jacq. International journal of Biology Research, 2(3): 20-22, 2017.

- Abirami, R. G., & Kowsalya, S. (2011). Nutrient and nutraceutical potentials of seaweed biomass *Ulva lactuca* and *Kappaphycus alvarezii*. *Nong Ye Ke Xue Yu Ji Shu*, 5(1).
- Abirami, R. G., & Kowsalya, S. (2017). Quantification and correlation study on derived phenols and antioxidant activity of seaweeds from Gulf of Mannar. *Journal of Herbs, Spices & Medicinal Plants*, 23(1), 9-17.
- Ashwini, S., & Shantaram, M. (2016). Preliminary phytochemical and biochemical composition of different solvent extracts of red seaweed *Gracilaria corticata* from Surathkal beach, Karnataka in India. *International Journal of Life Sciences and Technology*, 9(13), 106.
- Bacon A.S, Ball, J. L., Malhotra, R. M., Leong, P., (2000). The importance of recognising *Streptococcus milleri* as a cause of orbital cellulitis. *Eye*, 14(5), 814-815.
- Hwang S., Kolanjinathan, K., & Stella, D. (2009). Antibacterial activity of marine macro algae against human pathogens. *Recent Research in Science and Technology*, 1(1).
- Cai Y Z, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Journal of life science*, 74: Pg no: 2157-2184, 2004.
- Cai Y Z, Sun M, Corke H. Antioxidant activity of betalains from plants of the *Amaranthaceae*. *Journal of Agricultural food chemistry*, 51: Pg no: 2288-2294, 2003.
- Dayton, P. K., & Hessler, R. R. (1972, March). Role of biological disturbance in maintaining diversity in the deep sea. In *Deep Sea Research and Oceanographic Abstracts* (Vol. 19, No. 3, pp. 199-208). Elsevier.
- DhanaRajan M S, Hebsibah Elsie B. Evaluation of antimicrobial activity and phytochemical screening of *Gelidium acerosa*. *Journal of Pharmaceutical sciences and research* 2 (11), 704, 2010.
- Dodson.R.J., Klenk, H. P., Clayton, R. A., Tomb, J. F., White, O., Nelson, K. E., Ketchum, K. A., & Richardson, D. L. (1998). Erratum: The complete genome sequence of the

hyperthermophilic, sulphate-reducing archaeon *Archaeoglobus fulgidus*. *Nature*, 394(6688), 101-101.

- Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*, 4(7): Pg no: 685-688, 2005.
- Ellsworth, D. S., Reich, P. B., Naumburg, E. S., Koch, G. W., Kubiske, M. E., & Smith, S. D. (2004). Photosynthesis, carboxylation and leaf nitrogen responses of 16 species to elevated pCO<sub>2</sub> across four free-air CO<sub>2</sub> enrichment experiments in forest, grassland and desert. *Global Change Biology*, 10(12), 2121-2138.
- Elsayed, M., Elmegeed, D. F. A., Ghareeb, D. A., & El-Saadani, M. (2014). Phytochemical constituents and bioscreening activities of green algae (*Ulva Lactuca*). *International Journal of Agricultural Policy and Research*, 2(11), 373-378.
- Elsie, B. H., & DhanaRajan, M. S. (2010). Evaluation of antimicrobial activity and phytochemical screening of *Gelidium acerosa*. *Journal of Pharmaceutical sciences and research*, 2(11), 704.
- Fatma Chaari, Khawla Ben Jeddou, Sameh Maktouf, Oumèma Nouri-Ellouz, Claire Boisset Helbert, Raoudha Ellouz Ghorbel. Structural, functional, and antioxidant properties of water-soluble polysaccharides from potatoes peels. *Journal of Food Chemistry* 205, 97105, 2016.
- Fatma, B., Fatiha, M., Elattafia, B., & Noureddine, D. (2016). Phytochemical and antimicrobial study of the seeds and leaves of *Peganum harmala* L. against urinary tract infection pathogens. *Asian Pacific Journal of Tropical Disease*, 6(10), 822-826.
- Fayaz, M., Namitha, K. K., Murthy, K. C., Swamy, M. M., Sarada, R., Khanam, S., & Ravishankar, G. A. (2005). Chemical composition, iron bioavailability, and antioxidant activity of *Kappaphycus alvarezzi* (Doty). *Journal of agricultural and food chemistry*, 53(3), 792-797.
- Gann R Y, Xu X R, Song F L, Kuang L, Li H B. Antioxidant activity and total phenolic content of medicinal plants

associated with prevention and treatment of cardiovascular and cerebrovascular diseases. Journal of Medicinal Plants Research, vol 4: Pg no:24382444, 2010.

- Gorban, J. M., & Topol'nyikova, N. V. (2003). Influence of *Spirulina* on the endocrine status and lipid peroxidation processes in irradiated rats;
- Graham, J. G., Quinn, M. L., Fabricant, D. S., & Farnsworth, N. R. (2000). Plants used against cancer—an extension of the work of Jonathan Hartwell. Journal of ethnopharmacology, 73(3), 347-377.
- Gribben, P. E., Byers, J. E., Clements, M., McKenzie, L. A., Steinberg, P. D., & Wright, J. T. (2009). Behavioural interactions between ecosystem engineers control community species richness. Ecology Letters, 12(11), 1127-1136.
- Hardy, F. G., Guiry, M. D., Arnold, H. R., & British Phycological Society. (2006). A check-list and atlas of the seaweeds of Britain and Ireland (p. 435). London: British Phycological Society.
- Hebsibah Elsie B, DhanaRajan M S, Evaluation of antimicrobial activity and phytochemical screening of *Gelidium acerosa*. Journal of Pharmaceutical sciences and research 2 (11), 704, 2010.
- Hornsey, I. S., & Hide, D. (1985). The production of antimicrobial compounds by British marine algae. IV. Variation of antimicrobial activity with algal generation. British Phycological Journal, 20(1), 21-25.
- Jeeva, S., Domettilla, C., Joselin, J., & (2013). Phytochemical analysis on some south Indian seaweeds. Journal of Chemical and Pharmaceutical Research, 5(4), 75-278.
- Jensen, Willam B. The origin of the Soxhlet Extractor. Journal of chemical Education, 84(12): Pg no: 1913-1914, 2007.
- Kähkönen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J. P., Pihlaja, K., Kujala, T. S., & Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. Journal of agricultural and food chemistry, 47(10), 3954-3962.
- Kähkönen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J. P.,

- Pihlaja, K., Kujala, T. S., & Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *Journal of agricultural and food chemistry*, 47(10), 3954-3962.
- Kannan, R., Neelamathi, E., (2016). Screening and characterization of bioactive compounds of *Turbinaria ornata* from the gulf of Mannar, India. *Journal Agricultural and Environmental Science*, 16(2), 243-251.
  - Kim Y S, and Shin D H, 2015. Volatile constituents from the leaves of *polygonum cuspidatum* and their antibacterial activities. *Microbiology* 22: Pg no :139-144.
  - Lekameera, R., Vijayabaskar, P., & Somasundaram, S. T. (2007). Potential antioxidant activity of brown alga *Lobophora varie*. *Seaweed Res Utiln*, 29(2), 55-61.
  - Levy, S. B. (2002). Factors impacting on the problem of antibiotic resistance. *Journal of Antimicrobial Chemotherapy*, 49(1), 25-30.
  - Liu, I., Yokoyama, H., Simpson, K. L., & Chichester, C. O. (1973). Isolation and identification of 2-hydroxy plectania xanthin from *Rhodotorula aurantiaca*. *Phytochemistry*, 12(12), 2953-2956.
  - Madhavi, D. L. Patel, S. J., Nimbalkar, S. S., Kulkarni, A. D., and (2011). Lipid oxidation in natural and nourishment frameworks. *Nourishment Science and Technology*, New York -marcel dekker-, 5-64.
  - Maikhuri, R. K., Rao, K. S., Chauhan, K., Kandari, L. S., Prasad, P., & Rajasekaran, C. (2003). Development of marketing of medicinal plants and other forest products-can it be a path way for effective management and conservation? *Indian Forester*, 129(2), 169178.
  - Manilal A, Sujith S, Kiran G S, Selvin J, Shakir C, Gandhimathi R and Panikkar M V N. Biopotentials of Seaweeds collected from Southwest coast of India. *Journal of Marine science and technology*, 17: Pg no: 67-73, 2007.
  - Mansuya, P., Aruna, P., Sridhar, S., Kumar, J. S., & Babu, S. (2010). Antibacterial activity and qualitative phytochemical

analysis of selected seaweeds from Gulf of Mannar region. Journal of experimental Sciences.

- Mc Hugh, D. J. (2001). Prospects for seaweed production in developing countries (FAO Fisheries Circular No. 968 FIIU/C968 (En). FAO, Rome.
- Mohamed, E. A., Morsy, G. M., Bekhet, E. K., & El Din, R. A. S. (2018). phytochemical screening for antibacterial compounds of some seaweed from coastal area of abu-qir, alexandria, egypt. Egyptian J. of Phycol. Vol, 19(48).
- Nabavi, S. M. B., Farasat, M., Khavari-Nejad, R. A., & Namjooyan, F. (2014). Antioxidant activity, total phenolics and flavonoid contents of some edible green seaweeds from northern coasts of the Persian Gulf. Iranian journal of pharmaceutical research: IJPR, 13(1), 163.
- Nawas, P., & Sujatha, R., Siva, D.(2019). Screening of phytochemical profile and antibacterial activity of various solvent extracts of marine algae *Sargassum swartzii*. World Scientific News, 115, 27-40
- Ndhlala, A. R., Kasiyamhuru, A., Mupure, C., Chitindingu, K., Benhura, M. A., & Muchuweti, M. (2007). Phenolic composition of *Flacourtie indica*, *Opuntia megacantha* and *Sclerocarya birrea*. Food Chemistry, 103(1), 82-87.
- Neelamathi, E., & Kannan, R. (2016). Screening and characterization of bioactive compounds of *Turbinaria ornata* from the gulf of Mannar, India. Journal Agricultural and Environmental Science, 16(2), 243-251.
- Ohtomo, Y., Ijiri, A., Ikegawa, Y., Tsutsumi, M., Imachi, H., Uramoto, G. I., & Tanikawa, W. (2013). Biological CO<sub>2</sub> conversion to acetate in subsurface coal-sand formation using a high-pressure reactor system. Frontiers in microbiology, 4, 361.
- Patel, S. J., Madhavi, D. L., Nimbalkar, S. S., Kulkarni, A. D., and (2011). Lipid oxidation in natural and nourishment frameworks. Nourishment Science and Technology New York -marcel dekker-, 5-64.

- Patra, J. K., Patra, A. P., Mahapatra, N. K., Thatoi, H. N., Das, S., Sahu, R. K., & Swain, G. C. (2009). Antimicrobial activity of organic solvent extracts of three marine macroalgae from Chilika Lake, Orissa, India. *Malaysian Journal of Microbiology*, 5(2), 128-131.
- Peters, Lannergård, J., von Eiff, C., Sander, G., Cordes, T., Seggewiß, J., G., & Hughes, D. (2008). Identification of the genetic basis for clinical menadione-auxotrophic small-colony variant isolates of *Staphylococcus aureus*. *Antimicrobial agents and chemotherapy*, 52(11), 4017-4022.
- Phondani, P. C., Negi, V. S., Bhatt, I. D., Maikhuri, R. K., & Kothiyari, B. P. (2011). Promotion of medicinal and aromatic plants cultivation for improving livelihood security:
- Pietta., Graf, B. L., Simmler, C., Kim, Y., Kuhn, P., Pauli, G. F., and Raskin, I. (2000). Biochemical portrayal and mitigating properties of an isothiocyanate-advanced (*Moringa oleifera*) seed remove. *Plos one*, 12(8), e0182658.
- Piyusha Suresh Shelar, Vijay Kumar Reddy, Gauri Suresh Shelar, Kavitha M, Praveen Kumar G, Vidya Sagar Reddy G (2012). Medicinal value of Seaweeds and its applications- a Review. *Continental Journal of Pharmacology and Toxicology Research* 5(2): Pg no: 1-22.
- Rao, K. S., Semwal, R. L., Maikhuri, R. K., Nautiyal, S., Sen, K. K., Singh, K., & Saxena, K. G. (2003). Indigenous ecological knowledge, biodiversity and sustainable development in the central Himalayas.
- Ravikumar, T. M., Sachindra, N. M. Sowmya, R., Vivek, R., Rathinaraj, K., & (2014). Optimization of enzymatic hydrolysis of shrimp waste for recovery of antioxidant activity rich protein isolate. *Journal of food science and technology*, 51(11), 3199-3207.
- Rindi, F., & Guiry, M. D. (2004). A long-term comparison of the benthic algal flora of Clare Island, County Mayo, western Ireland. *Biodiversity & Conservation*, 13(3), 471492.
- Robertson-Andersson, D. (2007). Biological and economical feasibility studies of using seaweeds in recirculation systems

in abalone farming (Doctoral dissertation, PhD thesis, Botany Department, University of Cape Town).

- Sabirin, F., Kazi, J. A., Ibrahim, I. S., & Rashit, M. M. A. A. (2015). Screening of seaweeds potential against oral infections. *Journal of Applied Sciences Research*, 11(5), 1-6.
- Sachindra, N. M. Sowmya, R., Ravikumar, T. M., Vivek, R., Rathinaraj, K., & (2014). Optimization of enzymatic hydrolysis of shrimp waste for recovery of antioxidant activity rich protein isolate. *Journal of food science and technology*, 51(11), 3199-3207.
- Sameera, M. (2010). Al Johani; Javed Akhter; Hanan Balkhy; Ayman El-Saed; Mousaad
- Sande, M. A., Ruiz-Rebollo, M. L., Sánchez-Antolín, G., García-Pajares, F., FernándezOrcajo, P., Velicia-Llames, R., & Caro-Patón, A. (2008). *Acalculous cholecystitis* due to *Salmonella enteritidis*. *World journal of gastroenterology: WJG*, 14(41), 6408.
- Sanger, G., Rarung, L. K., Damongilala, L. J., Kaseger, B. E., & Montolalu, L. A. D. Y. (2019, May). Phytochemical constituents and antidiabetic activity of edible marine red seaweed (*Halymenia durvillae*). In IOP Conference Series: Earth and Environmental Science (Vol. 278, No. 1, p. 012069). IOP Publishing.
- Sankar, C. V., Viju, N., Anitha, A., Vini, S. S., Satheesh, S., & Punitha, S. (2014). Antibiofilm activities of extracellular polymeric substances produced by bacterial symbionts of seaweeds.
- Satheesh Kumar bhandary, S., Biswas, A., Dhali, G. K., Chowdhury, A., Boyer, J. L., and Santra, A. (2013). Oxidative pressure and hepatic stellate cell initiation are entering occasions in arsenic instigated liver fibrosis in mice. *Toxicology and connected pharmacology*, 251(1), 59-69.
- Selim, R. E., Ahmed, S. M., El-Zemity, S. R., Ramses, S. S., & Moustafa, Y. T. (2015). Antifungal activity and seasonal variation of green alga (*Ulva lactuca*) extracts. *Asian Journal of Agriculture and Food Sciences* (ISSN: 2321-1571), 3(05).

- Singh, G., Tamboli, E., Acharya, A., Kumarasamy, C., Mala, K., & Raman, P. (2015). Bioactive proteins from Solanaceae as quorum sensing inhibitors against virulence in *Pseudomonas aeruginosa*. *Medical hypotheses*, 84(6), 539-542.
- Smit, A. J. (2004). Medicinal and pharmaceutical uses of seaweed natural products: a review. *Journal of applied phycology*, 16(4), 245-262.
- Sothornvit, R., Olsen, C. W., McHugh, T. H., & Krochta, J. M. (2007). Tensile properties of compression-molded whey protein sheets: determination of molding condition and glycerol-content effects and comparison with solution-cast films. *Journal of Food Engineering*, 78(3), 855-860.
- Sujatha, R., Siva, D., & Nawas, P. (2019). Screening of phytochemical profile and antibacterial activity of various solvent extracts of marine algae *Sargassum swartzii*. *World Scientific News*, 115, 27-40.
- Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos, L Byrne D H. Comparison of ABTs, DPPH, FRAP and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food composition analysis*, vol 19: Pg no: 669-675, 2006.
- Urbano, M. G., & Goni, I. (2002). Bioavailability of nutrients in rats fed on edible seaweeds, Nori (*Porphyra tenera*) and Wakame (*Undaria pinnatifida*), as a source of dietary fibre. *Food Chemistry*, 76(3), 281-286.

## Antibacterial Activity of a Traditional Medicinal Plant *Pentatropis Microphylla* Wight & Arn (Apocynaceae)

**Dr. R. Prema**

Head and Associate Professor,

Department of Botany

Arulmigu Palaniandavar Arts College for Women, Palani,  
Tamil Nadu, India

### Abstract

The primary objective of this study is to assess the antimicrobial properties of the species *Pentatropis microphylla*. The study species was made in to fine powder and extracted with petroleum ether, ethyl acetate and methanol using soxhlet extractor and filtered. Freshly prepared nutrient agar medium are used for the culture of bacteria *P. microphylla* generally showed inhibitory activity against the growth of bacteria like *Bacillus thuringiensis*, *Escherichia coli*, *Bacillus subtilis* and *Klebsiella pneumonia*. The result obtained confirm the therapeutic potency of the study species. *P. microphylla* of Anthiyur Forest of Erode district. The extracts of this plant possess compounds of activity of inhibitory and can be used as antimicrobial agent in new drugs for the therapy of infectious diseases caused by pathogens.

**Keywords:** *Pentatropis microphylla*, *Bacillus thuringiensis*, *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumonia*.

### Introduction

Plants are one of the most important sources of medicine and plant derived compounds (phytochemicals) have been attracting much interest as natural alternatives to synthetic compounds. Nowadays herbal drugs are prescribed widely even when their biologically active compounds are unknown because of their effectiveness, minimal side effects in clinical experience and

relatively low cost (Valiathan, 1998). The studies of World Health Organization (WHO) indicate that over 30% of world's plant species have at one time or another been used for medicinal purposes. The medicinal value of plant is due to the presence of certain secondary metabolites. The application of plants as medicine dates back to prehistoric period. The early civilization reveals that a considerable number of drugs used in modern medicine have figured in ancient manuscripts such as the Rig, Veda, the Bible, the Quran, the Iliad, the Odyssey and the History of Herodotus. Over 600 years ago, the ancient Chinese were the first to use the plants of natural vegetation as the source of medicine. In India, in ayurvedic system of medical practice, barks of plants have been in medicinal use for over 3000 years. Charaka and Susruta, two of the earliest Indian authors had sufficient knowledge on the properties of the Indian medicinal plants.

Despite the local medicinal usage of the species, *P.microphylla* no studies were carried out for confirming their medicinal uses. Therefore, in the present study, antibacterial studies were performed to confirm their healing properties. For that, various extracts of the useful parts of the species *viz.*, leaf, stem bark and root were tested against certain pathogenic bacteria.

## Materials and methods

The information gathered from local residents and through a review of existing literature revealed that in the study species, *P. microphylla*, the leaf, stem bark and root parts hold medicinal uses and hence used for medicinal purposes. Therefore, in the present study antibacterial properties were analyzed by using these parts against the bacteria selected.

### Collection and processing of plant parts

Fresh leaf, stem bark and root parts of the study species were collected from the Anthiyur forests of Erode district and brought to the laboratory by keeping them in ice box. The fresh

materials were washed under running tap water, air dried and then homogenized to fine powder and stored in air tight bottles.

### Preparation of plant extracts

To know the medicinal importance, the shade dried plant parts of the study species was made into a fine powder of 40 mesh size using the pulverize separately. Following that, 100g of the powder was filled in the filter paper and successively extracted using 500 mL solvents *viz.*

Petroleum ether, ethyl acetate and methanol separately using the Soxhlet extractor for 8 – 10 hours (Gafner *et al.*, 1985). Then the extracts were filtered separately through Whatman No.1 filter paper to remove all undissolved matter, including cellular materials and other constituents that are insoluble in the extraction of solvents.

### Antibacterial activity of the plant extracts

A vast number of experiments were carried out to show the antibacterial efficacy of the plant extracts to cure large number of pathogenic diseases. Antibacterial activity of petroleum ether, ethyl acetate and methanol extracts of leaf, stem bark and root parts of the study species were determined by disc diffusion method (Bauer *et al.*, 1966).

### Collection and maintenance of Bacterial strains

The following Bacterial strains were used in the present study:

#### Bacterial strains

**G (+)ve bacteria** - *Bacillus subtilis* and *B. thuringiensis*.

**G (-)ve bacteria** - *Klebsiella pneumonia* and *Escherichia coli*.

These bacteria were obtained from the Department of Microbiology, Hindustan College of Arts and Science, Coimbatore. The bacterial strains were maintained at 4°C on nutrient agar and potato dextrose agar slants respectively and kept in refrigerator prior to subculture.

## Media used

Freshly prepared nutrient agar medium and potato dextrose agar (PDA) medium were used for the culture of bacteria respectively.

### Composition of Nutrient agar medium

Constituents	Amount
Peptone	5.0g
Beef extract	3.0g
Agar	15.0g
Distilled water	1000mL
pH	7.0

### Composition of PDA medium

Constituents	Amount
Potato	200.0g
Dextrose	20.0g
Agar	15.0g
Distilled water	1000mL
pH	6.2

## Method

The culture media were prepared and autoclaved at 121°C at 15 p.s.i. for 20 minutes and stored in refrigerator. The media were melted before the process of inoculation. The clean dry sterile Petri dishes were poured with nutrient agar medium (for bacteria). Four numbers of 10 mL broths were prepared separately for nutrient agar medium in test tubes and plugged with cotton and autoclaved.

The test tubes were labelled with the bacteria to be inoculated. The bacterial strains were inoculated onto the nutrient broth under aseptic conditions and incubated at  $37 \pm 0.5^{\circ}\text{C}$  for 18 hours. After incubation, the bacteria were smeared on the nutrient agar plate respectively using a sterile cotton swab.

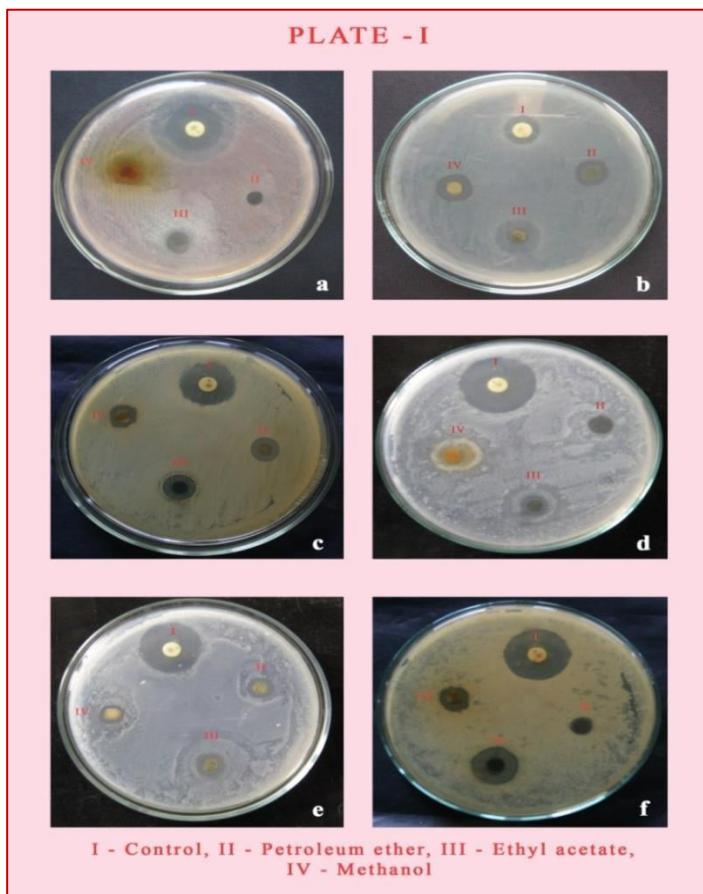
A sterile disc of 6 mm diameter was loaded with known quantity of 10 mg of dried crude extracts. These discs were placed on the surface of the media. The positive controls antibiotic and tetracycline were used at the concentration, 0.1 mg/10 mL of distilled water each and maintained by loading on discs. Then the Petri dishes were incubated at  $37 \pm 0.5^{\circ}\text{C}$  for 24 to 48 hours. The diameter of inhibition zone was measured. Triplicates were maintained for all tests.

## Results

### Antibacterial activity

The antibacterial activity of the extracts of the two studied species was assayed *in vitro* by disc diffusion method against four bacterial pathogens. Tables 33-38 exhibited the data on bacterial growth inhibition by various alcoholic extracts of leaf, stem bark and root parts of two studied plant species. The antibacterial activity of all the alcoholic leaf extracts of the study species, *P. microphylla* generally showed inhibitory activity against the growth of *B. thuringiensis* and *E. coli* (Plate - Ia). However, towards *B. subtilis* and *K. pneumoniae*, all these extracts showed activity with less pronounced manner (Table 1).

The antibacterial activity of certain alcoholic stem bark extracts of *P. microphylla* is given in Table 2. It shows that generally, all extracts have significant activity against the three bacteria viz., *B. subtilis* (Plate - Ib) *B. thuringiensis* and *K. pneumonia* and it was less against the other bacteria, *E. coli*. Similarly, the root extracts of this species has showed significant inhibitory activity against the two bacteria viz., *B. thuringiensis* and *E. coli* (Table 3 and Plate Ic). Further, it was noted that the inhibitory activity was noteworthy against the bacteria, *B. subtilis* and *K. pneumoniae* also.



**Figure 1.** Antibacterial activity of the different solvent extracts

## Discussion

Successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Traditional healers use primarily water as the solvent but in the present study it was found that plant extracts in organic solvents provided more consistent antibacterial activity. These observations can be rationalized in terms of the polarity of the compounds being extracted by each solvent and in addition to their intrinsic bioactivity by their ability to dissolve or diffuse in the different media used in the assay. The results of the present study on the antibacterial activities of various organic chemical extracts of leaf, stem bark and root parts of the studied species against the colonial growth of four bacterial species presented in Tables 1 - 3. The study revealed that generally the inhibitory activity is pathogen-specific and depends on the plant parts and solvents used for the extraction of secondary metabolites of inhibition property.

For the species, *Pentatropis microphylla* almost all extracts of leaf, stem bark and root parts in general have the considerable antibacterial activity against the four bacterial species investigated (Tables 1 - 3).

**Table 1:** Antibacterial activity of certain alcoholic leaf extracts of the species, *P. microphylla*.

Plant extract	Diameter of zone inhibition (mm)			
	Gram positive bacteria		Gram negative bacteria	
	<i>B. subtilis</i>	<i>B. thuringiensi</i>	<i>K. pneumoniae</i>	<i>E. coli</i>
Petroleum ether	-	10.76 ± 0.62	-	12.45 ± 0.60
Ethyl acetate	12.13 ± 0.70	14.33 ± 0.80	8.03 ± 0.15	11.67 ± 0.35
Methanol	-	10.07 ± 0.21	10.93 ± 0.40	11.23 ± 0.45

\* Tetracycline

**Table 2:** Antibacterial activity of certain alcoholic stem bark extracts of the species, *P. microphylla*.

Plant extract	Diameter of zone inhibition (mm)			
	Gram positive bacteria		Gram negative bacteria	
	<i>B. subtilis</i>	<i>B. thuringiensis</i>	<i>K. pneumoniae</i>	<i>E. coli</i>
Standard*	20.77 ± 0.20	18.06 ± 0.56	11.33 ± 0.42	16.12 ± 0.61
Petroleum ether	11.87 ± 0.15	10.16 ± 0.57	9.16 ± 0.37	-
Ethyl acetate	12.13 ± 0.61	11.03 ± 0.61	17.23 ± 0.58	14.16 ± 0.66
Methanol	11.16 ± 0.47	14.17 ± 0.60	13.73 ± 0.75	8.06 ± 0.30

\* Tetracycline

**Table 3:** Antibacterial activity of certain alcoholic root extracts of the species, *P. microphylla*.

Plant extract	Diameter of zone inhibition (mm)			
	Gram positive bacteria		Gram negative bacteria	
	<i>B. subtilis</i>	<i>B. thuringiensis</i>	<i>K. pneumoniae</i>	<i>E. coli</i>
Standard*	20.23 ± 0.49	26.67 ± 0.61	12.17 ± 0.93	21.06 ± 0.70
Petroleum ether	-	10.63 ± 0.65	-	9.93 ± 0.40
Ethyl acetate	7.87 ± 0.90	13.67 ± 0.61	-	11.97 ± 0.55
Methanol	17.03 ± 0.76	14.87 ± 0.85	10.03 ± 0.45	10.03 ± 0.35

\* Tetracycline

The overall study on antibacterial activity reports that the plant species containing active compounds of inhibitory action substantially. The beneficial medicinal effects of these plant materials typically results from the combinations of secondary products present in these plant species. The heterogeneity of these secondary compounds in wild species is reported to be wide (Balandrin *et al.*, 1985). Based on this concept, it is explained that the study species due to heterogeneity of secondary compounds owing to their wildness could be with higher antibacterial activity.

The higher antibacterial activity of alcoholic extracts of the present study species may further indicates that the antibacterial principles/chemical constituents which are either polar or non polar can be effectively extracted only through the organic solvent medium (Eseawi and Srour, 2000; Raskin *et al.*, 2002; Aiyelaagbe *et al.*, 2007; Zakaria *et al.*, 2010; Rahul *et al.*, 2011). Many early studies also reported the effective inhibitory activity of alcoholic solvents against the growth of the pathogenic microbes (Thomas *et al.*, 1999; Reddy *et al.*, 2001; Erdogan, 2002; Ates and Erdogan, 2003; Nair *et al.*, 2005; Mohan *et al.*, 2005; Poonkothai and Saravanan, 2008; Salam *et al.*, 2013; Silvia *et al.*, 2013; Deepak *et al.*, 2014; Subba Lakshmi; Pullaiah, 2015). The poor antibacterial activity of some extracts might be attributed to the extracting capacity of solvent and the concentration of the active ingredients in the extracts (Akporie and Olorungbon, 2011).

## Conclusion

From the present investigation, the results obtained confirm the therapeutic potency of the studied plant species of Anthiyur forest of Erode district, *Pentatropis microphylla* prescribed in traditional medical practice by local people. Further, it supports the folkloric usage of these plants and suggests that their alcoholic extracts possess compounds of activity of inhibitory and they can be used as antibacterial agents in new drugs for the therapy of infectious diseases caused by pathogens. The most active extracts can be subjected to isolation of the therapeutic antimicrobial compounds and undergo further pharmacological evaluation.

## References

- Abdellatef, E. and M.M. Khalafalla, 2008. Ethylene inhibitors promote *in vitro* regeneration of medium staple cotton (*Gossypium hirsutum* L.) cv. Barac B- 67. Advances in Natural and Applied Sciences, 2(3): 178-184.
- Abrie, A.L. and J. van Staden, 2001. Micropropagation of the endangered *Aloe polyphylla*. Plant Growth Regulation 33: 19-23.
- Adekunle, A.A. and A.M. Ikumapayi, 2006. Antifungal property and phytochemical screening of the crude extracts of *Funtumia elastica* and *Mallotus oppositifolius*. West Indian Med. J. 55(4): 219-223.
- Adesegun, S.A., A. Fajana, C.I. Orabueze and H.A.B. Coker, 2009. Evaluation of antioxidant properties of *Phaulopsis fasciculata* C.B.C.I. (Acanthaceae). Alternat Med. 6(2): 227-231.
- Balakrishnan, N.P. and M. Mohanan, 1991. Flora of the Nilgiri Biosphere Reserve. E.E.C. News 7-9.
- Balakrishnan, N.P. and R. Ansari, 1990. *Enumeration of the flora of the Nilgiri Biosphere Reserve*. Report submitted to MoEF, New Delhi (unpublished).
- Banerjee, S., J. Thirupathi, P.C. Verma, P.D. Dwivedi, S.P.S. Khanuja and G.D. Bagchi, 2004. Thidiazuron- induced high-frequency shoot proliferation in *Cineraria maritime* Linn. Curr. Sci. 87:1287-1290.
- Chopra, H. 1959. Medicinal Herbs of Chhattisgarh, India Having Less known Traditional Uses. Retrieved from: <http://www.Botanical.com>.
- Christine, J. and B.C. McCarthy, 2002. Spatial and temporal variability of herbaceous vegetation in an eastern deciduous forest. Plant Ecol. 164: 37-48.
- Christophe, W. and D. Pharm, 2002. Ethnopharmacology of medicinal plants: Asia and the Pacific, Humana press, p. 1-69.
- Epand, R.F. 2007. Bacterial lipid composition and the antimicrobial efficacy of cationic steroid compounds. Biochimica et Biophysica Acta, 1768(10): 2500-2509.

- Erdogan, O.T. 2002. Antibacterial activities of some plant extracts used in folk medicine. *Pharmaceutical Biol.* 40: 269-273.
- Eseawi, T. and M. Srour, 2000. Screening some Palestinian medicinal plants for antibacterial activity. *J. of Ethanopharmacol.* 70: 343-349
- Janovska, D., K. Kubikova and L. Kokoska, 2003. Screening for antimicrobial activity of some medicinal plant species of traditional Chinese medicine. *Czech J. Food Sci.* 21(3): 107-110.
- Jansen, A.M., J.J.C. Cheffer and A.B. Svendsen, 1987. Antimicrobial activity of essencial oils: a 1976-1986 literature review. Aspects of test methods. *Planta Med.* 40: 395-398.
- Kumar, A. 1992. Somatic embryogenesis and high frequency plantlet regeneration in callus cultures of *Thevetia peruviana*. *Plant Cell Tissue and Organ Culture* 31: 47-50.
- Kumar, S. and K.V. Bhavanandan, 1988. Micropropagation of *Plumbago rosea* Linn. *Plant Cell Tiss. Org. Cult.* 15:275 – 278.
- Kumaraswamy, M., S. Balasubramanya and M. Anuradha, 2009. Germplasm conservation of patchouli (*Pogostemon cablin* Benth.) by encapsulation of *in vitro* derived nodal segments. *Int. J. Biodivers. Conserv.* 1(8): 224-230.
- Lal, N., S. Ahuja, A.K. Kukreja and B. Pandey, 1988. Clonal propagation of *Picrorrhiza kurroa* Royle ex Benth. by shoot tip culture. *Plant Cell Rep.* 7: 202-205.
- Lans, C.A. 2006. Ethnomedicines used in Trinidad and Tobago for urinary problems and diabetes mellitus. *J. Ethnobiol. Ethnomed.* 13(2): 45.
- Larrondo, J.V., M. Agut and M.A. Calvo-Torras, 1995. Antimicrobial activity of essences from labiates. *Microbios.* 82: 171-172.
- Locher, C.P., M.T. Burch, H.F. Mower, J. Berestecky, H. Davis, B. Van Poel, A. Lasure, D.A. Vanden Berghe and A.J. Vlietinck, 1995. Anti-microbial activity and anti-complement activity of extracts obtained from selected Hawaiian medicinal plants. *J. Ethanopharmacol.* 49: 23-32.

- Lu, C.Y. 1993. The use of thidiazuron in tissue culture. *In vitro Cellular Developmental Biology* 29: 92-96.
- Madhavan, M. and J.P. Joseph, 2001. *In vitro* organogenesis and somatic embryogenesis in *Datura metel* Linn. *Plant Cell Biotechnology and Molecular Biology*, 2,(3&4):125 – 132.
- Mandal, J. and U. Laxminarayana, 2014. Indirect shoot organogenesis from leaf explants of *Adhatodavasica* Nees. *SpringerPlus*, 3: 648.
- Manickam, V.S., R.E. Mathavan and R. Antonisamy, 2000. Regeneration of *Ginseng* plantlet from stem callus. *Plant Cell Tiss. Org. Cult.* 62: 181-185.
- Mao, A.H., A. Wetten, M. Fay and P.D.S. Caligari, 1995. *In vitro* propagation of *Clerodendrum colebrookianum* Walp: a potential natural anti-hypertension medicinal plant. *Plant Cell Rep.* 14: 493-496.
- Maragatham, C. and A. Panneerselvam, 2010. *In vitro* studies on the effect of precursors for the production of secondary metabolites in *Sida cordifolia*. *Plant Archives*, 10(1): 145-149.
- Nadeem, A.S., M. Mujeeb, M. Rashid, K. Hussain. 2013. Synthetic seed production; its relevance and future panorama. *American J. of Pharmtech Res.* 3(3): 382-397.
- Nadkarani, A.K. 1976. *Indian Materia Medica*, Vol. I Bombay; Popular Prakashan Pvt. Ltd p.430.
- Nagaraja, Y.P., V. Krishna and K.R. Maruthi, 2003. Rapid micropropagation of *Andrographis alata* through leaf culture. *Plant Cell Biotech. and Mole. Biol.*4: 117-124.
- Nagulendran, K.R., S. Velavan, R. Mahesh and V. Hazeena Begum, 2007. *In vitro* antioxidant activity and total polyphenolic content of *Cyperus rotundus* rhizomes. *E-Journal of Chemistry*. 4(3): 440-449.

## Formulation and Evaluation of Herbal Toothpaste from *Mimosa pudica* for Gingivitis

Keerthiga S and Shanmugavadi M,  
PG and Research Department of Biotechnology,  
Dr. N.G.P. Arts and Science College,  
Coimbatore, Tamil Nadu, India

### Abstract

Herbal treatments are becoming more and more popular worldwide. Due to its ability to prevent cavities and other dental issues, herbal toothpastes made of natural ingredients are currently seen as preferable to chemical, synthetic formulations when it comes to oral hygiene. The main concern is to determine whether herbal toothpastes might effectively cure dental caries, periodontal damage, bad breath, dry mouth, gum disease, and tartar. Eliminating any synthetic ingredients that are often present in toothpaste formulations is crucial for the development of stable and functionally successful toothpaste. Toothpaste containing herbs has drawn a lot of interest as a gingivitis reducer. While herbal toothpaste seems to be equally as effective as non-herbal toothpaste, it is no longer superior to toothpaste containing fluoride, such as sodium lauryl sulfate. For those looking to reduce their exposure to chemicals that may be harmful to their dental health as well as their general health, organic toothpaste is a wise and healthier option. *Mimosa pudica* has been used traditionally to cure a variety of conditions, including dental cavities, sinusitis, and dysentery. The Soxhlet apparatus was used to make the ethyl acetate extract of the plant. Studies on phytochemicals revealed the presence of steroids, alkaloids, phenols, flavonoids, and saponins. The existence of alkaloids was verified by thin-layer chromatography. When *Streptococcus mutans* was used to assess the sample's antibacterial activity, it revealed a zone of inhibition of 16 mm/100 µl. Unlike toothpaste made with chemical substances, the herbal toothpaste that is developed won't cause

any adverse effects. Thus, the present study revealed the potential of *Mimosa pudica* against gingivitis.

**Keywords:** *Mimosa pudica*, *Herbal Tooth paste*, *Streptococcus mutans*, *gingivitis*, *antibacterial activity*.

## Introduction

The use of any plant material for therapeutic or disease-treating purposes is the definition of herbal medicine. The World Health Organization (WHO) estimates that about 80% of people on Earth primarily get their medical care from natural medicinal substances. The usage of herbal medicine therapy dates back a very long way. Moreover, it was proposed that more than 35,000 plant species from various global human civilizations be employed in scientific studies. Strong antibacterial, antiviral, antifungal, and anticancer properties are possessed by several of them. For thousands of years, people have utilized medicinal plants to treat ailments, flavor and preserve food, halt the spread of disease, and handle other health-related concerns. Plants produce secondary metabolites, which are generally responsible for the biological characteristics of widely utilized plant species. Compounds originating from plants control microbial development in a range of settings (Hosseinzadeh, et al., 2015). Maintaining good dental health, including preventing cavities, gingivitis, periodontal disorders, and bad breath, is known as oral hygiene. Practicing proper dental hygiene can help prevent mouth infections. Mouth infections are caused by yeast and plaque-forming bacteria. Using herbal and herbal-based toothpaste is one of the most essential aspects of dental health care, and it has been used since ancient times (Ersoy et al., 2008).

A complex mixture of abrasives, surfactants, pH buffers, tartar-controlling compounds (such fluoride), humectants (to prevent dryness and enhance the pleasant mouth feel), and binders (to give them rigidity and form) goes into making toothpaste (Dange et al., 2014). The fluorides and surfactants that are used in the toothpaste are bad for health when used

continuously. Consequently, teeth pastes made of herbs are used to prevent this effect. Consumers favor herbal tooth paste because they choose natural sources over synthetic ones now days (Sheen *et al.*, 2001). Natural toothpastes are devoid of fluoride and triclosan. Natural ingredients like as extracts of lemon, eucalyptus, rosemary, chamomile, sage, and myrrh, as well as specific mineral salts like sodium chloride and fluoride, are often included in them.

*M. pudica* L., a prickly, creeping herb, either annual or perennial shrub belongs to the Fabaceae family. The intricate leaves of the plant droop and curl inward when touched, yet they soon unfold again. Furthermore, the plant can be easily exploited to meet consumer demands for interesting therapeutic properties without losing genetic diversity or going extinct—features that have deterred the mass production and consumption of many other natural products for medical purposes (Saraiva *et al.*, 2015).

Analgesics, anti-inflammatory, hypoglycemic, diuretic, astringent, antispasmodic, and blood-purifying are a few of *M. pudica*'s therapeutic qualities (Gilani AH and Atta-Ur Rahman, 2005). It has been used in the conventional medical system to treat tumors, alopecia, constipation, diarrhea, leprosy, dysentery, and insomnia in addition to a number of urogenital infections (Chatterjee and Prakash, 2000). Some studies suggest that *M. pudica* may have potential antidepressant and anxiolytic (anxiety-reducing) effects. The plant's compounds may interact with neurotransmitters in the brain, helping to alleviate symptoms of depression and anxiety.

*M. pudica* L. has the potential to be a useful natural mouthwash with antibacterial properties for medical caries prevention. To lessen the production of oral biofilm in people at high risk for caries, it can be combined with other ingredients to create an antibacterial dentifrice or prepared as an oral herbal mouth rinse. Hence the present study aims to develop a herbal tooth paste using *M. pudica* plant extract and also to evaluate its potential against gingivitis.

## Materials and Methods

### 1. Collection of *Mimosa pudica* and Authentication

The *M. pudica* plant was collected from Ambilikai, Dindugal district. The plant sample was identified and authenticated at Botanical Survey of India, Tamil Nadu Agricultural University (Ministry of Environment, Forest and Climate change), Coimbatore.

### 2. Processing and Extraction

The collected plant was washed thoroughly with running tap water and once in distilled water. Then it was allowed to shadow dry in room temperature for 3-4 days to reduce the moisture content and later dried in hot air oven at 60°C for 48 hours. The dried plant was powdered by using a mechanical blender. Store it in an airtight container for further analysis. The polar solvent used for the extraction process is ethyl acetate. About 20g of *Mimosa pudica* powder was weighed and mixed with 250ml of ethyl acetate. For the extraction of the plant sample Soxhlet apparatus was used. Extracted sample was stored at 4°C for further use. (Ranjeet Kumar *et al.*, 2013)

### 3. Phytochemical Analysis of extract

Qualitative phytochemical screening of *Mimosa pudica* ethyl acetate extract was carried out to identify the presence of alkaloids, flavonoids, proteins, glycosides, saponins, tannins, phenols, steroids, terpenoids, resins and carbohydrates. (Ranjeet Kumar *et al.*, 2013)

#### (i) Test for alkaloids [Wagner's Test]

2ml of crude extract was treated with 3-5 drops of Wagner's reagent and observed for the formation of reddish brown precipitate (or colouration) which shows the presence of alkaloids.

**(ii) Test for alkaloids [Dragendorff's Test]**

5ml of crude extract was treated with 2ml of HCl and 1ml Dragendorff's reagent. Appearance of red (or orange) precipitate indicates the presence of alkaloids.

**(iii) Test for flavonoids [Ammonium test]**

4ml of sample was treated with Few drops of 10% ammonium solution and heated for about 2mins. Appearance of fluorescence yellow colour indicates the presence of flavonoids.

**(iv) Test for flavonoids [Lead acetate test]**

2ml of crude extract was treated with few drops of 10% lead acetate solution. Formation of milky white precipitate indicates the presence of flavonoids.

**(v) Test for glycosides (Keller killani test)**

To 1 ml of extract, 2ml of glacial acetic acid containing one drop of 5% ferric chloride and 1 ml of concentrated sulphuric acid were added. Appearance of reddish brown colour at the junction of the two liquid layers indicates the presence of glycosides.

**(vi) Test for steroids**

2ml of crude extract was treated with 2ml of chloroform and 2ml of concentrated sulphuric acid. Formation of red or yellowish green shows the presence of steroids.

**(vii) Test for terpenoids**

2ml of crude extract was treated with 2ml of chloroform, 2ml of concentrated sulphuric acid and acetic anhydride then heated for 2 minutes. Development of reddish brown colour indicates the presence of terpenoids.

**(viii) Test for saponins [Foam test]**

1 ml of crude extract and 3 ml of distilled water was taken in test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

**(ix) Test for tannin [Ferric chloride test]**

1 ml of crude extract was mixed with ferric chloride solution. Development of dark green colour indicates the presence of tannins.

**(x) Test for resins**

2ml of crude extract was treated with 5ml of acetic anhydride solution and 1ml of concentrated sulphuric acid. Development of orange to yellow colour indicates the presence of resins.

**(xi) Test for carbohydrates [Benedict's test]**

0.5ml of crude extract was added with 0.5ml of Benedict's reagent and heated gently using water bath for 2minutes at 80°C. Formation of orange red precipitate indicates the presence of carbohydrates.

**(xii) Test for carbohydrates [Iodine test]**

2ml of crude extract was treated with 2ml of Iodine solution. Development of dark blue to purple colour indicates the presence of carbohydrates.

**(xiii) Test for phenol**

2ml of crude extract was mixed with drop of 5% ferric chloride solution. Formation of dark green colour indicates the presence of phenol.

**(xiv) Test for protein [Millon's test]**

1ml of crude extract was treated with 2ml of Millon's reagent. Formation of white precipitate which turns red when heating indicates the presence of protein.

**(xv) Test for protein [Ninhydrin test]**

1ml of crude extract treated with 2ml of 0.2% ninhydrin solution. Then while boiling it turns into violet colour which indicates the presence of protein.

## 5. Antibacterial Activity

The antimicrobial activity of *Mimosa pudica* ethyl acetate extract was tested against Gram positive oral infection causing organisms, *Streptococcus mutans* by using agar well diffusion method. Bacterial inoculum was grown in nutrient broth overnight. Muller Hinton Agar plates were prepared.

The test organism was swabbed on the Muller Hinton Agar plates and well was made by gel puncher. The extract was poured in the well at concentrations of 50 $\mu$ l, 100 $\mu$ l, Tetracycline (Positive control) and Ethyl acetate (Negative control) were also added into the separate plate and incubated at 37°C for 24 hours. The zone of inhibition was measured with a ruler after 24 hours incubation. (Chitra *et al.*, 2012)

## 6. Formulation of toothpaste

4ml of ethyl acetate extract of *Mimosa pudica* was mixed with all the other ingredients (Clove -2g, Cinnamon -2g, Orange peel - 2g, Honey - 5 drops, Coconut oil - 2 drops, Calcium carbonate - 5g) to clean mortar for mixing until it becomes paste consistency. Preparation of the toothpaste should be carried out under a clean and sterilized surface to avoid future contamination. The toothpaste was transferred to the respective tube. (Rathi *et al.*, 2022).

## 7. Physical Examinations of Toothpaste (Oluwasina *et al.*, 2023)

### Color:

Formulated toothpaste was evaluated for its color. By visually color was checked.

### Odour and taste:

Odour was found by smelling the toothpaste. Taste was checked manually by testing the formulation.

### Smoothness:

The smoothness was tested by rubbing the paste formulation between the fingers.

### pH:

pH of the formulated herbal toothpaste was determined by using a pH meter.

### Sharp and edge abrasive particle testing:

Extrude the content for 10-15 cm long on the butter paper, then the samples were tested by pressing it along its entire length by a finger for the presence of hard and sharp-edged abrasive particles.

### Moisture content:

5g of toothpaste was heated in an oven at 105°C for 6 hours. Moisture content was calculated between the initial and final weights.

Moisture content = Original sample weight - dry sample weight/original sample weight × 100.

### Determination of Spread ability:

1 g of sample was placed at the center of the glass plate and another glass plate was placed over it and 2kg weight was placed at the center of the plate to avoid sliding of the plate. Diameter of

the paste in cm was measured after 30 minutes. Repeated 3 times and average were reported.

## Foamability

The foamability of formulated toothpaste was evaluated by taking a small amount of formulation with water in measuring cylinder. The initial volume was noted and then shaken for 10 times. Final volume of foam was noted.

- Foaming power = VI-V2
- VI- Volume in ml of foam with water
- V2-Volume in ml of water only

### 1. Antibacterial activity of the toothpaste

Antibacterial activity of toothpaste was done using Muller Hinton agar against *S. mutans*. The well diffusion method was used to determine the antibacterial activity of the toothpaste. 1g of toothpaste was dissolved in 1ml of ethyl acetate solution. 100 $\mu$ l of sample was poured in the well along with positive control (tetracycline) and negative control (ethyl acetate). The plates were incubated at 37°C for 24 hours. (Senthilkumar *et al.*, 2022).

## Result and Discussion

### 1. Collection and Identification of Plant Sample

The *Mimosa pudica* leaves were collected from Ambilikai, Dindugul, Tamil Nadu and authenticated by the Botanical Survey of India (BSI) in "Tamil Nadu Agriculture University" Coimbatore and India.

A voucher specimen has been deposited at the Herbarium in the Botany Department of Tamil Nadu Agriculture University for further reference.

Name of species	Class	Order	Family	Genus	Identification
<i>Pudica</i>	<i>Magnoliopsida</i>	Fabales	Fabaceae	<i>Mimosa</i>	BSI/SRC/5/23/2023-24/Tech

**Table 1: Identification of plant****Figure 1: *Mimosa pudica***

## 2. Extraction of Plant Sample:

Shade dried plant sample was extracted with ethyl acetate using the Soxhlet apparatus which was maintained at the boiling point of 70°C. The plant extract was stored at 4°C for further use.

## 3. Phytochemical analysis of plant extract:

Preliminary phytochemical analysis of *Mimosa pudica* ethyl acetate extract showed the presence of alkaloids, tannins, terpenoids, phenols, protein, resins, saponin and steroids whereas Flavonoids, glycosides and carbohydrate were found to be absent. Ethanol extract of the plant also showed the presence of tannins, protein and steroids (Ranjeet *et al.*, 2013). Tamilarasi and Ananthi (2012), also reported the presence of alkaloids, glycosides, protein, steroids, flavonoids and phenols in the ethanol extract. The methanol extract of the plant showed the presence of all the mentioned phytochemicals except saponins and cardiac glycosides whereas water extract showed the presence of steroids,

phenols, glycosides and terpenoids (Chitra et al., 2012). Hence, these phytochemicals are responsible for the therapeutic activity of the *Mimosa pudica*.

**Table 2:** Phytochemical analysis of *Mimosa pudica* extract

S.No	Test	Results
1.	Protein	+
2.	Carbohydrates	-
3.	Alkaloids	+
4.	Phenols	+
5.	Tannins	+
6.	Flavonoids	-
7.	Saponins	+
8.	Glycosides	-
9.	Steroids	+
10.	Terepnoids	+
11.	Resin	+

#### 4. Antibacterial Activity of *Mimosa pudica* ethyl acetate extract

The plant extracts prepared from ethyl acetate solvents were assessed for their antibacterial activity using agar well diffusion method. The ethyl acetate extract of *Mimosa pudica* showed the maximum zone of inhibition of 12 mm at 100µl concentration against oral infection causing microorganism *S. mutans* whereas the positive control tetracycline showed 20mm zone of inhibition. Similarly, 100µl ethanolic extract showed good antibacterial activity against *Bacillus subtilis* (20mm), *Pseudomonas aeruginosa* (17mm) and *Klebsiella pneumonia* (17mm) (Tamilarasi and Ananthi,

2012). Chitra *et al.*, (2012) reported that the methanolic extract of *Mimosa pudica* showed good antibacterial activity against *Bacillus subtilis* (10.5mm), *Staphylococcus aureus* (11mm), *Klebsiella pneumoniae* (11.5mm).

**Table 3: Antibacterial activity of *Mimosa pudica ethyl acetate* extract against *S.mutans***

Volume of the sample	Zone of inhibition
100µl	12mm
50µl	10mm
Positive control ( tetracycline)	20mm



**Figure 2: Antibacterial activity of *Mimosa pudica* extract against *S.mutans***

## 5. Formulation of Toothpaste:

All the ingredients in the prescribed measurement were mixed together until it reaches as a paste. Mainly the *Mimosa pudica* extract is used as a gargle to treat oral cavity, dental plaque, bad breath, gingivitis, bleeding gums, inflammation in the mouth and toothache. Cinnamon is used to reduce infections and help to fight tooth decay and bad breath. Clove reduces pain to care for a toothache. It may reduce swelling and irritation in the affected

area. Calcium carbonate is an abrasive and binding agent. Honey is used as a sweetening agent and it also acts a barrier, contributing to the prevention of infections in the mouth. Coconut oil acts as a foaming agent in the toothpaste. Weight all the ingredients and into a clean mortar for mixing until it becomes paste consistency.



**Figure 3: Formulation of herbal toothpaste**

## **6. Physical examination of Toothpaste**

Organoleptic evaluation was done by sensory and visual inspection of appearance, color, odor, taste, texture, pH, hard & sharp-edged abrasive particles, moisture content, spread ability, and foam ability. The herbal toothpaste prepared using aloe Vera gel, clove oil, Neem powder, pomegranate peel powder also showed the all the desirable characteristics which is suitable for the herbal toothpaste (Senthilkumar *et al.*, 2022). The herbal toothpaste incorporated with *Mimosa pudica* ethyl acetate extract contains neutral pH, enough moisture content, good foaming and spreading ability. So it can be used as a good alternative for the chemical pastes in market.

**Table 4:** Organoleptic and physiochemical examination of herbal toothpaste

Parameter	Results
Appearance	Semisolid
Color	Beige color
Odor	Characteristic
Taste	Sour
Texture	Smooth
PH	7.2
Hard-&sharp-edged particles	Absent
Moisture content	4.8
Spread ability	Easily spreadable
Foam ability	Good

## 7. Antibacterial Activity of Herbal Toothpaste

100 $\mu$ l of the herbal toothpaste mix showed a potent antibacterial activity of 16mm against the test organism *Streptococcus mutans* which is close to the positive control tetracycline (20mm). Toothpastes were made using the extracts of *Allium cepa* L skin chaff, *Azadirachta indica* A. seed, and *Tetrapleura tetraptera* pod extracts. Among all, *Allium cepa*-based toothpaste has the best antimicrobial activities against the tested bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*) ( Oluwasina *et al.*, 2023). Toothpaste prepared using aloe Vera gel, clove oil, Neem powder, pomegranate peel powder also showed good antibacterial activity against *S. aureus* (Senthilkumar *et al.*, 2022).

**Table 5:** Antibacterial activity of toothpaste *S.mutans*

Volume of sample	Zone of inhibition in mm
100µl	16 mm
Positive (tetracycline)	20 mm

**Figure 4:** Antibacterial activity of herbal toothpaste

## Conclusion

The oral cavity is the principal pathway through which the body is exposed to the external environment and considered to be the gateway of infections affecting teeth, gum, mucosa and tongue. The present study was conducted to determine the ability of *M. pudica* against oral infection causing pathogens. Most of the biologically active phytochemicals were present in the ethyl acetate extract of *M. pudica*. *M. pudica* ethyl acetate extract was tested against *S. mutans* which showed 11mm zone of inhibition. The herbal toothpaste prepared using this extract showed a pH of 7.2, good foaming, acceptable taste, odour and good spreading ability. This herbal toothpaste also inhibited the growth of *S. mutans*. It is a good attempt to establish such herbal toothpaste containing orange peel extract, Cinnamon and Clove extract which helps in reducing bacterial growth in the mouth. Further studies are warranted to improve the stability of the formulation, prove the efficacy and safety of the formulated toothpaste and to improve the antimicrobial activity of the toothpaste.

## Reference

- Chatterjee A, Prakash SC. The Treatise of Indian Medicinal Plants. CSIR, New Delhi: Publications and Information Directorate. 2000; 2: 65–66.
- Chitra G, Athira KA and Anitha CT. Phytochemical Screening and Antibacterial Activity of *Mimosa pudica* L. and *Mimosa invisa* L. Against Selected Microbes. Nat. Env. & Poll. Tech. 2012;11(3): 431-433.
- Dange VN., Magdum CS., Mohite SK., Nitalikar MM., Shid SJ. International Journal of Universal Pharmacy and Bio Sciences. 2014; 3 (6): 68-79.
- Ersoy M, Tanalp J, Ozel E, Cengizlier R and SoymanM. The allergy of toothpaste, a case report. Allergollmmunopathol. 2008;36: 368-370.
- Gilani AH, Atta-Ur Rahman. Trends in ethnopharmacology. J Ethnopharmacol. 2005; 100:43–9
- Hosseinzadeh, S., Jafarikukhdan, A., Hosseini, A. and Armand, R. The application of Medicinal Plants in Traditional and Modern Medicine: A Review of *Thymus vulgaris*. International Journal of Clinical Medicine. 2015; 6(9): 635-642.
- Nikita M. Rathi, Shital V. Sirsat, Sanket S. Toshniwal, Nikita T. Zagare, Shaikh Fazil Shaikh Mahamad. Formulation and Evaluation Study on Herbal Toothpaste : A Review. International Journal of Novel Research and Development. 2022;7(4):968-974.
- Olugbenga Oludayo Oluwasina, Suleiman Oladokun Idris, Clement Olusola Ogidi, Festus O. Igbe. Production of herbal toothpaste: Physical, organoleptic, phyto-compound, and antimicrobial properties. Heliyon. 2023;9: e13892.
- Ranjeet Kumar Ranjan, Sathish Kumar M, Seethalakshmi I and Rao MRK. Phytochemical analysis of leaves and roots of *Mimosa pudica* collected from Kalingavaram, Tamil Nadu. J. Chem. Pharm. Res. 2013; 5(5):53-55.
- Saraiva ME, de Alencar-Ulisses AVR, Ribero DA, de Olivera LGS, de Macedo DG, de Sousa FFS, de Menezes IRA, Sampaio

EVSB, Souza MMA. Plant species as a therapeutic resource in areas of the savanna in the state of Pernambuco, Northeast Brazil. *J Ethnopharm.* 2015; 171: 141153.

- Senthilkumar KL, Venkateswaran S, Vasanthan A, Chiranjeevi P, Mohamed N, Dinesh S, Neshkumar KLS. Formulation development and evaluation of novel herbal toothpaste from natural source. *International Journal of Pharmaceutical Chemistry and Analysis* 2022;9(1):17-21.
- Sheen S., Pontefract H., Moran J. The benefits of toothpaste-real and imagined the effectiveness of toothpaste in control of plaque, gingivitis, periodontitis, calculus and oral malodour. *Dent Update.* 2001; 28(3):144-147.
- Tamilarasi T and Ananthi T. Phytochemical Analysis and Anti Microbial Activity of *Mimosa pudica* Linn. *Research Journal of Chemical Sciences.* 2012; 2(2), 72-74.

## Unveiling the Comprehensive Role of $\beta$ - Carotene in Human Health

**Dencili Verginiya L<sup>1</sup> and Dr. Jancy Rani D<sup>2</sup> and Dr. Sridevi D<sup>3</sup>**

<sup>1</sup>M.Sc. Food and Nutrition, Dept of Food Science and Nutrition,  
Dr. N.G.P Arts and Science College, Coimbatore.

Email: dencilidencili11@gmail.com

<sup>2</sup>Assistant Professor, Dept of Food Science and Nutrition,  
Dr. N.G.P Arts and Science College, Coimbatore.

Email: jancyranifsn@gmail.com

<sup>3</sup>Professor and Head, Dept of Food Science and Nutrition,  
Dr. N.G.P Arts and Science College, Coimbatore.

Email: drsridevi@drngpasc.ac.in

### Introduction

$\beta$ -Carotene, a key carotenoid with provitamin A activity, is crucial for human health due to its role as a precursor to vitamin A. Found abundantly in colorful fruits and vegetables such as carrots, sweet potatoes, and leafy greens,  $\beta$ -carotene contributes significantly to dietary intake and overall nutritional status (Wang *et al.*, 2021). The importance of  $\beta$ -carotene is underscored by its involvement in essential physiological functions, including vision, immune response, and skin health (Tang, 2010).

$\beta$ -Carotene, a naturally occurring carotenoid found in many fruits and vegetables, has garnered significant attention for its potential role in cancer prevention and therapy. As a precursor to vitamin A,  $\beta$ -carotene is essential for various physiological processes, including immune function and cellular differentiation (Wang *et al.*, 2021). Its potent antioxidant properties have been extensively studied for their ability to neutralize free radicals, thus reducing oxidative stress and potentially lowering the risk of cancer (Bohn *et al.*, 2022).

Beyond its function as a vitamin A precursor,  $\beta$ -carotene possesses potent antioxidant properties, which have been extensively studied for their potential to protect cells from oxidative damage and reduce the risk of chronic diseases. Recent studies have highlighted the role of  $\beta$ -carotene in lowering the incidence of certain cancers, cardiovascular diseases, and neurodegenerative disorders (Bohn *et al.*, 2022). However, the efficacy and safety of  $\beta$ -carotene supplementation remain controversial, with some large-scale studies indicating limited benefits and potential risks associated with high-dose supplements (Tanaka *et al.*, 2020).

This review aims to provide a comprehensive overview of the current understanding of  $\beta$ -carotene's role in cancer prevention and treatment. By synthesizing the latest research findings, this review seeks to clarify the impact of  $\beta$ -carotene on cancer and identify directions for future research.

## Physical Properties

**Molecular Structure and Composition:**  $\beta$ -Carotene ( $C_{40}H_{56}$ ) is a highly unsaturated hydrocarbon consisting of 40 carbon atoms and 56 hydrogen atoms. It belongs to the class of carotenoids known as tetraterpenes. The molecule is composed of eight isoprene units, forming a linear chain with conjugated double bonds, which are responsible for its light-absorbing properties and antioxidant activity (Britton, 2020).

## Solubility and Stability:

$\beta$ -Carotene is lipophilic and insoluble in water but soluble in organic solvents such as ethanol, acetone, and chloroform. This solubility characteristic is crucial for its bioavailability and application in food and pharmaceutical formulations.  $\beta$ -Carotene is sensitive to light, oxygen, and heat, which can cause degradation and loss of activity. Therefore, its stability is a major concern in its extraction, processing, and storage (Nishino *et al.*, 2017).

## Chemical Properties

**Antioxidant Activity:** One of the most significant chemical properties of  $\beta$ -carotene is its antioxidant activity. The molecule can quench singlet oxygen and neutralize free radicals, thus protecting cells and tissues from oxidative damage. This antioxidant activity is attributed to the extensive conjugated double bond system, which allows  $\beta$ -carotene to donate electrons and stabilize reactive species (Stahl & Sies, 2022).

**Pro-vitamin A Activity:**  $\beta$ -Carotene is a precursor to vitamin A (retinol) in the human body. It is cleaved by the enzyme  $\beta$ -carotene 15,15'-monooxygenase to produce two molecules of retinal, which can be further reduced to retinol or oxidized to retinoic acid, an important regulator of gene expression. This conversion is essential for maintaining vision, immune function, and skin health (Wang *et al.*, 2021).

**Chemical Reactivity and Degradation:** The chemical reactivity of  $\beta$ -carotene is influenced by factors such as light, heat, and oxygen. Exposure to these elements can lead to isomerization and oxidative degradation, resulting in loss of color and nutritional value. The main degradation products include epoxides, apocarotenals, and other oxidized derivatives. These reactions are a concern during the processing and storage of  $\beta$ -carotene-rich foods and supplements (Rodriguez-Amaya, 2016).

## Distribution in Plant Tissues

$\beta$ -Carotene is widely distributed in fruits and vegetables, often imparting orange, yellow, and red hues. Carrots (*Daucus carota*) are one of the richest sources, with concentrations ranging from 4,000 to 6,000  $\mu\text{g}/100 \text{ g}$ . Sweet potatoes (*Ipomoea batatas*) and pumpkins (*Cucurbita spp.*) also contain high levels, making them significant contributors to dietary  $\beta$ -carotene intake. Leafy greens such as spinach (*Spinacia oleracea*) and kale (*Brassica oleracea*) contain  $\beta$ -carotene along with other carotenoids, although their

green chlorophyll masks the carotenoid pigments (Khoo *et al.*, 2019).

In addition to edible parts,  $\beta$ -carotene is also present in flowers and seeds. Marigold flowers (*Tagetes spp.*) are known for their high  $\beta$ -carotene content, which is utilized in natural colorant production. Certain seeds, such as those of red palm oil (*Elaeis guineensis*), contain significant amounts of  $\beta$ -carotene, contributing to the orange-red color of the oil. The distribution in flowers and seeds is less explored but represents an important area for natural pigment extraction (Rodriguez-Amaya, 2016).

## Breast Cancer

Breast cancer remains one of the most prevalent and deadly cancers among women worldwide. Despite advances in treatment, there is a continuous search for novel therapeutic strategies that can improve patient outcomes and reduce side effects. Recent studies have explored the potential of  $\beta$ -carotene as a complementary treatment for breast cancer, examining its ability to modulate oxidative stress, influence cellular signaling pathways, and enhance the efficacy of conventional therapies (Dimitrov *et al.*, 2021).

## Lung Cancer

Initial epidemiological studies indicated an inverse relationship between  $\beta$ -carotene intake and lung cancer risk. Populations consuming diets rich in fruits and vegetables, and hence higher levels of  $\beta$ -carotene, appeared to have lower incidences of lung cancer (Michaud *et al.*, 2000). These findings suggested that  $\beta$ -carotene's antioxidant properties might protect against the oxidative damage implicated in lung carcinogenesis.

In the CARET study, high-risk individuals, including smokers and asbestos-exposed workers, received  $\beta$ -carotene and retinol supplements. Surprisingly, the trial was halted prematurely due to an observed increase in lung cancer incidence and mortality among the supplemented group compared to the placebo group

(Omnenn *et al.*, 1996). Similarly, the ATBC study, conducted among male smokers in Finland, reported an increased incidence of lung cancer and mortality in participants receiving  $\beta$ -carotene supplements (Albanes *et al.*, 1996). These unexpected results prompted a reevaluation of  $\beta$ -carotene's role in lung cancer.

A Research has explored the complex relationship between  $\beta$ -carotene and cancer, with mixed findings. While some epidemiological studies suggest an inverse association between  $\beta$ -carotene intake and the incidence of certain cancers, particularly lung and skin cancers, clinical trials have produced inconsistent results (Tanaka *et al.*, 2020). High-dose  $\beta$ -carotene supplementation, especially in smokers, has been associated with an increased risk of lung cancer, highlighting the need for a nuanced understanding of its effects (Omnenn *et al.*, 1996).

## Skin Cancer

$\beta$ -Carotene also modulates immune responses, enhancing the skin's ability to repair and regenerate after UV exposure. It can stimulate the production of immune cells, such as T-lymphocytes and natural killer cells, which play a crucial role in identifying and eliminating cancerous and pre-cancerous cells. This immunomodulatory effect further contributes to the prevention of skin cancer development (Moeinvaziri *et al.*, 2021).

Some epidemiological studies indicate that higher dietary intake of  $\beta$ -carotene is associated with a reduced risk of skin cancer, particularly non-melanoma skin cancers (NMSC) such as basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). For instance, a cohort study by Wang *et al.* (2021) found that individuals with higher  $\beta$ -carotene intake had a lower incidence of BCC and SCC. These findings support the hypothesis that  $\beta$ -carotene's antioxidant and immunomodulatory properties may contribute to skin cancer prevention.

Vitamin A derived from  $\beta$ -carotene is involved in the regulation of cell differentiation and proliferation. Retinoic acid,

an active metabolite of vitamin A, binds to nuclear receptors that control the expression of genes involved in cell growth and differentiation. This regulation is vital for maintaining normal skin cell turnover and preventing the uncontrolled proliferation characteristic of cancer (Gudas, 2012).

Combining  $\beta$ -carotene with other antioxidants, such as vitamins C and E, may enhance its protective effects against oxidative stress and skin damage. These combinations are being explored in both dietary and topical formulations to provide comprehensive protection against UV-induced skin damage (Offord *et al.*, 2002).

## Cognitive Function

$\beta$ -Carotene's primary mechanism in supporting cognitive function is its antioxidant activity. The brain is particularly vulnerable to oxidative stress due to its high oxygen consumption and lipid-rich environment. Free radicals and reactive oxygen species (ROS) can damage neuronal cells, leading to cognitive decline and neurodegenerative diseases such as Alzheimer's and Parkinson's.  $\beta$ -Carotene can neutralize these free radicals, reducing oxidative damage and potentially slowing the progression of cognitive impairment (Huang *et al.*, 2021).

Beyond its antioxidant and anti-inflammatory effects,  $\beta$ -Carotene may directly influence brain function through its conversion to vitamin A. Retinoic acid, the active form of vitamin A, plays a crucial role in neurogenesis, synaptic plasticity, and neurotransmitter synthesis. These processes are essential for learning, memory, and overall cognitive function.  $\beta$ -Carotene's contribution to maintaining adequate vitamin A levels may therefore support cognitive health (Tanprasertsuk *et al.*, 2022).

Several observational studies have explored the relationship between dietary  $\beta$ -carotene intake and cognitive function. The results generally support a protective role for  $\beta$ -carotene. For example, a study by Yuan *et al.* (2020) involving

older adults found that higher dietary intake of  $\beta$ -carotene was associated with better cognitive performance and a lower risk of cognitive decline. These findings suggest that  $\beta$ -carotene, through its antioxidant and neuroprotective properties, may help maintain cognitive function as individuals age.

Longitudinal studies have also provided valuable insights. The Nurses' Health Study, which followed a large cohort of women over several decades, reported that higher  $\beta$ -carotene intake was associated with slower rates of cognitive decline. This association remained significant even after adjusting for other dietary factors and lifestyle variables, indicating a potential independent effect of  $\beta$ -carotene on cognitive health (Devore *et al.*, 2010).

## Skin Health

One of the primary ways  $\beta$ -carotene benefits skin health is through its antioxidant properties, which help neutralize free radicals generated by ultraviolet (UV) radiation. UV radiation is a major factor in skin aging and carcinogenesis, as it induces the production of reactive oxygen species (ROS) that can damage DNA, proteins, and lipids in skin cells (Stahl & Sies, 2012).  $\beta$ -Carotene's ability to quench singlet oxygen and other ROS helps reduce oxidative stress and protect skin cells from UV-induced damage.

A study by Grether-Beck *et al.* (2017) demonstrated that supplementation with  $\beta$ -carotene significantly increased skin's resistance to UV-induced erythema, commonly known as sunburn. This protective effect was attributed to the accumulation of  $\beta$ -carotene in the skin, which enhances its antioxidant capacity and reduces the extent of UV-induced oxidative damage.

The oxidative stress from UV radiation also accelerates the skin aging process, leading to wrinkles, loss of elasticity, and uneven pigmentation. By mitigating oxidative damage,  $\beta$ -carotene can slow down the aging process and maintain youthful skin. A

review by Wang *et al.* (2020) highlighted that regular consumption of β-carotene-rich foods or supplements could improve skin texture and reduce the appearance of fine lines and wrinkles, promoting overall skin health.

β-Carotene also plays a crucial role in enhancing skin barrier function, which is essential for protecting against pathogens, toxins, and excessive water loss. Vitamin A, derived from β-carotene, regulates the growth and differentiation of keratinocytes, the primary cells in the epidermis. This regulation is vital for maintaining a healthy and functional skin barrier (Moeinvaziri *et al.*, 2021).

## Cataract Prevention

Cataracts, characterized by the clouding of the eye's lens, are another major cause of visual impairment. Observational studies have shown an inverse relationship between dietary β-carotene intake and the risk of cataract formation. A study by Christen *et al.* (2014) found that higher dietary intake of β-carotene was associated with a significantly lower risk of developing cataracts in a large cohort of women. This protective effect is likely due to β-carotene's antioxidant properties, which help prevent oxidative damage to the lens proteins.

Vitamin A, derived from β-carotene, is crucial for the visual cycle. It combines with opsin to form rhodopsin, a pigment in the photoreceptor cells of the retina that is essential for vision in low-light conditions. Rhodopsin undergoes a cycle of bleaching and regeneration in response to light, and adequate vitamin A levels are necessary to maintain this cycle. β-Carotene supplementation can help ensure sufficient vitamin A availability, thereby supporting optimal visual function (Johnson *et al.*, 2022).

Increasing the dietary intake of β-carotene through foods such as carrots, sweet potatoes, spinach, and kale is a practical and effective strategy for improving eye health. These foods provide not only β-carotene but also other essential nutrients that work

synergistically to promote eye health. A diet rich in these colorful fruits and vegetables can help maintain optimal vision and reduce the risk of eye diseases (Wang *et al.*, 2020).

## References

- Albane, D., Heinonen, O. P., Taylor, P. R., Virtamo, J., Edwards, B. K., Rautalahti, M., & Huttunen, J. K. (1996). Alpha-Tocopherol and beta-carotene supplements and lung cancer incidence in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study: effects of base-line characteristics and study compliance. *Journal of the National Cancer Institute*, 88(21), 1560-1570.
- Bohn, T., Desmarchelier, C., Dragsted, L. O., Nielsen, C. S., Stahl, W., Rühl, R., & Keijer, J. (2022). Host-microbe interactions and modulation of human metabolism and health by carotenoids: insights from mechanistic studies and intervention trials. *Trends in Food Science & Technology*, 120, 15-26.
- Britton, G. (2020). Carotenoids: Volume 4: Natural Functions. Birkhäuser.
- Christen, W. G., Liu, S., Schaumberg, D. A., & Buring, J. E. (2014). Dietary carotenoids, vitamins C and E, and risk of cataract in women: a prospective study. *Archives of Ophthalmology*, 126(1), 102-109.
- Devore, E. E., Kang, J. H., Breteler, M. M., & Grodstein, F. (2010). Dietary intakes of berries and flavonoids in relation to cognitive decline. *Annals of Neurology*, 69(6), 939-949.
- Dimitrov, N. V., Kandaraki, C., & Oganesian, N. (2021).  $\beta$ -Carotene and breast cancer: From prevention to treatment. *Antioxidants*, 10(8), 1212.
- Grether-Beck, S., Marini, A., Jaenicke, T., & Krutmann, J. (2017). Photoprotection of  $\beta$ -carotene: A review on molecular mechanisms and clinical evidence. *International Journal of Vitamin and Nutrition Research*, 87(1-2), 2-14.
- Gudas, L. J. (2012). Emerging roles for retinoids in regeneration and differentiation in normal and disease states.

Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids, 1821(1), 213-221.

- Huang, Y., Li, W., He, Z., Wu, M., Li, L., Zhu, X., ... & Zhang, H. (2021). Dietary β-carotene intake and risk of cognitive decline: A meta-analysis of prospective studies. *Journal of Alzheimer's Disease*, 79(3), 1113-1123.
- Johnson, E. J., Vishwanathan, R., Rasmussen, H. M., & Barua, A. B. (2022). The importance of carotenoids for ocular health and the role of biomarkers. *Nutrients*, 14(4), 878.
- Khoo, H. E., Prasad, K. N., Kong, K. W., Jiang, Y., & Ismail, A. (2019). Carotenoids and their isomers: Color pigments in fruits and vegetables. *Molecules*, 24(23), 4329.
- Michaud, D. S., Feskanich, D., Rimm, E. B., Colditz, G. A., Speizer, F. E., Willett, W. C., & Giovannucci, E. L. (2000). Intake of specific carotenoids and risk of lung cancer in 2 prospective US cohorts. *The American Journal of Clinical Nutrition*, 72(4), 990-997.
- Moeinvaziri, M., Farahi, A., & Darvishi, M. (2021). Beta-carotene: A review on its potential effects on immune cells in inflammatory conditions. *Journal of Cellular Physiology*, 236(8), 5805-5821.
- Nishino, H., Murakoshi, M., & Satomi, Y. (2017). Cancer prevention by carotenoids. In *Carotenoids: Volume 5: Nutrition and Health* (pp. 123-134). Birkhäuser.
- Offord, E. A., Gautier, J. C., Avanti, O., & Scaletta, C. (2002). Photoprotective potential of lycopene, beta-carotene, vitamin E, vitamin C, and carnosic acid in UVA-irradiated human skin fibroblasts. *Free Radical Biology and Medicine*, 32(12), 1293-1303.
- Omenn, G. S., Goodman, G. E., Thornquist, M. D., Balmes, J., Cullen, M. R., Glass, A., & Barnhart, S. (1996). Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. *New England Journal of Medicine*, 334(18), 1150-1155.
- Rodriguez-Amaya, D. B. (2016). Natural food pigments and colorants. *Current Opinion in Food Science*, 7, 20-26.

- Stahl, W., & Sies, H. (2022). Bioactivity and protective effects of natural carotenoids. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, 1867(11), 166108.
- Tanaka, T., Shnimizu, M., & Moriwaki, H. (2020). Cancer chemoprevention by carotenoids. *Molecules*, 17(3), 3202-3242.
- Tang, G. (2010). Bioconversion of dietary provitamin A carotenoids to vitamin A in humans. *The American Journal of Clinical Nutrition*, 91(5), 1468S-1473S.
- Tanprasertsuk, J., Scott, T. M., & Johnson, E. J. (2022). Vitamin A and cognitive function. *Advances in Nutrition*, 13(4), 849-866.
- Wang, X. D., Tang, G., Fox, J. G., & Krinsky, N. I. (2021). Enzymatic conversion of  $\beta$ -carotene into vitamin A: biochemistry, molecular biology, and mechanisms. *Progress in Lipid Research*, 84, 101126.
- Yuan, C., Chen, H., Wang, H., & Yang, Y. (2020). Higher dietary carotenoid intake associated with better cognitive performance in middle-aged and older adults: A meta-analysis of observational studies. *Nutrients*, 12(7), 2090.

## An Overview of Bioactive Phytochemicals in *Hordeum vulgare* (Barley)

Jenisha. W, Sathya Devi. V, Yogeetha. G,  
Sasikala C & Vidhya N

Department of Microbiology,  
Dr.N.G.P Arts and Science College,  
Coimbatore. Tamil Nadu, India

### Abstract

*Hordeum vulgare L.*, or barley, is one of the oldest cereal crops in the world. The potential use of barley in human diets is of great interest. Barley possesses a high concentration of  $\beta$ -glucan, fibre, and vitamin E, among other beneficial metabolites. It is also widely recognized for being an abundant source of phytochemical derivatives, including proanthocyanidins, chalcones, flavones, phenolic acids, and flavanols. Phenolic substances have antioxidant and anti-inflammatory properties, making them valuable dietary components. Thus, barley's contain high phytochemical content may be primarily responsible for its health advantages. In this review paper available information regarding barley phytochemicals and their Isolation, characterization, synthesis and their ability to treat major nutrition-related disorders such as cancer, cardiovascular disease and diabetes.

### 1.0 Introduction

One of the most important food grains has been barley (*Hordeum vulgare*, family Poaceae) since ancient times. With a global cultivation area of nearly 70 million hectares, it ranks fourth among cereals in terms of output volume(Badr *et al.*,2000) Barley can be categorized as a two-row, six-row, spring or winter crop that is hulless or hulled. Barley can also be categorized as normal, waxy, or high amylose starch, high lysine, high  $\beta$ -glucan, or proantho-cyanidin-free (Schulte *et al.*,2009) based on the makeup

of the grain. Because it includes more proteins, lipids, and soluble dietary fiber, barley without the hull has a higher nutritional value than barley with the hull. Different varieties of barley have unique physical and chemical properties that impact how they process and how well they work in the end. About 65% of produced barley is used for animal feed, 33% for malting, and only 2% for direct human consumption (Sullivan *et al.*, 2013). Global barley cultivation in 2014 exceeded 48 million hectares, with a harvest of 144 million metric tons the United States produced 3.8 million tons on 988,660 hectares of land (Shewry *et al.*, 2014). Regular barley consumption may lower the chance of developing some diseases, including chronic heart disease (Sullivan *et al.*, 2013; Annapurna *et al.*, 2011 and Bayset *et al.*, 2011), colon cancer (Dongowski *et al.*, 2002; Finn *et al.*, 2008), high blood pressure (Behallet *et al.*, 2006), and gallstones (Hoang *et al.*, 2011; Zhang *et al.*, 1990), as reported by epidemiological research. It has been demonstrated that barley helps to maintain a healthy colon (Kanauchi *et al.*, 1999), stimulate the immune system (Tada *et al.*, 2009) and overall strengthen the immune system (Kemp *et al.*, 2014).

The natural existence of the bioactive components of vitamins, minerals, fibre, and other phytochemicals is thought to have these medicinal potentials. Interestingly, among the myriads of bioactive substances present in barley, fibre component, especially  $\beta$ -glucan fibre, is mainly credited for barley's health benefits (Baik *et al.*, 2008; Thondreet *et al.*, 2011 & Agostini *et al.*, 2015). Additionally, phytochemicals are crucial role in preventing the developing the chronic disease (Seo *et al.*, 2015). The primary phytochemicals in barley that have been associated with health benefits are lignans, phenolic acids, flavonoids, sterols, vitamin E (tocols), and folates (Malik *et al.*, 2012).

In addition to serving as defensive mechanisms against infections, parasites, and predators the phenolic compound play a vital role and also contribute to the color of plants (Lattanzio *et al.*, 2006) Sterols and tocots are mainly components of plant oils that provide benefits such as protection against toxins,

neurological diseases like Alzheimer's disease, and diabetes (Bartłomiej *et al.*, 2012; Shahidi *et al.*, 2010). When it comes to the quantity and variety of phytochemicals it contains, barley holds its own against other common cereal grains like wheat, oats, rye, and rice. . In addition, barley has some unique phytochemical properties, such as the presence of all eight tocol vitamins, which are usually not complete in some cereals (Panfili *et al.*, 2003). We summarized recent findings about the phytochemical components of barley and their ability to modulate parameters related to human health, analysed the ideas and limitations of recent studies, and suggested directions for future research because of the high potential benefits of phytochemicals on human healthanalysed the limitations and ideas of recent studies, and proposed directions for future research.

## 2.0 Phytochemicals in Barley

A variety of phytochemicals, or non-nutrient components, are present in barley in variable amounts that are often influenced by environmental or genotypic factors, or by the combination of the two factors (Panfili *et al.*, 2003). Phytochemicals in barley may exist in free, conjugated, or bound forms and are categorized into several major classes, including phenolic acids, flavonoids, lignans, tocols, phytosterols, and folates (Fogarasi *et al.*, 2015).

### 2.1. Flavonoids

It is an phytochemical compounds with a C<sub>6</sub>-C<sub>3</sub>-C<sub>6</sub> skeleton (2 aromatic rings joined by a 3-carbon link). They may also be UV-B absorbing substances (Tossiet *et al.*, 2012], which offer defence against UV rays in reaction to stress caused by "excess light" (Agati *et al.*, 2011), (Bashandy *et al.*, 2009). Based on clinical research, flavonoids may be the bioactive ingredient in cereal grains that helps to moderate a number of disorders, such as cancer and heart disease (Tang *et al.*, 2016), (Gani *et al.*, 2012).In barley grains, the main forms of flavonoids are flavanols, anthocyanins, and proanthocyanidins (polymers of flavonoids).The pericarp of barley

grains contains flavanols and anthocyanins, which are primarily found as glycoside derivatives such as cyanidin-3-glucoside, penidin-3-glucoside, and delphinidin-3-glucoside, (Abdel-Aal *et al.*, 2006). According to research, blue and purple barley grains have the highest flavonoid content among all barley kinds. Flavonoids in barley grains are generally correlated with the depth of color (Liu *et al.*, 2013). Water-soluble vacuolar pigments called anthocyanins are the most known type of flavonoids found in barley. They mostly cause purple or blue tints in the colour of the kernels in the pericarp or aleurone layers of barley grain.

## 2.2. Phenolic Acids

The outer layers of the barley kernel are representing the phenolic acids, which are the predominant phenolic category of phytochemicals in the grain (Dykes *et al.*, 2007). They have been divided into two categories: cinnamic acid and benzoic acid and their derivatives (Quinde Z *et al.*, 2006). Because phenolic acids include unsaturated carboxylic groups, they have been associated with a reduced risk of chronic disease (Pandey *et al.*, 2009). Due to its high phenolic acid content, barley especially the hulled variety may also be a great dietary source of free radicals and other naturally occurring antioxidants with potential (Zhao *et al.*, 2008).

The bound form of barley has the largest quantities of phenolic acids, followed by the conjugated and free forms, in that order (Bonoli *et al.*, 2004). While the bound forms are esterified to cell wall components such lignin, cellulose, arabinoxylans, polysaccharides, and hemicelluloses, the free forms are frequently found in the outer region of the pericarp (Tang *et al.*, 2016). Between 4.6 µg/g and 23 µg/g in free form, 86 µg/g and 198 µg/g in conjugated form, and 133 µg/g and 523 µg/g in bound form, barley's phenolic acid concentrations roughly come up into these ranges, while the total phenolic acid concentration ranges between 604 µg/g and 1346 µg/g (Holtekjølen *et al.*, 2006) Major phenolic acids found in barley are ferulic acid (FA; 27% of dry

matter), vanillic acid (28%), syringic acid (17%), and p-coumaric acid (22%), in free forms .Gamel *et al.*,2012).

### 2.3. Tocols

In barley, a class of lipid-soluble phytochemicals known as tocopherols and tocotrienols together are present. Tocols are known for their antioxidant capabilities, particularly their ability to prevent lipid peroxidation in biological membranes (Niki *et al.*, 1985). Cereals include tocols that, in addition to their antioxidant qualities, have anticancer and cancer suppression effects (Sen *et al.*, 2017), stimulate the immune system (Becker *et al.*,2013), reduce the risk factors for cardiovascular diseases (CVD) (Tiwari *et al.*, 2009), and encourage the induction of apoptosis (Suman *et al.*, 2013). The capacity of tocols to remove carotid artery stenosis, or atherosclerotic blockages, may lower the risk of stroke, which is one of the most remarkable findings concerning them (Upadhyay *et al.*, 2000).

As barley contains a high concentration and a good distribution of all eight physiologically active vitamins, it is one of the greatest cereal sources of tocols (Moreau *et al.*, 2007). It is composed of eight chemical forms:  $\alpha$ -tocopherol ( $\alpha$ -T),  $\beta$ -tocopherol ( $\beta$ -T),  $\gamma$ -tocopherol ( $\gamma$ -T),  $\delta$ -tocopherol ( $\delta$ -T), and their four corresponding unsaturated tocotrienols:  $\alpha$ -tocotrienol ( $\alpha$ -T3),  $\beta$ -tocotrienol ( $\beta$ -T3),  $\gamma$ -tocotrienol ( $\gamma$ -T3),  $\delta$ -tocotrienol ( $\delta$ -T3). Tocol analysis was performed using a method specifically designed for cereals, when compared to other reported methods, proved to provide a more accurate qualitative and quantitative determination of all eight forms of tocol in barley samples (Panfili *et al.*, 2003). While tocotrienols are generally found in the endosperm and pericarp portion of barley grains, most of the tocopherols in barley are found in the germ fraction (Zielinski *et al.*, 2006).

## 2.4. Lignans

Natural polyphenols called lignans are found in a wide range of plants and act as organic defense compounds. Their structural and functional resemblance to  $17\beta$ -estradiol makes them bioactive as phytoestrogens. Numerous pharmacological benefits, including antioxidant, anticancer, antiviral, antibacterial, insecticidal, fungistatic, estrogenic, and antiestrogenic properties, as well as protection against coronary heart disease, have been associated with lignans (Saarinen *et al.*, 2013). Smedset *et al.* 2007, reported that barley contains a number of lignans, with the major ones being pinoresinol, medioresinol, syringaresinol, lariciresinol, cyclolariciresinol, secoisolariciresinol, secoisolariciresinol-sesquilignan, matairesinol, oxomatairesinol, and 7 hydroxymatairesinol. These contents are estimated to be 71 mg/100 g, 22 mg/100 g, 140 mg/100 g, 133 mg/100 g, 28 mg/100 g, 42 mg/100 g, 24 mg/100 g, 42 mg/100 g, 28 mg/100 g, and 541 mg/100 g, respectively. As minor lignans, the concentrations of todolactol,  $\alpha$ -conidengrin acid, nortrachelogenin, and lariciresinol-sesquillgnan are around 127 mg/100 g, 33 mg/100 g, 15 mg/100 g, and 6.6 mg/100 g, individually.

## 2.5. Phytosterols

Due to the increased consumption of cereals, they are the main dietary source of phytosterols. An important micronutrient for human health is phytosterol. Prior research has demonstrated the potential advantages of regular dietary plant sterol consumption, including lowered cholesterol levels in the blood, protection against cardiovascular disease, and anticancer properties. (Patch, Rao & Janezic *et al.*, 1992; Tapsell, Williams, & Gordon *et al.*, 2006). Phytosterols found in HB grains are primarily found in free and esterified forms, such as phenolic acid esters, fatty acid esters, and steroid glucose or acetyl glycoside esters (Gani, Wani, Masoodi, & Hameed *et al.*, 2012).

The distribution of phytosterols in kernels is uneven, with the outer layers having a higher concentration (between 82.0 and 115.3 mg/100 g). In barley, phytosterol comprises just 1-3 percent phytostanol. Based on the research, the levels of phytostanol in ten different types of barley ranged from 10 to 30 µg/g. For the variety of barley under study (Lampi *et al.*, 2004) measured the stanol contents of campestanol (11 µg/g) and sitostanol (5 µg/g).

## 2.6. Folates

Water-soluble B vitamins called folates have the same biological function as folic acid. The natural folate content of cereals is thought to be substantial, despite a lack of evidence regarding the folate content of HB. It is thought that the distribution of folate in barley grains is uneven, with more of it found in the bran than in the endosperm (Giordano *et al.*, 2016). Folate is a component of numerous metabolic pathways and possesses the same biological activity as folic acid (Romano *et al.*, 1995). Although not much is known about folate in barley, cereals are nonetheless thought to be a significant source of the vitamin (Andersson *et. al.*, 2008). The total folate content of 10 genotypes of barley (518–789 ng/g of dry mass), which was found to be near to that of rye (574–775 ng/g, n = 10), greater than that of wheat (323–774 ng/g, n = 130) (Piironen *et al.*, 2008), and oat (495–604 ng/g, n = 5) (Shewry *et al.*, 2008). Similar folate contents of 598–664 ng/g were similarly reported by Edelmann *et al.* (2013) for the five hulled Finnish barley cultivars under investigation.

## 3. Phytochemicals of Barley on Human Health

Barley grains are high in therapeutic chemicals such as β-glucan, tocots, and resistant starch. Resistance training methods have been demonstrated to lower blood cholesterol levels. It has been demonstrated that starch lowers blood sugar and enhances gut health (Geng *et al.*, 2022). Barley is a valuable food since it is packed with fiber, vitamins, and minerals, among other beneficial ingredients. There are minerals like iron, zinc, magnesium, and

phosphorus; there's also contain vitamin E and the B-complex vitamins. Barley has a lot of beta-glucan, a type of soluble fiber that has been related to lower cholesterol and a lower risk of heart disease. It has been demonstrated that the antioxidants in barley strengthen immunity and fight against the harm caused by free radicals. Certain studies have connected the antioxidant content of barley to a decreased risk of cancer, especially colon cancer.

### 3.1. Cardiovascular Disease (CVD)

One of the main causes of death in the US is CVD. In 2011, the total number of deaths linked to CVD was 229.6 per 100,000 Americans; by 2013, heart disease alone was responsible for almost one out of every seven deaths in the country. High blood cholesterol is now acknowledged to be a major risk factor for the development of CVDs (Mozaffarian *et al.*, 2016). A natural antioxidant's ability to prevent oxidative processes caused by these substances can be produced through the daily consumption of antioxidant-rich foods like polyphenols, which can also improve cardiovascular health (Frei *et al.*, 2012).

Dietary restrictions to decrease cholesterol intake and/or the use of a class of medication called statins to lower serum cholesterol are recent techniques for lowering the risk of CVDs. Barley kernels are beneficial to cardiovascular health since they are high in phytosterols. Eating barley grains high in phytonutrients could reduce the need for medication or dietary restrictions in order to moderate cardiovascular disease.

In the intestinal lumen, barley phytosterols have the ability to compete with cholesterol for the production of micelles, preventing cholesterol absorption and promoting secretion and cholesterol regulation instead. Research indicates that at moderate dosages of 15 mL/d (1 tablespoon/d), the quantities of total phytosterols in barley oils are sufficient (0.18–1.44 g/15 g oil) to considerably cut low-density lipoprotein (LDL) cholesterol (Moreau *et al.*, 2007). It has been found that lignans, which are also

contained in barley, serve as potent antioxidants that are superior to vitamin E and comparable to FA. Additionally, it has been noted that they reduce the risk of CVD (Prasad *et al.*, 2000).

### 3.2. Blood Sugar-Lowering Effect

Humans use glucose more efficiently when they eat meals that contain barley, according to recent studies. While processing or mixing a grain with other foods may impact its glycemic index (GI), barley typically has a GI of 34–70, which is lower than other cereals. Studies have indicated that barley grains, particularly those that are amylose-only and contain 99% amylose starch, can significantly contribute to the production of very low GI meals. The effectiveness of barley meals in lowering blood sugar levels is thought to be related to both  $\beta$ -glucan and the ratio of amylose to amylopectin. Glycemic response to barley diets is usually caused by a combination of these two variables. When researchers fed barley to mice with and without diabetes, they found that all of the mice had better glycemic tolerance (Geng *et al.*, 2022) found that after feeding diabetic mice a diet containing 70.83% barley, their fasting glucose levels returned to normal. It was researched how barley meals affected the health of individuals in India.

The results showed that four weeks of consuming 100 g of whole barley increased the area of the 3-h blood glucose curve from 107.9 to 91.5 mg/dL (Minehira *et al.* 2001) examined the impact of  $\beta$ -glucan on the prandium glucose metabolism of healthy persons. They proposed that the slowed glycemic response and increased intestinal viscosity following a meal high in  $\beta$ -glucans would result in delayed or decreased glucose absorption (Minehira *et al.*, 2001). The large intestine of healthy people contains combinations of starches called resistant starches, which include anti-digestive enzymes. These starches resemble macromolecular polymers. Resistant starch, found in bread made from barley, has been connected to a decrease in blood sugar (Lockyer *et al.*, 2017). Resistant starch, found in bread made from barley, has been linked to a lowering in blood sugar.

### 3.3. Anticancer Activity

Several studies have suggested that barley grains may shield human cells against carcinogens (Ferrazzano *et al.*, 2011). Studies show that the chlorophyll in barley grains interacts with carcinogens to reduce their effects in a dose-dependent way. Additionally, the anti-oxidant properties of barley grains aid in the battle against cancer by preventing free radicals from damaging the body's DNA (Nishino *et al.*, 2005). Additionally, selenium has antioxidant properties that guard against the harm that free radicals a type of chemical cause to cells. This kind of damage could increase the risk of getting cancer. Consuming a diet high in plant-based fiber, such as barley, may also help reduce the risk of colorectal cancer. Moreover, certain data indicates that  $\beta$ -glucan fibers can have anticancer and innate immune benefits. Although it is often used as a supplement, scientists are investigating ways to use it in cancer immunotherapy treatments (Ferrazzano *et al.*, 2011).

## 4. Isolation of Phytochemicals in Barley and Their Properties

Barley is one of the most important cereal crops cultivated in all agricultural regions across the world. Barley is high in proteins, and carbohydrates, including Beta-glucan, lipids, vitamins, and minerals. Consuming barley could reduce total cholesterol and low-density lipoprotein cholesterol. Barley also contains antioxidant qualities and may help manage blood sugar levels. It is thought to be a beneficial supplement to a balanced diet for persons at risk cardiovascular disease.

### 4.1. Steeping

The Steeping operation is the most critical stage in malting. To provide homogeneous malt, It is necessary to achieve even moisture content across the grain bed. Most barely requires a steeping regime that taken them to 42-46% moisture.

## 4.2. Polishing

The pearl barley is polished on machines similar to those used for pearlizing but equipped with stones made of hard white sandstone instead of emery composition. The average yield of barley is 67% of the whole barley.

## 4.3. Milling Machine

Cylindrical mill stones. @450rpm.Abrasive disc with 6-8 abrasives with carborendum or emery rolls.

# 5. Properties of Barley and Their Nutrition Value

Barley is a cereal grain used in bread, beverages, stews, and other dishes. As a whole grain, barley provides fiber, vitamins, and minerals. These nutrients may enhance heart health, help prevent cancer, reduce inflammation, and more.

## 5.1. Vitamin and Minerals

Barley's potassium, folate, iron, and vitamin B-6 content, together with its lack of cholesterol, all support cardiovascular functions.

## 5.2. Sodium and Pottassium

The American Heart Association Trusted Source (AHA) recommends avoiding foods that are very high in sodium, such as fast foods. Instead, consuming vegetables, fruits, grains, and other potassium-rich foods may help maintain healthy blood pressure.

## 5.3. Bone Health

The phosphorus, calcium, copper, magnesium, and zinc in barley all contribute to improved bone structure and strength. For example, zinc plays a role in bone mineralization and development. Calcium, copper, magnesium, and phosphorus,

meanwhile, contribute to bone health and are essential for maintaining a strong skeletal system.

## 5.4. Dietary Tips

Barley is a versatile grain with a nutty flavor and a chewy, pasta-like texture. People can prepare it in various ways. In the sections below, we list some preparation methods for different types of barley.

## 6. Characterization of Phytochemicals in Barley

Barley contains an assortment of phytochemicals (non-nutrient components) in varying concentrations usually determined by genotypic or environmental factors, or the interaction of both factors (Malik *et al* 2012). Phytochemicals in barley may exist in free, conjugated, or bound forms and are categorized into several major classes, including phenolic acids, flavonoids, lignans, tocots, phytosterols, and folates (Fogarasi *et al* 2015).

### 6.1. Phenolic Acid

The phenolic acids are the simplest polyphenols in terms of chemical structure. Phenolic acids are carboxylic acids derived from either benzoic or cinnamic acid (Pandey *et al*, 2009). They are a major class of secondary metabolites in plants, and they play significant roles in plant defense and human health. (Quinde-Axtell, *et al*, 2006)

#### 6.1.1. Types of Phenolic Acids

Different types of phenolic acids Phenolic acids are roughly classified into two primary categories: Hydroxybenzoic Acids include gallic acid, p-hydroxybenzoic acid, protocatechuic acid, vanillic acid, and syringic acid. They have a C6-C1 skeleton, which is produced from benzoic acid. Hydroxycinnamic acids include caffeine, ferulic acid, p-coumaric acid, sinapic acid, and chlorogenic acid.

## 6.1.2. Characteristics of Phenolic Acid

Antioxidant action, phenolic acids protect cells by reducing free radicals and oxidative stress. Anti-inflammatory effects include modulating inflammatory pathways and decreasing pro-inflammatory mediator production. Phenolic acids have antimicrobial properties, preventing the growth of bacteria, fungus, and viruses. Anti-Cancer Activity: Cancer cells can be induced to apoptosis and their growth inhibited. Phenolic acids have cardioprotective effects via decreasing inflammation and oxidative stress.

## 6.2. Flavanoids

Flavonoids are a diverse group of phytonutrients (plant chemicals) found in many fruits and vegetables, including barley. (Tossi *et al*, 2012)

### 6.2.1. Types of Flavonoids in Barley

Flavonols Some examples include quercetin and kaempferol. Properties include strong antioxidants, anti-inflammatory and anti-cancer properties. Flavones include Luteolin and Apigenin. Properties include antioxidants, anti-inflammatory properties, and potential benefits for cardiovascular health. Some examples of anthocyanins include cyanidin and delphinidin. Properties: Antioxidant, anti-inflammatory, and eye health-promoting.

## 7. Synthesise of Phytochemicals in Barley

The synthesis of phytochemicals in barley involves complex biochemical pathways that are regulated by various enzymes and environmental factors. Barley (*Hordeum vulgare*) produces a wide array of phytochemicals, including phenolic acids, flavonoids, lignans, and tocots.

## 7.1. Phenolic Acids

The shikimate pathway transforms simple carbohydrates into aromatic amino acids (phenylalanine and tyrosine), which is the starting point for phenolic acid production. **Phenylpropanoid Pathway:** The enzyme phenylalanine ammonia-lyase (PAL) deaminates amino acids to create cinnamic acid, a precursor for numerous phenolic acids. Important Enzymes Phenylalanine Ammonia-Lyase (PAL) converts phenylalanine to Cinnamic Acid. Cinnamate-4-Hydroxylase (C4H) converts cinnamic acid to p-coumaric acid. 4-Coumarate Ligase (4CL) converts p-coumaric acid to p-coumaroyl-CoA. (Delcour and Hoseney *et al.*, 2009) Hydroxylases and methyltransferases modify phenolic structures to yield ferulic, sinapic, and caffeic acids.

## 7.2. Flavonoids

The Synthesis Process Phase-wise, the phenolic acid production and the phenol phenylpropanoid pathway are similar. P-coumaroyl-CoA and malonyl-CoA are converted to chalcone by Chalcone Synthase (CHS), which catalyzes the first committed step in the biosynthesis of flavonoids. Key intermediate naringenin is produced by Chalcone Isomerase (CHI) from chalcone. important enzymes Chalcone Oxidase (CHS) Invertase Isomerase (CHI) Dihydroflavonols are produced when flavanones are converted to their 3-hydroxylase (F3H) form. Leuco-anthocyanidins are produced when dihydroflavonols are converted by Dihydroflavonol 4-Reductase (DFR) (Holopainen-mantila, Tapani, *et al.*, 2007) Synthesizes anthocyanidins (ANS) from leuco-anthocyanidins.

## 7.3. Lignans

Similar to phenolic acids and flavonoids, the phenylpropanoid pathway starts here. **Linking Responses:** To create the lignan structure, two molecules of p-coumaroyl-CoA or similar chemicals are connected. Important enzymes CCR stands for Cinnamoyl-CoA Reductase (Andersson, Lampi, *et al.*, 2008)

Alcohol Dehydrogenase with Cinnamyl Base (CAD) Protein called Dirigent (DIR): controls how phenylpropanoid units are stereoselectively coupled to create lignan.

#### 7.4. Tocols (Vitamin E)

**Route Pathway of Methylerythritol Phosphate (MEP):** The plastidial MEP route, which yields the isoprenoid precursors, is used to manufacture protocols. In order to create the tocopherol or tocotrienol structure, homogentisate and phytol diphosphate (also known as geranylgeranyl diphosphate) must condense (Gutierrez-Gonzalez, *et al.*, 2013). This process is catalyzed by prenyl transferase (PT).

**Cyclization and Methylation:** To create distinct forms of tocopherols and tocotrienols, additional alterations include cyclization and methylation processes.

**Important Enzymes:** Phytol transferase homogentisate (HPT): This enzyme produces 2-methyl-6-phytolbenzoquinol from homogentisate. Tocopherol Cyclase (TC): Produces the tocopherol ring structure from the intermediate. Methyltransferases: Generate α-, β-, γ-, and δ-tocopherols and tocotrienols by adding methyl groups.

### 8. Environmental and Genetic Factors

**Light:** UV light can enhance the synthesis of certain phenolic compounds and flavonoids.

**Nutrients:** Soil nutrients and fertilizers can influence the levels of various phytochemicals.

**Stress Conditions:** Biotic (pathogens) and abiotic (drought, salinity) stress can trigger the production of defense-related phytochemicals.

**Genetic Variability:** Different barley varieties have different capacities to synthesize phytochemicals.

**Genetic Engineering:** Advances in biotechnology allow for the manipulation of specific genes to enhance the production of desirable phytochemicals.

## References

- Abdel-Aal, E. S. (2012). M., Choo, T.-M., Dhillon, S. and Rabalski, I. Free and bound phenolic acids and total phenolics in black, blue and yellow barley and their contribution to free radical scavenging capacity. *Cereal Chem.*, 89, 198-204.
- Adlercreutz, H. (2007). Lignans and human health. *Critical Reviews in Clinical Laboratory Sciences*, 44(5–6), 483–525.
- Agati, G., Biricolti, S., Guidi, L., Ferrini, F., Fini, A., & Tattini, M. (2011). The biosynthesis of flavonoids is enhanced similarly by UV radiation and root zone salinity in *L. vulgare* leaves. *Journal of plant physiology*, 168(3), 204-212.
- Andersson, A. A., Lampi, A. M., Nystrom, L., Piironen, V., Li, L., Ward, J. L., & Åman, P. (2008). Phytochemical and dietary fiber components in barley varieties in the HEALTHGRAIN diversity screen. *Journal of agricultural and food chemistry*, 56(21), 9767-9776.
- Cavallero, A., Gianinetti, A., Finocchiaro, F., Delogu, G., & Stanca, A. M. (2004). Tocols in hull-less and hulled barley genotypes grown in contrasting environments. *Journal of Cereal Science*, 39(2), 175-180.
- Dykes, L., & Rooney, L. W. (2007). Phenolic compounds in cereal grains and their health benefits. *Cereal foods world*, 52(3), 105-111.
- Edelmann, M., Kariluoto, S., Nyström, L., & Piironen, V. (2013). Folate in barley grain and fractions. *Journal of Cereal Science*, 58(1), 37-44.
- Frei, B. (Ed.). (2012). *Natural antioxidants in human health and disease*. Academic Press.
- Geng, L., Li, M., Zhang, G., & Ye, L. (2022). Barley: a potential cereal for producing healthy and functional foods. *Food Quality and Safety*, 6, fyac012.
- Goupy, P., Hugues, M., Boivin, P., & Amiot, M. J. (1999). Antioxidant composition and activity of barley (*Hordeum vulgare*) and malt extracts and of isolated phenolic

compounds. *Journal of the Science of Food and Agriculture*, 79(12), 1625-1634.

- Hallikainen, M. A., Sarkkinen, E. S., & Uusitupa, M. I. (2000). Plant stanol esters affect serum cholesterol concentrations of hypercholesterolemic men and women in a dose-dependent manner. *The Journal of nutrition*, 130(4), 767-776.
- Hendriks, H. F. J., Weststrate, J. A., Van Vliet, T., & Meijer, G. W. (1999). Spreads enriched with three different levels of vegetable oil sterols and the degree of cholesterol lowering in normocholesterolaemic and mildly hypercholesterolaemic subjects. *European Journal of Clinical Nutrition*, 53(4), 319-327.
- Jones, P. J., Raeini-Sarjaz, M., Ntanios, F. Y., Vanstone, C. A., Feng, J. Y., & Parsons, W. E. (2000). Modulation of plasma lipid levels and cholesterol kinetics by phytosterol versus phytostanol esters. *Journal of lipid research*, 41(5), 697-705.
- Khalid, W., Arshad, M. S., Jabeen, A., Muhammad Anjum, F., Qaisrani, T. B., & Suleria, H. A. R. (2022). Fiber-enriched botanicals: A therapeutic tool against certain metabolic ailments. *Food Science & Nutrition*, 10(10), 3203-3218.
- Kim, M. J., Hyun, J. N., Kim, J. A., Park, J. C., Kim, M. Y., Kim, J. G., & Chung, I. M. (2007). Relationship between phenolic compounds, anthocyanins content and antioxidant activity in colored barley germplasm. *Journal of Agricultural and Food Chemistry*, 55(12), 4802-4809.
- Lockyer, S., & Nugent, A. P. (2017). Health effects of resistant starch. *Nutrition bulletin*, 42(1), 10-41.
- Miettinen, T. A., Puska, P., Gylling, H., Vanhanen, H., & Vartiainen, E. (1995). Reduction of serum cholesterol with sitostanol-ester margarine in a mildly hypercholesterolemic population. *New England Journal of Medicine*, 333(20), 1308-1312.
- Moreau, R. A., Flores, R. A., & Hicks, K. B. (2007). Composition of functional lipids in hulled and hulless barley infarctions obtained by scarification and in barley oil. *Cereal chemistry*, 84(1), 1-5.

- Mustafa, S. B., Akram, M., Muhammad Asif, H., Qayyum, I., Hashmi, A. M., Munir, N., & Ahmad, S. (2019). Antihyperglycemic activity of hydroalcoholic extracts of selective medicinal plants *Curcuma longa*, *Lavandula stoechas*, *Aegle marmelos*, and *Glycyrrhiza glabra* and their polyherbal preparation in alloxan-induced diabetic mice. *Dose-Response*, 17(2), 1559325819852503.
- Niki, E., Kawakami, A., Saito, M., Yamamoto, Y., Tsuchiya, J., & Kamiya, Y. (1985). Effect of phytol side chain of vitamin E on its antioxidant activity. *Journal of Biological Chemistry*, 260(4), 2191-2196.
- Obadi, M., Sun, J., & Xu, B. (2021). Highland barley: Chemical composition, bioactive compounds, health effects, and applications. *Food Research International*, 140, 110065.
- Patch, C. S., Tapsell, L. C., Williams, P. G., & Gordon, M. (2006). Plant sterols as dietary adjuvants in the reduction of cardiovascular risk: Theory and evidence. *Vascular Health and Risk Management*, 2(2), 157.
- Prasad, K. (2000). Oxidative stress as a mechanism of diabetes in diabetic BB prone rats: effect of Secoisolariciresinol Diglucoside (SDG). *Molecular and cellular biochemistry*, 209, 89-96.
- Rhee, Y. (2016). Flaxseed secoisolariciresinoldiglucoside and enterolactone down-regulated epigenetic modification associated gene expression in murine adipocytes. *Journal of Functional Foods*, 23, 523-531.
- Rhee, Y. (2016). Flaxseed secoisolariciresinoldiglucoside and enterolactone down-regulated epigenetic modification associated gene expression in murine adipocytes. *Journal of Functional Foods*, 23, 523-531.
- Romano, P. S., Waitzman, N. J., Scheffler, R. M., & Pi, R. D. (1995). Folic acid fortification of grain: an economic analysis. *American journal of public health*, 85(5), 667-676.
- Sahoo, K., & Sharma, A. (2023). Understanding the mechanistic roles of environmental heavy metal stressors in regulating

ferroptosis: adding new paradigms to the links with diseases. *Apoptosis*, 28(3), 277-292.

- Sen, C. K., Khanna, S., & Roy, S. (2007). Tocotrienols in health and disease: the other half of the natural vitamin E family. *Molecular aspects of medicine*, 28(5-6), 692-728.
- Smeds, A. I., Eklund, P. C., Sjoholm, " R. E., Willfor, " S. M., Nishibe, S., Deyama, T., & Holmbom, B. R. (2007). Quantification of a broad spectrum of lignans in cereals, oilseeds, and nuts. *Journal of Agricultural and Food Chemistry*, 55(4), 1337-1346.
- Tang, Y., Zhang, B., Li, X., Chen, P. X., Zhang, H., Liu, R., & Tsao, R. (2016). Bound phenolics of quinoa seeds released by acid, alkaline, and enzymatic treatments and their antioxidant and  $\alpha$ -glucosidase and pancreatic lipase inhibitory effects. *Journal of agricultural and food chemistry*, 64(8), 1712-1719.
- Tossi, V., Lombardo, C., Cassia, R., & Lamattina, L. (2012). Nitric oxide and flavonoids are systemically induced by UV-B in maize leaves.
- Upadhyay, J., & Misra, K. (2009). Towards the interaction mechanism of tocopherols and tocotrienols (vitamin E) with selected metabolizing enzymes. *Bioinformation*, 3(8), 326.
- Zielinski, H. (2008). Tocotrienols: distribution and sources cereals-role in human health. *Tocotrienols: Vitamin E Beyond Tocopherols*. Boca Raton: CRC Press. p, 23-42.

## Targeting Anthranilate Synthase from *Malassezia Globosa* from Tryptophan Pathway with the Chemical Constituents from *Albizia amara* Extract

Subhashini, S Rithik, N Tharun Kumar,  
R Loganathan, and M Jeyam

Department of Biotechnology,

Dr. G.R. Damodaran College of Science,

Coimbatore. Tamil Nadu, India

Department of Bioinformatics,

Bharathiar University, Coimbatore, Tamil Nadu, India

Corresponding author: subhashini.r@grd.edu.in

### Abstract

Yeast causes superficial mycoses most frequently by *Malassezia globosa*. Treating microbial infections is increasingly challenging due to the associated side effects of antifungal drugs. Novel treatments for important medical areas focus on promising drug targets, a primary focus for the pharmaceutical industry. These advancements have significantly accelerated the drug discovery process and have led to the development of more target-based therapies. Researchers assess crude extracts, isolated, or synthesized compounds for their efficacy against various infectious diseases. Screening of *Albizia amara* extracts resulted in more phytochemicals in the chloroform extract and it is effective against *M. globosa*. Anthranilate synthase is the initial enzyme in the tryptophan biosynthesis pathway where the enzyme is involved in the production of aromatic amino acids. The absence of this enzyme in higher eukaryotic organisms makes it a desirable target. The three-dimensional structure of anthranilate synthase in *M. globosa* is not available in the PDB database. Hence, its structure is modeled, validated, and simulated. Interaction studies of the compounds from crude extract revealed hexadecanoic acid, 2-

hydroxy-1-(hydroxymethyl) ethyl ester had a high affinity for anthranilate synthase indicating its potential and further evaluated for treating *Malassezia* infections.

**Keywords:** *Malassezia globosa*, *Albizia amara*, drug target, metabolic pathway, physiochemical properties, anthranilate synthase, modeling, molecular interaction

## Introduction

The lipophilic yeasts *Malassezia*, formerly known as *Pityrosporum*, are commensals of the normal human skin microbiome. There are 17 species in this genus, which belongs to the phylum *Basidiomycota* (class: *Malassezio mycetes*). Species of *Malassezia* are distributed on seborrheic skin, including the face, scalp, and thorax. Except *M. pachydermatis*, all *Malassezia* species are dependent on exogenous lipids since they lack the fatty acid synthase gene (Glatz *et al.*, 2015). According to Grice and Dawson (2017), there may be variances in the global distribution of *Malassezia* species. Amongst, *M. globosa* is one of the most common species in Asia (Prohicet *et al.*, 2016; Theelen *et al.*, 2018).

The transition of an organism from a commensal state to a pathogenic potential is due to both endogenous and external stimuli (Guého *et al.*, 1998). Moreover, they engage in direct or indirect interactions with nearly every cell component of the normal epidermis, such as keratinocytes, Langerhans cells, melanocytes, and the host immune system (Glatzel *et al.*, 2015; Grice and Dawson, 2017). This could result in several skin conditions such as seborrheic dermatitis, atopic dermatitis, *pityriasis versicolor*, dandruff, etc (Ashbee and Evans, 2002; Cafarchia and Otranto, 2008; Guého *et al.*, 1998). Treatment involves the use of ketoconazole, selenium sulfide, zinc pyrithione, ciclopiroxolamine, and climbazole (Schwartz *et al.*, 2010). However, termination of the treatment often leads to the reoccurrence of the symptoms (Crespo and Delgado, 2002).

This highlights the necessity for new antifungal agents to address issues caused by *Malassezia*. Therefore the need is to

discover new antifungal targets (protein) and antifungal compounds for effective treatment. Genome sequencing has resulted in the discovery of more new targets for developing drugs. The traditional method of drug discovery is time-consuming, labour-intensive, and costly (Thomford *et al.*, 2018). Moreover, advanced experimental methods have greatly improved our understanding of protein and protein-ligand complex structures. Precise target identification is paramount to the drug discovery process, playing a crucial role in combating diseases and drug-resistant microorganisms.

Drug targets can be either inhibited or activated by drug molecules like small organic compounds, antibodies, and therapeutic proteins. For effective treatment, drug targets must exhibit high potency and specificity to ensure strong effects on a particular biological pathway while minimizing impacts on other pathways. In modern drug discovery, the focus is on finding potential drugs for existing therapeutic targets, which are then tested for effectiveness. This exploration of drug targets sparked a greater interest in discovering novel targets, as noted by Ohlstein *et al.* (2000) and Terstappen and Reggiani (2001). Thus the focus in drug development has been shifted to the computational approaches for identifying drug targets which minimize time and cost (Naorem *et al.*, 2022; Kaur *et al.*, 2023).

Tryptophan, an essential aromatic amino acid with a heterocyclic indole ring belongs to the family of alpha-amino acids involved in the synthesis of proteins. Biosynthesis of tryptophan occurs exclusively in plants and microbes. Many bacteria and fungi utilize the shikimate pathway, which plays a role in the interaction between the host and pathogen during infections. Pathogens utilize this pathway to produce compounds essential for their growth and metabolism. The seven-step pathway via shikimate generates chorismate, the precursor for the three aromatic amino acids (phenylalanine, tyrosine, and tryptophan), and is also the initial compound for the biosynthesis of folates, including tetrahydrofolate or vitamin B9.

Anthranoate synthase (AS) is the initial enzyme in tryptophan biosynthesis and it belongs to the glutamine amidotransferase family which catalyzes the conversion of chorismate into anthranilate (Morollo and Eck, 2001; Tang *et al.*, 2001; Lin *et al.*, 2001). The formation of this compound transfers ammonia from glutamine to chorismate, resulting in the production of glutamate and pyruvate.

In this reaction, ammonia catalyzes a 1,4-nucleophilic substitution, leading to the removal of pyruvate (Parthasarathy *et al.*, 2018). The enzyme also shares evolutionary origins with other chorismate metabolizing enzymes such as salicylate synthase, aminodeoxychorismate synthase, and isochorismate synthase, owing to the similarity in reaction chemistry and polypeptide folding (Bulloch *et al.*, 2004; He *et al.*, 2004; He and Toney, 2006). Synthesis of tryptophan is absent in animals. Therefore, they must obtain this essential nutrient from their diet, which can be broken down or converted into other necessary compounds, such as neurotransmitters. In spite, it contains multiple attractive targets for the development of herbicides and antimicrobials. Using different computational strategies, anthranilate synthase (MGL\_0538) from the tryptophan pathway of *M. globosa* is reported as a drug target (Subhashini and Jeyam, 2017).

Medicinal plants are a natural asset and their medicinal value lies in the compounds termed phytocompounds. In contrast to synthetic drugs, antimicrobials of plant origin are not associated with many side effects, have enormous therapeutic potential, and are available at low cost (Nisaret *et al.*, 2017). *Albizia amara* (Roxb.) B.Boivin (Basionym: *Mimosa amara* Roxb.) belonging to the family *Mimosaceae* is one of the most considered species in ayurvedic medicine. Different parts of this plant are used as folk remedies for treating various conditions like common cold, diarrhoea, intestinal ailments, dandruff, wounds, skin diseases, poisonous bites, piles, leprosy, leucoderma, boils, etc., (Ayyanar and Ignacimuthu, 2005; Subhashini and Jeyam, 2013). The plant was reported for anticancer, antiarthritic, antiinflammatory, antihyperlipidemic,

larvicidal, and hepatoprotective activities (Akilandeswari *et al.*, 2009; Indravathi and Pullaiah, 2013). The present study utilized the *in vitro* approaches to identify phytochemicals and the active plant extract against *M. globosa*. Further, anthranilate synthase was subjected to different computational methods that aid in understanding the binding orientations with the compounds of *A. amara* which has been widely considered in designing the inhibitor molecules.

## Materials and Methods

### **Preparation of *Albizia amara***

Fresh leaves of *A. amara* (Roxb.) B. Boivin (Family: Leguminosae; Tamil name: Usilai), *Phyla nodiflora* (L.) Greene (Family: Verbenaceae; Tamil name: Poduthalai), was collected in the Coimbatore region. The collected plant sample was washed, shade-dried, and powdered.

The powdered sample weighing about 80g was extracted with 500ml of each solvent sequentially with petroleum ether, n-hexane, chloroform, ethyl acetate, methanol, and water using the Soxhlet apparatus. The obtained extracts were evaporated and the water extract was concentrated under reduced pressure at 40°C. Each extract obtained was stored in an airtight container with proper labeling and preserved at 4° C for further studies (Saranraj *et al.*, 2011). The extracts were then used for various tests to determine their potential bioactivity.

### **Screening of phytochemicals**

*Albizia amara* extracts were subjected to qualitative analysis for the identification of various classes of chemical constituents using standard prescribed methods (Harborne, 1984; Kokate *et al.*, 2005).

### (i) Test for proteins and amino acids

**Biuret test:** To 1 ml of the extract, an equal volume of 40% sodium hydroxide solution and two drops of 1% copper sulphate solution was added. The appearance of violet colour indicates the presence of protein.

**Ninhydrin test:** To 1 ml of the extract, add 2 drops of freshly prepared 0.2% ninhydrin reagent and heat. The appearance of pink or purple colour indicates the presence of proteins, peptides, or amino acids.

### (ii) Test for carbohydrates

**Fehling's test:** Five ml of Fehling's solution was added to 0.5 mg of the extract and boiled in a water bath. The formation of a yellow or red precipitate indicates the presence of reducing power.

**Benedict's test:** About 5 ml of Benedict's solution was added to 0.5 mg of the extract and boiled in a water bath. The appearance of red, yellow, or green precipitate indicates the presence of reducing sugars.

### (iii) Test for alkaloids

**Wagner's test:** To 1 ml of the extract, a few drops of Wagner's reagent were added and the formation of a reddish-brown precipitate indicates the presence of alkaloids.

### (iv) Test for flavonoids

**Shinoda Test:** About 1 ml of the extract, a pinch of magnesium turnings, and 1-2 drops of concentrated hydrochloric acid were added. The formation of pink colour indicates the presence of flavonoids.

**Lead acetate test:** To 1 ml of the extract, a few drops of 10% lead acetate solution was added. The appearance of a yellow colour precipitate indicates the presence of flavonoids.

#### (v) Test for phenols and tannins

**Lead acetate test:** To 1 ml of the extract, a few ml of 1% lead acetate solution was added and the formation of precipitate indicates the presence of tannins and phenolic compounds.

**Ferric chloride test:** To 1 ml of the extract, a few ml of 5% ferric chloride was added. The development of dark bluish-black colour indicates the presence of tannins.

**Sodium hydroxide test:** A small quantity of extract was dissolved in 0.5 ml of 20% sulphuric acid solution. Followed by the addition of a few drops of aqueous sodium hydroxide solution, it turns blue indicating the presence of phenols.

#### (vi) Test for steroids and sterols

**Salkowski's test:** The extract was dissolved in 2 ml of chloroform and an equal volume of concentrated sulphuric acid was added along the sides of the test tube.

The upper layer turns red and the lower layer turns yellow with green fluorescence representing the presence of the steroids and sterols in the extract.

#### (vii) Test for saponins

##### **Honey comb test:**

5 ml of the extract was taken in a test tube and a few drops of 5% sodium bicarbonate solution were added. The mixture was shaken vigorously and kept for 3 minutes. The formation of honey comb-like froth indicates the presence of saponins.

##### **Foam test:**

About 1 ml of the extract was diluted with 20 ml distilled water and shaken well in a graduated cylinder for 15 min. The formation of foam to a length of 1cm indicates the presence of saponins and steroids.

### (viii) Test for glycosides

**Glycosides test:** The extract was dissolved in 1 ml of water and then aqueous sodium hydroxide solution was added to this. The formation of yellow colour indicates the presence of glycosides.

### Evaluation of antifungal activity

The antifungal effect of *A. amara* extracts against *M. globosa* was evaluated by agar well diffusion method. Sabouraud dextrose agar (SDA) plates were overlaid with the olive oil and the prepared inoculum was spread on the surface of the agar plate with the sterile swab. About 4 wells of 5 mm diameter in each plate were cut using a sterile cork borer.

Each extract was separately dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 100 mg/ml. The plant extract at different concentrations (500, 1000, 1500, and 2000 µg/ml) was carefully added into each well separately and allowed to diffuse at room temperature for 2 hours and incubated at 32° C. Ketoconazole (10 µg/ml) and the DMSO was used as controls. The antimicrobial agent diffuses in the agar medium and its activity was evaluated by measuring the diameter of the inhibition zone (in mm). Different extract activity was analyzed using one-way analysis of variance with GraphPad Prism 7.03, statistical software (GraphPad Software, La Jolla California, USA) and reported as mean ± standard deviation of three independent experiments (Prabhamanju *et al.*, 2012).

### Gas Chromatography-Mass Spectrometry (GC-MS) analysis

About 2 µl aliquot was injected into a Fisons GC8000 series coupled to an MD800 MS with a quadrupole mass analyzer. Chromatography was performed using the DB5-MS column where the injection temperature was set at 230° C and helium flowed at a rate of 1 ml/min. After a 5-minute solvent delay at 70° C, the oven temperature was raised to 260° C to maintain isocratic condition at a rate of 70° C/min. Ion trace integration was performed using the mass target-finding method to identify characteristic peaks.

Interpretation of the GC-MS mass spectrum was conducted using the database of the National Institute of Standards and Technology (NIST). The spectrum of the unknown component was compared with the spectra of known components stored in the NIST library to document the compound name (Hemamalini *et al.*, 2013).

## Unknown structure modeling, validation, and physicochemical properties

Anthranilate synthase of *M. globosa* lacks an experimentally determined structure in the PDB database. To model its structure, its sequence (with the ID XP\_001732763) was retrieved from the National Center for Biotechnology Information (NCBI) website at <https://www.ncbi.nlm.nih.gov>. The target (unknown) sequence was compared to the protein database (<http://blast.ncbi.nlm.nih.gov>) to find similar known sequences (templates) using the Basic Local Alignment Search Tool (BLAST). The template identified for the enzyme is 1QDL\_A which showed less than 40% identity, increased e-value and its query coverage in the template was also less.

When the sequence identity between the target and template decreases, it can generate less accurate structural models with errors (Khor *et al.*, 2015). This could be because these proteins are hypothetical or uncharacterized. An alternative protein structure prediction method, I-TASSER (Iterative Threading ASSEmbly Refinement) was utilized for modeling the enzyme due to the low percentage of identity (<https://zhanglab.ccmb.med.umich.edu/I-TASSER>). Finally, the correct protein model is assessed by the confidence score (C-score) (Roy *et al.*, 2010). The conformation of the best model was further validated by Ramachandran plot using PDBsum generate (<http://www.ebi.ac.uk/thornton-srv/databases/pdbsum/Generate.html>). Subsequently, various characteristics of the protein sequences, such as molecular weight, amino acid composition, and other physicochemical properties were analyzed using ProtParam.

## Molecular simulation, Druggability site prediction, and ADME

Molecular dynamics (MD) were conducted for a better understanding of the stability of the modeled protein and its intramolecular conformations with Desmond Molecular Dynamics System, version 3.1 of Schrodinger, at 10 ns timescale. The distance between the protein and the box wall was set to be more than 5 Å and the steepest descent minimization method is used with a maximum of 3000 steps.

The root-mean square deviation (RMSD) and root-mean square fluctuation (RMSF) of the backbone were calculated throughout the simulation (Shivakumar *et al.*, 2010; Gunasekaran *et al.*, 2016). The druggability site of the modeled target based on the top-ranked site score in the SiteMap was used. The structure of the ligands present in PubChem was retrieved in .sdf format (<https://pubchem.ncbi.nlm.nih.gov>). Analyzed the pharmacokinetic and physicochemical properties of the compounds identified in the extract using QikProp (v4.3).

## Molecular docking

The target was imported to the Maestro (v10.1) package in Schrödinger. For docking, the protein preparation wizard uses two components namely pre-processing and refinement utility. The modeled proteins were assigned bond order, the addition of missed connectivity, and the hydrogen atom. All water molecules, ligands, metal ions, and cofactors were also removed in the .pdb file format (Furnham *et al.*, 2014). Next, all the ligands were prepared with LigPrep (v3.3) using the OPLS-2005 force field.

The molecular docking analysis was performed using Grid-based Ligand Docking Energetics (Glide v6.6) extra precision (XP) mode from Schrödinger (Friesner *et al.*, 2004; 2006). Finally, the best confirmation docked with the molecule selected based on docking score (G-Score) and docking energy value.

## Results and Discussion

The prevalence of *M. globosa* in India was reported by many authors (Dutta *et al.*, 2002; Kindo *et al.*, 2004; Chaudhary *et al.*, 2010; Kaur *et al.*, 2013; Shah *et al.*, 2013; Kalyani *et al.*, 2014; Archana *et al.*, 2015). As mentioned by Kavitha *et al.*, (2016), the selected organism grew well in the SDA plate overlaid with olive oil, a rich medium for cultivating yeast, including *Malassezia*.

### Qualitative analysis of the *Albizia amara* extract

The plant chosen for the research, *A. amara*, is shown in Fig. 1. The preliminary phytochemical screening of plants is significant in detecting the active principles that exist in medicinal plants exhibiting broad spectrum of pharmacological properties. These comprise alkaloids, terpenoids, phenolics, tannins, steroids, and volatile oils according to Okwu (2005). Investigation of phytochemical analysis of different extracts of *A. amara* leaf indicated the presence of alkaloids, flavonoids, phenols, tannins, steroids, saponins, and glycosides. The chloroform extract revealed the presence of most of the phytochemicals (Table 1).

Qualitative analysis of different extracts such as hydro-methanolic, petroleum ether, toluene, chloroform, methanol, and ethanol extracts from the leaves of this plant indicated the presence of alkaloids in all the extracts except petroleum ether and toluene.

In addition, the presence of steroids in the chloroform extract revealed significant antimicrobial activity. Similarly, the chromatographic fractions obtained from the leaf extract with a polar solvent revealed the highest antimicrobial activity (Baltazar and Nshimo, 2010). The antifungal activity result obtained by Praveen *et al.* (2011) corresponds with the result obtained in the current study.



**Fig. 1. *Albizia amara***

**Table 1:** Phytochemical screening of *Albizia amara* leaf extract

S. No.	Phytochemical constituents	Name of the tests	Indication of phytochemical constituents in different solvent extracts					
			PE	n-H	CF	EA	ME	AQ
1	Protein	Biuret test	-	-	+	-	-	-
		Ninhydrin test	-	-	+	-	-	-
2	Carbohydrates	Fehling's test	-	-	+	-	-	-
		Benedict's Test	-	-	+	-	+	-
3	Alkaloids	Wagner's Test	+	+	+	+	+	+
4	Flavonoids	Shinoda	+	+	+	+	+	+
		Lead	-	-	+	-	-	-

		Acetate						
5	Phenols and Tannins	Ferric Chloride	-	-	+	+	+	+
		Lead Acetate	-	-	+	-	-	+
		Sodium Hydroxide	-	-	+	-	-	-
6	Steroids and Sterols	Salkowski's Test	-	-	+	-	-	+
7	Saponins	Honey Comb Test	-	-	+	-	-	-
		Foam Test	-	-	-	-	+	+
8	Glycosides	Glycosides Test	+	+	+	+	+	+

PE=Petroleum Ether; n-H=Hexane; CF=Chloroform; EA=Ethyl Acetate; ME=Methanol; AQ=Aqueous;

+ indicates present and

- indicates absent

### Antifungal activity of extracts against *M. globosa*

Despite the discovery of numerous antimicrobial drugs, many undiscovered plants with medicinal properties are still present globally (Madureira, 2008). Therefore, there is a growing focus on using plants as a potential source for managing human diseases (Jahan *et al.*, 2011). The discovery of additional natural antimicrobials has prompted scientists to explore the effectiveness of inhibitory compounds derived from plant extracts. In the literature survey, there were no reports of the plant selected for this study against *M. globosa*, hence it is worthwhile to explore its activity.

Amongst all extracts, *A. amara* chloroform extract was found to be very effective in inhibiting the growth of the tested organisms with a maximum zone of inhibition ranging from  $11.0 \pm$

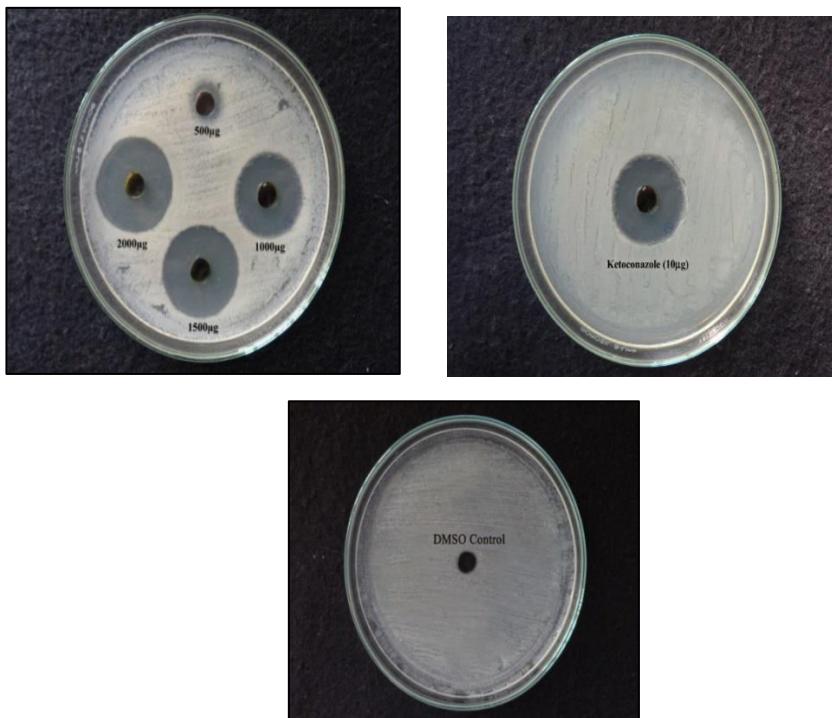
0.0 to  $25.6 \pm 0.6$  mm for *M. globosa* with a significance level of  $p<0.05$  (Table. 2). On the other hand, n-hexane and ethyl acetate extract of this plant exhibited no activity. Also, the petroleum ether extract of this plant showed activity with the last three concentrations tested. Methanol and aqueous extracts obtained from this plant revealed moderate activity. Praveen *et al.* (2011) found that the chloroform extract of *A. amara* exhibited higher antimicrobial activity when compared to other extracts, aligning with the current results. The absence of any zone against any microorganism in the negative control indicates the lack of influence of the medium on the studied effect.

The positive control (Ketoconazole) produced the zone against *M. globosa* and the inhibition was recorded as  $26.3 \pm 0.6$  mm, respectively(Fig 2).This indicates the effectiveness of this antifungal drug against *Malassezia* as reported already (Gupta *et al.*, 2001; Faergemann *et al.*,2006; Squire and Goode, 2002).

**Table 2:** Antifungal activity of *A. amara* extracts against *M. globosa*

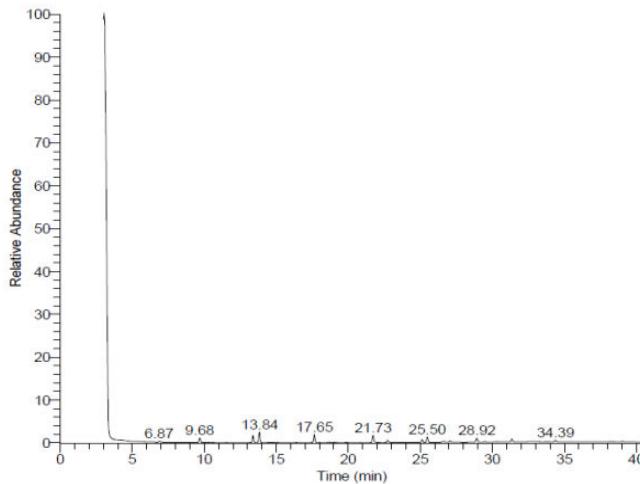
Conc . in $\mu\text{g}/\text{ml}$	Petrole um ether	n- Hexa ne	Chlorof orm	Ethyl acetate	Methanol	Aque ous	Ketocon azole ( $\mu\text{g}/\text{ml}$ )
<b>Inhibition of <i>M. globosa</i> in mm</b>							
500	-	-	$11.0 \pm 0.0$	-	$11.0 \pm 0.0$	-	$26.3 \pm 0.6$
1000	$10.0 \pm 0.0$	-	$20.6 \pm 0.6$	-	$13.3 \pm 0.6$	$10.33 \pm 0.58$	
1500	$12.3 \pm 0.6$	-	$23.3 \pm 0.6$	-	$17.0 \pm 0.0$	$12.0 \pm 0.0$	
2000	$15.0 \pm 0.0$	-	$25.6 \pm 0.6$	-	$18.6 \pm 0.6$	$13.67 \pm 0.58$	

Values are expressed as Mean  $\pm$  SD (n=3); p value < 0.05



**Fig. 2.** Antifungal activity of *A. amara* extract, ketoconazole and DMSO extract against *M. globosa*

**GC-MS analysis of the extract :** Natural compounds either in the pure form or as in the crude extract provide unlimited opportunities for the identification of hits to leads and then to drugs. The GC-MS analysis led to the identification of different compounds in the chloroform extract of *A. amara* is presented as spectral peaks (Fig. 3). A total of 27 bioactive components in the extract, along with their molecular formula, retention time, and peak area, are detailed in Table 3. GC-MS analysis resulted with different compounds in seeds (Kokila *et al.*, 2014) and roots (Karim *et al.*, 2016) of this plant.



**Fig. 3.** GC-MS analysis of the *A. amara* extract

**Table 3:** Chemical constituents in the chloroform extract of *A. amara*

S. No.	RT	Compound Name	Probability	Molecular Formula	Molecular weight	Peak Area
1	6.97	N-(2-Methyl-2H-tetrazol-5-yl)-acetamide	10.17	C <sub>4</sub> H <sub>7</sub> N <sub>5</sub> O	141	0.19
2	13.84	2-tert-Butyl-4-isopropyl-5-methylphenol	29.03	C <sub>14</sub> H <sub>22</sub> O	206	3.98
3	17.65	E-15-Heptadecenal	14.81	C <sub>17</sub> H <sub>32</sub> O	252	3.09
4	18.65	Tetradecanoic acid	55.03	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>	228	0.2
5	19.85	6,10,14-trimethylpentadecan-2-one	87.58	C <sub>18</sub> H <sub>36</sub> O	268	0.19
6	22.73	Hexadecanoic acid	78.19	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256	0.88
7	22.79	1-Methoxy-2-tert-butyl-6-methylbenzene	35.59	C <sub>12</sub> H <sub>18</sub> O	178	0.52

8	23.83	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	84.26	C <sub>17</sub> H <sub>24</sub> O <sub>3</sub>	276	0.16
9	25.14	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	66.3	C <sub>20</sub> H <sub>40</sub> O	296	1.21
10	25.5	1-Docosanol,methyl ether	7.51	C <sub>23</sub> H <sub>48</sub> O	340	2.31
11	26.59	Octadecanoic acid	67.4	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284	0.84
12	27.07	9,12,15-Octadecatrienoic acid	46.7	C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>	278	0.66
13	27.14	Bis[2-(1-hydroxy-1-methylethyl)-4-(1,1-dimethylethyl-6-(carbethoxy)phenyl] sulfide	60.53	C <sub>32</sub> H <sub>46</sub> O <sub>7</sub> S	574	0.24
14	25.5	1-Tetracosanol	5	C <sub>24</sub> H <sub>50</sub> O	340	2.31
15	29.49	9-Octadecenamide	21.85	C <sub>18</sub> H <sub>35</sub> NO	281	0.33
16	30.34	3-Octadecenoic acid, DMOX derivative	10.09	C <sub>22</sub> H <sub>41</sub> NO	335	0.28
17	32.71	Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl)ethyl ester	50.46	C <sub>19</sub> H <sub>38</sub> O <sub>4</sub>	330	0.45
18	33.12	1,11-Bis(methoxycarbonylethyl)-10,2-dihydroxycycloicosane	25.24	C <sub>28</sub> H <sub>48</sub> O <sub>6</sub>	480	0.31
19	33.93	1-Docosanol, formate	14.42	C <sub>23</sub> H <sub>46</sub> O <sub>2</sub>	354	0.15

RT=Retention time

## Physicochemical properties of anthranilate synthase

Identifying targets from various metabolic pathways of an organism or a disease, especially those unique to pathogens, is crucial for discovering potential drug targets (Kanehisa *et al.*, 2002). Mahendran *et al.* (2017) reported nine enzymes identified through the investigation of metabolic pathways.

These enzymes, lacking a three-dimensional structure, were modeled computationally to discover potential ligands against *Treponema pallidum*. Several researchers have widely used the KEGG database for analysing genetic and protein data (Arvind *et al.*, 2013; Hosen *et al.*, 2014; Singh *et al.*, 2016) in the identification of target. This approach is suitable for finding the remedy against disease-causing organisms, such as *Klebsiella pneumoniae* (Bhaskaret *et al.*, 2016) and *Mycobacterium tuberculosis* (Daisy *et al.*, 2013).

Various physicochemical properties were predicted using ProtParam (Table 4). The *in vivo* half-life is a prediction of the time taken by the protein to become half of the amount in a cell after its synthesis. The ProtParam relates the half-life of the protein to the identity of its N-terminal residue.

In this study, the N-terminal residue was methionine (MET) and the half-life was predicted to be 30 hrs which shows the higher stability of the protein in the cell. The protein's molecular weight is determined by adding the average isotopic masses of the amino acids in the protein and one water molecule. Isoelectric point (pI) is a point at which an amino acid net charge becomes zero and this is determined by the pH of the surrounding aqueous environment.

The theoretical pI is valuable for creating a buffer system for protein purification using two-dimensional gel electrophoresis, where proteins are separated based on their isoelectric focusing and molecular weight. A pI value less than 7 indicates an acidic protein, while a pI greater than 7 indicates a basic protein; a pI of 7 indicates a neutral protein (Roy *et al.*, 2010). The pI value of

anthranilate synthase is below 7 which indicate the acidic nature (table 4).

The extinction coefficient (EC) of the protein indicates the amount of light absorbed at particular wavelength (default 280 nm used by ProtParam) and this value is calculated with reference to tyrosine, tryptophan and cysteine.

The EC helps in predicting whether the protein can be analysed by using Ultraviolet (UV) spectroscopy or not. When the ProtParam returns value, it shows the presence of tyrosine, cysteine or tryptophan residues and indicates that it can be analysed using UV spectroscopy (Roy *et al.*, 2010). The protein accounted for this analysis returned value and it can be measured by the experimental method (Table 4).

The instability index (II) measures the stability of the protein *in vitro*. A protein is considered stable if its II value is less than 40 and *thein vivo* half-life is more than 16 hours. A protein is classified as unstable if its II value is greater than 40 and *thein vivo* half-life is less than 16 hours. Furthermore, protein instability can be attributed to the higher occurrence of amino acids such as methionine, glutamine, proline, glutamic acid, and serine (Ibrahim *et al.*, 2017). However, anthranilate synthase values are stable in both conditions (Table 7).

The aliphatic index of a protein denotes the thermostability of the globular proteins based on the volume of the protein filled by aliphatic side chains like isoleucine, alanine, valine and leucine. The high aliphatic index indicates that these proteins may be stable for a wide range of temperature. The positive score of GRAVY indicates the hydrophobicity and the negative score designates the protein can better interact with water (Ibrahim *et al.*, 2017). In the present study, anthranilate synthase is hydrophilic (Table 4).

**Table 4:** Physicochemical properties of the putative targets from *M. globosa*

Putative targets	Total AA	MW	N-T AA	H-L (hrs)	pI	EC	II	AI	GRA VY
Anthranoilate synthase	502	55579.64	Methionine	30	6.0 4	2533 0	39.3 4	92.5 9	-0.149

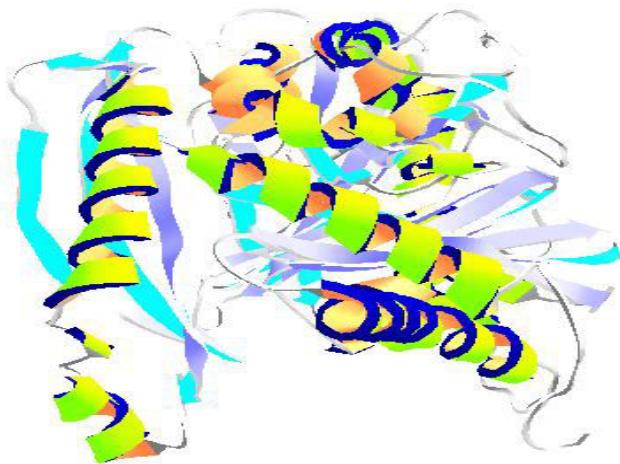
AA=Amino acids; MW=Molecular weight; N-T=N-Terminal amino acid; H-L=Half-life; pI=Isoelectric point; EC=Extinction coefficient; II=Instability index; AI=Aliphatic index; GRAVY=Grand average of hydropathicity

### Protein structure prediction and validation

A threading-based approach was utilized due to the limited homology between the selected target of *M. globosa* and their templates, despite the absence of an evolutionary relationship between them. Generally, the model with the maximum C-score will be chosen (Roy *et al.*, 2010; Zhang, 2008; Irfan and Abid, 2015; Yang *et al.*, 2015) indicating better model quality influenced by the significant threading alignment and structural assembly refinement convergence. The usefulness of the predicted models relies on their accuracy which can be achieved by root mean square deviations (RMSD).

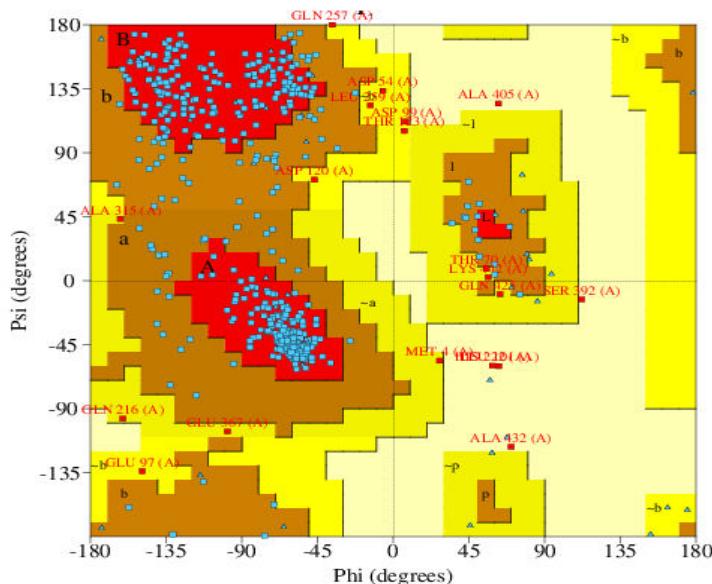
Models generated through threading typically have an RMSD range of 2-5 Å. A TM-score > 0.5 indicates protein pairs with similar folds, while a TM-score < 0.17 signifies randomly selected protein pairs with gapless alignment (Roy *et al.*, 2010). Here, anthranilate synthase resulted in 3 models where the C-score of the first model was 1.6 with the estimated TM and RMSD scores being  $0.88 \pm 0.07$  and  $4.7 \pm 3.1$  Å respectively. The predicted model of the enzyme is represented in Fig. 4. The 3D model of lysyl oxidase constructed using I-TASSER revealed the top five models with varied C-scores. The first model structure of this protein, with a C-score of -2.75, TM score of  $0.40 \pm 0.13$ , and RMSD score of  $13.6 \pm 4.0$  Å, was chosen (Mishra *et al.*, 2017). Likewise, the structure of

the human desmocollin-2 protein was modeled using this server, and the first model with the highest C-score of -2.17, TM-score of  $0.46 \pm 0.15$ , and RMSD score of  $14.1 \pm 3.8 \text{ \AA}$  was chosen (Rohini *et al.*, 2012).



**Fig. 4.** Modeled structure of anthranilate synthase from *M. globosa*

The quality of a good model is typically determined by the percentage of residues within favoured regions. Ramachandran plot analysis for the computationally predicted protein structure showed the residues in the most favoured regions of about 75% as indicated by Rahman *et al.*, (2008). In this study, the modeled protein structure of the target signifies 77 % of their amino acid residues in the most favoured region (Fig. 5). Consequently, these structures were chosen for a molecular dynamics simulation study to assess their stability. The druggability sites predicted by the modeled proteins are presented in table 5.



PROCHECK statistics		
Ramachandran Plot statistics		
	No. of residues	%-age
Most favoured regions [A,B,L]	337	76.9%**
Additional allowed regions [a,b,l,p]	82	18.7%
Generously allowed regions [-a,-b,-l,-p]	14	3.2%
Disallowed regions [XX]	5	1.1%*
-----	-----	-----
Non-glycine and non-proline residues	438	100.0%
End-residues (excl. Gly and Pro)	2	
Glycine residues	39	
Proline residues	26	
-----	-----	-----
Total number of residues	505	

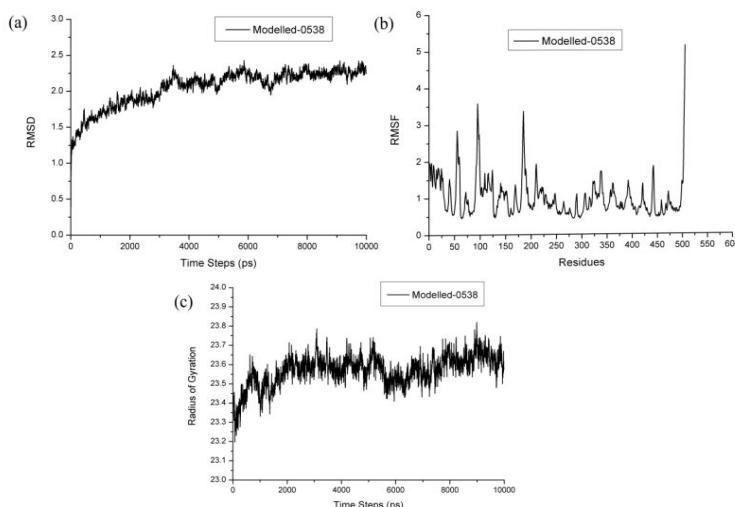
**Fig. 5.** Ramachandran plot analysis of the anthranilate synthase

**Table 5.** Druggability sites predicted for the targets

Name of the target	Predicted active sites
<b>Anthranilate synthase</b>	ARG437; ASP439; LEU65; GLU66; SER67; ASN69; THR70; ARG73; GLY75; TYR77; SER78; PHE79; PHE122; MET121; TYR282; PRO281; PRO298; GLU299; THR300; GLN303; VAL305; HIS312; ILE314; ASN371; GLU373; PHE375; GLU367; SER368; THR382; SER383; PHE384; VAL435; GLY436; ASP444; ASP446; <b>GLY468</b> ; VAL447; CYS448; <b>GLU478</b>

### Molecular simulation

The explicit solvent molecular dynamics simulation of the modeled anthranilate synthase shows considerable stability during simulation. The RMSD stabilized at 2.2 Å after the 6th nanosecond and the RMSF of the protein with reduced fluctuations found around the loop region (Figure 6 a-c).



**Fig. 6.** (a) RMSD Graph; (b) RMSF Graph; (c) Radius of Gyration Graph of the Modeled Anthranilate Synthase

The enzyme UDP-N-acetyl glucosamine pyrophosphorylase from *Moniliophthora perniciosa* was computationally predicted with 78.6% of amino acids within energetically allowed regions. Subsequently, the model underwent molecular dynamics simulation, resulting in a refined structure (Junior *et al.*, 2011).

### Molecular Interaction between anthranilate synthase and extracted compounds

Pathways unique to microbial cells and are not found in mammalian cells, highlighting the distinct biochemical processes in different organisms. One such key pathway in microorganisms is the L-tryptophan (L-Trp) biosynthesis (Fernandes *et al.*, 2015; Wellington *et al.*, 2017 and Lott, 2020). The enzymes involved in the tryptophan biosynthetic pathway are valuable as drug targets because its end product is one among the essential amino acids (Smith *et al.*, 2001; Webby *et al.*, 2010). The chemical structures of all GC-MS compounds, budmunchiamine (reported compound in *A. amara*), and the standard (ketoconazole) used for the docking analysis were imported to the Glide in Schrodinger. The docking analysis performed indicated that only 4 compounds resulted with better G-Scores and found to interact with anthranilate synthase.

These compounds are hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethylester (-5.369), 1,11-is methoxy carbonyl ethenyl -10,2-dihydroxy cycloicosane (-4.729), and bis[2-(1-hydroxy-1-methyethyl)-4-(1,1-dimethylethyl-6-(carbethoxy) phenyl sulfide (-4.603), and N-(2-Methyl-2H-tetrazol-5-yl)-acetamide (-4.034). Additionally, ketoconazole had a G-Score of -7.447, which was lower than that of the reference compound budmunchiamine (-4.949) (Table 6). Of all the above compounds, hexadecanoic acid,2-hydroxy-1- (hydroxymethyl) ethyl ester showed the better interaction at -34.232 kcal/mol with the amino acid residues such as GLU 478, GLY 468 of the target (Fig. 7). The identified

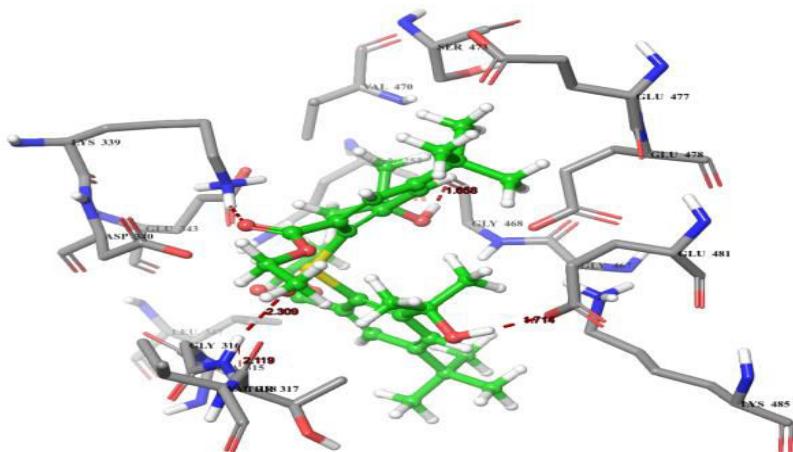
compound possess antibacterial activity was confirmed in *Chromobacterium violaceum* (Venkatramanan et al., 2020).

**Table 6:** Interaction between anthranilate synthase and compounds

S. No.	Name of the compounds	Docking score (G-Score)	Docking energy (kcal/mol)	Number of h-bonds	H-bond distance (Å)	Interacting amino acid residues
1	Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl)ethyl ester	-5.369	-34.232	3	1.906 2.092 2.220	GLU478 (O)...(H) GLY468 (O)...(H) GLY468 (H)...(O)
2	1,11-bis(methoxycarbonylethenyl)-10,2-dihydroxycycloicosane	-4.729	-48.116	2	2.089 2.149	THR407 (O)...(H) THR317 (H)...(O)
3	Bis[2-(1-hydroxy-1-methyethyl)-4-(1,1-dimethyethyl-6-(carbethoxy)phenyl] sulfide	-4.603	-53.142	4	1.658 1.714 2.309 2.119	GLY468 (O)...(H) GLU481 (O)...(H) THR317 (H)...(O) THR317 (H)...(O)
4	N-(2-Methyl-2H-tetrazol-5-yl)-acetamide	-4.034	-25.287	2	1.997 2.102	TYR431 (H)...(N) GLY466 (O)...(H)
5	3-Octadecenoic acid, DMOX derivative	-3.984	-31.65	1	1.762	THR407 (O)...(H)
6	2-tert-Butyl-4-isopropyl-5-methylphenol	-3.811	-24.703	1	1.600	ILE314 (O)...(H)

7	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	-3.57	-27.87	1	2.283	THR317 (H)...(O)
8	9,12,15-Octadecatrienoic acid	-3.42	-34.662	2	1.804 2.079	TYR431 (H)...(O) ARG452 (H)...(O)
9	9-Octadecenamide	-3.034	-29.324	1	2.122	TYR431 (H)...(O)
10	6,10,14-Trimethylpentadecan-2-one	-2.990	-26.659	1	1.629	TYR431 (H)...(O)
11	1-Docosanol, methyl ether	-2.972	-32.001	1	961	GLN252 (H)...(O)
12	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-E]]-	-2.646	-33.336	1	1.928	GLY406 (O)...(H)
13	1-Methoxy-2-tert-butyl-6-methylbenzene	-2.511	-21.397	1	2.397	ARG452 (H)...(O)
14	Tetradecanoic acid	-1.958	-27.679	3	1.945 2.059 2.312	TYR431 (H)...(O) ARG452 (H)...(O) GLY466 (H)...(O)
15	Octadecanoic acid	-1.828	-28.903	3	1.944 2.082 2.333	TYR431 (H)...(O) ARG452 (H)...(O) GLY466 (H)...(O)
16	1-Tetracosanol	-1.825	-35.312	2	1.864 1.925	ARG491 (H)...(O) ASN484 (O)...(H)
17	1-Docosanol, formate	-1.638	-35.607	2	2.113	TYR431 (H)...(O)

					2.332	ARG452 (H)...(O)
18	E-15-Heptadecenal	-1.604	-26.986	1	2.147	ARG452 (H)...(O)
19	Hexadecanoic acid	-1.462	-29.004	2	1.621 1.925	TYR431 (H)...(O) ARG452 (H)...(O)
20	Ketoconazole (C <sub>26</sub> H <sub>28</sub> Cl <sub>2</sub> N <sub>4</sub> O <sub>4</sub> )	-7.447	-54.116	2	1.886 1.657	VAL474 (H)...(O) GLU229 (O)...(H)
21	Budmunchiamine (C <sub>27</sub> H <sub>56</sub> N <sub>4</sub> O)	-4.949	-44.404	1	2.073	GLU299 (O)...(H)



**Fig. 7.** Interactions between hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester and 1-anthranilate synthase

## Pharmacokinetic properties of compounds

QikProp predicts required physicochemical descriptors of any compounds based on the Lipinski's rule of 5, which is important for rational drug design. This rule assesses if a compound has pharmacological properties that would make it an effective drug, evaluating its drug likeness (Lipinski *et al.*, 1997; 2001). The molecular weight range for the compound is accepted within 130.0 to 500.0. The acceptable range for solvent volume is between 500.0 and 2000.0. The estimated h-bond donors (range: 0.0-6.0) and acceptors (range: 2-20) represent the h-bonds donated and accepted by the solute to and from water molecule. The pharmacological parameter QPlogPo/W estimates the absorption of the compound within the body and the acceptable range is -2.0 to 6.5. Oral human absorption below 25% is considered poor, while absorption above 80% is considered good (Tripathi *et al.*, 2012). Additionally, other pharmacological properties like solubility (QPlogS) and skin permeability (QPlogKp) were examined as reported by Shaikh and Siu (2016).

In this analysis, the molecular weights of all compounds were within the range of 141-500, except for bis[2-(1-hydroxy-1-methylethyl)-4-(1,1-dimethylethyl-6-(carbethoxy)phenyl] sulfide (574.771) and ketoconazole (531.438). Here, the molecular volumes of the GC-MS compound are within the normal range of values.

All compounds had acceptable h-bond donors. On the contrary, The h-bond acceptors were within the acceptable range for all the compounds but not satisfied by 2-tert-butyl-4-isopropyl-5-methylphenol, 1-methoxy - 2 - tert- butyl- 6- methylbenzene, 2 hexadecen-1-ol, 3, 7, 11, 15-tetramethyl-, [R-[R\*,R\*-(E)]] , 1docosanol,methyl ether, and 1-tetracosanol. The partition coefficient of octanol and water (QPlogPo/W) property was unsatisfactory for only 8 GC-MS compounds and these were not found to be interacted with targets.

All the GC-MS compounds were predicted to have low solubility, leading to low oral bioavailability. Comparatively, the budmunchiamine and ketoconazole were predicted with high solubility than GC-MS compounds. The obtained logK<sub>p</sub> value of all the compounds indicates low skin permeability. Here, 1-docosanol, methyl ether, and 1,4-(propane-1',3'-diyl)-2,3: 4,5-bis([b]quinoxalino)cyclohexane with the higher values than the other GC-MS compounds indicated that these compounds may be highly permeable to the skin. Overall, 25 GC-MS compounds had high human oral absorption percentages, while bis[2-(1-hydroxy-1-methylethyl)-4-(1,1-dimethylethyl-6-(carbethoxy)phenyl] sulfide and N-(2-methyl-2H-tetrazol-5-yl)-acetamide showed < 80% absorption. The percentage of human oral absorption for budmunchiamine and ketoconazole was less than 80%.

In the present analysis, the molecular weight of all the compounds were in the range of 141-500 except bis[2-(1-hydroxy-1-methylethyl)-4-(1,1-dimethylethyl-6-(carbethoxy) phenyl] sulfide and ketoconazole (574.771; 531.438). The h-bond donors were within the acceptable range for all the compounds. The h-bond acceptors were within the acceptable range for all the compounds but this property is not satisfied for 2-tert-butyl-4-isopropyl-5-methylphenol, 1-methoxy-2-tert-butyl-6-methylbenzene, 2-hexadecen-1-ol,3,7,11,15-tetramethyl-[R-[R\*,R\*-E]], 1-docosanol, methyl ether, and 1-tetracosanol. The partition coefficient of octanol and water (QPlogPo/W) property was not satisfied only for 8 GC-MS compounds. All the GC-MS compounds were predicted with low solubility which causes low oral bioavailability. Comparatively, the budmunchiamine and ketoconazole were predicted with high solubility than GC-MS compounds.

The obtained logK<sub>p</sub> value of all the compounds represents low skin permeability for the skin. Here, 1-docosanol, methyl ether, and 1,4-(propane-1',3'-diyl)-2,3: 4,5-bis([b]quinoxalino)cyclohexane with the higher values than the other GC-MS compounds indicated that these compounds may be highly permeable to the

skin. Overall, the percentage of human oral absorption of all the compounds was high for 25 GC-MS compounds and 2 GC-MS compounds namely bis[2-(1-hydroxy-1-methylethyl)-4-(1,1-dimethylethyl-6-(carbethoxy)phenyl] sulfide and N-(2-methyl-2H-tetrazol-5-yl)-acetamide showed < 80%. The percentage of human oral absorption was found >80% for ketoconazole but <80% for budmunchiamine (Table 10).

**Table 7:** Physicochemical properties of the selected compounds

S. N o.	Name of the Compounds	Molecul ar weight	Molecu lar volume	Hydr ogen donors	Hydro gen accepto rs	QPlo gPo/ W	QPlogS	QPlog Kp	Percent Human Oral Absorp tion
1	N-(2-Methyl-2H-tetrazol-5-yl)-acetamide	141.132	525.71	1	6	-0.759	-0.88	-4.133	68.712
2	2-tert-Butyl-4-isopropyl-5-methylphenol	206.327	821.633	1	0.75	3.79	-4.03	-1.705	100
3	E-15-Heptadecenal	252.439	1163.039	0	2	5.379	-6.117	-1.578	100
4	Tetradecanoic acid	228.374	1007.806	1	2	4.514	-4.71	-2.346	95.997
5	6,10,14-Trimethylpentadecan-2-one	268.482	1188.956	0	2	5.74	-6.314	-1.267	100
6	Hexadecanoic acid	256.428	1129.036	1	2	5.291	-5.604	-2.154	87.586
7	1-Methoxy-2-tert-butyl-6-methylbenzene	178.274	705.457	0	0.75	3.224	-3.541	-0.957	100
8	7,9-Di-tert-butyl-1-oxaspiro(4,5)dec a-6,9-diene-2,8-dione	276.375	977.272	0	5	2.738	-3.554	-2.651	100
9	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-[R-[R*,R*-E]]-	296.535	1292.888	1	1.7	6.33	-6.961	-1.164	100

10	1-Docosanol, methyl ether	340.632	1551.299	0	1.7	7.929	-12.503	3.197	100
11	Octadecanoic acid	284.481	1250.261	1	2	6.07	-6.504	-1.962	92.149
12	9,12,15- Octadecatrienoic acid	278.434	1124.849	1	2	5.339	-4.934	-2.051	87.849
13	Bis[2-(1-hydroxy- 1-methylethyl)-4- (1,1- dimethylethyl)-6- (carbethoxyphenyl)] sulfide	574.771	1695.561	2	9.5	5.19	-4.069	-2.182	60.727
14	1-Tetracosanol	354.658	1613.397	1	1.7	8.382	-9.2	-0.352	100
15	9- Octadecenamide	281.481	1240.551	2	2.5	4.391	-4.843	-2.116	100
16	3-Octadecenoic acid	335.572	1436.722	0	2.5	7.412	-8.202	-0.176	100
17	Hexadecanoic acid,2-hydroxy- 1- (hydroxymethyl) ethyl ester	330.507	1376.633	2	5.4	4.509	-5.845	-2.237	100
18	1,11- Bis(methoxycarbonyl- bonylethylene)- 10,2- dihydroxycyclo eicosane	480.684	1603.988	2	7.4	5.387	-3.847	-0.987	100
19	1-Docosanol, formate	354.615	1585.268	0	2	7.91	-9.451	-1.51	100
20	Budmunchiamine	452.766	1723.281	1	8.5	3.921	-0.831	-4.166	75.618
21	Ketoconazole	531.438	1549.446	0	8.25	4.373	-4.237	-1.48	92.165

## Conclusion

The chemical constituents identified from the chloroform extract of *A. amara* namely hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester appear to be potential candidates for investigating their anti-*Malassezia* effects. The docking studies clearly indicated its interaction with the active site of the target could be evaluated further for the effective treatment against *Malassezia globosa*.

## References

- Akilandeswari S., Senthamarai R., Valarmathi R., Savarinsha JA., and Selvan AT. Evaluation of anti-inflammatory and anti-arthritis activity of *Albizialebbeck* and *Albiziaamara* extracts. Biomed. 2009;4(3): 295-302.
- Archana BR., Beena PR., and Kumar S. Study of the distribution of *Malassezia* species in patients with pityriasisversicolor in Kolar region. Indian Journal of Dermatology. 2015; 60(3): 321.
- Arvind A., Jain V., Saravanan P., and Mohan CG. Uridine monophosphate kinase as potential target for tuberculosis: from target to lead identification, Interdisciplinary Sciences: Computational Life Sciences. 2013; 5(4): 296-311.
- Ashbee HR., and Evans EG. Immunology of diseases associated with *Malassezia* species. Clinical Microbiological Reviews. 2002; 15(1): 21-57.
- AyyanarM., and Ignacimuthu S. Medicinal plants used by the tribals of Tirunelveli hills, Tamil Nadu to treat poisonous bites and skin diseases. NOPR: Indian Journal of Traditional Knowledge. 2005; 4(3): 229-236.
- BaltazaryG., and Nshimo CM. *In vitro* antimicrobial activity of *Albiziaamaraleaves* from Lindi region, Tanzania. Tanzania Journal of Natural & Applied Sciences. 2010; 1(1): 35-42.
- Bhaskar BV., Babu TMC., and Rajendra W. Homology modeling and development of dihydrodipiconate reductase inhibitors of *Klebsiella pneumonia*: A computational approach. International Journal of Current Pharmaceutical Research. 2016; 8(3): 71-76.

- Bulloch EM., Jones MA., Parker EJ., Osborne AP., Stephens E., Davies GM., Identification of 4-amino-4-deoxychorimsate synthase as the molecular target for the antimicrobial action of, (6S)-6-fluoroshikimate. *J. Am. Chem. Soc.* 2004; 126: 9912-9913.
- Cafarchia C., and Otranto D. The pathogenesis of *Malassezia* yeasts. *Parassitologia*. 2008; 50(1):65-67.
- Chaudhary R., Singh S., Banerjee T., and Tilak R. Prevalence of different *Malassezia* species in *pityriasis versicolor* in central India. *Indian Journal of Dermatology, Venereology, Leprology*. 2010; 76(2): 159-64.
- CrespoEV., and Delgado VF. *Malassezia* species in skin diseases. *Current Opinion in Infectious Diseases*. 2002; 15(2): 133-142.
- Daisy P., NivedhaRP., and Bakiya RH. *In silico* drug designing approach for biotin protein ligase of *Mycobacterium tuberculosis*. *Asian Journal of Pharmaceutical and Clinical Research*. 2013; 6(1): 103-107.
- Dutta S., Bajaj AK., Basu S., and Dikshit A. Pityriasisversicolor: Socioeconomic and clinic-mycologic study in India. *International Journal of Dermatology*. 2002; 41(11): 823-824.
- Faergemann J., Ausma J., and Borgers M. In vitro activity of R126638 and ketoconazole against *Malassezia* species. *Acta Dermato-Venereologica*. 2006; 86(4): 312-315.
- Fernandes JD., Martho K., Tofik V., Vallim MA., and Pascon RC. The role of amino acid permeases and tryptophan biosynthesis in *Cryptococcus neoformans*. *PloS One*. 2015; 10(7): e0132369.
- Friesner RA., Banks JL., Murphy RB., Halgren TA., Klicic JJ., Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. *Journal of Medicinal Chemistry*. 2004; 47(7): 1739-1749.
- Furnham N., Holliday GL., de Beer TA., Jacobsen JO., Pearson WR., and Thornton JM. The catalytic site atlas 2.0: cataloguing catalytic sites and residues identified in enzymes. *Nucleic Acids Research*. 2014; 42: D485-489.

- Glatz M., Bosshard PP., Hoetzeneker W., and Schmid-Grendelmeier P. The role of *Malassezia* spp. in atopic dermatitis. *J. Clin. Med.* 2015; 4: 1217-1228.
- Grice EA., and Dawson TL. Host-microbe interactions: *Malassezia* and human skin. *Curr. Opin. Microbiol.* 2017; 40: 81-87.
- Gueho E., Boekhout T., Ashbee HR., Guillot J., Van Belkum A., Faergemann J. The role of *Malassezia* species in the ecology of human skin and as pathogens. *Med. Mycol.* 1998; 36: 220-229.
- Gunasekaran D., Sridhar J., Suryanarayanan V., Manimaran NC., and Singh SK. Molecular Modeling and structural analysis of nAChR variants uncovers the mechanism of resistance to snake toxins. *Journal of Biomolecular Structure and Dynamics.* 2016; 35(8): 1654-1671.
- Gupta AK., Kohli Y., Faergemann J., Summerbell RC. Epidemiology of *Malassezia* yeasts associated with *pityriasis versicolor* in Ontario, Canada. *Medical Mycology.* 2001; 39(2): 199-206.
- Harborne JB. A guide to modern techniques of plant analysis. In: *Phytochemical Methods*. Chapman and Hall Publications, New York, 2<sup>nd</sup> ed., 1984; 1-288.
- He Z., and Toney MD. Direct detection and kinetic analysis of covalent intermediate formation in the 4-amino-4-deoxychorismate synthase catalyzed reaction. *Biochemistry.* 2006; 45: 5019-5028.
- He Z., Stigers Lavoie KD., Bartlett PA., and Toney MD. Conservation of mechanism in three chorismate-utilizing enzymes. *J. Am. Chem. Soc.* 2004; 126: 2378-2385.
- Hemamalini G., Jithesh P., and Nirmala P. Phytochemical analysis of leaf extract of plant *Acacia nilotica* by GCMS method. *Advances in Biological Research.* 2013; 7(5): 141-144.
- Hosen MI., Tanmoy AM., Mahbuba DA., Salma U., Nazim M., Islam MT., and Akhteruzzaman S. Application of a subtractive genomics approach for *in silico* identification and characterization of novel drug targets in *Mycobacterium*

tuberculosis F11. Interdisciplinary Sciences: Computational Life Sciences. 2014; 6(1): 48-56.

- Ibrahim KS., Gurusubramanian G., Zothansanga., Yadav RP., Senthil Kumar N., Karutha Pandian S., Borah P., and Mohan S. Protein Sequence Analysis: In: *Bioinformatics-A Student Companion*. Springer, Singapore. 2017; 149-189.
- IndravathiG., and Pullaiah T. *In vitro* propagation studies of *Albiziaamara* (Roxb.) Boiv. African Journal of Plant Science. 2013; 7(1): 1-8.
- Jahan, FI., Md. Hasan RUI., Jahan R., Seraj S., Chowdary AR., Md. Islam T., Khatun Z., and Rahmatullah M. A comparison of medicinal plant usage by folk medicinal practitioners of two adjoining villages in Lalmonirhat district, Bangladesh. *American-Eurasian Journal of Sustainable Agriculture*. 2011; 5(1): 46-66.
- Junior MCS., Gonçalves PA., Taranto AG., Koblitz MGB., Góes-Neto A., Purification, characterization and structural determination of UDP-N-acetylglucosamine pyro phosphorylase produced by *Moniliophthora perniciosa*. *The Journal of the Brazilian Chemical Society*. 2011; 22(6): 1015:1023.
- Kalyani M., Bhuvaneshwari G., Narasimhalu AS., Shameem Banu M., Renu., and Jayakumar S. Characterization and *in-vitro* susceptibility of *Malassezia* species in *pityriasis versicolor* cases from a tertiary care centre. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. 2014;5(1): 585-592.
- Kanehisa M., Goto S., Kawashima S., and Nakaya A. The KEGG databases at GenomeNet. *Nucleic Acids Research*. 2002; 30(1): 42-46.
- Karim M., Tohami E., Rahman AF-E., and Hunida E. GC-MS analysis and biological activity of different fractions of Sudanese *Albizia amara* (Vohl)Benth. Roots. *International Journal of Advanced Research*. 2016; 4(8): 1806-1814.
- Kaur H., Modgil V., Chaudhary N., Mohan B., Taneja N. Computational Guided Drug Targets Identification against Extended-Spectrum Beta-Lactamase-Producing Multi-Drug

Resistant Uropathogenic *Escherichia coli*. *Biomedicines*. 2023; 11(7): 2028.

- Kaur M., Narang T., Bala M., Gupte S., Aggarwal P., and Manhas A. Study of the distribution of *Malassezia* species in patients with pityriasisversicolor and healthy individuals in tertiary care hospital, Punjab. *Indian Journal of Medical Microbiology*. 2013; 31(3): 270-274.
- Kavitha K., Usha MG., Murugesh., Chandrashekhar NR. Distribution of *Malassezia* species in patients with pityriasisversicolor and healthy individuals in south India. *Journal of Evidence Based Medicine and Healthcare*. 2016; 3(34): 1627-1631.
- Khor BY., TyeGJ., Lim TS., and Choong YS. General overview on structure prediction of twilight-zone proteins. *Theoretical Biology and Medical Modelling*. 2015; 12(15): 1-11.
- Kindo AJ., Sophia SK., Kalyani J., and Anandan S. Identification of *Malassezia* species. *Indian Journal of Medical Microbiology*. 2004; 22(3): 179-181.
- Kokate CK., Purohit AP., and Gokhale SB. *Pharmacognosy*. NiralinPrakashan, Pune, 13<sup>th</sup> edition. 2005; 109-113.
- Kokila K., DeepikaPriyadarshini S., and Sujatha V. Antioxidant, antibacterial and GC-MS analysis of *Albizia amara* leaves and seed extracts-A comparison. *Indo American Journal of Pharmaceutical Research*. 2014; 4(4): 1928-1938.
- Lin X., Xu S., Yang Y., Wu J., Wang H., Shen H., et al. Purification and characterization of anthranilate synthase component I (TrpE) from *Mycobacterium tuberculosis* H37Rv. *Protein Expr. Purific*. 2009; 64: 8-15.
- Lott JS. The tryptophan biosynthetic pathway is essential for *Mycobacterium tuberculosis* to cause disease. *BiochemSoc Trans*. 2020; 48: 2029-2037.
- Madureira MDC. Rediscovering traditional medicine. *Spore*. 2008; 136: 16-17.
- Mahendran R., Jeyabasker S, Manoharan S., Francis A., and Shah U. *In silico* metabolic pathway analysis and docking analysis of *Treponema pallidum* subs. *Pallidium nichols* for

potential drug targets. *Asian Journal of Pharmaceutical and Clinical Research.* 2017; 10(5): 261-264.

- MorolloAA., and Eck MJ. Structure of the cooperative allosteric anthranilate synthase from *Salmonella typhimurium*. *Nature Struc. Biol.* 2001; 8: 243-247.
- Naorem RS., Pangabam BD., Bora SS., Goswami G., Barooah M., Hazarika DJ., Fekete C. Identification of Putative Vaccine and Drug Targets against the Methicillin-Resistant *Staphylococcus aureus* by Reverse Vaccinology and Subtractive Genomics Approaches. *Molecules.* 2022; 27(7): 2083.
- Nisar B., Aeysha S., and SyedaLaila R. Comparison of medicinally important natural products versus synthetic drugs – a short commentary. *Natural Products Chemistry & Research.* 2017; 6:2
- Ohlstein EH., Jr. RuffoloRR., and Elliott JD. Drug discovery in the next millennium. *Annual Review of Pharmacology and Toxicology.* 2000; 40: 177-191.
- Okwu DE. Phytochemical, vitamins and mineral contents of two Nigerian medicinal plants. *International Journal of Molecular and Advance Sciences.* 2005; 1(4): 375-381.
- Parthasarathy A., Cross PJ., Dobson RCJ., Adams LE., Savka MA., and Hudson AO. A three-ring circus: Metabolism of the three proteogenic aromatic amino acids and their role in the health of plants and animals. *Front. Mol. Biosci.* 2018; 5:29.
- Prabhamanju M., GokulshankarS., Sharma NK., Babu K., and Chiranjeevi A. Anti-fungal activity of selected plant extracts against *Malassezia globosa*. *International Journal of Advanced Scientific and Technical Research.* 2012; 5(2): 162-168.
- Praveen P., Thippeswamy S., Mohana DC., and Manjunath K. Antimicrobial efficacy and phytochemical analysis of *Albizia amara* (Roxb.) Biov. An indigenous medicinal plant against some human and plant pathogenic bacteria and fungi. *Journal of Pharmacy Research.* 2011; 4(3): 832-835.
- Prohic A., JovovicSadikovic T., Krupalija-Fazlic M., and Kuskunovic-Vlahovljak S. *Malassezia* species in healthy skin

and in dermatological conditions. *Int. J. Dermatol.* 2016; 55: 494-504.

- Rahman MBA., Zulkifli MF., Murad AMA., Mahadi NM., Basri M., Rahmanand RNZA., Salleh AB. *Ab-initio* protein structure prediction of *Leucosporidium antarcticum* antifreeze proteins using I-TASSER simulations. *1<sup>st</sup> WSEAS International Conference on Biomedical Electronics and Biomedical Informatics (BEBI '08)*, Rhodes, Greece. 2008; 23-28.
- Rohini K., Srikumar PS., and Anbarasu K. Structural modeling of human desmocollin2 using I-TASSER methods, *International Conference on Biological and Life Sciences*. 2012; 40:150-153.
- Roy A., Kucukural A., and Zhang Y. I-TASSER: a unified platform for automated protein structure and function prediction. *Nature Protocol*. 2010; 5(4): 725-738.
- Saranraj P., Sakthi SS., and Geetha M. Pharmacological screening of *Datura metel* and *Acalypha indica* for its antifungal activity against pathogenic fungi. *International Journal of Pharmaceutical science and health care*. 2011; 2(1): 15-29.
- Schwartz JR., Cardin CW., and Dawson TL. Seborrheic dermatitis and Dandruff. In: *Textbook of Cosmetic Dermatology*, London: Martin Dunitz, Ltd. 2010; pp. 230-241.
- Shah A., Koticha A., Ubale M., Wanjare S., Mehta P., and Khopkar U. Identification and speciation of *Malassezia* in patients clinically suspected of having *Pityriasis versicolor*. *Indian Journal of Dermatology*. 2013; 58(3): 239.
- Shivakumar D., Williams J., Wu Y., Damm W., Shelley J., and Sherman W. Prediction of absolute solvation free energies using molecular dynamics free energy perturbation and the OPLS Force Field. *Journal of Chemical Theory and Computation*. 2010; 6(5): 1509-1519.
- Singh S., Singh DB., Singh A., Gautam B., Ram G., Dwivedi S., Ramteke PW. An approach for identification of novel drug targets in *Streptococcus pyogenes* SF370 through pathway analysis. *Interdisciplinary Sciences: Computational Life Sciences*. 2016; 8(4): 388-394.

- Smith DA., Parish T., Stoker NG., Bancroft GJ. Characterization of auxotrophic mutant of *Mycobacterium tuberculosis* and their potential as vaccine candidates. *Infect Immun.* 2001; 69: 1142-1150.
- Squire RA., and Goode K. A randomised, single-blind, single-centre clinical trial to evaluate comparative clinical efficacy of shampoos containing ciclopiroxolamine (1.5%) and salicylic acid (3%), or ketoconazole (2%, Nizoral) for the treatment of dandruff/seborrhoeic dermatitis. *The Journal of Dermatological Treatment.* 2002; 13(2): 51-60.
- Subhashini R., and Jeyam M. Computational identification of putative drug targets in *Malassezia globosa* by subtractive genomics and protein cluster network approach. *International Journal of Pharmacy and Pharmaceutical Sciences.* 2017; 9(9): 215-221.
- Subhashini R., and Jeyam M. Traditional medicinal plants used in the healing of skin related problems in Coimbatore district. *World Journal of Pharmaceutical Research.* 2013; 2(6); 2111-124.
- Tang X-F., Ezaki S., Atomi H., and Imanaka T. Anthranilate synthase without an LLES motif from a *hyperthermophilic carchaeon* is inhibited by tryptophan. *Biochem. Biophys. Res Comm.* 2001; 281: 858-865.
- Terstappen GC., and Reggiani A. *In silico* research in drug discovery. *Trends in Pharmacological Sciences.* 2001; 22(1): 23-26.
- Theelen B., Cafarchia C., Gaitanis G., Bassukas ID., Boekhout T., and Dawson, TL. *Malassezia* ecology, pathophysiology, and treatment. *Med. Mycol.* 2018; 56: S10-S25.
- Thomford NE., Senthebane DA., Rowe A., Munro D., Seele P., Maroyi A., Dzobo K. Natural Products for Drug Discovery in the 21st Century: Innovations for Novel Drug Discovery. *Int J Mol Sci.* 2018; 19(6):1578.
- Venkatramanan M., Sankar Ganesh P., Senthil R., Akshay J., Veera Ravi A., Langeswaran K., Vadivelu J., Nagarajan S., Rajendran K., and Shankar EM. Inhibition of Quorum Sensing and Biofilm Formation in *Chromobacterium violaceum* by Fruit

Extracts of *Passiflora edulis*. ACS Omega. 2020;5(40): 25605-25616.

- Webby CJ., Jiao W., Hutton RD., Blackmore NJ., Baker HM., Baker EN., Jameson GB., and Parker EJ. Synergistic allosteric regulatory network for the control of aromatic amino acid biosynthesis in *Mycobacterium tuberculosis*. The Journal of Biological Chemistry. 2010; 285(40): 30567-30576.
- Wellington S., Nag PP., Michalska K. A small-molecule allosteric inhibitors of *Mycobacterium tuberculosis* tryptophan synthase. Nat Chem Biol. 2017; 13: 943-950.

## Web References

<https://www.ncbi.nlm.nih.gov>

<http://blast.ncbi.nlm.nih.gov>

<http://web.expasy.org/protparam>

<https://zhanglab.ccmb.med.umich.edu/I-TASSER>

<http://www.ebi.ac.uk/thornton-srv/databases/pdbsum/Generate.html>

<https://pubchem.ncbi.nlm.nih.gov>

## Comprehensive Review of PCOS: Genetic Roots, Gut Health, and Cutting-Edge Treatments in the Indian Context

Sreesaila N P<sup>1</sup>, Dr Nimmi O S<sup>2</sup>

<sup>1</sup>Research Scholar, Department of Biotechnology,

<sup>2</sup>Assistant Professor, Department of Biotechnology,  
Nehru Arts and Science College, Coimbatore.

### Abstract

Polycystic Ovary Syndrome (PCOS) is a multifactorial endocrine disorder affecting women of reproductive age, characterized by hyperandrogenism, ovulatory dysfunction, and polycystic ovaries and this disorder is surprisingly increasing among Indian women. This chapter explores the intricate genetic, microbiological, and phytopharmaceutical dimensions of PCOS and recent advancements in the field. Genetic studies have identified several key genes, including AR, FSHR, INS, DENND1A, and THADA, contributing to the syndrome's pathophysiology. The gut microbiota's role is increasingly recognized, with dysbiosis linked to systemic inflammation and insulin resistance, exacerbating PCOS symptoms. Phytopharmaceuticals, such as inositol, cinnamon, spearmint, and curcumin, offer promising therapeutic benefits, enhancing insulin sensitivity and reducing androgen levels. Recent research highlights novel biomarkers like Anti-Müllerian Hormone (AMH) for early diagnosis and innovative therapies beyond metformin, such as GLP-1 receptor agonists. Comprehensive lifestyle interventions, including diet, exercise, and behavioral therapy, are essential for effective management. Advancements in artificial intelligence are poised to revolutionize PCOS diagnosis and personalized treatment plans. This chapter underscores the importance of an integrated approach to understanding and managing PCOS, paving the way for improved outcomes and quality of life for affected women.

**Keywords:** PCOS, FSHR, AMH, Microbiota, Dysbiosis

## Introduction

PCOS is a prevalent condition characterized by hyperandrogenism, ovulatory dysfunction, and polycystic ovaries. It is closely linked with metabolic syndromes such as insulin resistance and obesity. While the exact etiology of PCOS remains unclear, genetic, environmental, and lifestyle factors are all implicated. Polycystic Ovary Syndrome (PCOS) affects a significant number of Indian women, presenting unique challenges in diagnosis and management within the cultural and healthcare context of India. Studies indicate a high prevalence of PCOS among Indian women, attributed to genetic predispositions and lifestyle factors such as diet and sedentary habits. The syndrome's manifestations, including irregular menstrual cycles, hirsutism, and infertility, can have profound socio-cultural implications in a society where fertility and marital expectations often intersect. Access to healthcare and awareness about PCOS remain variable across different regions, impacting timely diagnosis and treatment initiation. Additionally, cultural stigmas surrounding reproductive health may hinder open discussions and proactive management of PCOS symptoms. Addressing these complexities requires tailored healthcare approaches that integrate genetic insights, lifestyle modifications, and culturally sensitive interventions to improve outcomes and quality of life for Indian women affected by PCOS.

## Literature Review

Polycystic Ovary Syndrome (PCOS) is a complex endocrine disorder affecting up to 10% of women of reproductive age worldwide (Azziz *et al.*, 2016). It is characterized by hyperandrogenism, menstrual irregularities, and polycystic ovaries, often accompanied by insulin resistance and metabolic disturbances (Teede *et al.*, 2010). Genetic predisposition plays a significant role in the pathogenesis of PCOS. Family and twin studies have demonstrated a heritable component, with estimates of heritability ranging from 40% to 70% (Day *et al.*, 2018). Genome-wide association studies (GWAS) have identified several

susceptibility loci associated with PCOS, including but not limited to the genes encoding the androgen receptor (AR), follicle-stimulating hormone receptor (FSHR), and insulin signaling pathway genes such as INS and IRS (Day *et al.*, 2018; Hayes *et al.*, 2015).

AR gene variants, such as the CAG repeat polymorphism, have been linked to increased androgen levels and the severity of hyperandrogenism in PCOS (Xita *et al.*, 2008). Additionally, polymorphisms in FSHR have been associated with alterations in follicular development and ovulatory dysfunction, contributing to the phenotype of PCOS (Wang *et al.*, 2012).

Recent research has focused on the role of gut microbiota in the pathogenesis of PCOS. Dysbiosis, characterized by alterations in microbial composition and diversity, has been observed in women with PCOS compared to healthy controls (Torres *et al.*, 2019). Specific changes include an increase in *Bacteroides* and a decrease in beneficial bacteria like *Bifidobacterium*, which are associated with metabolic disorders and inflammation (Lindheim *et al.*, 2016). The gut microbiota influences PCOS through various mechanisms, including modulation of insulin sensitivity, inflammation, and sex hormone metabolism (Torres *et al.*, 2019). Short-chain fatty acids (SCFAs), produced by gut bacteria from dietary fiber, play a crucial role in regulating glucose metabolism and insulin sensitivity (Torres *et al.*, 2019).

Phytopharmaceuticals derived from plants offer potential therapeutic options for managing PCOS symptoms. Myo-inositol and D-chiro-inositol, two naturally occurring inositols, have been extensively studied for their ability to improve insulin sensitivity and ovarian function in women with PCOS (Pizzo *et al.*, 2014). In a randomized controlled trial, myo-inositol supplementation was shown to restore spontaneous ovulation and improve metabolic parameters in women with PCOS (Unfer *et al.*, 2012). Other phytopharmaceuticals such as cinnamon, spearmint, and curcumin have demonstrated promising effects in reducing insulin

resistance, lowering androgen levels, and improving menstrual regularity in women with PCOS (Kort and Lobo, 2014; Grant *et al.*, 2012; Arentz *et al.*, 2014).

Recent advancements in PCOS research encompass novel diagnostic markers, therapeutic strategies, and technological innovations. Anti-Müllerian hormone (AMH) has emerged as a reliable biomarker for ovarian reserve and PCOS diagnosis (Dewailly *et al.*, 2014). Innovations in therapeutic approaches include the investigation of GLP-1 receptor agonists and sodium-glucose cotransporter-2 (SGLT2) inhibitors for managing insulin resistance and metabolic dysfunction in PCOS (Pasquali and Pelusi, 2020).

## Genetics of PCOS

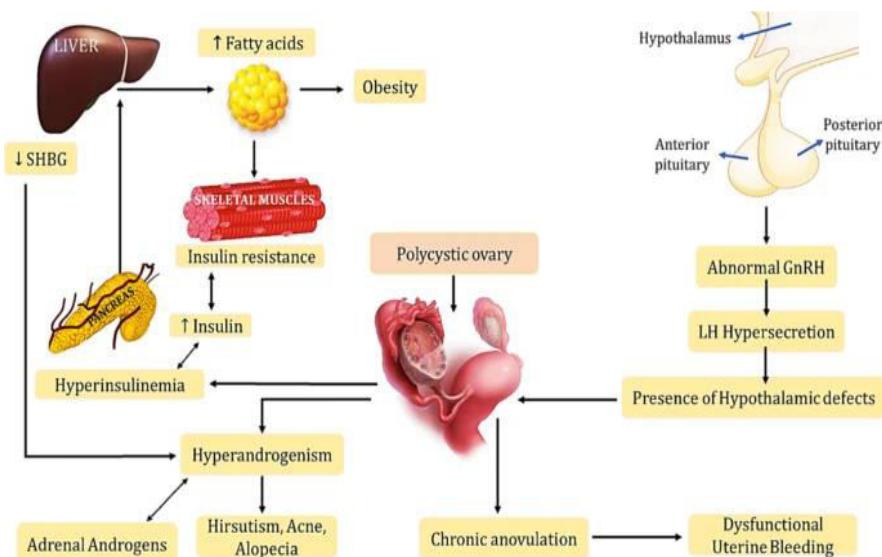
Genetic predisposition plays a crucial role in PCOS, with familial clustering and twin studies underscoring its heritability. Key genetic components include:

- **Androgen Receptor (AR) Gene:** Variants in the AR gene are associated with increased androgen levels.
- **Follicle-Stimulating Hormone Receptor (FSHR) Gene:** Polymorphisms affect follicular development and ovulation.
- **Insulin Gene (INS) and Insulin Receptor Substrate (IRS):** These genes contribute to insulin resistance, a hallmark of PCOS.
- **Genome-Wide Association Studies (GWAS):** GWAS have identified multiple loci linked to PCOS, such as DENND1A, THADA, and LHCGR.

Recent research has identified over 150 susceptibility loci associated with PCOS, highlighting the complex genetic landscape. Variants in genes related to steroidogenesis, gonadotropin signaling, and metabolic pathways contribute to PCOS pathophysiology. Additionally, epigenetic modifications, such as DNA methylation and histone modifications, play a significant role in regulating these genes, affecting their expression and function.

**Table 1:** Key Genetic Polymorphisms Associated with PCOS

Gene	Polymorphism	Effect
AR	CAG repeat length	Hyperandrogenism
FSHR	Ser680Asn	Altered follicular response
INS	VNTR	Insulin resistance
DENND1A	rs2479106	Follicular development and ovulation
THADA	rs13429458	Metabolic and reproductive functions

**Figure 1:** Genetic Pathways Involved in PCOS

Implications of Polycystic ovary syndrome in women's life. Polycystic ovary syndrome (PCOS) can significantly impact women's health. It involves excessive release of gonadotropin-releasing hormone (GnRH) from the brain, leading to elevated androgen levels and ovarian cysts due to increased luteinizing hormone (LH) secretion. Additionally, reduced sex hormone-

binding globulin (SHBG) and increased adrenal androgens can also contribute to hyperandrogenism in PCOS. Abbreviations: LH, luteinizing hormone; GnRH, gonadotropin-releasing hormone; PCOS, polycystic ovary syndrome, SHBG; sex hormone-binding globulin.

## Gut Microbiota and PCOS

The gut microbiota has been increasingly recognized for its role in PCOS pathophysiology. Dysbiosis can influence metabolic and inflammatory pathways, exacerbating PCOS symptoms.

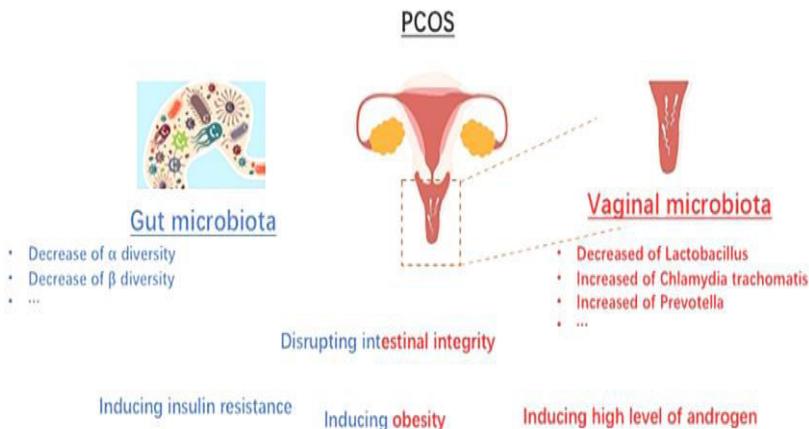
- **Gut-Brain Axis:** Interactions between the gut microbiota and the central nervous system can affect hormonal and metabolic regulation.
- **Inflammation and Insulin Resistance:** Altered gut microbiota composition can promote systemic inflammation and insulin resistance.
- **Short-Chain Fatty Acids (SCFAs):** SCFAs produced by gut bacteria influence glucose metabolism and insulin sensitivity.

It is important to note that various types of microbes in the human body play a role in maintaining balance, especially in regulating the immune system. On one hand, with increasing research on the role of gut microbes in health, we are gaining a better understanding of their interactions with the immune system. Researchers have found that the gut microbiota primarily influences the intestinal immune system. Among the components of the intestinal immune system, myeloid cells are considered the first responders, and several studies have suggested that gut microbiota have an impact on intestinal macrophages.

Regulating the gut-brain axis

Inducing chronic inflammatory state

Changing immune homeostasis

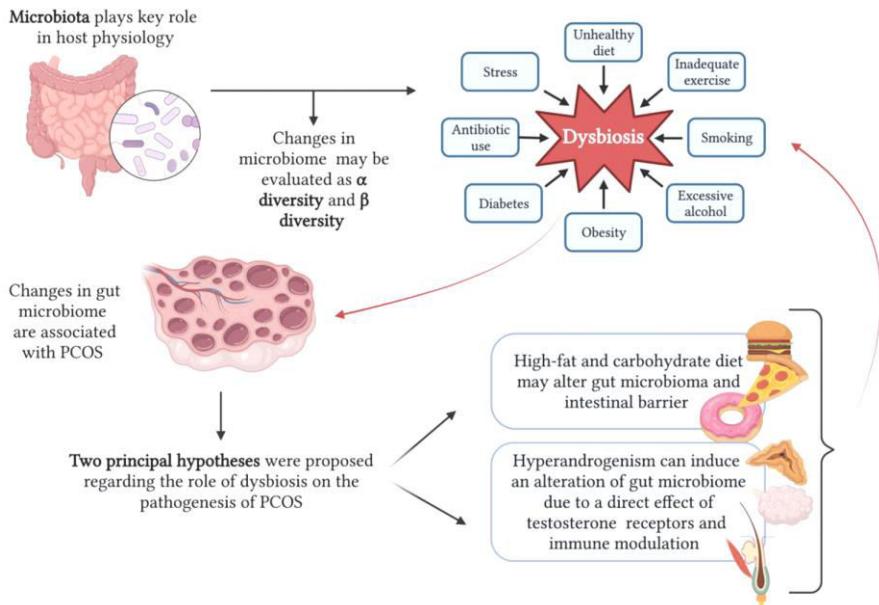


**Figure 2: Gut Microbiota Composition in PCOS**

Studies have shown that germ-free animals exhibit various immune deficiencies in the intestines, such as underdeveloped gut-associated lymphoid tissues, reduced numbers of gut-associated Th17 cells, lower counts of IgA-producing B cells, and fewer intraepithelial CD8+ T cells. Increasing evidence suggests that the immune system impacted by the vaginal microbiota is linked to diseases in the vaginal area.

The vaginal bacteria community may be influenced by the gut microbiota due to the proximity of the female genital tract to the intestine. Additionally, the vaginal microbiota is regulated by chemical changes and hormonal fluctuations. Its main role is to prevent infections and maintain an immuno-tolerant environment. Disruption of Lactobacillus dominance can alter immune homeostasis, causing the production of pro-inflammatory cytokines and abnormal immune cell recruitment.

Polycystic Ovary Syndrome (PCOS) is increasingly recognized as not solely a hormonal disorder but also involving dysregulation of the gut microbiota, termed microbiota dysbiosis.



**Figure 3:** Pathways Linking Gut Microbiota and PCOS

Emerging research suggests that women with PCOS exhibit alterations in the composition and diversity of their gut microbiota compared to healthy individuals. Dysbiosis in PCOS is characterized by reduced microbial diversity and an imbalance in specific bacterial taxa, notably an increase in *Firmicutes* and a decrease in *Bacteroidetes*. These changes contribute to metabolic disturbances and chronic low-grade inflammation seen in PCOS, exacerbating insulin resistance and hormonal imbalances.

The gut microbiota influences host metabolism through various mechanisms, including fermentation of dietary fibers to produce short-chain fatty acids (SCFAs), which regulate glucose and lipid metabolism. Moreover, dysbiosis-induced alterations in gut permeability may lead to increased circulating levels of

lipopolysaccharides (LPS), triggering systemic inflammation and further impairing insulin sensitivity. Understanding the intricate relationship between PCOS and gut microbiota dysbiosis opens avenues for novel therapeutic strategies targeting the gut microbiome to alleviate metabolic and reproductive symptoms associated with PCOS.

**Table 1:** Examples of how different steroids interact with gut microbiota Steroids Modulation of steroids by gut microbiota

Steroids	Modulation of steroids by gut microbiota	Effect of steroids on gut microbiota
<b>Sex hormones</b>	<ul style="list-style-type: none"> <li>- Deconjugation by enzymes including sulfatase and glucuronidases</li> <li>- Biotransformation of unconjugated hormones</li> </ul>	<ul style="list-style-type: none"> <li>- Enhances intestinal alkaline phosphatase (IAP) activity</li> <li>- Facilitates intraluminal transport of IgA which neutralizes pathogenic bacteria</li> <li>- Regulates plasma immunoglobulin levels and B cell function</li> </ul>
<b>Corticosteroids</b>	<ul style="list-style-type: none"> <li>- Regulate intestinal corticosteroid production in response to stress</li> <li>- Biotransform into 21-dehydroxylated products or androgens</li> <li>- Influence GR activity</li> </ul>	<ul style="list-style-type: none"> <li>- Mediate the effects of stress through modulation of the microbiome</li> <li>- Modulate the oral microbiome in a metatranscriptomic study</li> <li>- Modulate infant intestinal microbiota composition with altered maternal cortisol level</li> </ul>
<b>Bile acids</b>	<ul style="list-style-type: none"> <li>- Deconjugates by bile salt hydrolase (BSH)</li> <li>- Conversion of primary into secondary bile acids by 7- dehydroxylation, 12<math>\alpha</math>-dehydrogenation and desulfation</li> </ul>	<ul style="list-style-type: none"> <li>- Exhibit antimicrobial and cytotoxic properties by inducing membrane damage or through FXR</li> <li>- Regulate gut microbiota composition through FXR</li> </ul>

Vitamin D3	<ul style="list-style-type: none"> <li>- Increase 25-hydroxyvitamin D and biosynthesis of 7-dehydrocholesterol by probiotics and prebiotics respectively</li> <li>- Upregulates VDR expression by probiotics</li> <li>- Hydroxylate and activate vitamin D3 by cytochrome enzymes</li> </ul>	<ul style="list-style-type: none"> <li>- Increase gut microbial diversity and Akkermansia abundance</li> <li>- Elevate tight junction protein expression and improves intestinal barrier function</li> <li>- Enhance the production of antimicrobial peptides</li> </ul>
------------	--	--

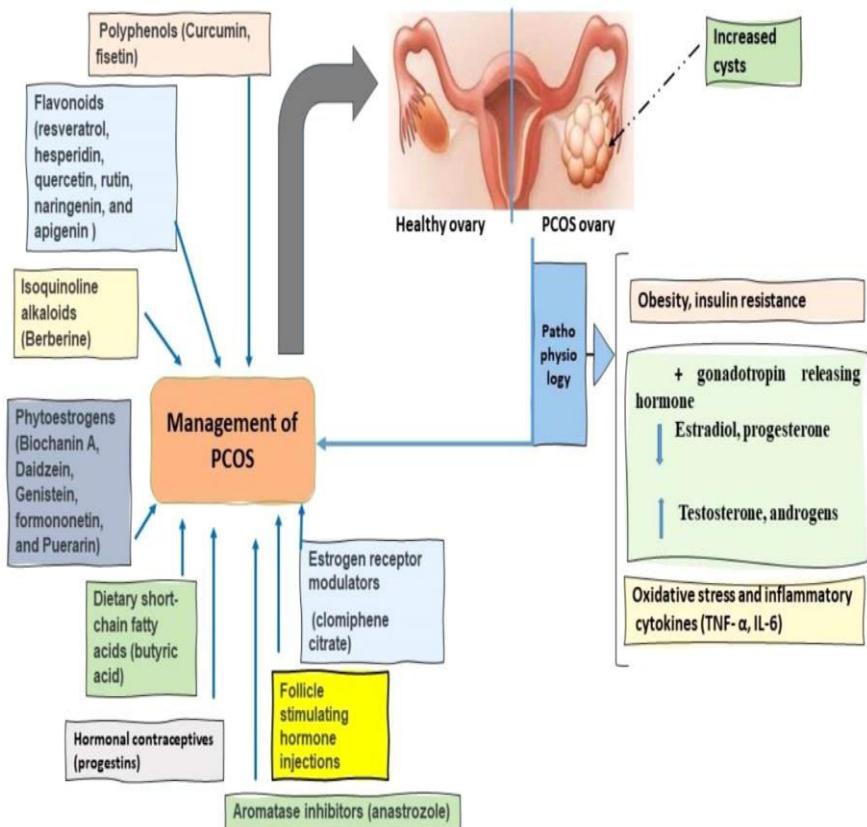
## Phytopharmaceutical Studies in PCOS

Phytopharmaceuticals, derived from medicinal plants, offer promising therapeutic options for PCOS. Key compounds include:

- **Inositol:** Myo-inositol and D-chiro-inositol enhance insulin sensitivity and improve ovulatory function.
- **Cinnamon:** Reduces insulin resistance and improves menstrual regularity.
- **Spearmint:** Exhibits anti-androgenic properties, reducing hirsutism and improving hormonal profiles.
- **Curcumin:** Anti-inflammatory and antioxidant effects help alleviate metabolic and reproductive symptoms.

**Table 2:** Phytopharmaceuticals and their Effects on PCOS Symptoms

Phytopharmaceutical	Active compound	Effects
Inositol	Myo – inositol	Improves insulin sensitivity and ovulation
Cinnamon	Cinnamaldehyde	Reduces insulin resistance, and improves cycles.
Spearmint	Menthol	Decreases androgen levels, reduces hirsutism
Curcumin	Curcuminoids	Anti-inflammatory, antioxidant effect.



**Figure 4:** Phytopharmaceuticals and their Mechanisms of Action

Certain isolated phytochemicals like curcumin, berberine, rutin, resveratrol, quercetin, hesperidin, and others have potential to treat PCOS. Additionally, natural compounds such as *Vitex agnus-castus*, *Cinnamomum cassia*, *pomegranate juice*, and *spearmint tea* are effective in treating symptoms in PCOS patients. These phytochemicals and herbal drug formulations may be used as alternatives to metformin and clomiphene citrate for managing PCOS, either alone or alongside conventional therapy. Further research is needed to determine the appropriate dosage and duration of therapy and to address safety, quality, and efficacy concerns.

## Recent Advancements in PCOS Research

Recent research in PCOS focuses on novel diagnostic markers and therapeutic approaches.

- **Anti-Müllerian Hormone (AMH):** Elevated AMH levels are a reliable marker for PCOS, aiding in early diagnosis.
- **Innovative Therapies:** Beyond metformin, new agents like GLP-1 receptor agonists are being explored for their efficacy in managing insulin resistance.
- **Lifestyle Interventions:** Comprehensive lifestyle changes including diet, exercise, and weight management are pivotal in PCOS treatment.
- **Artificial Intelligence (AI):** AI is being utilized to develop predictive models for PCOS diagnosis and personalized treatment plans.

Advancements in understanding the molecular mechanisms underlying PCOS have led to the identification of novel therapeutic targets. For instance, research on the role of microRNAs in PCOS has opened new avenues for potential treatments.

Additionally, advancements in omics technologies, such as genomics, proteomics, and metabolomics, are providing deeper insights into the complex interplay of genetic and environmental factors in PCOS.

## Lifestyle Interventions and Patient Management

Lifestyle interventions play a crucial role in the management of PCOS. A multidisciplinary approach, including dietary modifications, physical activity, and behavioral therapy, can significantly improve metabolic and reproductive outcomes.

- **Dietary Modifications:** A low glycemic index (GI) diet, rich in fiber and whole foods, can help manage insulin resistance and reduce inflammation. Specific dietary

patterns, such as the Mediterranean diet, have shown beneficial effects in PCOS management.

- **Physical Activity:** Regular physical exercise improves insulin sensitivity, aids in weight management, and enhances overall well-being. Both aerobic exercises and resistance training are recommended.
- **Behavioral Therapy:** Addressing psychological aspects, such as stress and anxiety, is essential. Cognitive-behavioral therapy (CBT) and stress management techniques can improve quality of life and adherence to lifestyle changes.

**Table 3:** Lifestyle Interventions for PCOS Management

Intervention	Description	Benefits
Low GI Diet	Diet low in refined carbs and sugars	Improves insulin sensitivity, reduces weight
Mediterranean Diet	Rich in fruits, vegetables, and fish	Anti-inflammatory, improves metabolic health
Aerobic Exercise	Cardiovascular activities	Enhances insulin sensitivity, weight loss
Resistance Training	Weight lifting, resistance bands	Increases muscle mass, boosts metabolism
Cognitive Behavioral Therapy (CBT)	Psychological counseling	Reduces stress, improves mental health

Lifestyle interventions play a crucial role in the management of Polycystic Ovary Syndrome (PCOS), addressing both its metabolic and psychological aspects. A low glycemic index (GI) diet, focused on reducing refined carbohydrates and sugars, improves insulin sensitivity and aids in weight management, which are pivotal in mitigating the insulin resistance commonly observed in PCOS. The Mediterranean diet, abundant in fruits, vegetables, and fish rich in omega-3 fatty acids, not only supports metabolic health but also exerts anti-inflammatory effects, potentially reducing chronic inflammation associated with PCOS.

Regular aerobic exercise, such as brisk walking or cycling, enhances insulin sensitivity and promotes weight loss, which can help regulate menstrual cycles and improve fertility outcomes. Complementing aerobic exercise, resistance training with weights or resistance bands stimulates muscle growth, increases metabolic rate, and aids in weight control. Additionally, cognitive behavioral therapy (CBT) offers essential psychological support, helping women manage stress, enhance coping mechanisms, and improve overall mental well-being amidst the challenges of living with PCOS. Together, these lifestyle interventions form a comprehensive approach to PCOS management, addressing its multifaceted nature and empowering individuals to take proactive steps towards better health outcomes.

## Conclusion

PCOS is a multifaceted disorder influenced by genetic, microbiotic, and environmental factors. Advances in understanding its pathophysiology and exploring phytopharmaceutical treatments hold promise for improved management strategies. Ongoing research into genetics, gut microbiota, and novel therapeutic approaches will be crucial in enhancing outcomes for women with PCOS. Comprehensive lifestyle interventions, including diet, exercise, and behavioral therapy, are vital components of effective patient management.

## References

- Arentz S, Abbott JA, Smith CA. Combined lifestyle and herbal medicine in overweight women with polycystic ovary syndrome (PCOS): a randomized controlled trial. *Phytother Res.* 2017; 31(9):1330-1340.
- Azziz R, Carmina E, Chen Z. Polycystic ovary syndrome. *Nat Rev Dis Primers.* 2016; 2:16057.
- Day FR, Hinds DA, Tung JY. Causal mechanisms and balancing selection inferred from genetic associations with polycystic ovary syndrome. *Nat Commun.* 2018; 9(1):222.

- Dewailly D, Andersen CY, Balen A. The physiology and clinical utility of anti-Müllerian hormone in women. *Hum Reprod Update*. 2014;20(3):370-385.
- Goodarzi, M. O., Dumesic, D. A., Chazenbalk, G., & Azziz, R. (2011). Polycystic ovary syndrome: Etiology, pathogenesis and diagnosis. *Nature Reviews Endocrinology*, 7(4), 219-231. <https://doi.org/10.1038/nrendo.2010.217>.
- Grant P, Ramasamy S. An open-label randomized controlled trial examining the effect of curcumin on hepatic insulin resistance and the expression of proinflammatory cytokines in overweight/obese individuals with type 2 diabetes and nonalcoholic fatty liver disease. *J Clin Endocrinol Metab*. 2012; 97(6):2058-2069.
- Kort DH, Lobo RA. Preliminary evidence that cinnamon improves menstrual cyclicity in women with polycystic ovary syndrome: a randomized controlled trial. *Am J Obstet Gynecol*. 2014; 211(5):487.e1-6.
- Legro, R. S., Arslanian, S. A., Ehrmann, D. A. (2013). Diagnosis and treatment of polycystic ovary syndrome: An Endocrine Society clinical practice guideline. *Journal of Clinical Endocrinology & Metabolism*, 98, (12), 4565-4592. <https://doi.org/10.1210/jc.2013-2350>.
- Lindheim L, Bashir M, Münzker J. Alterations in gut microbiome composition and barrier function are associated with reproductive and metabolic defects in women with polycystic ovary syndrome (PCOS): a pilot study. *PLoS One*. 2016; 11(4):e0153035.
- March, W. A., Moore, V. M., Willson, K. J., et al. (2010). The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. *Human Reproduction*, 25, (2), 544-551. <https://doi.org/10.1093/humrep/dep399>.
- Pasquali R, Pelusi C. Gynecological aspects of metabolic syndrome. *Nat Rev Endocrinol*. 2020; 16(3):139-150.
- Pizzo A, Laganà AS, Barbaro L. Comparison between effects of myo-inositol and D-chiro-inositol on ovarian function and

metabolic factors in women with PCOS. *Gynecol Endocrinol.* 2014; 30(3):205-208.

- Shi, Y., Zhao, H., Shi, Y. (2012). Genome-wide association study identifies eight new risk loci for polycystic ovary syndrome. *Nature Genetics*, 44,(9), 1020-1025. <https://doi.org/10.1038/ng.2384>
- Teede HJ, Misso ML, Costello MF. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. *Hum Reprod.* 2018; 33(9):1602-1618.
- Torres PJ, Siakowska M, Banaszewska B. Gut microbial diversity in women with polycystic ovary syndrome correlates with hyperandrogenism. *J Clin Endocrinol Metab.* 2018; 103(4):1502-1511.
- Tremellen, K., & Pearce, K. (2012). Dysbiosis of gut microbiota (DOGMA) - A novel theory for the development of polycystic ovarian syndrome. *Medical Hypotheses*, 79,(1), 1-5. <https://doi.org/10.1016/j.mehy.2012.04.016>.
- Unfer V, Carlomagno G, Papaleo E. Hyperinsulinemia and PCOS: role of myo-inositol and D-chiro-inositol. *Climacteric.* 2012; 15(1):72-76.

## Cutting-Edge Nano Herbal Medicines, Phytochemical Products, Innovations and Applications

**Dr. K. Kavithaa<sup>1\*</sup>, Dr. P. Senthilkumar<sup>2</sup> and Aadersh Shibu<sup>3</sup>**

<sup>1</sup>Assistant Professor, Department of Biotechnology,  
Hindusthan College of Arts & Science, Coimbatore-28.

<sup>2</sup>Associate Professor & Head (i/c), Department of Biotechnology,  
Hindusthan College of Arts & Science, Coimbatore-28.

<sup>3</sup>Department of Biotechnology,  
Hindusthan College of Arts & Science, Coimbatore-28.  
Corresponding Author Email id: k.kavithaa@hicas.ac.in

### Abstract

Herbal remedies were used by generations all over the globe; in particular, they are highly sought after in India. Herbal treatments are an affordable substitute for traditional medical care. They have made a substantial contribution to rural livelihoods, and many people engage in the gathering and exchange in the gathering and exchange of medicinal plants in addition to traditional healers who use herbal remedies. Due to its capacity to treat a variety of illnesses with fewer adverse effects, the demand for herbal medicines has grown. The increased health benefits of phytochemical products such as flavonoids, phenolic acids, carotenoids, and fatty acids have led to a global increase in their popularity. They have distinct chemical compositions that are linked to their varied physicochemical qualities and various uses in food industries. It is crucial to establish a novel drug delivery system (NDDS) to overcome several obstacles, including low bioavailability, in vivo stability, aqueous insolubility, intestinal absorption, and non-specific sites of activity. Herbal medications have a greater chance of treating chronic illnesses like cancer and other debilitating diseases when Nanoscience is incorporated into traditional medicine as an NDDS. Depending on the characteristics

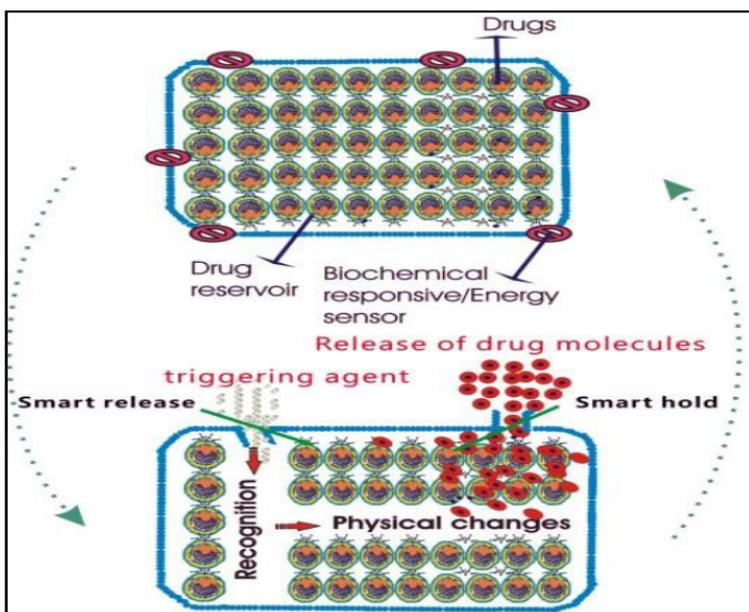
of the nanoparticles, new approaches such as polymer, magnetic, and metallic nanoparticle synthesis can be used to produce the desired results. With the use of cutting-edge technologies, it will be possible to characterize nanoparticles and ascertain the toxicity profiles associated with their chemical and physical characteristics. The synthesis of nanoparticles and the potential impact of nanotechnology on intelligent herbal drugs, the uses of phytochemical products, and their application will be briefly covered in this chapter.

**Keywords:** *Herbal medicine, NDDS- Novel Drug Delivery System, Phytochemical Products, Nanotechnology*

## Introduction

Ayurveda is a traditional medicinal discipline practised in India. [1]. Because of its potential therapeutic effect and reduced drawbacks when set side by side to other medications, herbal medicines have gained recognition from doctors and patients. In addition, they increase the medicine's bioavailability [2]. Herbal remedies were long disregarded for the creation of innovative formulations because they lacked scientific support and were difficult to process. The experimental needs of herbal medicines in developing novel drug delivery systems, something like solid dispersion, liposomes, microemulsions, nanoparticles, and solid lipid nanoparticles, can be met by contemporary phytopharmaceutical research. The fabrication of innovative drug delivery methods for herbal formulations is extremely difficult due to the intricacy of the active ingredients. Because, most standard dosage forms only allow a particular quantity of the allocated dose to reach the intended site and most drugs are diffused throughout the body based on their physicochemical and biochemical properties, they have low therapeutic value [3]. Targeted drug delivery, lowers frequency of dosage, decreases elimination while boosting solubility and adsorption. As Target based drug delivery is an important component of the novel drug delivery system (NDDS) for herbal medicines.

Nanoparticles are regarded as one of the more significant NDDS. By using nanoparticles to target specific organs, herbal medicines can be administered more effectively, safely, and with more targeted drug delivery. Fig 1, to put it more clearly nanotechnology is the architecting and production of compounds at the molecular and atomic level. Keeping in note of the size restriction, structures as small as several hundred nm are commonly called nanotechnology. It is the application and control of matter on a microscopic level. Atoms and molecules behave differently at this size and produce a range of intriguing and surprising outcomes. Studies on nanotechnology and nano-science have proliferated in several product domains in recent years. It offers chances for the innovation and productivity of materials, particularly those for pharmacological aspects, where traditional methods might run out of steam [4]



**Fig 1:** Target site-based release of drug molecules. [5]

## 1.1. Novel drug delivery system for herbal treatment:

Because of its many uses for people, NDDS is intended in addressing the shortcoming of the conventional herbal medication arrangement.

- By directing herbal medicines to specific organs, nanoparticles can enhance drug delivery, safety, efficacy, selectivity, solubility, and frequency of dosage.
- Herbal medicines can be targeted with nanoparticles to a specific organ, improving its solubility, safety, efficacy, selectivity, and frequency of dosage.
- Drugs are delivered in nanoparticle size, which increases their surface area and speeds up their absorption into the bloodstream.
- Decrease in toxicity without compromising therapeutic benefits.
- The improved penetration and stability of NPs passing through Blood-Brain Barrier (BBB).

## 1.2. Phytochemical products

Fruits such as citrus fruits are familiar foods for individual health because of their exceptionally high concentrations of nutritional components and phytochemicals that promote health, such as fatty acids, pectin, carotenoids, and especially polyphenols [6].

The potential health-promoting functions of citrus fruits, with their high antioxidant capacity and associated phytochemicals and nutrients, has garnered more notice in recent times, coinciding with the community's growing attention in plant antioxidants. Numerous epidemiological studies findings showed a link between eating citrus fruit and a lower risk of developing chronic illnesses like diabetes, heart disease, and cancer [7,8].



**Fig 2:** Phenotype and Genotype of the major citrus varieties and hybrid [9]

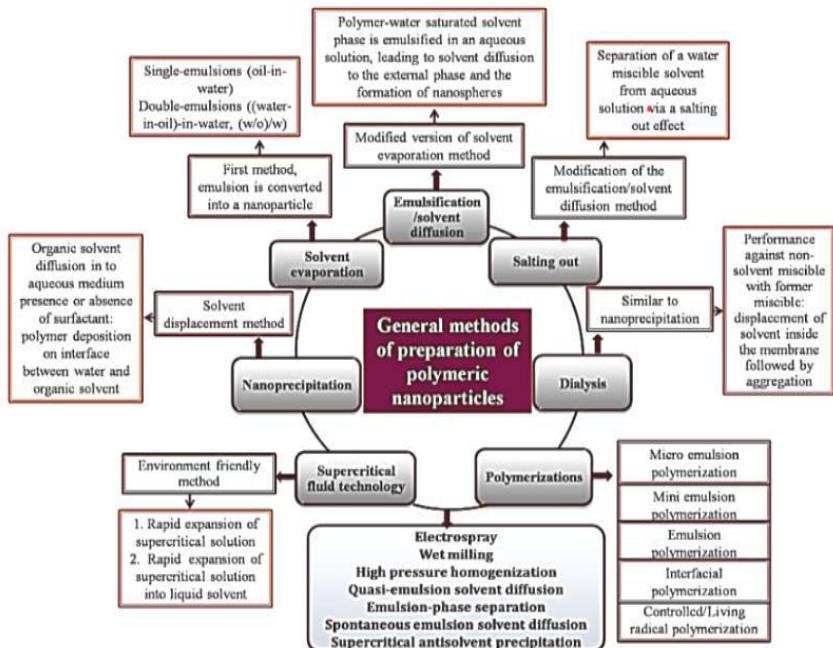
Furthermore, it has been discovered that citrus fruits and their byproducts contain some medicinal elements with pharmacological effects. In particular, citrus peel and pulp have been shown to have anti-pathologic result on treating sore throats, coughs, earaches, and puking; The process of steam-distillation was implemented to obtain the extracts of Citrus fruits which can be used as sedatives and tonics. Results shown to have high free radical scavenging and anti- Fungal activity of certain phytochemicals present in essential oil derived from peels of citrus fruits [10,11].

## Methods

### 2.1 Methods for Nanoparticles Synthesis:

#### 2.1.1 Polymer nanoparticle

Particles in the range of 10-1000nm that are solid and colloidal are called polymer nanoparticles. There two main subtypes of polymer nanoparticles namely Nanospheres and Nano-capsules. These Nanoparticles are made directly from monomers through polymerization, or premade polymers. Many techniques are being employed including dialysis, solvent evaporation, salting out, supercritical fluid evaporation, and the quick expansion of supercritical solution [12].



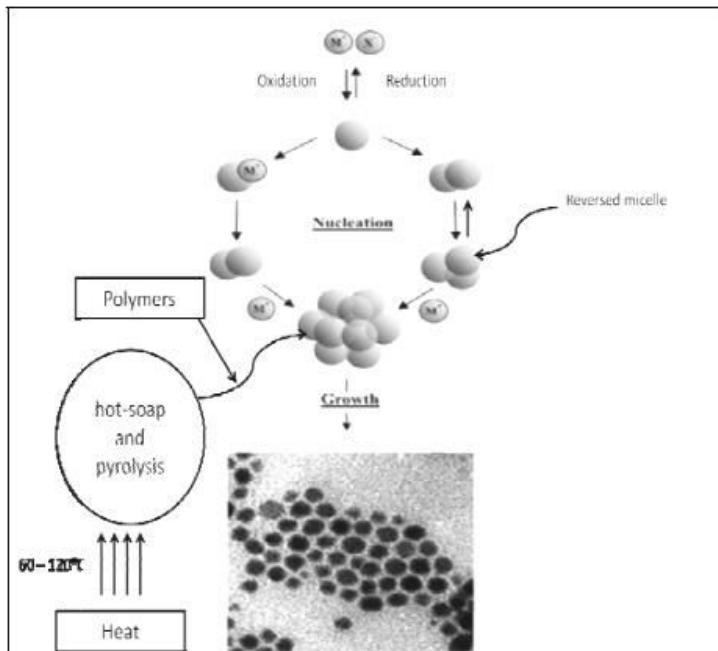
**Fig 3:** Schematic representation of synthesis of Polymeric nanoparticles [5]

Numerous considerations, including categories of polymeric organisation, the application, the size, etc., were reminded when selecting the technique. Any one of such methods can be used to create polymeric nanoparticles, and their effective therapeutic action has been demonstrated. A diagrammatic representation (Figure 3) of how polymeric nanoparticles can be synthesised.

## 2.1.2 Metallic Nanoparticles

The term “metal nanoparticle” refers to nanosized metals with length, breadth, and thickness ranging from 1 to 100 nm. There are certain techniques which are implemented for the synthesis of Metallic-Nanoparticles using a combination liquid phase technique, including chemical reduction, sol gel, and reversed miscelle. Noble Prize winner chemical reduction

techniques were used to continually manufacture spherical-shaped and sized nanoparticles [13].



**Fig 4:** Schematic Representation of synthesis of Metallic Nanoparticle [5]

Figure 3 depicts the schematic representation of how metallic nanoparticle is synthesised. Because of their unique properties, which include enormous surface enriches, distinctive electronic structure between molecules and metallic states, and processing of higher quantity of lower co-ordination sited, metal nanoparticles are widely employed.

These find application in radiofrequency techniques for tumor catabolism by heat, magnetic separation of labelled cells and other biotic entities, delivery of therapeutic drugs, genes, and radionuclides, and contrast agents for magnetic resonance imaging.

### 2.1.3. Magnetic Nanoparticle

Pure metals such Cobalt, Iron, and Nickel along with metal alloys like Iron-platinum and Cobalt-platinum were used to generate magnetic nanoparticles [14]. It is achievable to get particle sizes of about 3nm with the help of using magnetic nanoparticles. Particles as small as less than atom size allow us to generate recording media with a recording density of up to 1 Tb/in<sup>2</sup>. Numerous techniques have been documented a few of which microemulsion, thermal decomposition, solvothermal, coprecipitation, sonochemistry, colloidal approach, and combustion synthesis [15].

One of MNP's main uses is in bio-separation, where targeted biomolecules are conjugated to MNPs carried out with specific receptors. Using an applied magnetic field these complexes can be easily attracted and extracted from pristine mixture. As compared to commonly used methods like centrifugation and filtration it is much quicker and easier. Additionally, biosensing, medication administration, magnetic resonance imaging, and hyperthermia all make use of this technology [16].

## 2.2. Methods for Phytochemical Extraction

The Action process, In the pharmaceutical industry, the phrase "extraction" refers to the process of separating medicinally active components from plant (and animal) tissues using specific solvents and conventional techniques. The plant products that are obtained in this way are rather complicated mixes of metabolites that can be used externally or orally.

They can be obtained in liquid or semi-solid state, or in dry powder form once the solvents are removed. These comprise the groups of preparations referred to as pilular (semisolid) extracts, tinctures, decoctions, infusions, fluid extracts, and powdered extracts.

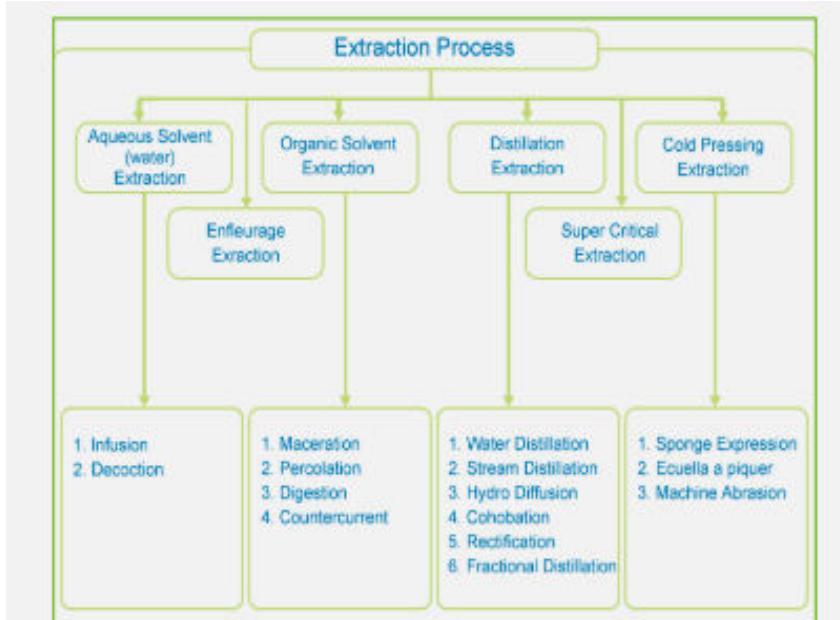


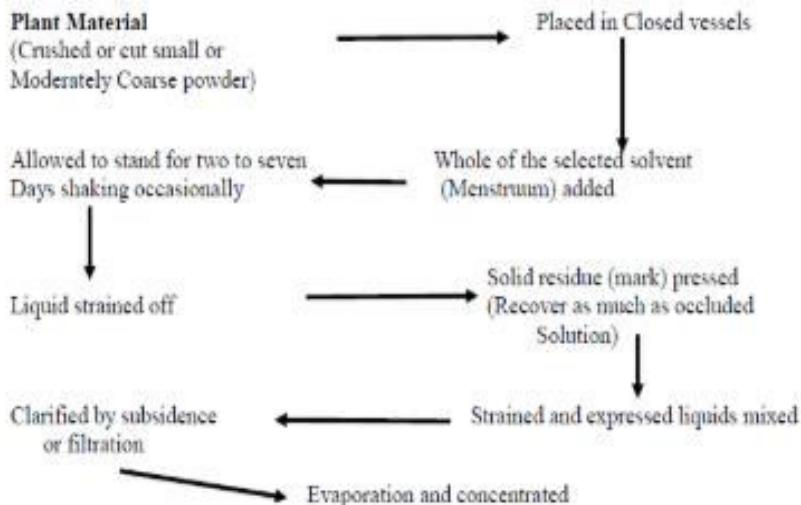
Fig 5: Methods for extraction process of phytochemicals [18]

In general, there are several methods for extracting of phytochemicals from the plants: maceration, infusion, percolation, digestion, decoction, hot continuous extraction (Soxhlet), aqueous-alcoholic extraction by fermentation, counter-current, extraction with microwave assistance, ultrasound (sonication), supercritical fluid , and phytonic (using hydrofluorocarbon solvents). Expression, hydrolytic maceration followed by distillation, steam distillation, water and steam distillation, and enfleurage (cold fat extraction) are some of the hydro distillation procedures that can be used for fragrance plants. [17].

### 2.2.1. Maceration

Plant drugs, either whole or coarsely powdered, are macerated (for fluid extract) by keeping them in conduct with the solvent in a stoppered container for a predetermined amount of time. Agitation on a regular basis until the soluble stuff dissolves.

The most appropriate use case for the technique is with thermolabile medications.



**Fig 6.** Maceration Process (Steady- State Extraction) [18]

### 2.2.2. Decoction

This process involves boiling the crude medication in water for 15 minutes, chilling, filtering, and then extracting the heat and water-soluble components putting enough cold water through the medication to generate the necessary volume.

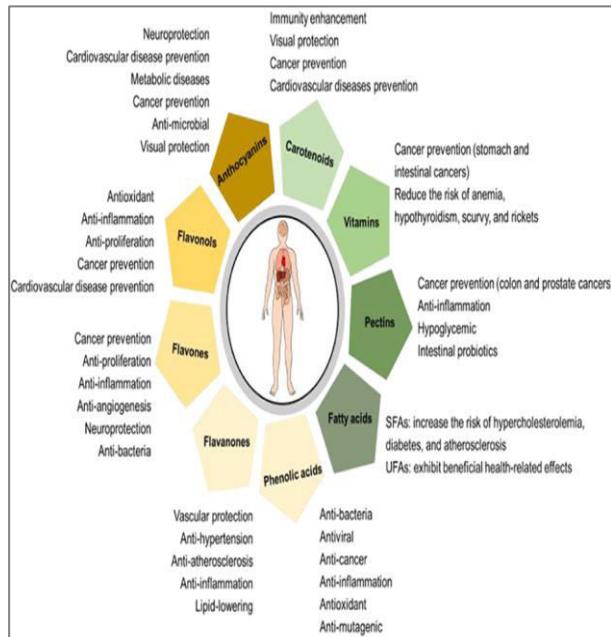
### 2.2.3. Plant tissue homogenization

Researches have employed plant tissue homogenization in solvent extensively. Fresh plant parts, either wet or dry, are ground into tiny particles in a blender, a solvent of specific quantity was added, further it was rapidly mixed for 10mins, later on it was kept for 24 hours. After that the extract was filtered. To find the concentration, the filtrated extract was dried under low pressure and redissolved in the solvent.

## 2.2.4. Infusion

It is a diluted mixture of the crude medications easily soluble ingredients. The solids are macerated in either cold or boiling water for the brief duration to produce fresh infusions.

## 2.3. Potential therapeutic-promoting effects of phytochemical in citrus.



**Fig 7:** Phytochemical compounds and its therapeutic properties citrus [19]

## 2.4. Nanoparticle formulations and their pharmacological actions.

Different Nanoparticles expression and therapeutic actions have been analysed. This chapter covers the activity Nanoparticle for the generation of herbal expression, Therapeutic use of particular drugs to increase the bioavailability and medicinal effect [20].

**Table 1:** Nanoparticle formulation and their pharmacological actions [5]

SL No.	Nanoparticle Name	Functionalization	Uses	Method of synthesis
1	Curcumin	Anticancer	Potent Anticancer and Antitumor.	Wet-milling technique.
2	Paclitaxel	Antineoplastic	Acts against several tumours, ovarian and breast cancers.	Nanoprecipitation.
3	Berberin	Anticancer	Inflammation and several cancers.	Emulsion, Ionic gelation.
4	Camptothecin	anticancer	Potent anticancer	Encapsulated with hydrophobically modified glycol.
5	Ginkgo biloba	Alzheimer's dementia	Acts against loss of memory, thinking, language, behaviour.	Combination of Dry and wet process. (Gas-phase and liquid-phase grinding)
6	Triptolide	Anti-arthritis	Inflammatory and autoimmune diseases, especially for rheumatoid arthritis.	Nano encapsulation
7	Salvia miltiorrhiza	Anti-hyperlipidaemia	Cerebrovascular diseases, improve blood stasis.	Phospholipid complex loaded.
8	Quercetin	Anti-oxidant	Potent anticancer	Gelatin and chitosan loaded.
9	Breviscapine	Anti-cardiovascular	Cerebrovascular and cardiovascular diseases also against pulmonary fibrosis.	Lipid encapsulation.
10	Naringenin	Antioxidant, Anti-inflammatory.	Acts against several tumours and hepatoprotective.	Nano precipitation.
11	Dodder	Antioxidant	Acts against carcinogenesis and ageing also used as hepatoprotective.	Nano precipitation.
12	Silymarins	Hepatoprotective	Several liver diseases, breast cancer.	Cold homogenization.
13	Genistein	Antioxidant	Used in cardiovascular diseases, breast and uterine cancer also in osteoporosis.	Nano emulsion and chitosan microsphere.
14	Centellaasiatica	Anxiolytic	Acts as anti-anxiety, also used in leprosy, cancer, syphilis and allergy.	Ionic gelation.
15	Annual mugwort	Antimalarial	Also used for Asthma	Hydrophilic encapsulation.

## Significant advantages of Nanoparticles

Significant of Nanoparticles are given below,

- ✓ Enhanced Drug Solubility
- ✓ Improved therapeutic efficacy
- ✓ Specific targeting
- ✓ Boost stability
- ✓ Decreased toxicity
- ✓ Controlled release

## Nano Herbal Medicines and its Applications

Nano herbal medications represent a novel method blending the old understanding of herbal therapies with current nanotechnology. These treatments involve the manufacturing of herbal extracts or chemicals into nano-sized particles, typically ranging from 1 to 100 nanometres. This reduction in particle size boosts the bioavailability and efficacy of herbal components, making them more potent and targeted in their action.

The use of nano herbal medicines spans several industries including healthcare, pharmaceuticals, and cosmetics. In healthcare, these formulations offer promising alternatives for medication administration, permitting controlled release and targeted distribution of herbal ingredients to specific tissue or cells, thereby avoiding adverse effects and maximizing therapeutic outcomes [21]. Furthermore, when traditional herbal therapies have demonstrated therapeutic effects, nano herbal medications hold promise in the treatment of chronic conditions such as tumor, diabetes, cardiovascular condition. Nano herbal formulations in cosmetics present chances to create skincare products with enhanced efficacy and absorption, meeting the growing desire for sustainable and natural beauty solution. Overall, nano herbal medicines offer a potential new direction for the pharmaceutical and healthcare sectors by combining cutting-edge nanotechnology with traditional herbal medicine to successfully treat modern health issues.

## Phytochemical Products and its Applications

Plants are the source of phytochemical products, which are a wide range of substances with possible health advantages. The vivid colour, flavors, and scents of plant meals are a result of these bioactive compounds, which are widely distributed in fruits, vegetables, herbs, and other botanical sources. More significantly, they have a variety of therapeutic qualities that make them indispensable compounds as natural treatments to promote health and prevent disease is one of their main uses. Anti-oxidant, Anti-inflammatory, Anti-bacterial, and Anticancer effects have been established by compounds such as polyphenols, flavonoids, carotenoids, and alkaloids [23]. Thus, the use of phytochemical-rich supplements, herbal extracts, and functional goods has increased in order to promote general health and lower the risk of chronic illness such as cancer, heart disease, and other diseases.

Phytochemicals are valuable resources for drug research and development in the pharmaceutical industry. The fact that so many contemporary medications are either structurally inspired by or derived from plant molecules shows how phytochemicals can be used therapeutically to address a wide range of illnesses. Furthermore, phytochemicals are essential to the formulation of herbal and botanical medications, which provide safer and more organic substitutes for synthetic treatments [24]. Products containing phytochemicals are highly valued in the cosmetics industry due to their ability to improve skin tone. In skincare formulations, plant extracts high in vitamins, antioxidants, and other bioactive components are used to hydrate, nourish, and revitalize the skin. In order to maintain skin health and fight indications of age, phytochemicals with anti-ageing and UV-protective properties are also added to sunscreen, anti-ageing creams, and other beauty products. All things considered; phytochemicals products are a rich source of bioactive substances with a variety of uses in numerous industries. Their adaptability and capacity to enhance health and well-being highlight their importance in contemporary medicine, cosmetics, and food

technology, spurring continued study and development into use of plants to improve human health and appearance.

## Conclusion

Recently, there has been increased interest in herbal medications due to their potential to treat nearly all illness. The use of herbal medications is, however, restricted by a number of issues, including limited oral absorption, poor solubility, poor bioavailability, instability, and unexpected toxicity. Nanoparticles can be extremely important in solving such issues. Thus, several nanoparticles exhibit promise for use in the delivery of herbal medications with improved therapeutic outcomes. Numerous techniques including solvent evaporation, sequential simplex optimization, homogenization, and wet and dry precipitation, were used to generate the herbal drugs coated with nanoparticles. Nanoparticle drug carriers enhance the pharmacokinetic and bio-distribution of therapeutic agent due to their tiny particle measurement and greater surface area to volume ratio. They can transverse the blood barrier, enhance the solubility of hydrophobic substances, and boost their stability in addition to their site-specific activity.

Citrus fruits are eaten in vast quantities all over the world because of their delicious Flavors and appealing scents. Citrus and citrus-derived products have a unique flavour and range of medicinal and nutraceutical potentials, including anti-inflammatory, antimicrobial, anticancer, neuroprotective effect, and other biological properties. These potentials are attributed to the high abundance of phytochemicals. Citrus fruits vary greatly in terms of its phytochemical and nutritional composition depending on the variety, fruit portion, maturity, production region, and numerous other environmental factors. Selecting the tight solvent is crucial for removing different phytochemicals from plants. Non-standard extraction techniques can cause the phytochemicals in the plants to degrade and can also cause variances, which makes the process non-reproducible.

## References

- Chakraborty, K., Shivakumar, A.R., & Ramachandran, S. (2016). Nano-technology in herbal medicines: A review. *International Journal of Herbal Medicine*, 4, 21-27.
- Chen, Y., Lin, X., Park, H., & Greever, R. (2009). Study of artemisinin nanocapsules as anticancer drug delivery systems. *Nanomedicine: nanotechnology, biology and medicine*, 5(3), 316-322.
- Deepak Yadav, D. Y., Suruchi Suri, S. S., Choudhary, A. A., Mohd Sikender, M. S., Hemant, H., Beg, M. N., ... & Mohd Asif, M. A. (2011). Novel approach: herbal remedies and natural products in pharmaceutical science as nano drug delivery systems.
- Edeoga, H. O., Okwu, D. E., &Mbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African journal of biotechnology*, 4(7), 685-688.
- Geckeler, K. E., & Stirn, J. (1993). Polyreaktionen – Mechanismen, Systematik, Relevanz. *Naturwissenschaften*, 80, 487-500.
- Gence, L., Servent, A., Poucheret, P., Hiol, A., &Dhuique-Mayer, C. (2018). Pectin structure and particle size modify carotenoid bioaccessibility and uptake by Caco-2 cells in citrus juices vs. concentrates. *Food & function*, 9(6), 3523-3531.
- Grosso, G., Galvano, F., Mistretta, A., Marventano, S., Nolfo, F., Calabrese, G., & Scuderi, A. (2013). Red orange: experimental models and epidemiological evidence of its benefits on human health. *Oxidative medicine and cellular longevity*, 2013.
- Iwaki, T., Kakihara, Y., Toda, T., Abdullah, M., & Okuyama, K. (2003). Preparation of high coercivity magnetic FePt nanoparticles by liquid process. *Journal of Applied Physics*, 94(10), 6807-6811.
- Kesarwani, K., & Gupta, R. (2013). Bioavailability enhancers of herbal origin: An overview. *Asian Pacific journal of tropical biomedicine*, 3(4), 253-266.

- Kharisov, B. I., Dias, H. R., Kharissova, O. V., Vázquez, A., Pena, Y., & Gomez, I. (2014). Solubilization, dispersion and stabilization of magnetic nanoparticles in water and non-aqueous solvents: recent trends. *RSC Advances*, 4(85), 45354-45381.
- Kumar, K., & Rai, A. K. (2012). Miraculous therapeutic effects of herbal drugs using novel drug delivery systems. *International Research Journal of Pharmacy*, 3(2), 27-30.
- Li, D. C., Zhong, X. K., Zeng, Z. P., Jiang, J. G., Li, L., Zhao, M. M., & Gao, Y. X. (2009). Application of targeted drug delivery system in Chinese medicine. *Journal of Controlled Release*, 138(2), 103-112.
- Liu, S., Lou, Y., Li, Y., Zhang, J., Li, P., Yang, B., & Gu, Q. (2022). Review of phytochemical and nutritional characteristics and food applications of *Citrus* L. fruits. *Frontiers in nutrition*, 9, 968604.
- Liu, S., Lou, Y., Li, Y., Zhang, J., Li, P., Yang, B., & Gu, Q. (2022). Review of phytochemical and nutritional characteristics and food applications of *Citrus* L. fruits. *Frontiers in nutrition*, 9, 968604.
- Mukherjee, P. K., Pitchairajan, V., Murugan, V., Sivasankaran, P., & Khan, Y. (2010). Strategies for revitalization of traditional medicine. *Chinese Herbal Medicines*, 2(1), 1-15.
- Murthy, K. C., Jayaprakasha, G. K., Safe, S., & Patil, B. S. (2021). *Citrus limonoids* induce apoptosis and inhibit the proliferation of pancreatic cancer cells. *Food & Function*, 12(3), 1111-1120.
- Scalbert, A., Johnson, I. T., & Saltmarsh, M. (2005). Polyphenols: antioxidants and beyond. *The American journal of clinical nutrition*, 81(1), 215S-217S.
- Schwarz, J. A., Contescu, C. I., & Putyera, K. (Eds.). (2004). Dekker encyclopedia of nanoscience and nanotechnology (Vol. 5). CRC press.
- Sharma K, Mahato N, Lee YR. Extraction, characterization, and biological activity of citrus flavonoids. *Rev Chem Eng*. (2019) 35:265-84. doi: 10.1515/revce-2017-0027

- Sohn, B. H., & Cohen, R. E. (1997). Processible optically transparent block copolymer films containing superparamagnetic iron oxide nanoclusters. *Chemistry of materials*, 9(1), 264-269.
- United Nations Industrial Development Organization, Handa, S. S., Khanuja, S. P. S., Longo, G., & Rakesh, D. D. (2008). Extraction technologies for medicinal and aromatic plants. *Earth, Environmental and Marine Sciences and Technologies*.
- Velavan, S. (2015). Phytochemical techniques-a review. *World Journal of Science and Research*, 1(2), 80-91.
- Wang, F., Zhao, C., Yang, M., Zhang, L., Wei, R., Meng, K., & Zheng, J. (2021). Four citrus flavanones exert atherosclerosis alleviation effects in ApoE-/-mice via different metabolic and signaling pathways. *Journal of Agricultural and Food Chemistry*, 69(17), 5226-5237.
- Williamson, E. M. (2001). Synergy and other interactions in phytomedicines. *Phytomedicine*, 8(5), 401-409.

## Nano Herbal Medicines, Phytochemical Products and Applications

Dr. M. Velammal

Department of Chemistry,  
Yadava College, Madurai - 14

### Abstract

The biologically active compounds present in plants are called phytochemicals. These phytochemicals are derived from various parts of plants such as leaves, flowers, seeds, barks, roots and pulps. These phytochemicals are used as sources of direct medicinal agents. Herbal medicines have been used all over the world from last many years. Especially in India, there is wide market for herbals. They have less adverse effects as compared with modern medicines. They have fewer side effects than modern medications; herbal medicines have been utilized extensively throughout history and are now recognized by both doctors and patients for having a superior therapeutic value.

Nanoparticles are the newly discovered technology in the drug discovery and it has the property of self-targeting in the sense that without the bond of a specific ligand, the nanoparticles can be used for targeting, due to their recognizable small size, at the infected pathological areas. The natural nanoparticles are colloidal framework with herbal particles changing in size from 1 to 1000 nm. Nano-sized medication conveyance frameworks of natural medications have a likely future for improving the movement and conquering issues related with plant drugs. The new approach in herbs as nanotechnology have a sound future which has a scientific approach to deliver the component in sustained manner which increase the patient compliance and avoid repeated administration.

**Keywords:** *Phytochemicals, Herbal medicine, Nano technology, Nano particles, Herbal plants*

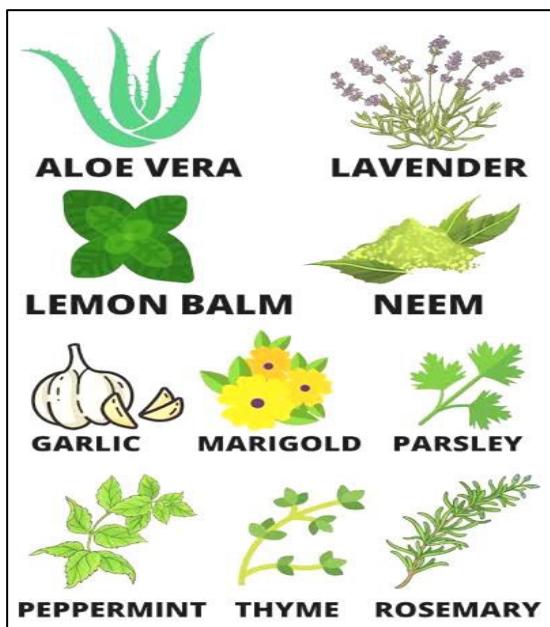
## Introduction

From last many years, herbal remedies and natural products are being used to cure the diseases. Unlike widely used allopathic system, the herbal remedies have thousands of constituents that all work simultaneously against the diseases. Nanoparticles are particles between 1 and 100 nanometers in size. In nanotechnology, a particle is defined as a small object that behaves as a whole unit with respect to its transport and properties. Particles are further classified according to diameter. Herbs and herbal treatments have been utilized to treat illnesses since the dawn of time. The action of herbal medications relies upon by and large capacity of an assortment of dynamic segments, as all the constituents give synergistic activity and hence improve the helpful worth. Incorporation of herbal drugs in the delivery system also helps to increase in solubility, enhanced stability, protection from toxicity, enhanced pharmacological activity, improved tissue macrophage distribution, sustained delivery and protection from physical and chemical degradation.

Phytochemicals are the chemicals that present naturally in plants. Now- a-days these phytochemicals become more popular due to their countless medicinal uses. Phytochemicals play a vital role against number of diseases such as asthma, arthritis, cancer etc. unlike pharmaceutical chemicals these phytochemicals do not have any side effects. The study of phytochemicals has been instrumental in the discovery of new plant natural products which are of commercial values in various industries such as the traditional and complementary medicine systems, pharmaceutical industries, nutraceuticals, and dietary supplement industries. Phytochemicals have been in existence since time immemorial and are known to be responsible for the organoleptic properties (color, taste, flavor, aroma, and odor) of plants, such as the smell of garlic, ginger, and the deep purple color of blueberries.

## Characteristics of Herbal Plants

India has a rich and unique collection of flora, with an estimated 45,000 plant species, among which are numerous species of medicinal plants spread over many different geographical and climatic zones. Many of these species have been used in the traditional medicine systems of Ayurveda, Unani, and others. There is a growing global trend toward the evaluation of medicinal plants for the presence of potentially useful bio-active materials, and this trend has been encouraged by the limitations in uses of synthetic compounds as antibiotics and antivirals, partly because the latter are generally more expensive and less accessible than traditional materials, and also because of the emergence of drug-resistant microbes.



**Fig 1:** Medicinal Plants

**Table 1:** Some of the medicinal plants and their characteristics

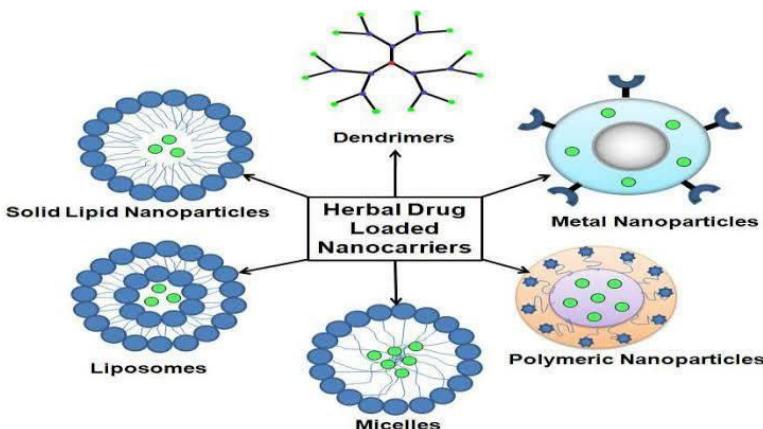
S.No	Family/Species	Plant Part	Traditional Use
1.	Acanthaceae / <i>Rungia repens</i> Nees	Aerial parts	Juice applied on the skin to treat skin diseases including itching of the skin.
2.	Acanthaceae / <i>Rhinacanthus communis</i> Nees	Aerial parts	Paste of the leaves is applied externally to treat eczema and herpes
3.	Apocynaceae / <i>Wrightia tinctoria</i> R. Br.	Aerial parts	Leaves are used to treat various skin disorders including herpes, psoriasis and nonspecific dermatitis.
4.	Aristolochiaceae / <i>Aristolochia indica</i> L.	Aerial parts	Paste of the aerial part is mixed with neem leaf and burned, the fumes are inhaled to treat migraine.
5.	Asclepiadaceae / <i>Pergularia daemia</i> (Forsskal) Chiov.	Aerial parts	Decoction from the aerial part is taken internally to get relief from fever
6.	Asclepiadaceae / <i>Gymnema sylvestre</i> R. Br.	Aerial parts	Powder made from the aerial parts is taken internally to treat diabetes. Traditional Ayurvedic medicine uses <i>Gymnema</i> to treat a variety of other disorders as well, including digestion problems, cough, constipation, and malaria.
7.	Asteraceae/ <i>Wedelia chinensis</i> (Osbeck) Merr.	Aerial parts	Widely used in India to treat jaundice and other liver and gall bladder ailments.
8.	Asteraceae/ <i>Sphaeranthus indicus</i> L.	Aerial parts	Paste is applied externally to treat skin disease and leprosy.

9.	Bombacaceae <i>Durio zibethinus</i> L.	Leaves	Leaf juice is applied on the head of a patient with fever. The leaves are employed in medicinal baths to treat jaundice. Decoctions of the leaves and fruits are applied to swellings and skin disease.
10.	Boraginaceae/ <i>Trichodesma indicum</i> R.Br.	Aerial parts	The traditional healers use this herb in treatment of diseases related to urinary system and also to treat patients having the problem of piles specially the bleeding piles.
11.	Cleomaceae / <i>Cleome pentaphylla</i> L.	Aerial parts	The juice of the leaves has been used to relieve earache and itching.
12.	Caesalpiniaceae / <i>Caesalpinia bonduc</i> (L.) Roxb.	Aerial parts	The seeds are bitter and they are useful in treating inflammation, cough, asthma, leprosy, skin diseases, dysentery, colic and intestinal worms
13.	Caesalpiniaceae / <i>Cassia alata</i> L.	Leaves	Crushed leaves are rubbed on ringworm-affected skin, for immediate relief.
14.	Convolvulaceae/ <i>Evolvulus alsinoides</i> L.	Aerial parts	Decoction made from the aerial part is taken internally to treat dysentery, falling and graying of hair and to treat fever.
15.	Cucurbitaceae / <i>Mukia maderaspatana</i> (L.) M. Roemer	Aerial parts	The leaves are used as a poultice in treating skin eruptions. It is used internally in the treatment of gonorrhea.
16.	Euphorbiaceae/ <i>Ricinus communis</i> L.	Aerial parts	The decoction of the leaf is used as hair tonic/alopecia.
17.	Fabaceae / <i>Clitoria ternatea</i> L.	Aerial parts	Paste made from the aerial part is taken internally to treat pulmonary tuberculosis.
18.	Clusiaceae/ <i>Garcinia mangostana</i> L.	Aerial parts	A portion of the rind is steeped in water overnight and the infusion is given as a remedy for chronic diarrhea in adults and children.

19	Lamiaceae/ <i>Leucas aspera</i> Spr.	Aerial parts	Leaves and flowers are used for inhalation through nose to cure migraine. In addition, two drops of the juice of the flowers is useful as a nasal drop. Juice of the leaves is used as local application to treat psoriasis, chronic skin eruptions and chronic rheumatism.
----	---	--------------	---

## Herbal nanoparticles

Nanoparticles can be used to target the herbal medicine to individual organ which enhance the selectivity, drug delivery, efficacy and protection. Nanoparticles can be used to enhance the herbal drug solubility and help to localize the drug in a specific site thus resulting in better potency. The herbal nanoparticles are colloidal system with herbal particles varying in size from 1 to 1000 nm. Herbal medicines have been recognized by physicians and patients due to their potential medicinal effect and also their fewer unwanted secondary effect as compared with modern medicines. The modern phyto-pharmaceutical research can solve the scientific needs of herbal medicines in developing novel drug delivery systems, such as nanoparticles, micro emulsion, matrix system, solid dispersion, liposomes and solid lipid nanoparticles.



**Fig 2:** Herbal Nanoparticles

## Role of Nanoparticles

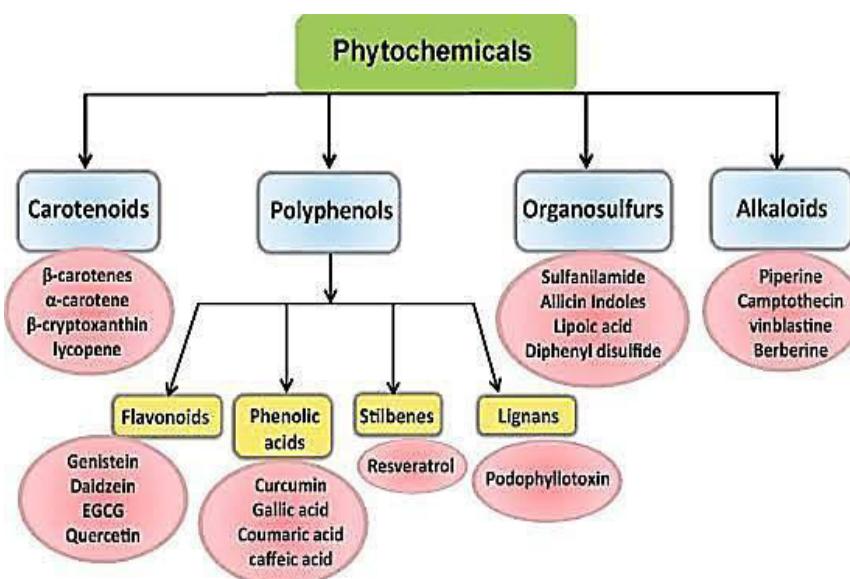
To transport the drug in the small particle size that increases the entire surface area of the drugs administering quicker dissolution in the blood drug delivery system is targeted in a specific manner. Suffusion of the drugs across epithelial and endothelial barriers to transport the drugs at sites of action.

- The high surface area to volume ratio of nanoparticles gives a tremendous driving force for diffusion, mainly at elevated temperatures. Sintering can take place at lower temperatures, over shorter time scales than for larger particles
- Suspensions of nanoparticles are possible since the interaction of the particle surface with the solvent is strong enough to overcome density differences, which otherwise usually result in a material either sinking or floating in a liquid
- Nanoparticles also frequently possess unexpected optical properties as they are small
- The drug delivery method is specifically targeted
- Because it is smaller than liposomes and micro spheres can effortlessly flow through the bone's sinusoidal gaps, marrow and spleen versus other organ systems with long period of circulation
- Nanoparticles improve a drug's or protein's resistance to enzymatic breakdown
- They provide a considerable advancement above current practices. The administration routes of oral and intravenous (IV) in terms of efficacy and efficiency

## Phytochemicals

Phytochemicals are simply plant-derived chemicals. The word "phyto" comes from the Greek word plant. It is used to refer

to the secondary metabolites produced by plants. The metabolites are usually synthesized as a measure for self-defense against insects, pests, pathogens, herbivores, ultraviolet exposure, and environmental hazards. Phytochemicals differ from the essential nutrients (primary metabolites) such as the carbohydrates, proteins, fats, minerals, and vitamins that are needed for the day to day maintenance of the plants. Sometimes, phytochemicals are used to refer to functional foods with antioxidant properties, nutraceuticals, phytonutrients, anti-nutrients, phytotoxins, and so forth.



**Fig 3: Phytochemicals**

### Functions of Phytochemicals in the Living Organisms

Phytochemicals perform quite a number of roles in the living organisms and the mechanism by which they accomplish it has not been fully understood. However, phytochemical functions as:

- Antioxidants by preventing oxidative damage of important biomolecules such as nucleic acids, proteins, and fats.
- Antimicrobial agents: antibacterial, antifungal, antiviral, anti-trypanocidal agents.
- Stimulation of immune system.
- Modulation of detoxifying enzymes.
- Anti-inflammatory functions.
- Reduction of platelet aggregations.
- Physiological activities such as interfering with the binding of
- Pathogens to cell receptors.

## Progress in Phytochemical Research

- **As nanoparticle (NP) synthetic precursors:** Phytochemicals can act as cheap and raw materials for different classes of nanoparticle (NP) synthesis. Phytochemicals can be selectively utilized to synthesize the NP of interest, for example, having red emission, having good ability to carry drugs, and so forth.
- **As a fabricating source of nanoparticles (NPs):** Literature goes in favor of the phytochemicals as NPs surface decorating agents for enhancing the phytochemical activity of the related NPs. It is also very interesting that this field of research is nascent, and requires a lot of research which would definitely boost both the areas of phytomedicine and nanotechnology.
- **Antitumor effect:** Cancer is a leading cause of deaths worldwide. Phytochemicals could contribute to the cancer treatment in the following ways:
- **Drugs source:** certain important chemicals, such as taxol can directly be isolated from plants, and could be potentially used as an anticancer agent.
- Along with a source of drugs isolation, phytochemicals could also be used to obtain NPs having anticancerous effects, as well as NPs using as agents for anticancerous drugs delivery, as well as photoluminescent agents in their treatment.

## Conclusion

Nanoparticles are an emerging field in which new and innovative tools are being developed to tackle issues of water, air, and soil pollution. Nanomaterials are being functionalized with organic and inorganic materials to make them more useful for biosensing, environmental remediation, disease diagnosis, and much more. Nanotechnology talk is moving out of its comfort zone of scientific discourse. Due to their potential to heal practically all diseases, herbal medicines are currently receiving increased attention. However, the use of herbal medications is constrained by number of issues, including poor solubility, poor bioavailability, limited oral absorption, instability, and unpredictable toxicity. Nanotechnology has developed appealing therapies for the pharmaceutical industry that will deal with the issue posed by herbal medications in order to solve such issues. It is anticipated that the effectual and valuable relevance of the natural products and herbal remedies being applied with the nanocarrier will enhance the significance of existing drug delivery systems.

## References

- Anand P, Kunnumakkara AB, Newman RA. Bioavailability of Curcumin: Problems and Promises, Mol Pharmaceutics, 2007; 4(6): 807-818.
- Anani K, Hudson JB, de Souza C, Akpagana K, Tower GHN, Arnason JT, Gbeassor M (2000): Investigation of medicinal plants of Togo for antiviral and antimicrobial activities. Pharm Biol 38: 40–45.
- Ayyanar M, Ignacimuthu S (2005): Traditional knowledge of Kanitribals in Kouthalai of Tirunelveli hills, Tamil Nadu, India. J Ethnopharmacol 102: 246–255.
- Bisht S, Feldmann G, Soni S, Ravi R, Karikar C and Maitra A, Polymeric nanoparticles encapsulated curcumin (nanocurcumin): a novel strategy for human cancer therapy, J Nanobiotechnol, 2007; 5(3): 2-18.

- Gao D. Drug design for cancer: Gold nanoparticle liposomehybrids for drug delivery and monitoring. *Drug Des* 2013;2:2.
- Gupta D, Nguyen P, Yu M. Nanoparticles for superior pharmacokinetics and enhanced efficacy. *J Dev Drugs* 2014;3:2.
- Hudson JB (1995): The phytochemical approach to antiviral chemotherapy. In: Chessin M, eds., *Antiviral Proteins in Higher Plants*: Boca Raton, FL, CRC Press, pp. 161–174.
- Hudson JB, Towers GHN (1999): Phytomedicines as antivirals. *Drugs of the Future* 24: 295–320.
- Jain SK (1964): The role of botanist in folklore research. *Folklore* 5: 145–150. Kamboj VP (2000): Herbal medicine. *Curr Sci* 78: 35–39.
- Jain SK (1994): Ethnobotany and research on medicinal plants in India. *Ciba Found Symp*, 185: 153–64; discussion 164-168.
- Li Y, Dong L, Jia A, Chang X, Xue H. Preparation and characterization of solid lipid nanoparticles loaded traditional chinese medicine. *Int J Biol Macromol*, 2006; 38: 296–9.
- Lopes CM. Therapeutics delivery: Innovationstechnology approaches. *Drug Des*, 2014;3:3.
- Mehmood Z, Ahmad I, Mohammad F, Ahmad S (1999): Indian medicinal plants a potential source for anticandidal drugs. *Pharm Biol*. 37: 237–242.
- Pal DC, Jain SK (1998): *Tribal Medicine*. Naya Prokash, 206, Bidhan Sarani, Calcutta, India, p. 316.
- Perumal Samy R, Ignacimuthu S (2000): Antibacterial activity of some folklore medicinal Plants used by tribals in Western Ghats of India. *J Ethnopharmacol* 69: 63–71.
- Perumal Samy R, Ignacimuthu S, Sen A (1998): Screening of 34 Indian medicinal plants for antibacterial properties. *J Ethnopharmacol* 62: 173–181.
- Rusin A, Krawczyk Z, Grynkiewicz G, Gogler A, Zawisza-Puchalka J of genistein, their properties and possible application, *Acta Biochim Pol*, 2010; 57: 23-34.
- Shirwaikar A, Shirwaikar A, Prabu SL, Kumar GA. Herbal excipients in novel drug delivery systems. *Indian journal of*

pharmaceutical sciences. 2008 Jul; 70(4):415.

- Wu XY, Lee PI. Preparation and characterization of thermal- and pH-sensitive nanospheres. Pharm Res. 1993;10:1544-7.
- Yadav D, Suri S, Choudhary AA, Sikender M, Hemant K, Beg NM, et al. Novel approach: Herbal remedies and natural products in pharmaceutical science as nano drug delivery systems. Int J Pharm Tech. 2011; 3:3092-116.
- Zhang JF, Hou SX, Liu HL. Comparison of preparing two polylactide nanoparticles loaded lipophilic anti-cancer herb drug by nanoprecipitation method. Zhongguo Zhong Yao Za Zhi, 2007; 32: 303-6.

## Exploring Potential *Herpes Simplex Virus* Inhibitors using Docking and ADMET Analysis of Phytocompounds from the Indian Medicinal Plants

G. Manigandan<sup>1</sup>, T. Raja<sup>2</sup>, S. Santhiya<sup>3</sup>, P. Ravikumar<sup>4\*</sup>

<sup>1</sup>. PG Student, Department of Biotechnology & Bioinformatics,  
Bishop Heber College (Autonomous),  
Tiruchirapalli-620017, Tamil Nadu, India.

<sup>2</sup>. Assistant Scientific Officer, Iyarvi Research Center for Bioinformatics  
(IRCB), Erode - 638452, Tamil Nadu, India.

<sup>3</sup>. Research Assistant, Iyarvi Research Center for Bioinformatics (IRCB),  
Erode - 638 452, Tamil Nadu, India.

<sup>4</sup>. Senior Scientist, Iyarvi Research Center for Bioinformatics (IRCB),  
Erode - 638 452, Tamil Nadu, India.

\*Corresponding Author: E-mail: headircb@gmail.com;  
Ph: +91-4285 – 227321/99445 27321

### Abstract

Herpes Simplex Encephalitis (HSE) is a rare neurological disorder characterized by inflammation of the brain (encephalitis). The present study was designed to find the potential phytocompounds from Indian medicinal plants against Herpes Simplex Virus, type - 1 (HSV, TYPE - 1) using *in silico* studies. The 3D structures of the phytocompounds were obtained from IMPPAT & PubChem database. The Lipinski rule of five for all the phytocompounds were tested using SwissADME. The 3D structure of the target protein was retrieved from the PDB database. The docking studies were performed using PyRx 0.8 and the results were analyzed using Discovery Studio 2021. From the results, the phytocompounds Betulinic acid, Friedlein and beta - Amyrin showed very good binding affinity like -9.8, -9.7 and -9.5 Kcal/mol, respectively. Toxicity studies were done for the best-interacted phytocompounds and the results showed that the compounds had very less toxicity. Hence, the present study

concludes that Betulinic acid, Friedlein from *Punica granatum* and beta - Amyrin from *Swertia chirata* may have a potential effect in the treatment of HSV.

**Keywords:** *Herpes Simplex Virus, Docking, PyRx, Discovery studio, Phytocompound*

## Introduction

Encephalitis is an inflammation of the brain parenchyma accompanied by neurologic impairment. When the pathogenic organism causes gross purulence, the term cerebritis is used. Infectious, autoimmune, and post-infectious encephalitis are the most prevalent causes (Venkatesan *et al.*, 2013). Viral encephalitis can be an uncommon consequence of widespread diseases (e.g., herpes virus infections) or a distinct presenting of rare viruses (eg, rabies virus infection). Encephalitis can occur alone or in combination with meningitis, myelitis, radiculitis, or neuritis (Dubey *et al.*, 2018). The most common causes of encephalitis are viral or autoimmune. Encephalitis has the following symptoms. Infections can also produce encephalopathy (abnormal mental state, disorientation, behavioural changes, agitation, or sleep-wake cycle disturbance) without causing direct effects on the central nervous system (CNS) brain tissue infection or inflammation (Gnann and Whiteley, 2017).

Infections with the herpes simplex virus (HSV) have been documented since ancient Greece (Roizman *et al.*, 2013). The clinical symptoms of these illnesses were widely recorded in the medical journals during the twentieth century. In the 1930s, life-threatening diseases such newborn HSV infections and herpes simplex encephalitis were first documented in the literature. Several early observations laid the groundwork for our current understanding of HSV. First, despite both humoral and cell-mediated immune responses, an individual might become infected again (leading to the recognition that the virus establishes latency and may recur upon various provocative stimuli to produce disease) Second, the distinction between HSV-1 and HSV-2 was

established. HSV-1 was formerly linked to infections above the belt, such as the mouth and eyes, whereas HSV-2 was linked to diseases below the belt, such as genital herpes. However, with an ever-increasing proportion of genital herpes caused by HSV-1, there is substantial overlap between the locations of HSV infection in modern times. In the United States, over half of individuals are seropositive for HSV-1, while around 15% of sexually active adults are infected with HSV-2 (McQuillan *et al.*, 2018, Looker *et al.*, 2017). Although vidarabine therapy was established in the late 1970s and was shown to reduce mortality from herpes simplex encephalitis, acyclovir was not demonstrated to be effective in the treatment of biopsy-confirmed herpes simplex encephalitis until much later (1986)(Whitley *et al.*, 1986). Our knowledge of the identification, treatment, and management of herpes virus encephalitis has vastly improved. First, since the early 1980s, the overall incidence of herpes simplex encephalitis (1 in 100,000 to 150,000 people) has remained stable (Whitley, 2004). Greater accuracy in incidence is unlikely to be attained in the absence of a systematic reporting system. Second, and perhaps more crucially, the use of PCR to identify HSV DNA in the CSF became the gold standard for diagnosing herpes simplex encephalitis, displacing brain biopsy as a valid diagnostic option (Rowley *et al.*, 1990).

HSV type-1 is definitely a public health emergency in India, defined as an initial beginning of fever and a change in mental state (including symptoms such as confusion, disorientation, coma, or inability to speak), and/or new onset of seizures (excluding mild febrile seizures) (Narain and Lal, 2014). The country has had recurrent outbreaks of encephalitis with unclear causes. In India, there were about 44,000 cases of encephalitis between 2008 and 2014, with approximately 6000 deaths, mostly in Uttar Pradesh and Bihar. Encephalitis has become more common in 2016, with over 125 children dying in one hospital in Gorakhpur alone (TOI, 2022).

Herpes simplex encephalitis symptoms appear gradually over several days, frequently without notice. Headaches, fevers,

and seizures are common early signs. Drowsiness with overall weakness (stupor) and confusion or disorientation is further symptoms. Affected persons may develop speech problems such as a decreased capacity to communicate by speech, writing, and/or signs (aphasia), a loss of sense of smell (anosmia), and memory loss after the initial symptoms manifest. Behavioral abnormalities such as hyperactivity or psychotic episodes might develop in rare circumstances. Herpes simplex encephalitis might have symptoms that are similar to meningitis. A stiff neck, altered reflexes, disorientation, convulsions, and paralysis are all possible signs.

Herpes simplex encephalitis patients may experience more severe symptoms such as loss of consciousness, hallucinations, and partial paralysis (hemiparesis). Herpes simplex encephalitis can damage the retina, the nerve-rich membrane that lines the inside of the eyes, causing inflammation (retinitis).

The previous study stated that Glycoprotein D (gD) binding to one of the virus's host receptor molecules, HVEM or nectin-1, initiates virus-host cell fusion. The conformational shift that gD adopts is crucial to this process. Because of this, the gD-host cell receptor complex can interact with both the gH/gL heterodimer complex. gH/gL can then take on a form that allows it to attach to gB, allowing gB to connect both viral and host cell membranes. The two membranes eventually fuse, allowing the viral capsid to enter the cell (Eisenberg *et al.*, 2012).

Acyclovir is the only antiviral medicine presently licenced by the FDA for the treatment of HSE. Acyclovir was tested in early clinical trials at dosages of 10 mg/kg every 8 hours (30 mg/kg/day) for 10 days (Kimberlin *et al.*, 1996).

*Justicia adhatoda* is a medicinal plant that belongs to the acanthaceae family and is widely used in Ayurvedic, Siddha, and Naturopathic medicine. It is a plant that may be found across Southeast Asia (Nikomtat *et al.*, 2008). It also has excellent bioactivities, such as antiallergic properties, Antidiabetic (Sydiskis

*et al.*, 1991), antioxidant (Tragooolpua *et al.*, 2007), antimicrobial, anti-ulcer (Verma *et al.*, 2008) and antiviral properties (Wagner, 1989). Medicinal herbs including *Acacia*, *Terminalia*, *Curcuma*, and *Terminalia mulleri* have antiviral effect against Herpes simplex viruses, as previously described (HSV). *In vitro* cell lines, the ethanolic extract of *Justiciae adathoda* showed strong cytotoxicity and antiviral activity against HSV-1. HSV-2 infection is inhibited by extracts from the medicinal plant *Phyllanthus urinaria* at the early stages of viral infection and establishment in the host, according to a research (Yoosook *et al.*, 1999). Furthermore, the aqueous extract of *Swertia chirata* was found to have antiviral activity against HSV-1.

In this work, the anti-herpetic efficacy of 25 Egyptian plant extracts was determined *in vitro*. Cell viability was used to assess antiviral efficacy against Herpes Simplex Virus type 1 (HSV-1). Only *Euphorbia coopire* (*Euphorbiaceae*) and *Morusalba* (*Moraceae*) extracts demonstrated substantial anti-herpetic efficacy (El-Toumy *et al.*, 2018).

In this study, the twelve different Indian medicinal plants like *Adhatoda zylanica*, *Andrographis paniculata*, *Carissa carandas*, *Curcuma longa*, *Ocimum gratissimum*, *Ocimum tenuiflorum*, *Phyllanthus urinaria*, *Punica granatum*, *Scorparia dulcis*, *Swertia chirata*, *Syngium aromaticum* and *Terminalia catappa* were selected to find the potential ability for treating the infection caused by *Herpes Simplex Virus-1*.

## Materials and Methods

### Ligand selection

Using literature & IMPPAT database (Mohanraj *et al.*, 2018), around 301 phytochemical compounds were selected from the different Indian medicinal plants like *Justicia adhatoda*, *Eugenia caryophyllus*, *Scorpia dulcis*, *Terminalia cattapa*, *Curcuma longa*, *Phyllanthus urinaria*, *Andrographis paniculata* (Anandharaj, 2020), *Swertia chirata*, *Carissa carandas*, *Ocimum tenuiflorum*, *Ocimum gratissimum*, *Punicagranatum* (Jadhav *et al.*, 2012) for treating Herpes Simplex Encephalitis (Type - 1). The 3D structures of these

compounds were retrieved from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) and using SwissADME (Daina *et al.*, 2017) they were subjected to test Lipinski Rule of Five. From the results, 195 compounds obeyed Lipinski Rule of Five and these compounds were taken for the study.

### Target protein selection and preparation

The target protein Glycoprotein D (gD) belonging to gD gene was found in the literature for HSE (HSV TYPE - 1) (Antoine *et al.*, 2013). Glycoprotein D (gD) is a structural component of the herpes simplex virus (HSV) envelope which is essential for virus entry into host cells. Chinese hamster ovary (CHO-K1) cells are one of the few cell types which are nonpermissive for the entry of many HSV strains (Whitbeck *et al.*, 1997).

The 3D structure of this target protein was retrieved from the PDB database (<https://www.rcsb.org>). The UniProt ID of this target protein was taken from the Uniprot database (<https://www.uniprot.org/>).

### Docking studies

Docking studies for the target protein gD and the phytocompounds (ligands) were done using PyRx 0.8 software (Trott and Olson, 2010). The target protein was further prepared for docking studies using this software. All the ligands were uploaded using Open Babel option in the PyRx 0.8. The grid was generated and the docking studies were performed using Vina wizard option in the PyRx 0.8. The values of binding affinity were saved in Excel file. The results were analyzed using Discovery Studio 2021 and the 2D & 3D docked images were taken. In the results, the lowest binding affinity indicates good result.

### ADMET and CYP Properties

ADMET and CYP properties were tested for all the best-interacted phytocompounds using SwissADME (Daina *et al.*, 2017). Lipinski, BBB (Blood - Brain Barrier), HIA (Human Intestinal Absorption), PGP (P-glycoprotein), XLogP3, TPSA (Topological

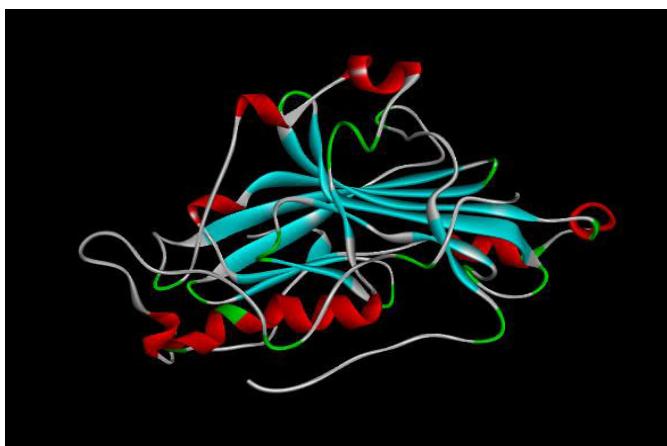
Polar Surface Area), LogS, Fraction Csp3, Rotatable bonds, CYP enzyme inhibitor properties, Skin permeation and Bioavailability score were evaluated for all the best-interacted compounds.

## Results and Discussion

### Ligand and Target protein selection

The 3D structure of ligands (Phytocompounds) was retrieved from the Pubchem database. The 3D structure of the target protein GlycoProtein D (gD) belonging to gD gene was obtained from PDB database and its PDB ID is 2C36.

The 3D structure of the target protein is shown in figure 1.



**Figure 1:** The 3D structure of Target protein gD

### Docking Studies

Docking studies for the target protein Glycoprotein D (gD) and the Phytocompounds (Ligands) were done using Pyrx 0.8 software. In the results, all the 10 compounds were interacted with the target protein and all the results are shown in table 1 and the 2D & 3D interaction of phytocompounds with the target protein are shown in figure 2-9.

**Table 1:** Interaction of Phytocompounds with the target protein

S .No	PubChe m (CID)	Compound Name	Plant Name	Binding Affinity (Kcal/mol)	No.of Bonds	Interacting Residues	Bond Lengt h (Å)
1	2371	Betulic acid	<u>Punicagranatum</u>	-9.8	4	GLN27 ASN227 ILE224 ARG36	2.36 2.72 2.30 2.53
2	244297	Friedlein	<u>Punicagranatum</u>	-9.7	1	ILE224	2.11
3	225689	beta.-Amyrin	<u>Swertia chirata</u>	-9.5	1	HIS295	2.17
4	10105195 5	Dulcioic acid	<u>Scoparia dulcis</u>	-9.3	2	GLN300 HIS295	2.15 2.51
5	12314613	Terminolic acid	<u>Terminalia catappa</u>	-9.2	3	GLN27 HIS295 ASP30	2.05 1.88 2.46
6	3694932	10,11-dihydroxy-2,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,7,8,8a,10,11,12,13,14b-tetradecahydronicene-4a-carboxylic acid	<u>Punicagranatum</u>	-9.1	1	ASP30	2.60
7	71597391	Triterpenoids	<u>Scoparia dulcis</u>	-9	5	VAL34 ASP301 SER298 GLN300 PRO297	2.53 2.68 2.22 2.38 3.05
8	12305222	Hyptatic acid A	<u>Terminalia catappa</u>	-8.7	1	GLN27	2.87
9	4369270	Digitoxigenin	<u>Carissa carandas</u>	-8.5	4	GLY33 VAL34 ILE224 ASN227	2.09 2.67 4.59 1.91
10	10131673 8	Carindone	<u>Carissa carandas</u>	-8.3	3	GLN27 ARG36 ASP301	1.47 1.85 2.14
<b>Synthetic Drug</b>							
11	135398 513	Acylovir		-6.2	5	HIS295 GLN300 ASP301 ASN227 ILE224	2.39 2.98 2.14 2.12 1.98

From the results (Table 1), among other compounds, 10 compounds showed very good results with the target protein. Of which, the phytocompound Betulic acid showed very good binding affinity (-9.8 Kcal /mol) with the amino acid residues GLN27, ASN227, ILE224, ARG36 of the target protein. The phytocompound Friedlein also gave very good binding affinity of -9.7 Kcal /mol with the amino acid residue ILE224. The binding affinity -9.5 Kcal/ mol was observed between the phytocompound beta.-Amyrin and the amino acid residue HIS295 of target protein. Dulcioic acid and Terminolic acid showed binding affinity of -9.7 Kcal/mol with the amino acid residues GLN300, HIS295 and -9.2 Kcal/ mol with the amino acid residues GLN27, HIS295 and ASP30, respectively.

Among the other compounds, the lowest binding affinity - 8.3 Kcal /mol was observed between the phytocompound Carindone with the amino acid residue GLN27, ARG36 and ASP301 of target protein. Thus, in the results of the present study, all the phytocompounds showed very good binding affinity, of which, the phytocompounds Betulic acid, beta.-Amyrin and Friedlein showed the highest binding affinity among the other phytocompounds. Besides, the binding affinity of the Synthetic drug Acyclovir with the target protein was -6.2 kcal /mol and the interacted the amino acid residues were HIS295, GLN300, ASP301, ASN227 and ILE224.

Previously, Padma *et al.* (1998) found that ethanol extract from *Annona muricata* and aqueous extract from *Petunia nyctagineiflora* have the potential effect against HSV-1 (strains 753166 and A-16). Previous study reported that anti-herpetic action has also been shown in extracts produced from the green microalga *Haematococcus pluvialis*. HSV-1 reproduction was inhibited by extracts produced from this microalga using pressurized liquid extraction, which was hypothesized to be mediated by an inhibition in the virus's attachment to the host cell, the viral-cell fusion process, and/or virus entry into the cell (Santoyo *et al.*, 2012).

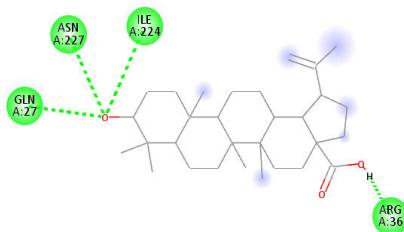
Previous study reported that Phenolic acids are shown to have considerable antiviral efficacy against HSV-1 (Medini *et al.*, 2016). Ismaeel *et al.* (2018) found that the presence of alkaloids, as well as various other phytocompounds was discovered during a preliminary screening of phytochemicals in the *Phaleria macarcropia* plant. These plant extracts have been reported to be harmless to Vero cells yet powerful antiviral agents against HSV.

Zandi *et al.* (2007) reported that the alga extract exhibiting antiviral action against HSV-1 was produced from *Cystoseira myrica* that significantly reduces the virus's replication. Previous study reported that the compounds produced from red macroalgae (*Sympphyocladia latiuscula*) has antiviral activity especially for HSV-1 (Park *et al.*, 2014).

The green algae *E. compressa* belongs to *Ulvaceae* family produced chemically modified polysaccharides has antiviral action against HSV-1 infection, according to another study (Lopes *et al.*, 2017).

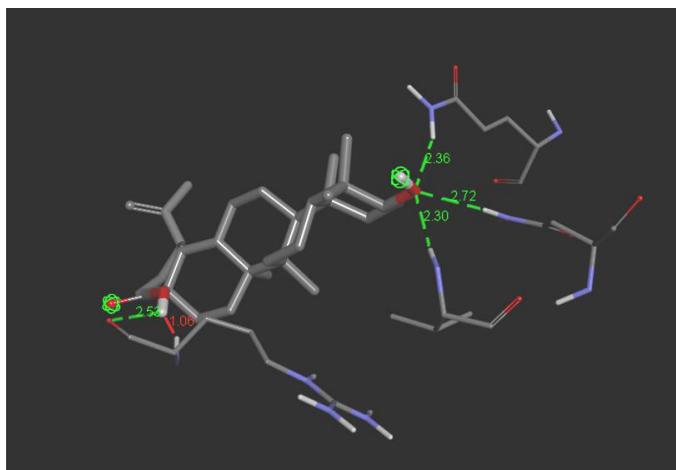
Similarly, in the present study Betulinic acid, Friedlein from *Punica granatum*, beta - Amyrin from *Swertia chirata*, Dulcioic acidfrom *Scorpiadulcis* and Terminolic acid from *Terminalia catappa* may have a potential effect in the treatment of HSV.

In the present study, when compared to synthetic drug (Acyclovir), the values of binding affinity were high in the all 10 best interacted phytocompounds. In which, the phytocompounds Betulinic acid, Friedlein, beta.-Amyrin, Dulcioic acid and Terminolic acid showed highest binding affinity among the other phytocompounds.

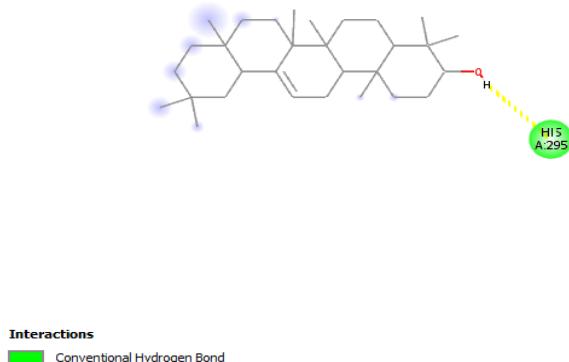


Interactions  
■ Conventional Hydrogen Bond  
■ Unfavorable Donor-Donor

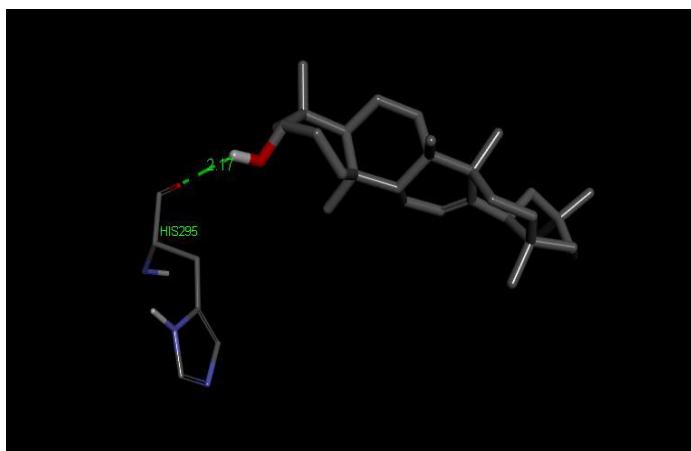
**Figure 2:** The 2D interaction of phytocompound Betulic acid with the target protein



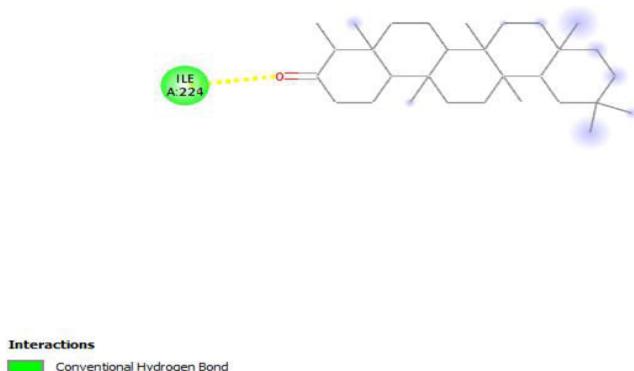
**Figure 3:** The 3D interaction of phytocompound Betulic acid with the target protein



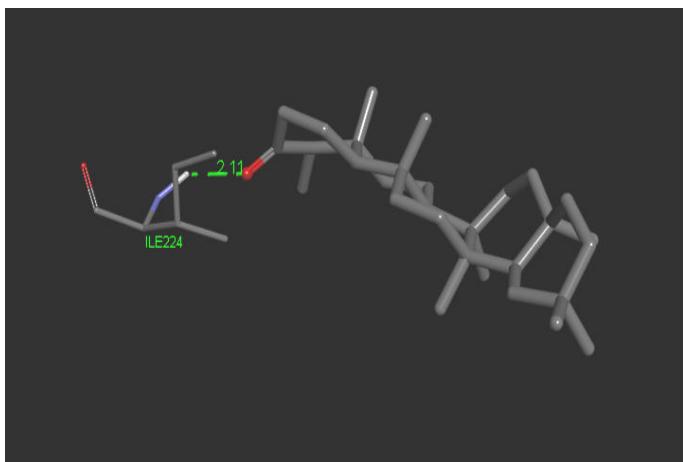
**Figure 4:** The 2D interaction of phytocompound Friedlein acid with the target protein



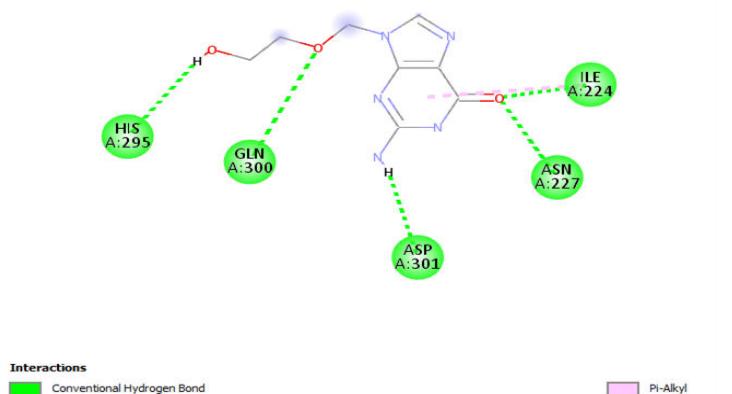
**Figure 5:** The 3D interaction of phytocompound Friedlein acid with the target protein



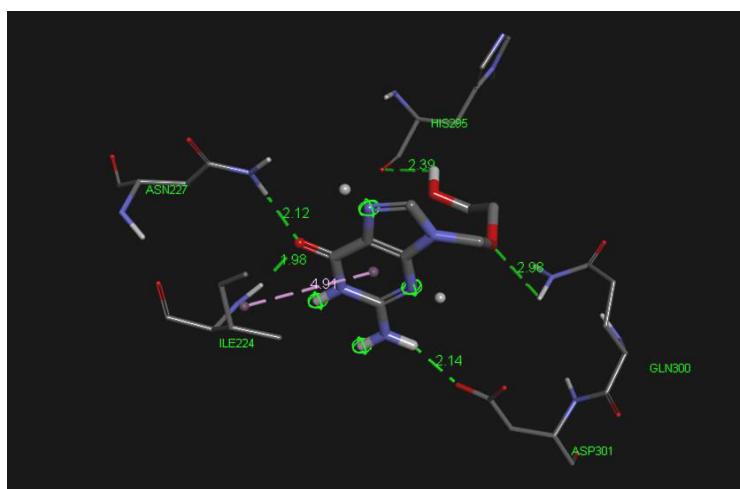
**Figure 6:** The 2D interaction of phytocompound beta-Amyrin acid with the target protein



**Figure 7:** The 3D interaction of phytocompound beta-Amyrin acid with the target protein



**Figure 8:** The 2D interaction of Synthetic drug Acyclovir with the target protein



**Figure 9:** The 3D interaction of Synthetic drug Acyclovir with the target protein

## ADMET and CYP properties

In the present study, ADMET properties were tested for the best interacted phytocompounds using SwissADME and the results were tabulated (table 2). From the results, all the best interacted phytocompounds obey Lipinski rule of five. Most of the compounds did not cross Blood – Brain Barrier (BBB) and had high Intestinal Absorption (HIA). Many phytocompounds predicted to be effluated from the CNS by P-glycoprotein. Among the 10 compounds, XLogP3 value of 6 compounds was within the range. TPSA (Topological Polar Surface Area) and Log S value of the most of 4 compounds were within the limit. In all the compounds, Fraction Csp3 value of all the 10 compounds were less than 0.25 and the value of other compounds were above this limit. Rotatable bonds of all the compounds were within the limit.

In the results of CYP properties, Except Betulinic acid, all the 10 compounds and the Synthetic drug Acyclovir does not inhibit the CYP450 enzymes and does not give any adverse reactions. Betulinic acid inhibits CYP2C9 the CYP enzyme. The value of log K<sub>p</sub> (Skin Permeant) is good for all compounds and A Bioavailability Score (ABS) is good for all the 10 compounds and for the Synthetic drug Acyclovir.

**Table 2:** ADMET Properties of Phytocompounds

S. N o	Pub Chem (CID)	Compound Name	Lipi nsk i	B B B	H I A	P G P-	XL OG P3	T P SA (Å)	Lo g S (E SO L)	Fra ctio n Cs p3	Rot atab le Bon ds
1	2371	Betulinic acid	Yes	No	Low	Yes	8.21	57.53	-7.71	0.90	2
2	244297	Friedlein	Yes	No	Low	NA	9.80	17.07	-8.66	0.97	0
3	225689	beta-Amyrin	Yes	No	Low	NA	9.15	20.23	-8.25	0.93	0

4	101051 955	Dulcioic acid	Yes	No	Low	Yes	7.56	57.53	-7.37	0.90	1
5	12314 613	Terminolic acid	Yes	No	High	NO	4.50	118.2	-6.70	0.90	2
6	36949 32	10,11-dihydroxy-2,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,7,8,8a,10,11,12,13,14b-tetradeca hydroperoxy-4a-carboxylic acid	Yes	No	High	NO	6.51	77.76	-5.30	0.90	1
7	71597 391	Triterpenoids	Yes	No	High	NO	3.94	97.99	-5.19	0.83	1
8	12305 222	Hyptatic acid A	Yes	No	High	NO	5.84	97.99	-6.42	0.90	2
9	43692 70	Digitoxigenin	Yes	Yes	High	NO	2.64	66.76	-3.76	0.87	1
10	10131 6738	Carindone	Yes	No	Low	NO	3.68	100.90	-5.21	0.77	2
<b>Synthetic drug</b>											
11	13539 8513	Acyclovir	Yes	No	High	NO	-1.56	119.0	-0.41	0.38	4

**Note:** Obey Lipinski: Yes means 0 violation & good, BBB (Blood - Brain Barrier): Yes means good, HIA (Human Intestinal Absorption):

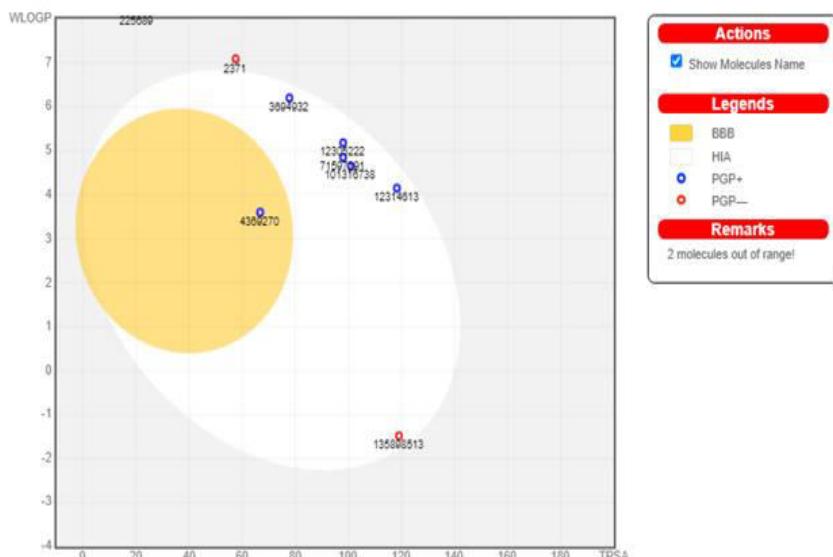
High means good, PGP- (Molecules predicted not to be effluated from the CNS by P-glycoprotein): Yes means good, Lipophilicity: XLOGP3 value between -0.7 and +5.0 means good, Polarity: TPSA between 20 and 130 Å<sup>2</sup> means good, Water Solubility (Log S scale: Insoluble < -10 < Poorly < -6 < Moderately < -4 < Soluble < -2 < Very < 0 < Highly): Log S value not higher than 6 means good, Saturation (Fraction Csp3): Fraction of carbons in the sp<sup>3</sup> hybridization not less than 0.25 means good, and Flexibility (Rotatable bonds): No more than 9 rotatable bonds means good.

**Table 3:** Cytochrome P450 properties of phytocompounds

S. no	PubChem (CID)	Compound Name	CY P1A 2 inhibitor	CY P2C 19 inhibitor	CY P2C 9 inhibitor	CY P2 D6 inhibitor	CY P3A 4 inhibitor	Log K <sub>p</sub> (Skin permeation)(cm/s)	A Bioavailability Score (ABS)
1	2371	Betulinic acid	No	No	Yes	No	No	-3.26	0.85
2	244297	Friedlein	No	No	No	No	No	-1.94	0.55
3	225689	beta-Amyrin	No	No	No	No	No	-2.41	0.55
4	101051 955	Dulcioic acid	No	No	No	No	No	-3.72	0.85
5	123146 13	Terminolic acid	No	No	No	No	No	-6.18	0.56
6	369493 2	10,11-dihydroxy-2,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,7,8,8a,10,11,12,13,14b-tetradecahydrodropicene-4a-carboxylic acid	No	No	No	No	No	-4.56	0.56
7	715973 91	Triterpenoids	No	No	No	No	No	-6.39	0.56

8	123052 22	Hyptatic acid A	No	No	No	No	No	-5.13	0.56
9	436927 0	Digitoxigenin	No	No	No	No	No	-6.71	0.55
10	101316 738	Carindone	No	No	No	No	No	-6.81	0.55
<b>Synthetic drug</b>									
11	135398 513	Acyclovir		No	No	No	No	-8.78	0.55

**Note:** Yes means the compound inhibits the CYP450 enzymes and gives unanticipated adverse reactions; No means the compound does not inhibit the CYP450 enzymes and does not give any adverse reactions; The more negative the log K<sub>p</sub>, the less skin permeant is the molecule; ABS 0.55 means it passes the rule of five & 0.17 means it fails the rule of five.



**Figure 10:** Boiled egg for all the compounds

## Conclusion

In the present study, the phytocompounds from different Indian medicinal plants and the target protein Glycoprotein D (gD) belonging to the gD gene were subjected to *in silico* docking analysis to find the potential inhibitors for HSV. In which, 36 compounds showed better results than the Synthetic drug Acyclovir. Among them, 10 compounds showed very good binding affinity. Toxicity studies were done for the 10 best-interacted phytocompounds and the results showed that the compounds had very less toxicity. Of which, the phytocompounds such as Betulinic acid, beta-Amyrin, Friedlein, Dulcioic acid and Terminolic acid showed the highest binding affinity among the other phytocompounds. Hence, the present study concludes that the Betulinic acid, Friedlein from *Punica granatum* and beta-Amyrin from *Swertia chirata* may give a potential effect in the treatment of HSV.

## References

- Antoine, T. E., Park, P. J., & Shukla, D. (2013). Glycoprotein targeted therapeutics: a new era of anti-herpes simplex virus-1 therapeutics. *Reviews in medical virology*, 23(3), 194-208.
- Daina, A., Michelin, O., & Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific reports*, 7(1), 42717.
- Dubey, D., Pittock, S. J., Kelly, C. R., McKeon, A., Lopez-Chiriboga, A. S., Lennon, V. A., & Flanagan, E. P. (2018). Autoimmune encephalitis epidemiology and a comparison to infectious encephalitis. *Annals of neurology*, 83(1), 166-177.
- Eisenberg, R. J., Atanasiu, D., Cairns, T. M., Gallagher, J. R., Krummenacher, C., & Cohen, G. H. (2012). Herpes virus fusion and entry: a story with many characters. *Viruses*, 4(5), 800-832.
- El-Toumy, S. A., Salib, J. Y., El-Kashak, W. A., Marty, C., Bedoux, G., & Bourgougnon, N. (2018). Antiviral effect of polyphenol rich plant extracts on herpes simplex virus type 1. *Food science and human wellness*, 7(1), 91-101.

- Gnann, J. W., & Whitley, R. J. (2017). Herpes simplex encephalitis: an update. *Current infectious disease reports*, 19, 1-12.
- Ismaeel, M. A. H. M. U. D., DYARI, H. R. E., NOR, N. S. M., YAACOB, W. A., & Ibrahim, N. A. Z. L. I. N. A. (2018). Anti-human herpesvirus type-1 activity of *Phaleriamacrocarpa* fruits methanol extract and fractions. *Malaysian Applied Biology*, 47(5), 31-40.
- Jadhav, P., Kapoor, N., Thomas, B., Lal, H., & Kshirsagar, N. (2012). Antiviral potential of selected Indian medicinal (ayurvedic) plants against herpes simplex virus 1 and 2. *North American journal of medical sciences*, 4(12), 641.
- Kimberlin, D. W., Lakeman, F. D., Arvin, A. M., Prober, C. G., Corey, L., Powell, D. A., & Whitley, R. J. (1996). Application of the polymerase chain reaction to the diagnosis and management of neonatal herpes simplex virus disease. *Journal of Infectious Diseases*, 174(6), 1162-1167.
- Looker, K. J., Magaret, A. S., May, M. T., Turner, K. M., Vickerman, P., Newman, L. M., & Gottlieb, S. L. (2017). First estimates of the global and regional incidence of neonatal herpes infection. *The Lancet Global Health*, 5(3), e300-e309.
- Lopes, N., Ray, S., Espada, S. F., Bomfim, W. A., Ray, B., Faccin-Galhardi, L. C., & Nozawa, C. (2017). Green seaweed *Enteromorpha compressa* (Chlorophyta, Ulvaceae) derived sulphated polysaccharides inhibit herpes simplex virus. *International J. Biological Macromolecules*, 102, 605-612.
- McQuillan, G., Kruszon-Moran, D., Flagg, E. W., & Paulose-Ram, R. (2018). Prevalence of herpes simplex virus type 1 and type 2 in persons aged 14-49: United States, 2015-2016.
- Medini, F., Megdiche, W., Mshvildadze, V., Pichette, A., Legault, J., St-Gelais, A., & Ksouri, R. (2016). Antiviral-guided fractionation and isolation of phenolic compounds from *Limonium densiflorum* hydroalcoholic extract. *Comptes Rendus. Chimie*, 19(6), 726-732.
- Mohanraj, K., Karthikeyan, B. S., Vivek-Ananth, R. P., Chand, R. B., Aparna, S. R., Mangalapandi, P., & Samal, A. (2018).

- IMPPAT: A curated database of Indian Medicinal Plants, Phytochemistry and Therapeutics. *Scientific reports*, 8(1), 4329.
- Narain, J. P., & Lal, S. (2014). Responding to the challenge of acute encephalitis syndrome/JE in India. *J Commun Dis*, 46(1), 1-3.
  - Nikomtak, J., Thongwai, N., Lumyong, S., & Tragoolpua, Y. (2008). Anti-viral activity of *Cissusre panda* Vahl. plant extract on herpes simplex virus.
  - Padma, P., Pramod, N. P., Thyagarajan, S. P., & Khosa, R. L. (1998). Effect of the extract of *Annona muricata* and *Petunia nyctagineiflora* on Herpes simplex virus. *Journal of ethnopharmacology*, 61(1), 81-83.
  - Park, H. J., Kurokawa, M., Shiraki, K., Nakamura, N., Choi, J. S., & Hattori, M. (2014). Antiviral activity of the marine alga *Sympyocladial atiuscula* against herpes simplex virus (HSV-1) in vitro and its therapeutic efficacy against HSV-1 infection in mice. *Biological and Pharmaceutical Bulletin*, 28(12), 2258-2262.
  - Roizman, B., Knipe, D., Whitley, R. (2013). Herpes Simplex Viruses. In Knipe D, Howley P (ed), *Fields Virology*, LWW, Philadelphia, 2:1823-1897.
  - Rowley, A. H., Wolinsky, S. M., Whitley, R. J., & Lakeman, F. D. (1990). Rapid detection of herpes-simplex-virus DNA in cerebrospinal fluid of patients with herpes simplex encephalitis. *The Lancet*, 335(8687), 440-441.
  - Santoyo, S., Ramírez-Anguiano, A. C., Aldars-García, L., Reglero, G., & Soler-Rivas, C. (2012). Antiviral activities of *Boletus edulis*, *Pleurotus ostreatus* and *Lentinus edodes* extracts and polysaccharide fractions against Herpes simplex virus type 1. *Journal of Food & Nutrition Research*, 51(4).
  - Saran, N., & Anandharaj, B. (2020). *In-vitro* Antiviral Activity of *Justiciae adhathoda* Plant Extracts against Human Herpes Simplex Virus Type-1. *Journal of Natural Remedies*, 21(7).
  - Sydiskis, R. J., Owen, D. G., Lohr, J. L., Rosler, K. H., & Blomster, R. N. (1991). Inactivation of enveloped viruses by anthraquinones extracted from plants. *Antimicrobial agents and chemotherapy*, 35(12), 2463-2466.

- Tragooolpua, Y., Jatisatiennr, A. (2007). Anti-herpes simplex virus activities of *Eugenia caryophyllus* (Spreng.) Bullock & S. G. Harrison and essential oil, eugenol. *Phytotherapy Research*, 21(12):1153-1158.
- Trott, O., & Olson, A. J. (2010). AutoDockVina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of computational chemistry*, 31(2), 455-461.
- Venkatesan, A., Tunkel, A. R., Bloch, K. C., Lauring, A. S., Sejvar, J., Bitnun, A., & Cherry, J. (2013). Case definitions, diagnostic algorithms, and priorities in encephalitis: consensus statement of the international encephalitis consortium. *Clinical Infectious Diseases*, 57(8), 1114-1128.
- Verma, H., Patil, P. R., Kolhapure, R. M., & Gopalkrishna, V. (2008). Antiviral activity of the Indian medicinal plant extract, *Swertia chirata* against *herpes simplex viruses*: A study by in-vitro and molecular approach. *Indian Journal of Medical Microbiology*, 26(4), 322-326.
- Wagner, H. (1989). Search for new plant constituents with potential antiphlogistic and antiallergic activity. *Plantamedica*, 55(03), 235-241.
- Whitbeck, J. C., Peng, C., Lou, H., Xu, R., Willis, S. H., Ponce de Leon, M., & Eisenberg, R. J. (1997). Glycoprotein D of *herpes simplex virus* (HSV) binds directly to HVEM, a member of the tumor necrosis factor receptor superfamily and a mediator of HSV entry. *Journal of virology*, 71(8), 6083-6093.
- Whitley, R. J. (2004). Herpes simplex virus. *Infections of the central nervous system*. 3rd ed. Philadelphia: Lippincott Williams & Wilkins, 123-44.
- Whitley, R. J., Alford, C. A., Hirsch, M. S., Schooley, R. T., Luby, J. P., Aoki, F. Y., & NIAID Collaborative Antiviral Study Group\*. (1986). *Vidarabine* versus acyclovir therapy in herpes simplex encephalitis. *New England Journal of Medicine*, 314(3), 144-149.
- Yoosook, C., Panpisutchai, Y., Chaichana, S., Santisuk, T., & Reutrakul, V. (1999). Evaluation of anti-HSV-2 activities of

*Barleria lupulina* and *Clinacanthus nutans*. Journal of ethnopharmacology, 67(2), 179-187.

- Zandi, K., Fouladvand, M., Pakdel, P., & Sartavi, K. (2007). Evaluation of in vitro antiviral activity of a brown alga (*Cystoseiramyrifica*) from the Persian Gulf against *herpes simplex virus* type 1. African Journal of Biotechnology, 6(22).

## In Silico Analysis of Phytocompounds from Indian Medicinal Plants for Treating Blood Cancer

P. Ravikumar<sup>1\*</sup> · R. Haripriya<sup>2</sup> · S. Malini<sup>3</sup> · T. Raja<sup>4</sup>

<sup>1</sup>. Senior Scientist, Iyarvi Research Center for Bioinformatics (IRCB), Erode - 638 452, TamilNadu, India.

<sup>2</sup>. Research Assistant, Iyarvi Research Center for Bioinformatics (IRCB), Erode - 638 452, Tamil Nadu, India.

<sup>3</sup>. PG Student, Department of Biotechnology & Bioinformatics, Bishop Heber College (Autonomous), Tiruchirapalli-620017, Tami Nadu, India.

<sup>4</sup>. Assistant Scientific Officer, Iyarvi Research Center for Bioinformatics (IRCB), Erode - 638 452, Tamil Nadu, India.

\*Corresponding Author: E-mail: headircb@gmail.com;  
Ph: +91-4285 – 227321/99445 27321

### Abstract

Blood cancer has been a growing concern during the last decade and requires early diagnosis to start proper treatment. The present study was designed to find the potential phytocompounds from Indian medicinal plants against Blood Cancer (BC) using *in silico* studies. The 3D structure of phytocompounds was obtained using the IMPPAT and PubChem databases. The Lipinski rule of five for all the phytocompounds was tested using SwissADME. The 3D structure of the target protein (Tyrosine protein kinase FLT3) was retrieved from the PDB database. The docking studies were performed using PyRx, and the results were analyzed using Discovery Studio 2021. From the results, the phytocompounds 7,4'-Dihydroxyflavone, Kaempferol, and Liquiritigenin showed very good binding affinity, like -8.6, -8.6, and -8.4 Kcal/mol, respectively. Toxicity studies were done for the best-interacted phytocompounds, and the results showed that the compounds had very less toxicity. Hence, the present study concludes that the 7,4'-Dihydroxyflavone, Kaempferol and

Liquiritigenin from *Glycine max*, may have the potential ability to treat Blood Cancer.

**Keywords:** *Blood Cancer, Phytocompound, Docking, SwissADME, Glycine max.*

## Introduction

Cancer is the uncontrolled proliferation of aberrant cells that can spread throughout the human body (Eid *et al.*, 2021). It is currently one among the world's leading causes of death. Study of Sung *et al.* (2021) estimated that there would be around 10,000,000 deaths annually and 19.3 million additional cases in 2020. The death rates of various cancers change depending on type of cancer. For example, lung cancer will account for 18% of deaths in 2020, while colorectal cancer will account for 9.4%, and liver disease, stomach cancer, & breast cancer will account for 8.3%, 7.7%, & 6.9% of deaths, respectively. Nearly ten percent of all cancer were diagnosed cases are blood cancers (Eid *et al.*, 2021). Early detection and prediction have long been thought to be wise approaches to prevent cancer fatalities around the world. According to the Leukemia& Lymphoma Association [3 T. L. L. Society.2016–2017], there are 1,290,773 people with blood cancer in the United States (US). Myeloma, leukaemia, lymphoma, and myelodysplastic syndromes are examples of prevalent blood malignancies. Blood malignancies impact blood cells, bone marrow, lymphatic system, and some other elements of the lymphatic system, to name a few.

AML's incidence frequency rises with age, starting at 1.3 per 100 000 people in a group of individuals under 65 years old. In a patient population over 65 years old, there were 12.2 instances per 100,000. Given current treatments, as many as 70% of individuals are 65 or older and have decreased survival odds, which are usually verified one year following diagnosis. Because of genetic causes, AML is more likely to occur in children. Men are slightly more affected than women. In the United States in 2019, there were around 61,780 new cases of leukaemia and 22,840 leukemia-related fatalities. The general consensus predicts a rise to

roughly 21,450 additional acute cases myeloid leukaemia (AML), predominantly in adults, and 10,920 fatalities from AML, almost all of whom are adults. Acute myeloid leukaemia has a 5 overall survival rate of only 27.40 percent. Acute Myeloid Leukemia develops in individuals with an underlying haematological condition or as a major side effect of earlier therapy, with contact to map of the area II, alkylating drugs, or radiation being key contributors (Grafone *et al.*, 2012).

Acute myeloid leukaemia (AML) is an uncommon hematological cancer that accounts for only 0.8% of all new cases of cancer in Australia. Workplace productivity is harmed by high morbidity and mortality due to worker turnover and premature death. Productivity was estimated utilizing productive output years of life (PALYs), a concept related to performance life years (QALYs) that accounts for productivity loss due to disease rather than poor health. AML will cost Australia \$1.43 billions of dollars in lost gross national product (\$971,000,000 in US dollars) during the next ten years, resulting in the loss of 7,600 decades of existence and 7,337 PALYs. Secondary analyses show that increasing survival rates by 20% might save almost AU\$52 million, and boosting return-to-work rates by 20% could save almost AU\$118 million based on current projections. Our research shows that while in low-incidence cancers, increased mortality and morbidity have significant consequences for decades of existence, productivity, and the economy. Better treatment procedures will very certainly lead to large financial gains. This emphasises the importance of funding research to enhance medicines (Ward *et al.*, 2021).

Acute myeloid leukaemia (AML) is a group of haematological disorders marked by the clonal proliferation of immature myeloid progenitor cells. One of the most common treatments for AML is chemotherapy. The establishment of medication tolerance of leukemic cells, which would be a severe impediment to disease therapy, affects the clinical result. Understanding of the mechanisms for resistance to various cytostatics and cytotoxic

medicines is essential for developing effective treatment methods for AML (Bobrova & Romanovskaya, 2021).

Metal complexes are widely used in cancer treatment. The many tuning variables (metals, receptor, and metal-ligand interaction) provide unique drug design opportunities, resulting in a large portfolio of metallodrugs with a greater range of activities and mechanisms of action than pure organic compounds. Clinically approved metallodrugs including cisplatin, carboplatin, and oxaliplatin are used to treat a wide variety of cancers and play an important part in combination therapy, such as immunotherapy. However, metallodrugs have poor pharmacokinetic, low rates of drug target accumulation, metal-mediated off-target reactivity, and drug resistance formation, all of which can restrict their efficacy and clinical translation (Peña *et al.*, 2022).

One of the most frequently mutated genes in human leukaemia is tyrosine kinase, a class III receptor tyrosine kinase (RTK) protein that plays a crucial regulatory role in healthy hematopoiesis (Bathula *et al.*, 2017). AML patients who are newly diagnosed with fms-like tyrosine kinase 3 (FLT3) gene mutations account for about 30% of cases. Although FLT3-ITDmut detrimental prognostic effect in AML has been amply demonstrated, FLT3-TKDmut prognostic importance is still up for debate (Daver *et al.*, 2021).

Leukemia has indeed been treated using a variety of medications. The hunt of safer and more efficient medications remains among the most difficult fields of study due to negative consequences of such therapy and drug resistance. As a result, novel therapeutic approaches are critical for bettering outcomes. Organic ingredients and their derivatives account for over half of the medications used to treat cancer today. Natural sources of anti-leukemic medicines have been found in medicinal plants. The cytotoxicity of these plants, as well as the mechanisms behind their toxicity to leukemic cells and isolated chemicals, were examined (Maher *et al.*, 2021).

Medicinal plants have played a significant role in the diagnosis and reduction of injury ailments since ancient times (Dar *et al.*, 2017). Moving forward to the twentieth century, several plant chemicals play an important role in cancer treatment. Vincristine & vinblastine, among example, are two anticancer alkaloids discovered in the 1960s from of the Periwinkle plant (*Catharanthus roseus*) and are used to treat a variety of tumours (Howes, 2018). Taxol, an anticancer medicine discovered in 1971 from the bark of the Pacific yew (*Taxus brevifolia*), is another key anticancer treatment. Natural products' vast biodiversity including, in particular, their complicated chemical composition, may explain why they are so actively explored as possible anticancer medicines. They can interact with a variety of molecular targets and affect a variety of biological pathways since they are made up of so many molecules (Turrini *et al.*, 2019, Catanzaro *et al.*, 2018, Mondal *et al.*, 2009, Turrini *et al.*, 2019, Turrini *et al.*, 2018, Turrini *et al.*, 2018).

In the current study, the different Indian medicinal plants like *Allium sativum*, *Zingiber officinale*, *Curcuma longa*, *Withania somnifera* and *Glycine max* were selected to find the potential phytocompounds for treating Acute Myeloid Leukemia (AML).

## Materials and Methods

### Ligand selection

Using literature & IMPPAT database (Mohanraj *et al.*, 2018), around 350 phytochemical compounds were selected from the different Indian medicinal plants like *Allium sativum*, *Curcuma longa*, *Glycine max*, *Withania somnifera*, *zingiber officinale* (Polu *et al.*, 2015)for treating Blood cancer Disease. The 3D structure of these compounds was retrieved from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) and using SwissADME (Daina *et al.*, 2017), they were subjected to test Lipinski Rule of Five. From the results, 335 compounds obeyed Lipinski Rule of Five and these compounds were taken for the study.

## Target protein selection

The target protein (Tyrosine protein kinase FLT3) belonging to gene FLT3 was found in the literature for Blood Cancer (Bathula *et al.*, 2017). The 3D structure of this target protein was retrieved from the PDB database (<https://www.rcsb.org>). The UniProt ID of this target protein was taken from the Uniprot database (<https://www.uniprot.org/>).

## Docking studies

Docking studies for the target protein (Tyrosine protein kinase FLT3) and the phytocompounds (ligands) were done using PyRx 0.8 software (Trott and Olson, 2010). The target protein was further prepared for docking studies using this software. All the ligands were uploaded using Open Babel option in the PyRx 0.8. The grid was generated and the docking studies were performed using Vina wizard option in the PyRx 0.8. The values of binding affinity were saved in XL file. The results were analyzed using Discovery Studio 2021 and the 2D & 3D docked images were taken. In the results, the lowest binding affinity indicates good result.

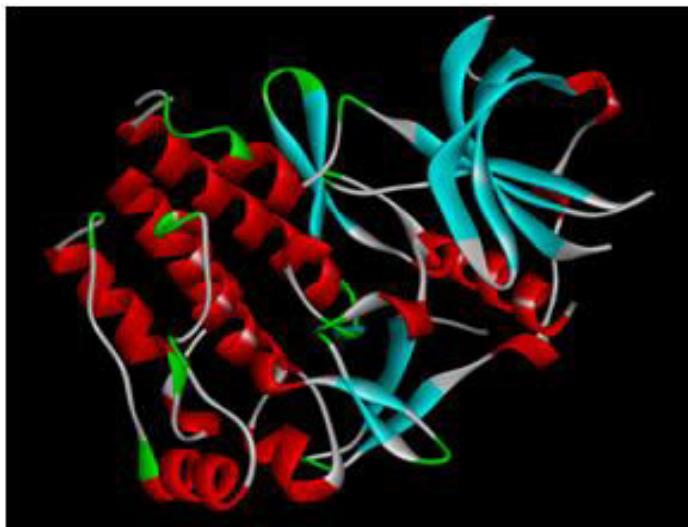
## ADMET & CYP properties

ADMET and CYP properties were tested for all the best-interacted phytocompounds using SwissADME (Daina *et al.*, 2017). Lipinski, BBB (Blood - Brain Barrier), HIA (Human Intestinal Absorption), PGP (P-glycoprotein), XLogP3, TPSA (Topological Polar Surface Area), LogS, Fraction Csp3, Rotatable bonds, CYP enzyme inhibitor properties, Skin permeation and Bioavailability score were evaluated for all the best-interacted compounds.

## Results

### Ligand and Target protein selection

The 3D structure of ligands (phytocompounds) was retrieved from the PubChem database. The 3D structure of the target protein (Tyrosine protein kinase FLT3) was obtained from PDB database and its PDB ID is 1RJB. The 3D structure of the target protein is shown in figure 1.



**Figure 1:** The 3D structure of Target protein  
(Tyrosine protein kinase FLT3)

### Docking studies

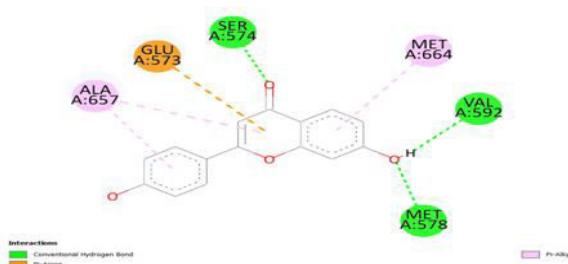
Docking studies for the target protein (Tyrosine protein kinase FLT3) and the phytocompounds (ligands) were done using PyRx 0.8 software. In the following results, all the 10 compounds were interacted with target protein. In which, all the results are showed in table 1 and the 2D & 3D interaction of phytocompounds and synthetic drug with the target protein are shown in figure 2-9.

**Table 1:** Interaction of Phytocompound with the target protein

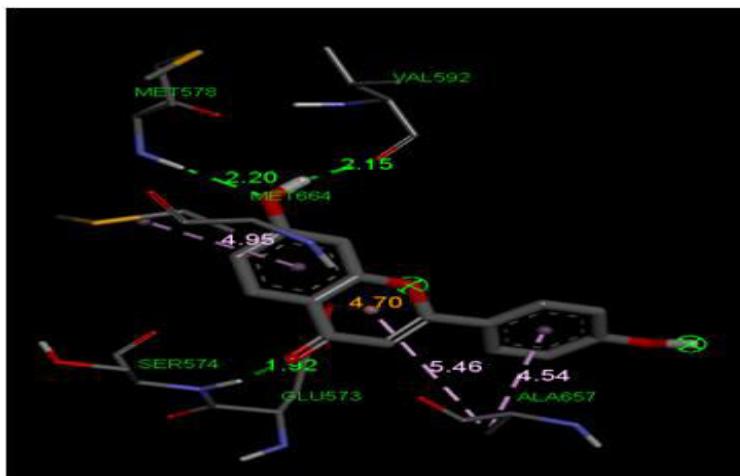
S. No.	PubChem (CID)	Compound Name	Plant Name	Binding Affinity (Kcal/mol)	No. of Bonds	Interacting Residues	Bond Length (Å)
1.	5282073	7,4'-Dihydroxyflavone	<i>Glycine max</i>	-8.6	7	ALA657 ALA657 SER574 GLU573 MET664 MET578 VAL592	4.54 5.46 1.92 4.70 4.95 2.20 2.15
2.	5280863	Kaempferol	<i>Glycine max</i>	-8.6	7	SER574 SER574 SER574 GLU573 ASP593 MET578 MET664	3.53 2.22 2.11 4.61 4.17 1.22 5.64
3.	114829	Liquiritigenin	<i>Glycine max</i>	-8.4	5	MET578 VAL592 MET664 SER574 ALA657	2.29 2.19 5.90 1.86 4.59
4.	5281707	Coumestrol	<i>Glycine max</i>	-8.4	8	VAL581 VAL581 VAL581 ILE867 ILE867 ILE867 LEU802 CYS807	4.62 3.94 4.86 3.01 5.20 3.70 3.00 2.14
5.	5280961	Genistein	<i>Glycine max</i>	-8.2	10	ALA657 GLU573 SER574 LEU576 GLN577 MET664 VAL592 VAL592 ASP593 ARG595	5.34 4.30 3.59 2.86 2.67 5.87 2.30 2.34 3.64 2.60

6.	10429233	Dihydrocurcumin	<i>Curcum a longa</i>	-8.1	11	GLN575 PRO851 ARG849 ILE836 TYR842 TYR842 ARG834 GLY831 GLY831 PHE621 PHE621	3.78 5.13 4.44 5.16 2.06 2.27 2.57 3.48 3.39 4.66 4.99
7.	442667	Anhydroglycinol	<i>Glycine max</i>	-8.1	6	MET664 LEU576 SER574 GLU573 GLU573 ALA657	5.84 2.78 3.68 4.23 4.15 4.90
8.	5469424	Demethoxycurcumin	<i>Curcum a longa</i>	-7.5	5	ARG810 PRO851 ARG849 TYR842 ILE836	3.03 3.38 3.00 1.97 3.68
9.	5322052	2,4,4'-Trihydroxychalcone	<i>Glycine max</i>	-7.4	7	ARG834 ILE836 GLU573 ARG849 LEU850 PRO851 ARG810	2.82 3.85 2.32 2.15 5.37 5.34 1.78
10.	10447050	1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one	<i>Curcum a longa</i>	-7.2	3	PRO851 ILE836 ARG810	3.24 3.76 3.49
<b>Synthetic drug</b>							
11.	24889392	Quizartinib	-	-8.6	7	ARG849 ARG849 ALA848 ILE836 PHE621 GLN575 TYR589	4.69 2.00 5.42 4.92 5.14 2.73 3.57

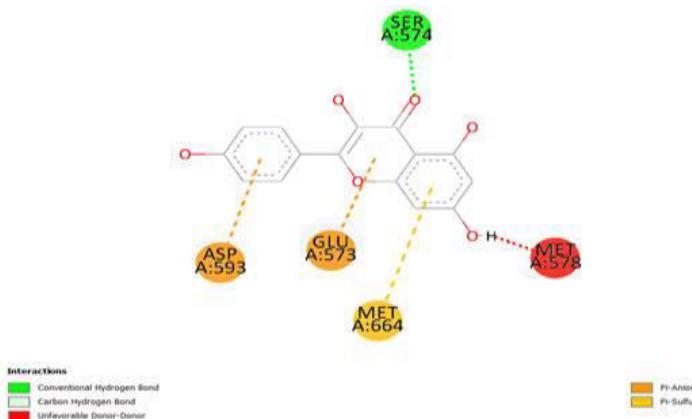
From the results (Table 1), among other compounds, 10 compounds showed very good results with the target protein. Of which, the phytocompound 7,4'-Dihydroxyflavone showed very good binding affinity (-8.6 Kcal/mol) with the amino acid residues ALA657, ALA657, SER574, GLU573, MET664, MET578, VAL592. The phytocompound Kaempferol also gave very good binding affinity of -8.6 Kcal/mol with the amino acid residues SER574, SER574, SER574, GLU573, ASP593, MET578, and MET664. The binding affinity -8.4 Kcal/mol was observed between the phytocompound Liquiritigenin and the amino acid residues MET578, VAL592, MET664, SER574, and ALA657 of target protein. Among the other compounds, the lowest binding affinity (-7.2 Kcal/mol) was observed between the phytocompound 1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one and the amino acid residues PRO851, ILE836, ARG810 of target protein. Besides, the binding affinity of the 2,4,4'-Trihydroxychalcone with the target protein was -7.4 Kcal/mol and the interacted the amino acid residues were ARG834, ILE836, GLU573, ARG849, LEU850, PRO851, ARG810. Thus, in the results of the present study, all the phytocompounds showed very good binding affinity when compared to the 1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one. Of which, the phytocompounds 7,4'Dihydroxyflavone, Kaempferol, Liquiritigenin and Coumestrol showed highest binding affinity among the other phytocompounds.



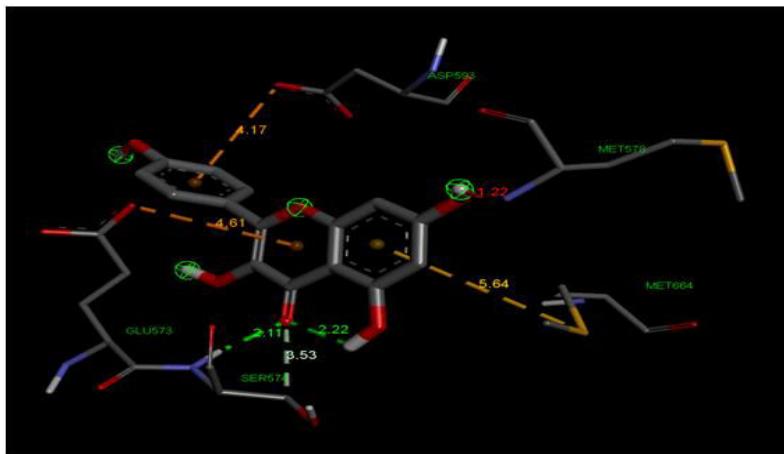
**Figure 2:** The 2D interaction of phytocompound 7,4'-Dihydroxyflavone with the target protein



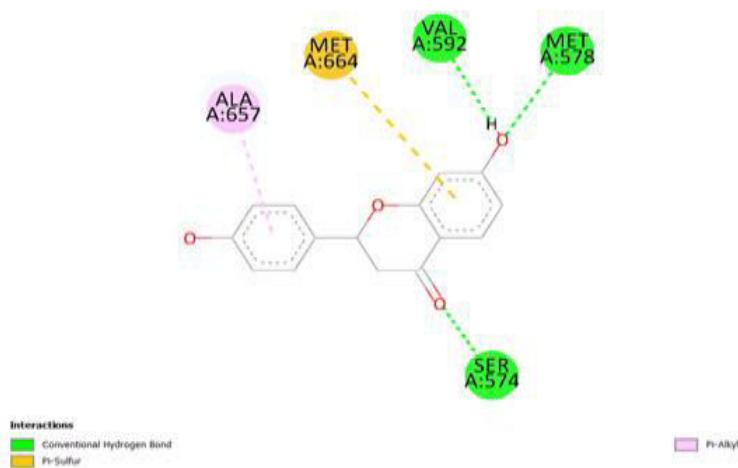
**Figure 3:** The 3D interaction of phytocompound 7,4'-Dihydroxyflavone with the target protein



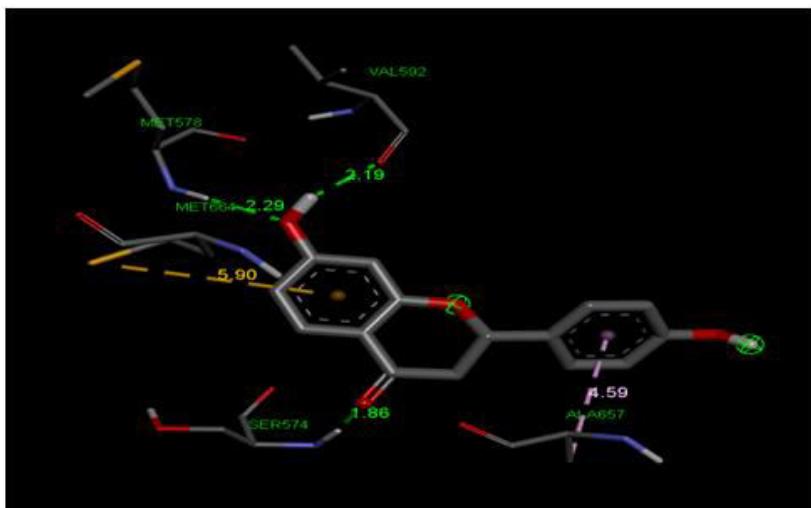
**Figure 4:** The 2D interaction of phytocompound Kaempferol with the target protein



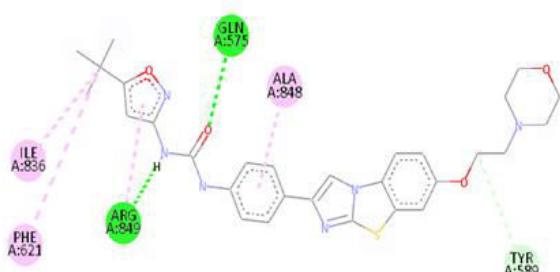
**Figure 5:** The 3D interaction of phytocompound Kaempferol with the target protein



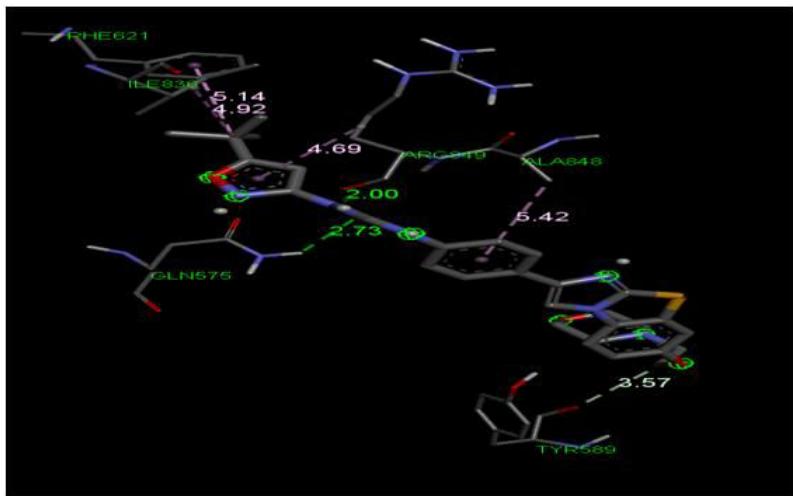
**Figure 6:** The 2D interaction of phytocompound Liquiritigenin with the target protein



**Figure 7:** The 3D interaction of phytocompound Liquiritigenin with the target protein



**Figure 8:** The 2D interaction of synthetic drug Quizartinib with the target protein



**Figure 9:** The 3D interaction of synthetic drug Quizartinib with the target protein

#### ADMET & CYP Properties

In the present study, ADMET properties were tested for the best interacted phytocompounds and Synthetic drug Quizartinib using SwissADME and the results was tabulated (table 2). From the results, all the best interacted phytocompounds obey Lipinski rule of five. Most of the compounds did not cross Blood Brain Barrier (BBB) and had high Intestinal Absorption (HIA). Many phytocompounds predicted to be effluated from the CNS by P-glycoprotein. Among the 10 compounds, XLogP3 values of 5 compounds were within the range. TPSA (Topological Polar Surface Area) and Log S value of the most of the compounds were within the limit. In all the compounds, Fraction Csp3 values of 2 compounds were less than 0.25 and the value of other compounds were above this limit. Rotatable bonds for most of the compounds were within the limit.

**Table 2:** ADMET Properties of Phytocompounds

S. N o	PubChem (CID)	Compound Name	Lipi nski	BBB	HIA	PGP -	XLO GP3	TPS A (Å)	Log S (ES OL)	Frac tion Csp3	Rotat able Bond s
1.	5282073	7,4'-Dihydroxyflavone	Yes	Yes	High	Yes	3.26	70.67	- 4.03	0.00	1
2.	5280863	Kaempferol	Yes	No	High	Yes	1.90	111.1 3	- 3.31	0.00	1
3.	114829	Liquiritigenin	Yes	Yes	High	No	2.30	66.76	- 3.28	0.13	1
4.	5281707	Coumestrol	Yes	No	High	Yes	3.10	83.81	- 3.87	0.00	0
5.	5280961	Genistein	Yes	No	High	Yes	2.67	90.90	- 3.77	0.00	1
6.	10429233	Dihydrocurcumin	Yes	No	High	Yes	3.01	93.06	- 3.77	0.24	9
7.	442667	Anhydroglynolin	Yes	Yes	High	No	2.82	62.83	- 3.78	0.07	0
8.	5469424	Demethoxycurcumin	Yes	No	High	Yes	3.32	83.83	- 3.92	0.10	7
9.	5057077	2,4,4'-Trihydroxychalcone	Yes	Yes	High	Yes	2.62	77.76	- 3.35	0.00	3
10.	28688976	1,7-Bis(4-hydroxyphe nyl)-1,4,6-heptatrien-3-one	Yes	No	High	No	3.80	95.83	- 4.80	0.21	4
<b>Synthetic drug</b>											
11.	24889392	Quizartinib	Yes	No	Low	No	5.64	134.4 0	- 6.63	0.34	10

**Note:** Obey Lipinski: Yes means 0 violation & good, BBB (Blood - Brain Barrier): Yes means good, HIA (Human Intestinal Absorption): High means good, PGP- (Molecules predicted not to

be effluated from the CNS by P-glycoprotein): Yes means good, Lipophilicity: XLOGP3 value between -0.7 and +5.0 means good, Polarity: TPSA between 20 and 130 Å<sup>2</sup> means good, Water Solubility (Log S scale: Insoluble < -10 < Poorly < -6 < Moderately < -4 < Soluble < -2 < Very < 0 < Highly): Log S value not higher than 6 means good, Saturation (Fraction Csp3):

S. No.	PubChe (CID)	Compound Name	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Log K <sub>p</sub> (Skin permeability cm/s)	A Bioavailability Score (ABS)
1	5282073	7,4' Dihydroxyflavon	Yes	No	No	Yes	Yes	-5.54	0.55
2	5280863	Kaempferol	Yes	No	No	Yes	Yes	-6.70	0.55
3	114829	Liquiritigenin	Yes	No	No	No	No	-4.07	0.55
4	5281707	Coumestrol	Yes	No	No	Yes	No	-5.98	0.55
5	5280961	Genistein	Yes	No	No	Yes	Yes	-6.05	0.55
6	5469424	Demethoxcurcumin	Yes	No	Yes	No	Yes	-6.01	0.55
7	442667	Anhydroglicinol	Yes	No	No	Yes	Yes	-5.85	0.55
8	10429233	Dihydrocurcumir	No	No	Yes	Yes	Yes	-6.42	0.55
9	5057077	2,4,4'-Trihydroxychalcone	Yes	No	Yes	No	Yes	-6.00	0.55
10	28688976	1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one	Yes	Yes	Yes	Yes	Yes	-5.97	0.55
<b>Synthetic drug</b>									
11	24889392	Quizartinib	No	Yes	Yes	Yes	Yes	-5.72	0.51

**Note:** Yes, means the compound inhibits the CYP450 enzymes and gives unanticipated adverse reactions; No means the compound does not inhibit the CYP450 enzymes and does not give any adverse reactions; The more negative the log K<sub>p</sub>, the less skin

permeant is the molecule; **ABS 0.55** means it passes the rule of five & **0.17** means it fails the rule of five.

The compound having high negative value ( $\log K_p$ ) has less skin permeation ability (Daina *et al.*, 2017). According to this statement, the compounds 7,4'-Dihydroxyflavone, Demethoxy curcumin, 1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one, Coumestrol, Dihydro curcumin, Anhydroglycinol, Genistein, Kaempferol, Liquiritigenin, 2,4,4'-Trihydroxy chalcone semicarbazide have less skin permeation ability. Besides, the bioavailability score of all the 10 compounds were good and they passed the rule of five.

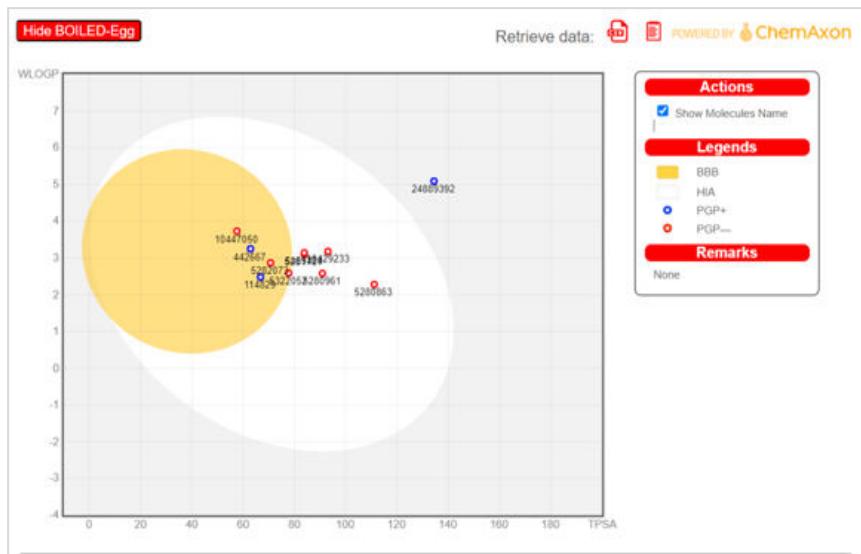


Figure 10: Boiled egg for all the compounds

**Note:** BBB: Points located in BOILED-Egg's yolk are molecules predicted to passively permeate through the blood-brain barrier. HIA- Points located in BOILED-Egg's white are molecules predicted to be passively absorbed by the gastrointestinal tract. PGP+: Blue dots are for molecules predicted to be effluated from the central nervous system by the P glycoprotein. PGP-: Red dots are for molecules predicted not to be effluated from the central nervous system by the P-glycoprotein.

## Discussion

FLT3 inhibitors have shown promising efficacies in aggressive AML. However, the duration of clinical response is short because of the rapid development of resistance. Novel next-generation FLT3 inhibitors are in active development to concur the resistance. Combining FLT3 inhibitors with other targeted agents are additional areas of investigation to minimize resistance to current FLT3 inhibitors. FLT3 is an important target in AML due to the high incidence of mutations resulting in constitutive signaling, and associated poor outcomes. The first-generation type I inhibitor midostaurin has shown benefit, and second-generation type I inhibitors such as gilteritinib show promise, as do novel combinations. The current status of this rapidly evolving field was summarized in this review, but new preclinical and clinical data continue to be rapidly generated, with the ultimate goal of successful targeted therapy for this common subset of AML patients who currently continue to have poor treatment outcomes. In the same way, the present study reported that 7,4'-Dihydroxyflavone, Kaempferol and Liquiritigenin from *Glycine max* may give a potential effect in the treatment of Blood Cancer.

## Conclusion

In the present study, the phytocompounds from different Indian medicinal plants and the target protein (Tyrosine protein kinase FLT3) were subjected for *in silico* docking analysis to find the potential inhibitors for BC. In which, 335 compounds showed better results than the Synthetic drug Quizartinib. Among them, 10 compounds showed very good binding affinity. Toxicity studies were done for the 10 best-interacted phytocompounds and the results showed that the compounds had very less toxicity. In which, the phytocompounds 7,4'Dihydroxyflavone, Kaempferol and Liquiritigenin showed highest binding affinity among the other phytocompounds. Hence, the present study concludes that the 7,4'-Dihydroxyflavone, Kaempferol and Liquiritigenin from *Glycine max* may give a potential effect in the treatment of Blood Cancer.

## References

- Bathula, R., Rani, S. S., & Devi, K. R. Modeling, Simulation, Docking Studies of Tyrosine Kinase Involved in Leukemia and Microwave Synthesis of Quinazoline Derivatives.
- Bobrova, N. M., & Romanovskaya, T. V. (2021). Mechanisms of Drug Resistance in Acute Myeloid Leukemia. *Biology Bulletin Reviews*, 11(Suppl 1), 32-46.
- Catanzano, E., Greco, G., Potenza, L., Calcabrini, C. & Fimognari, C. (2018). Natural products to fight cancer: A focus on *Juglans regia*. *Toxins*, 10(11), 469.
- Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific reports*, 7(1), 42717.
- Dar, R. A., Shahnawaz, M., Rasool, S., & Qazi, P. H. (2017). Natural product medicines: A literature update. *J phytopharmacol*, 6(6), 349-351.
- Daver, N., Venugopal, S., & Ravandi, F. (2021). FLT3 mutated acute myeloid leukemia: 2021 treatment algorithm. *Blood cancer journal*, 11(5), 104.
- Eid, M. M., Rashed, A. N. Z., Bulbul, A. A. M., & Podder, E. (2021). Mono-rectangular core photonic crystal fiber (MRC-PCF) for skin and blood cancer detection. *Plasmonics*, 16, 717-727.
- Grafone, T., Palmisano, M., Nicci, C., & Storti, S. (2012). An overview on the role of FLT3-tyrosine kinase receptor in acute myeloid leukemia: biology and treatment. *Onc. reviews*, 6(1).
- Howes, M. J. R. (2018). The evolution of anticancer drug discovery from plants. *The Lancet Oncology*, 19(3), 293-294.
- Maher, T., Ahmad Raus, R., Daddiouaissa, D., Ahmad, F., Adzhar, N. S., Latif, E. S., & Mohammed, A. (2021). Medicinal

plants with anti-leukemic effects: A review. *Molecules*, 26(9), 2741.

- Mohanraj, K., Karthikeyan, B. S., Vivek-Ananth, R. P., Chand, R. B., Aparna, S. R., Mangalapandi, P., & Samal, A. (2018). IMPPAT: A curated database of Indian Medicinal Plants, Phytochemistry and Therapeutics. *Scientific reports*, 8(1), 4329.
- Mondal, A., Gandhi, A., Fimognari, C., Atanasov, A. G., & Bishayee, A. (2019). Alkaloids for cancer prevention and therapy: Current progress and future perspectives. *European journal of pharmacology*, 858, 172472.
- Peña, Q., Wang, A., Zaremba, O., Shi, Y., Scheeren, H. W., Metselaar, J. M., & Lammers, T. (2022). Metallodrugs in cancer nanomedicine. *Chemical Society Reviews*, 51(7), 2544-2582.
- Polu, P., Nayanabhirama, U., & Khan, S. (2015). Herbal medicinal plants as an anticancer agent. *Annals of Phytomedicine*, 4(1), 37-45.
- Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global cancer statistics 2020: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians*, 71(3), 209-249.
- Trott, O., & Olson, A. J. (2010). AutoDockVina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of computational chemistry*, 31(2), 455-461.
- Turrini, E., Calcabrini, C., Tacchini, M., Efferth, T., Sacchetti, G., Guerrini, A., & Fimognari, C. (2018). *In vitro* study of the cytotoxic, cytostatic, and antigenotoxic profile of *Hemidesmus indicus* (L.) R. Br. (*Apocynaceae*) crude drug extract on T lymphoblastic cells. *Toxins*, 10(2), 70.
- Turrini, E., Catanzaro, E., Ferruzzi, L., Guerrini, A., Tacchini, M., Sacchetti, G. & Fimognari, C. (2019). *Hemidesmusindicus* induces apoptosis via proteasome inhibition and generation of

reactive oxygen species. *Scientific Reports*, 9(1), 7199.

- Turrini, E., Catanzaro, E., Muraro, M. G., Governa, V., Trella, E., Mele, V., & Fimognari, C. (2018). *Hemidesmusindicus* induces immunogenic death in human colorectal cancer cells. *Oncotarget*, 9(36), 24443.
- Turrini, E., Ferruzzi, L., & Fimognari, C. (2014). Natural compounds to overcome cancer chemoresistance: toxicological and clinical issues. *Expert Opinion on Drug Metabolism & Toxicology*, 10(12), 1677-1690.
- Ward, S. J., Lichtman, A. H., Piomelli, D., & Parker, L. A. (2021). Cannabinoids and cancer chemotherapy-associated adverse effects. *JNCI Monographs*, 2021(58), 78-85.

## Crafting Future Pharmaceuticals: The Fusion of AI, ML, and Plant-Derived Medicine Design

Kruthiga Natarajan<sup>1</sup>, Rajkuberan Chandrasekaran

<sup>1</sup>Department of Biotechnology,  
Karpagam Academy of Higher Education, Coimbatore, India.  
kuberan87@gmail.com

### Abstract

Artificial intelligence, particularly machine learning, holds promise in expediting pharmaceutical research by distilling valuable insights from the intricate web of data generated during drug discovery. Over recent years, AI/ML methodologies have demonstrated remarkable efficacy across various therapeutic domains, ushering in new benchmarks for drug development. While contemporary medicine remains pivotal in combatting numerous illnesses, the quest for effective treatments for chronic ailments persists. Furthermore, the side effects associated with many modern medications underscore the urgency for safer alternatives. Traditional plant-derived remedies offer a reservoir of potential, albeit their exploration methods have grown antiquated. Within this discourse, we delve into the fusion of AI with drug discovery, outlining a framework for leveraging AI in revitalizing the pursuit of alternative drug compounds from traditional botanical sources.

**Keywords:** Artificial intelligence, machine learning, Traditional plant-derived medicine

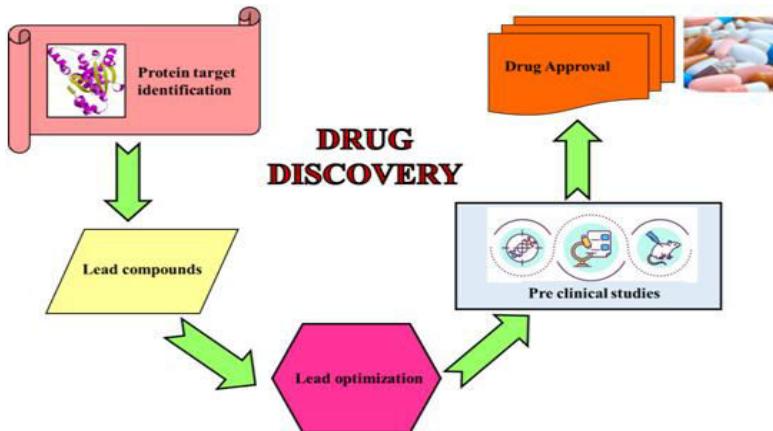
### Introduction

The incidence of several major diseases, ranging from cardiovascular disorders to various cancers and infectious illnesses, has experienced a notable decline since 1970 [1,2], contributing significantly to the global extension of average

lifespan [3]. This progress in healthcare and overall societal well-being primarily stems from the evolution of modern medicine, heavily reliant on chemically synthesized pharmaceuticals. The process of discovering chemically synthesized drugs typically initiates with the identification of a lead compound tailored to a specific drug target, followed by meticulous refinement, preclinical evaluations conducted across multiple animal models to assess both efficacy and safety parameters, culminating in rigorous human clinical trials (see Fig. 1) [4]. The trajectory from lead compound identification through optimization and completion of preclinical studies spans an average of 5 to 6 years, with merely approximately 1 in 5000 lead compounds progressing to human trials. Despite the prolonged and resource-intensive nature of this discovery process, alongside significant attrition rates, numerous modern medications continue to harbor substantial adverse effects, necessitating the exploration of alternative, safer therapeutic options [5]. Furthermore, the current approach to drug discovery has yet to provide an optimal solution for a myriad of chronic diseases, such as diabetes and certain cancers. Ongoing endeavors persist in the pursuit of alternative approaches for the discovery of lead compounds that are both effective and safer. One avenue for such exploration lies within plant-based traditional medicine, which presents a vast reservoir of potential knowledge [6,7].

However, in many instances, the active ingredients of plant-derived traditional medicines remain unidentified compared to modern medicine [8]. Even when the active ingredient is partially known, the standard dosage form often comprises a slurry of the entire plant or leaf extract [8], lacking precise definition (thus subject to variations in concentration based on factors like plant size and age). This extensive historical use of medicinal plant extracts suggests their potential as reservoirs for novel drug discovery when coupled with modern methodologies and principles. Plant extract consists of a multitude of chemical compounds and is accompanied by diverse forms of ancient medical documentation describing their usage; a robust, self-

learning automated system such as an AI platform will be indispensable in deciphering this wealth of data [9].



**Figure 1:** The key elements of the drug discovery process are outlined, including the utilization of the crystal structure of the protein sourced from the Protein Data Bank.

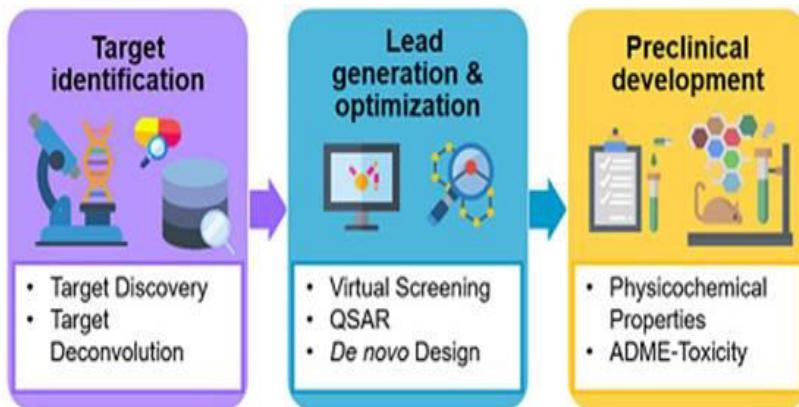
## Understanding the Role of Artificial Intelligence in Drug Design

AI functions as a computerized replication of human intelligence, utilizing acquired knowledge to address complex problems through the application or creation of rules, known as algorithms [11]. Creating new algorithms manually is often a time-consuming and challenging task for humans, a process that could be expedited with computer intervention. Among machine-learning algorithms, the neural network stands out for its loose resemblance to the functionalities of the human brain [12]. Although the neural network embodies AI, its functionalities lack precise definition, and its training requires human guidance [13]. With the continuous advancement of computing capabilities and the refinement of neural network algorithms, AI evolves rapidly [14]. Virtually every scientific field employs AI to enhance research outcomes, including the healthcare sector, where numerous major pharmaceutical companies are gradually integrating AI into their drug discovery efforts. For instance, Pfizer

utilizes the IBM Watson platform for immune-oncology drug discovery, while Sanofi has adopted Exscientia's AI platform for metabolic disease drug discovery, and Genentech utilizes the GNS healthcare AI platform for cancer drug development [15]. Notably, Sumitomo Dainippon Pharma of Japan successfully developed a drug molecule for obsessive-compulsive disorder within a year of adopting Exscientia's AI-enabled platform, marking the first AI-invented molecule to enter human trials [15]. This rapid identification of a suitable drug candidate for clinical trials within a short timeframe is noteworthy compared to conventional drug discovery processes. With appropriate adjustments, AI holds significant potential across various domains of drug discovery. The integration of AI with a database derived from plant-based traditional medicine could further transform the drug discovery landscape. This review explores the paradigm of AI integration across different stages of contemporary drug discovery and proposes a framework for an AI-driven platform tailored for drug discovery from plant-derived traditional medicine.

## Unveiling the Potential of AI and ML in Drug Discovery

Artificial intelligence and machine learning (AI/ML) have been integrated into various stages of the early drug discovery process, encompassing target identification, lead generation and optimization, and preclinical development (see Figure 2). In target discovery, AI-driven methodologies have been instrumental in amalgamating diverse datasets to unveil patterns, thereby elucidating the molecular mechanisms underlying diseases and drug responses. Within lead generation and optimization, AI/ML algorithms enhance scoring functions and quantitative structure-activity relationship (QSAR) models within virtual screening pipelines, facilitating the automation and refinement of de novo drug design processes. In preclinical development, AI/ML techniques are harnessed to construct predictive models of physicochemical properties through efficient processing of extensive chemical datasets, subsequently refining absorption, distribution, and metabolism, and excretion - toxicity (ADME-T) profiles [10].



**Figure 2:** The legend emphasizes the vital contribution of AI and ML in the drug discovery pipeline, enhancing decision-making and expediting the process across all stages. Key concepts like ADME are underscored, highlighting the importance of AI and ML methodologies.

## Harnessing Nature's Wisdom: The Promise of Plant-Derived Medicines

Discovering a new drug is consistently arduous. Once a drug target is identified, the primary challenge lies in discovering a highly specific lead compound to minimize the risk of off-target effects. Many chemically synthesized lead compounds exhibit various drug toxicities, rendering them unsuitable for final FDA approval. This limitation not only prolongs the discovery process but also leads to costly attrition. However, leveraging the extensive knowledge of plant-derived traditional medicine could help overcome this hurdle in highly specific lead compound discovery. Traditional medicine, particularly plant extracts, has been utilized for generations due to their generally well-tolerated nature. By isolating medicinal phytochemicals from plant extracts and purifying them, the risk of toxicities could be significantly mitigated. Hence, traditional medicine, primarily plant extracts, could serve as a valuable repository of knowledge in lead drug discovery, provided there is a reliable chemical database of

medicinal plants. One such example is the IMPPAT database, containing 9596 phytochemicals from 1742 Indian medicinal plants along with their therapeutic uses. Another comprehensive database, UMPDB, incorporates traditional, genomic, and chemical information of 1127 plants. Despite these databases containing information on numerous medicinal plants, the coverage remains limited compared to the vast number of plants documented in ancient therapeutic scripts. Therefore, expanding the inclusion of medicinal plants, their phytochemical information, and ancient therapeutic scripts is imperative [16, 17].

## Bridging the Gap: Integrating AI with Plant-Derived Medicine Design

Nearly every nation possesses its own reservoir of plant-derived traditional medicine. Among these, India, China, and Iran stand out for their rich medicinal heritage, collectively boasting hundreds of thousands of known medicinal plant species. Conducting a broad-spectrum, non-targeted mass-spectrometric analysis of each plant extract would yield extensive datasets, primarily composed of unidentified chemical signals, presenting an immense opportunity for novel discoveries. However, effectively leveraging these databases poses a challenge without knowledge of the structures of these unknown chemical signals. Hence, the initial step in drug design using traditional medicines involves elucidating the structures of these signals. Implementing AI-assisted structure elucidation, such as Computer-Assisted Structure Elucidation (CASE), can streamline this formidable task. CASE utilizes various statistical and ML methods to predict the structure of chemical signals. Kuhn et al. [18] demonstrated that different ML algorithms, including Random Forest, J48 decision tree, support vector machines, and HOSE codes, could accurately predict chemical structures from their nuclear magnetic resonance (NMR) spectra with minimal error. Li et al. [19] introduced a novel ML method, SubFragment-Matching, which employs a random forest algorithm to predict the structure of non-targeted mass-spectra.

## AI/ML applications in forecasting drug sensitivity and response

Enhancing targeted therapy response in complex diseases like cancer is the goal of personalized drug response prediction [20-22]. However, the challenge lies in the limited usage of candidate drugs in clinical settings and the diversity among cancer patients, making it difficult to customize therapy for each individual. Effective personalized treatment necessitates predictive methods capable of leveraging large, diverse, and sparsely sampled datasets. Utilizing precise AI/ML-based models incorporating *in vitro* and *in vivo* datasets can enhance the prediction of cancer cell response to specific compounds [23-27]. Various AI/ML models are employed to forecast drug sensitivity and anticancer drug response. Elastic net regression, ensemble-based methods, transfer learning, autoencoders, and multitask learning approaches have been extensively utilized in such endeavors. Further information regarding these AI/ML applications can be found in the Supporting Information [28-30].

## From Data to Drugs: The AI-Driven Path to Innovation

Upon understanding all the chemical compositions within a medicinal plant extract and their therapeutic implications, each compound within the extract dataset can undergo evaluation against the known drug targets associated with the diseases outlined in the therapeutic information. A sequence of protein-ligand interaction analyses can ascertain the binding affinity of the synthesized compounds. Nonetheless, this proves to be a time-intensive endeavor. The incorporation of CNN into protein-ligand interaction studies would favorably select chemical structures from this dataset based on the active site configuration, presenting a swifter and more methodical approach. Subsequently, it would compute the binding affinities and arrange them in order of priority, enabling the identification of the active constituents within a plant extract. Take garlic, for instance, recognized for its antidiabetic properties attributed to enhanced insulin secretion. Physiological investigations reveal that augmented insulin

secretion is primarily linked to escalated insulin gene expression and/or heightened insulin exocytosis. Employing nontargeted mass spectrometry coupled with AI-supported structure elucidation would anticipate the potential chemical composition of garlic extract. A series of protein-ligand interaction analyses incorporating all chemical structures of garlic extract with known targets for augmented insulin secretion, under CNN guidance, could uncover the active components of garlic or its antidiabetic attributes. Additionally, a modified version of the previously mentioned ReLeASE method could be employed [9].

## Advancing Towards Safer and More Effective Treatments: The AI Approach

Since ancient times, the therapeutic efficacy of plants has been widely acknowledged, with plant-derived medicines being utilized to treat various pathological conditions. These remedies are often administered as mixtures or concentrated extracts without the isolation of specific active compounds. However, modern medicine typically necessitates the isolation and purification of one or a few active compounds. Despite this, numerous global health challenges persist, including cancer, degenerative diseases, HIV/AIDS, and diabetes, for which modern medicine struggles to provide effective cures. In many cases, the isolation of an "active compound" has rendered it less effective. Drug discovery is a complex undertaking, requiring the evaluation of multiple parameters, including safety, pharmacokinetics, and efficacy, during the selection of drug candidates. The emergence of cutting-edge technologies, such as Artificial Intelligence, 'organ-on-chip' systems, and microfluidics technologies, has introduced automation into the drug discovery process, thereby accelerating the pace of drug discovery and facilitating the assessment of candidate compound safety, pharmacokinetics, and efficacy. Simultaneously, these technologies enable novel approaches to drug design and synthesis based on natural compounds. Recent advancements in analytical and computational techniques have opened up new

avenues for processing complex natural products and leveraging their structures to develop innovative drugs. We are currently witnessing the era of computational molecular design applied to natural products, with predictive computational software aiding in the discovery of molecular targets and derivatives of natural products. Looking ahead, the utilization of quantum computing, computational software, and databases in modeling molecular interactions and predicting drug development parameters such as pharmacokinetics and pharmacodynamics is expected to reduce the occurrence of false positive leads in drug development. This review explores the realm of plant-based natural product drug discovery and the pivotal role of innovative technologies in shaping next-generation drug discovery efforts [31].

## **Upsides and Downsides of AI and ML Implementation in Pharmaceutical Fields**

At present, there are no developed drugs that have incorporated AI methodologies. However, based on the progress outlined in this review, it is anticipated that it will take an additional 2-3 years for a drug to be brought to fruition utilizing AI techniques. Notably, experts strongly assert that AI will fundamentally alter the pharmaceutical landscape and revolutionize the drug discovery process. To effectively leverage AI in drug development, individuals must possess the expertise to train algorithms, necessitating domain-specific knowledge. This sets the stage for a conducive environment where AI and medicinal chemists can collaborate closely. While AI excels in analyzing vast datasets, medicinal chemists can contribute by training machines, establishing algorithms, or refining the analyzed data to expedite and enhance the accuracy of the drug development process. Despite the advantages AI offers in accelerating drug development, actual experiments remain indispensable. Furthermore, AI holds potential in aiding therapies like gene therapy, which are currently beyond our reach in healthcare. With AI, the prospect of amalgamating regenerative medicine with pharmacology and gene therapy becomes feasible,

presenting promising avenues for future medical interventions [32].

## Conclusion

Integrating AI into drug discovery offers vast potential for tackling challenging diseases, cutting R&D costs, and improving success rates. However, ongoing enhancements to AI platforms are vital, as demonstrated by recent research showing the importance of diverse data sources and robust governance. Pharmaceutical companies, with their expertise, are poised to lead in both creating quality databases and integrating them into advanced AI-driven drug discovery systems.

## Reference

- Ammad-Ud-Din M, Khan SA, Wennerberg K, Aittokallio T. Systematic identification of feature combinations for predicting drug response with Bayesian multi-view multi-task linear regression. *Bioinformatics*. 2017;33(14): i359-i368
- Carmona, F. and Pereira, A.M.S. (2013) Herbal medicines: old and new concepts, truths and misunderstandings. *Rev. Bras. Farmacognosia* 23, 379–385.
- Crimmins, E.M. (2015) Lifespan and healthspan: past, present, and promise. *Gerontologist* 55, 901–911
- DiMasi, J.A. et al. (2016) Innovation in the pharmaceutical industry: new estimates of R&D costs. *J. Health Econ.* 47, 20–33
- Dincer AB, Celik S, Hiranuma N, Lee S-I. DeepProfile: deep learning of cancer molecular profiles for precision medicine. *bioRxiv*. 2018:278739. <https://doi.org/10.1101/278739>
- Ding MQ, Chen L, Cooper GF, Young JD, Lu X. Precision oncology beyond targeted therapy: combining omics data with machine learning matches the majority of cancer cells to effective therapeutics. *Mol Cancer Res.* 2018;16(2): 269-278.
- Ding Z, Zu S, Gu J. Evaluating the molecule-based prediction of clinical drug responses in cancer. *Bioinformatics*. 2016;32(19):2891-2895.

- Fleming, N. (2018) How artificial intelligence is changing drug discovery. *Nature* 557 (7707), S55–S57
- Geeleher P, Cox NJ, Huang RS. Clinical drug response can be predicted using baseline gene expression levels and in vitro drug sensitivity in cell lines. *Genome Biol.* 2014;15(3):R47.
- Gent, E. (2020) Artificial intelligence is evolving all by itself. *Science* April 30
- Graim K, Friedl V, Houlahan KE, Stuart JM. PLATYPUS: a multiple-view learning predictive framework for cancer drug sensitivity prediction. *Pac Symp Biocomput.* 2019;24:136-147.
- Greenwood, B. (2014) The contribution of vaccination to global health: past, present and future. *Philos. Trans. R. Soc. London. Ser. B, Biol. Sci.* 369 (1645), 20130433–20130433
- Iorio F, Knijnenburg TA, Vis DJ, et al. A landscape of pharmacogenomic interactions in cancer. *Cell.* 2016;166(3): 740-754.
- Iwata M, Yuan L, Zhao Q, et al. Predicting drug-induced transcriptome responses of a wide range of human cell lines by a novel tensor-train decomposition algorithm. *Bioinformatics.* 2019;35(14):i191-i199. 277. Tan M. Prediction of anti-cancer drug response by kernelized multi-task learning. *ArtifIntell Med.* 2016;73:70-77.
- Kaplan, A. and Haenlein, M. (2020) Rulers of the world, unite! The challenges and opportunities of artificial intelligence *Bus. Horiz.* 63, 37–50
- Khan, S.R. et al. (2014) Current status and future prospects of toxicogenomics in drug discovery. *Drug Discovery Today* 19, 562–578
- Kit-Kay Mak, Mallikarjuna Rao Pichika (2019). Artificial intelligence in drug development: present status and future prospects. Volume 24, Issue 3, Pages 773-780.<https://doi.org/10.1016/j.drudis.2018.11.014>.
- Kuhn, S. et al. (2008) Building blocks for automated elucidation of metabolites: machine learning methods for NMR prediction. *BMC Bioinf.* 9 400–400

- Kumar, A. et al. (2018) Uttarakhand Medicinal Plants Database (UMPDB): a platform for exploring genomic, chemical, and traditional knowledge. *Data* 3, 7
- Li M, Wang Y, Zheng R, et al. DeepDSC: a deep learning method to predict drug sensitivity of cancer cell lines. *IEEE/ACM Trans Comput. Biol. Bioinform.* 2019;1. <https://doi.org/10.1109/tcbb.2019.2919581>.
- Li, Y. et al. (2020) Identification of metabolites from tandem mass spectra with a machine learning approach utilizing structural features. *Bioinformatics* 36, 1213– 1218.
- Lotter, W. et al. (2020) A neural network trained for prediction mimics diverse features of biological neurons and perception. *Nat. Mach. Intell.* 2, 210–219
- Mensah, G.A. et al. (2017) Decline in cardiovascular mortality: possible causes and implications. *Circ. Res.* 120, 366–380
- Mohanraj, K. et al. (2018) IMPPAT: a curated database of Indian medicinal plants, phytochemistry and therapeutics. *Sci. Rep.* 8 4329–4329
- Pan, S.-Y. et al. (2014) Historical perspective of traditional indigenous medical practices: the current renaissance and conservation of herbal resources. *Evidence-Based Complementary Altern. Med.* 2014, 525340
- Saifur R. Khan , Dana Al Rijjal , Anthony Piro , Michael B. Wheeler (2021). Integration of AI and traditional medicine in drug discovery .Volume 26, Issue 4, Pages 982-992. <https://doi.org/10.1016/j.drudis.2021.01.008>.
- Schneider, J. (2020) Human-to-AI coach: improving human inputs to AI systems. *Lect. Notes Comput. Sci.* 12080, 431–443
- Sharifi-Noghabi H, Zolotareva O, Collins CC, Ester M. MOLI: multi-omics late integration with deep neural networks for drug response prediction. *Bioinformatics*. 2019;35(14):i501-i509.
- Tan M, Ozgul OF, Bardak B, Eksioglu I, Sabuncuoglu S. Drug response prediction by ensemble learning and drug- induced gene expression signatures. *Genomics*. 2019; 111(5):1078-1088.
- Thomford, Nicholas Ekow, Dimakatso Alice Senthebane, Arielle Rowe, Daniella Munro, PalesaSeele, Alfred Maroyi, and Kevin

Dzobo. 2018. "Natural Products for Drug Discovery in the 21st Century: Innovations for Novel Drug Discovery" *International Journal of Molecular Sciences*, 19, no. 6: 1578. <https://doi.org/10.3390/ijms19061578>.

- Vatansever S, Schlessinger A, Wacker D, Kaniskan HÜ, Jin J, Zhou MM, Zhang B. (2021) Artificial intelligence and machine learning-aided drug discovery in central nervous system diseases: State-of-the-arts and future directions. *Med Res Rev*: 41(3):1427-1473. doi: 10.1002/med.21764. Epub 2020 Dec 9. PMID: 33295676; PMCID: PMC8043990.
- Yuan, H. et al. (2016), The traditional medicine and modern medicine from natural products. *Molecules* (Basel, Switzerland) 21, 559.