Phytochemical Investigation and Antioxidant Properties of Petrolium Ether Fruit Extract of *Ampelocissus barbata* (Vitaceae)

Course Code : PHARM-419

A Dissertation Submitted to the Department of Pharmacy, Dhaka International University, Dhaka, in Partial Fulfillment of the Requirement for Bachelor of Pharmacy (B. Pharm)

Submitted by

Badrunnahar Binty

Reg No: PH-24-19-111215

Session: 2019-20 (24th batch)

Knowledge is Power

DIU

Estd. 7th April, 1995

Department of Pharmacy

Dhaka International University, Dhaka,Bangladesh

March 2024

DEDICATION

Dedicated To

My

Reverend Parents

Dhaka International University

Department of Pharmacy

CERTIFICATE

This is to certify that the thesis entitled **“Phytochemical Investigation and Antioxidant Properties of Petrolium Ether Fruit Extract of *Ampelocissus barbata* (Vitaceae)”** is an original research work carried out for the partial fulfillment of the requirement for the Bachelor of Pharmacy (B. Pharm). None of the parts of this work has been submitted for any other degrees elsewhere. This original research work has been carried out and completed by Badrunnahar Binty, Registration No. PH-24-19-111215, Session: 2019-2020 (24th batch) under my supervision.

Approved as to the style and content.

Supervisor

Shahenul Islam

Lecturer

Department of Pharmacy

Dhaka International University

Approval

This is to certify that the project work has been submitted by Badrunnahar Binty, entitled **"Phytochemical Investigation and Antioxidant Properties of Petrolium Ether Fruit Extract of *Ampelocissus barbata* (Vitaceae)"** has been authorized by the Examination Committee for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (B. Pharm.) in the Department of Pharmacy, Dhaka International University, Bangladesh.

Board of Examiner (or Examination Committee)

………………………………… Chairman of the Committee

Name :

Designation :

University :

………………………………… Member

Name :

Designation :

University :

………………………………… Member

Name :

Designation :

University : ………………………………… Member

Name :

Designation :

University :

ACKNOWLEDGEMENT

All admiration and praises are solemnity due to Almighty Allah who is the Supreme Ruler of the Universe, whose mercy absolutely enabled me to complete this modest piece of work.

I am pleased and feel proud to express my deep appreciation, indebtedness and profound respect to my venerable supervisor Shahenul Islam, Lecturer, Department of Pharmacy, Dhaka International University, for his mastermind direction, constant supervision and support, optimistic counseling and continuous backup to carry out the research work as well as to prepare this dissertation.

I feel proud to express my heartiest gratitude to my reverend Chairman, Professor Farida Begum, Department of Pharmacy, Dhaka International University for providing all facilities, which helped me to perform the thesis work in a congenital and comfortable atmosphere.

It is also my great pleasure to convey my best regards and indebtedness to all my honorable teachers of the Department of Pharmacy, Dhaka International University, for their cooperation whenever I needed.

I would like to extend my thanks to my respected seniors, office stuffs, all of my friends, research students and adorable juniors for their help and assistance, friendly behavior and cooperation.

Finally, I would like to show supreme devotion to all who are made sacrifices for me, inspired and blessed me in every way.

The Author

ABSTRACT

This dissertation describes the biological investigations of *Ampelocissus barbata*, a plant belonging to the family Vitaceae. The fruits of the *Ampelocissus barbata* were extracted with methanol. The fraction of extract of Pet ether from *Ampelocissus barbata* fruits extract was used for the observation of Phytochemical evaluation, Antioxidant Activity Test. Phytochemical Test such as; Reducing sugar (Benedict's), Reducing sugar (Fehling's), Combined reducing sugar, Tannins (Ferric Chloride), Tannins (Potassium dichromate), Flavonoids, Saponin, Carbohydrates & Gums, Alkaloids (Dragendroff's), Glycoside, Proteins & Xanthoprotein. According to the Phytochemical study of the Fraction of extract of Pet ether from *Ampelocissus barbata*, the presence Tannins (Ferric Chloride) & Proteins & Xanthoprotein was revealed. The Antioxidant Tests such as; DPPH Scavenging Assay, Total Phenolic Content, Nitric Oxide (No) Scavenging Assay, Total Flavonoid Content, Total Tannin Content, Hydrogen Peroxide Scavenging Assay & Hydroxyl Radical Scavenging Assay. Antioxidant Test was revealed that the Fraction of extract of Pet ether from Ampelocissus barbata, extract exhibited high Total Phenolic Content and low Tannin Content and Flavonoid Content.

Total Tannin, Phenol and Flavonoid Content of fraction of extract of Pet ether from *Ampelocissus barbata* in Fruits Extract were found 21.36 mg GAE/g, 1817.5 mg GAE/g (gallic acid equivalent) and 3.5079 mg QE/g (quercetin equivalent) respectively. DPPH Scavenging Assay, Nitric Oxide (No) Scavenging Assay, Hydrogen Peroxide Scavenging Assay & Hydroxyl Radical Scavenging Assay were found IC_{50} 23.87 µg/ml, 24.4608 µg/ml, SC_{50} 29.2 µg/ml and SC_{50} 39.17 µg/ml.

The results suggest that the extract contains a diverse range of phytochemicals with potential antioxidant properties. As the plant remains an integral part in terms of the discovery of medicine thus the aim of the current work was to explore the Phytochemical profiles of the plant *Ampelocissus barbata* for Antioxidant activity.

Chapter One
Introduction

1.1 Overview :

The practice of diagnosis, the promotion of health, and the treatment and prevention of disease are all included in the definition of medicine as the science of healing [1] . It also refers to plant-based compounds, pharmaceuticals, and drugs that are used to treat a variety of illnesses and advance good health. According to the medical dictionary, medicine is both a drug and a method of illness prevention. Additionally, it is described as the investigation and management of general disorders or those affecting the body, particularly those that typically do not require surgical intervention [2] . In the 21st century, people are totally depended on medicine for various kinds of lifestyle diseases. There are different kinds of medical field such as Allopathy, Ayurveda and Homeopathy currently in from all over the world. People depend on those medical fields, according to their beliefs. Medicine is not a pure science, but is a science of probability. The progress of medicine mainly focuses on the science of healing and the development of science in the medical field. Its history is from prehistoric times to at present.

According to Gillian R Bentley medicines can be classified as [3]

a) Traditional medicine.

b) Modern medicine.

Traditional medicines are purely based on a natural way of treatment for all illness. These medicines are derived from plants and animals, besides that they are not usually processed. Modern medicines are being spread on biomedical science, genetics and medical technology, which are used to diagnose, treat and prevent injury and illness. One of the main medical processes in modern medicines is surgery. There are many new biological treatments being developed by using medicine and opened a new career for both women and men as nurses/compounders and physicians. These advances along with the growth in chemistry, genetics and lab technology led to modern medicine. [3]

1.2 History of Medicine:

The invention of medicine is undoubtedly considered the most precious and beneficial in human civilization. The history of medicine shows a remarkable journey of how we humans have approached different illnesses and diseases from the early periods till date. Some of the early medicine traditions come from Babylon, China, Egypt, and India. Though, there isn't much record of when and how plants were used as a healing agent. But, from ancient drawings discovered worldwide, it is believed that early humans used medicinal plants as healing agents [4].

Prehistoric Medicine

Early humans were unaware of the various diseases and their medications. Early humans used the trial and error method to discover the medicinal benefits of plants and herbs. They considered common cold and constipation as a part of human existence and treated it with various herbs. The unidentified diseases were believed to be supernatural or cast of spells. There's a common belief that in the world, the first doctors were sorcerers and magicians [5].

Magic and religious prayers played an essential role in prehistoric medicine. In ancient Mesopotamia, the people were not able to distinguish between magic and medicine. And if a person suffering from illness came to a doctor, the doctors would prescribe medicinal treatment and magical words to be recited. In the ancient era, the Babylonians, along with Egyptians, implemented diagnosis, physical examinations, and treatments. Early Egyptians were considered the healthiest with a notable healthcare system. [6]

Introduction

Traditional Medicine in India, China, and Japan

The Indian civilization was well developed in medicine with herbal treatments. The Atharvaveda, which belongs to the early iron age, throws light on the fact that early Indians used medications and ailments from herbs and other medicinal plants. [7]

The period from 800 BCE till 1000 BCE is considered the golden age of medicine in India with the introduction of medical treaties by Chakra, a physician, and Sushruta, a surgeon of ancient India. The knowledge of anatomy in ancient India was minimal as the Hindus were not allowed to cut dead bodies. The Indian physicians used all five senses in the diagnosis of diseases. The Indians were known to have identified around 700-800 medicinal plants in ancient times. They were also known for using animal parts for remedies. [8]

On the other hand, Chinese medicine was of great importance in history. The Chinese considered the human body to be made up of five elements: wood, fire, earth, metal, and water. As the Chinese's religious beliefs forbade them from tearing the dead bodies, therefore their anatomy knowledge was based on assumptions. According to the ancient Chinese Anatomy, the body contained five organs: heart, lungs, liver, spleen, and kidney, and the blood vessels contained blood and air. [8]

Japanese medicine, on the other hand, is considered interesting for its slow start and rapid modernization. Japanese medicine took a turn in 608 CE when few Japanese physicians were sent to China for study. The Chinese have a significant influence on the Japanese medical system. [9]

The oldest Japanese medical work dates back to 983 CE, which Tamba Yasuyori wrote. In his works, he has discussed different kinds of diseases and their treatment. These diseases and treatments are classified according to body parts. [9]

Introduction

Speaking of ancient medicine, you must have thought of the world's first doctor. The first doctor in the world was from the Egyptian civilization. According to the medical information given in the Edwin Smith Papyrus, which dates back to 3000 BC, Imhotep is credited with being the first doctor in the world. [10]

Modern Medicine

The Renaissance brought about a significant change in the history of medicine. The period between the 16th and 18th centuries was remarkable for medicine. This period saw a rapid increase in experimental investigations and advanced anatomy.

Even the first medicine was discovered during this period. Modern medicine started to emerge after the Industrial Revolution in the 18th century. At this time, there was rapid growth in economic activity in Western Europe and the Americas. Hippocrates is credited with being the man who invented medicine. He was a Greek physician who wrote the Hippocratic Corpus, a collection of seventy medical works. He is also accredited with the invention of the Hippocratic Oath for physicians. [11]

During the 19th century, economic and industrial growth continued to develop, and people made many scientific discoveries and inventions.

Scientists made rapid progress in identifying and preventing illnesses and in understanding how bacteria and viruses work.

However, they still had a long way to go regarding the treatment and cures of infectious diseases. [11]

1.3 Medicinal plant

Medicinal plants are widely used in non-industrialized societies, mainly because they are readily available and cheaper than modern medicines. The annual global export value of the thousands of types of plants with suspected medicinal properties was estimated to be US$2.2 billion in 2012. In 2017, the potential global market for botanical extracts and medicines was estimated at several hundred billion dollars [12]. Medicinal plants face both general threats, such as climate change and habitat destruction, and the specific threat of over-collection to meet market demand . Medicinal plants, also called medicinal herbs, have been discovered and used in traditional medicine practices since prehistoric times. Plants synthesize hundreds of chemical compounds for functions including defense against insects, fungi, diseases, and herbivorous mammals. Numerous phytochemicals with potential or established biological activity have been identified. However, since a single plant contains widely diverse phytochemicals, the effects of using a whole plant as medicine are uncertain. Further, the phytochemical content and pharmacological actions, if any, of many plants having medicinal potential remain unassessed by rigorous scientific research to define efficacy and safety [12]

The earliest historical records of herbs are found from the Sumerian civilization, where hundreds of medicinal plants including opium are listed on clay tablets. The Ebers Papyrus from ancient Egypt, c. 1550 BC, describes over 850 plant medicines. The Greek physician dioscorides, who worked in the Roman army, documented over 1000 recipes for medicines using over 600 medicinal plants in De materia medica, c. 60 AD; this formed the basis of pharmacopoeias for some 1500 years. Drug research makes use of ethnobotany to search for pharmacologically active substances in nature, and has in this way discovered hundreds of useful compounds. These include the common drugs aspirin, digoxin, quinine, and opium. The compounds found in plants are of many kinds, but most are in four major biochemical classes: alkaloids, glycosides, polyphenols, and terpenes. [13]

Fig 1.1 : Medicinal Plants

1.3.1 Characteristics of Medicinal Plants

Medicinal plants have many characteristics when used as a treatment, as follow:

Synergic medicine : The ingredients of plants all interact simultaneously, so their uses can complement or damage others or neutralize their possible negative effects.

Support of official medicine : In the treatment of complex cases like cancer diseases the components of the plants proved to be very effective.

Preventive medicine : It has been proven that the component of the plants also characterized by their ability to prevent the appearance of some diseases. This will help to reduce the use of the chemical remedies which will be used when the disease is already present i.e., reduce the side effect of synthetic treatment. [14]

1.3.2 Basis of modern medicine

Botanical drugs which form the basis for herbal remedies or phytomedicines include, for example: the herb of St John’s wort (Hypericum perforatum), used in the treatment of mild to moderate depression, the leaves of Ginkgo biloba, used for cognitive deficiencies (often in the elderly), including impairment of memory and affective symptoms such as anxiety, the flower heads of chamomile (Chamomilla recutita) used for mild gastrointestinal complaints and as an anti-inflammatory agent the leaves and pods of Senna (Cassia spp.) used for constipation. From the perspective of Pharmacognosy and rational phyto-therapy, such products lie alongside, and in some cases are, conventional pharmaceutical medicines. Herbal medicines are often considered to be part of complementary and alternative medicine (CAM), and the use of herbal medicine products (HMPs) and of CAM has increased across the developed world. [15]

Isolated compound from the plants are pure chemical entities, often used in the form of licensed medicines. They are sometimes produced synthetically and referred to as ‘nature identical’ (if that is the case), but were originally discovered from plant drugs. Examples include: morphine, from opium poppy (Papaver somniferum), used as an analgesic digoxin and other digitalis glycosides, from foxglove (Digitalis spp.), used to treat heart failure taxol, from the Pacific yew (Taxus brevifolia), used as an anticancer treatment quinine, from Cinchona bark (Cinchona spp.), used in the treatment of malaria galanthamine from Galanthus and Leucojum species, used in the management of cognitive disorders. [16]

1.3.3 Exploration of medicinal properties of plants

Since prehistoric times, the fields of botany and medicine have enjoyed an enduring and fruitful relationship. Whether in the sophisticated setting of a modern pharmaceutical laboratory or an herbalist’s hut on the banks of the Amazon River, plants provide a critical source of treatments for the myriad diseases that afflict humans. In either setting, the principal challenge of the medical practitioner is to distinguish plants that possess pharmaceutical properties from those that are toxic or medicinally inert. Although methods used to screen the plant kingdom for bioactive compounds have changed considerably over the course of human history, the invaluable selection of cures and therapeutics available to modern medicine is the product of a long history of pharmaceutical experimentation. [16]

The Past - We can only conjecture as to when and where the search for herbal remedies began. Archeological remains dating back sixty thousand years reveal that Neanderthals laid their dead to rest with plants that later became staples of ancient pharmacopoeias, such as millefoil (Achillea), St. Barnaby’s thistle (Centaurea), and joint fir (Ephedra). Whether these plants were actually used as medicines or simply served as a farewell gesture to the deceased may never be established with certainty. Preliterate cultures, whose lifestyles closely mirror those of our distant ancestors, maintain oral traditions of medical practice that depend primarily on native vegetation. By the time literacy developed into a basic means of human communication, the application of botanical lore to the practice of medicine was firmly established and systematized. Sumerian clay tablets and Egyptian papyri (2000 B.C.) describe ancient prescriptions and pharmacopeias in considerable detail. Many of the plants that appear in these early records are now known to possess highly bioactive constituents, evidence for which can be found in our own medicine, cabinets, Codeine, derived from the opium poppy and used as a narcotic analgesic in Nyquil, appears in medical traditions that predate modern pharmacology by thousands of years. Similarly, salicylate (aspirin) was originally extracted from willow bark, and ephedrine, the flu remedy in Vicks was derived from Ephedra. Approximately twenty-five percent of all modern prescriptions contain natural plant extracts, most of which were used in traditional medicine Moreover; a significant number of synthetic medicines are derived from plant products whose therapeutic qualities have only recently been improved by chemical tinkering. [16]

The Present - over the course of the following two centuries, the science of medicine became more sophisticated and specialized than any Renaissance herbalist could have imagined. Indeed, recent developments in the use of laser beams, ultrasonic waves, and genetic engineering continue to challenge the imagination of modern innovators. Surprisingly, this rapid progress in the medical sciences has also reaffirmed the continuing relevance of botany to medicine. Technical innovations have accelerated the search for medicinal substances in natural products by providing increasingly simple and economical methods for screening massive quantities of plant samples. During the last few decades, discoveries of plant products with antitumor, anti-malarial, antibiotic, and immune stimulating properties have demonstrated that we are far from exhausting the medicinal potential of botanical resources. Since the establishment of NCI (National Cancer Institute) in 1937, modern methods of medical research have isolated a number of plant products that have been successful in treating different types of human cancers. Examples include the anti-leukemic agent vincristine, derived from a tropical periwinkle (Catharanthus roseus), and the ovarian cancer therapeutic, Taxol, derived from the yew plant of the Pacific Northwest (Taxus brevifolia). Pharmacologically active compounds like these are identified by exposing crude extracts of plant tissues to living cultures of cancerous or HIV infected cells. If an extract exhibits an effect on diseased cells, the active constituent of the sample is isolated, chemically characterized, and subjected to clinical analysis. Up to ten years of study and hundreds of millions of dollars are required to demonstrate that a promising chemical agent is effective, safe to use, and economically producible. Since 1986 over 50,000 plant extractions have been screened at the NCI, of which fewer than ten have been identified as potentially useful drugs. (Cragg et al., 1995). Success rates have been particularly low in the search for antitumor agents. By comparison, the more recently initiated anti-AIDS research has been more promising: after only a decade, a number of potential anti-HIV compounds have been extracted from plants collected in distant continents (Ancistrocladus in Africa; Calophyllum in Malesia; Conospermum in Australia; Homolanthus in Samoa. All of these promising discoveries are presently under study by clinical physicians and toxicologists. Although modern medicine has yet to identify cures for AIDS, many cancers, and a host of other human maladies-arthritis, obesity, schizophrenia, parkinsonism, depression, to name just a few-potential pharmaceutical treatments for many of these conditions undoubtedly reside in the rich chemical diversity of the plant kingdom. [16]

Fig 1.2 : medicinal properties of plants

1.3.4 Role of medicinal plants in modern medicines

The use of natural products with therapeutic properties is as ancient as human civilization and, for a long time, mineral, plant and animal products were the main sources of drugs. Primitive peoples throughout the world made use of their indigenous flora as a source of medicines. Through the process of trial and error, these cultures examined and discovered many plants that produce unique molecular entities with valuable biological properties such as aspirin from willow bark (Salix alba), the anticancer alkaloid vincristine from the Madagascar periwinkle (Catharanthus roseus), and cardiac glycoside digitoxin from Digitalis species. [17]

Galantamine is a natural product discovered through an ethnobotanical lead and first isolated from Galanthus woronowii L. (Amaryllidaceae) and approved for the treatment of Alzheimer's disease. Tiotroprium is an inhaled anticholinergic bronchodilator, based on ipratropium, a derivative of atropine that has been isolated from Atropa belladonna L. (Solanaceae) and recommended for treatment of chronic obstructive pulmonary disease [17].

Exate can is an analog of camptothecin from Camptotheca acuminate Decne. (Nyssaceae) and developed as an anticancer agent. Galegine, an active anti-hyperglycemic agent isolated from the plant Galega officinalis L. provided the template for the synthesis of metformin and opened up interest in the synthesis of other biguanide-type ant diabetic drugs. [17]

1.4 The Importance of Medicinal Plants

It is estimated that 80 % of the people in Pakistan depend on plants to cure themselves, a 40% in China. In technologically advanced countries as the United States, it is estimated that 60 % of the population use medicinal plants habitually to fight certain ailments. In Japan there is more demand of medicinal plants than of "official" medicines. Modern medicine, through clinical tests, has been able to validate those plants that the tradition had used with the method of test and error. Many turned out to be been worth; others demonstrated to be innocuous; others, potentially dangerous. Biochemical tests have been the ones that determined the main components of the medicinal plants- the active principles. The capacity of the modern chemical industry to produce these principles without the aid of the plants does not suppose deny the importance that these have and will still have in the future. [18]

Among the main arguments in defense of the medicinal plants we have to mention the following:

Synergic medicine: It has been verified that in many cases the application of an isolated component has not had the wished effect, because it does not have the same curative power that it has when it is taken altogether with the rest of components, or because it has turned out to be toxic.

Backup of formal medicine: The treatment of very complex diseases can require in some cases the support of the medicinal properties of the plants or the derivatives that they provide. The importance of taxol, an obtained derivative of the yew of the Pacific (Taxus brevifolia)in the treatment of cancer and especially concerning breast cancer, has been approved by the same American F.D.

Preventive medicine: Finally, we do not have to forget the preventive character that the plants have regarding the appearance of diseases. In this sense the plants are better than the chemical remedies that are applied essentially when the disease has already appeared. It has been verified that natural food ingestion can prevent many pathologies. It has been admitted that the ingestion of vegetables with antioxidant properties, especially those that belong to the Brassicaceae group, like cabbages, radishes, etc. [19]

1.4.1 The medicinal plants of Bangladesh

South Asian countries have a large number of valuable medicinal plants naturally growing, mostly in fragile ecosystems that are predominantly inhibited by rural poor and indigenous community. In Bangladesh, 5,000species of angiosperm are reported to occur. The number of medicinal plants included in the “material medica” of traditional medicine in this subcontinent at present stands at about 2,000 More than 500 of such medicinal plants have so far been enlisted as growing in Bangladesh. Dhaka, Rajshahi, shylet and Chittagong division is rich in medicinal plants. [19]

1.5 Contribution of Medicinal Plants in Modern Medicine

The modern medicine refers to those medicinal preparations, which are scientifically by using modern technology and know how and which are in current use in modern pharmacopoeias for cure and management for disease.

Table 1.1: Plant derived medicinal substance occurring used in modern medicine. [20]

Drugs/Chemical	Action/Clinical use	Plant source
Atropine	Anticholinergic	*Atropa belladonna*
Arecoline	Anthelmintic	*Areca catechu*
Berberine	Bacillary dysentry	*Berberis vulgaris*
Bergenin	Antitussive	*Berberis vulgaris*
Caffeine	CNS stimulant	*Camellia sinensis*
Camphor	Rubefacient	*Cinnamomum acemphor*
Camptothecin	Anticancerous	*Camtotheca acuminate*
Calanolide A Calanollide B	AIDS	*Calophyllum lanigerum*
Codeine	Analgesic, sedative, antitussive	*Papaver somniferum Linn.*
Colchicine	Antiarthritic, antiinflammatory	*Colchicum autumnale Linn.*
Digitoxin, Digoxin	Cardiotonic	*Digitalis, purpura, Linn.* *Digitalis lanata Her*
Diosgenin	Expectorant, anti-inflammatory	*Dioscorea spp*
Hyoscine Hyoscyamine	Parasympatholytic, mydriatic, Antispasmodic	*Datura, hyoscyamus, scopolia,* *dubosia spp.*
Morphine	Sedative, narcotic, analgesic	*Papaver somniferum Linn.*

1.6 Phytochemical Screening

The plant kingdom represents an enormous reservoir of biologically active compounds with various chemical structures and protective/disease preventive properties (phytochemicals). These phytochemicals, often secondary metabolites present in smaller quantities in higher plants, include the alkaloids, steroids, flavonoids, terpenoids, tannins, and many others. Nearly 50% of drugs used in medicine are of plant origin, and only a small fraction of plants with medicinal activity has been assayed. There is therefore much current research devoted to the phytochemical investigation of higher plants which have ethnobotanical information associated with them. The phytochemicals isolated are then screened for different types of biological activity (Harborne et. al., 1998). [21]

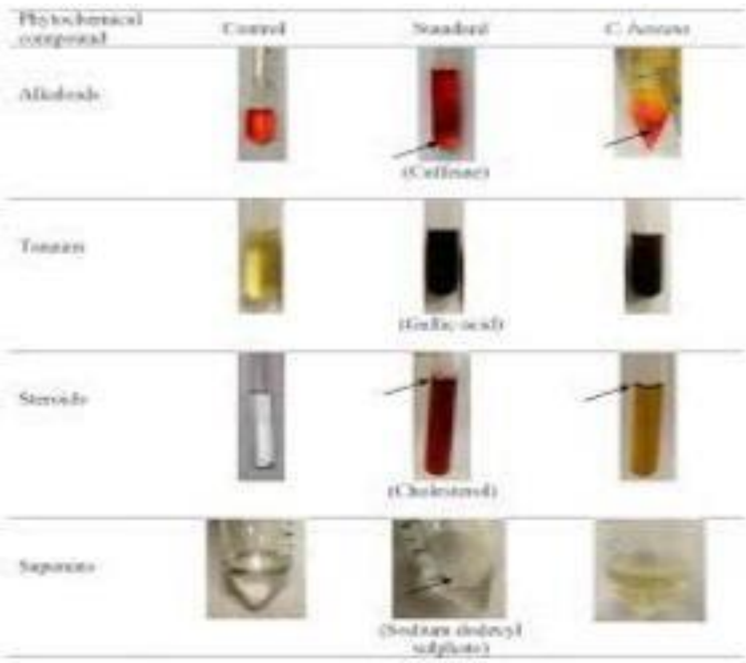

Fig 1.3 : Phytochemical Screening

1.7 Antioxidant Activity of Plant Extracts

Antioxidants are molecules that inhibit oxidation, a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells. They neutralize free radicals by donating electrons, thus preventing them from causing oxidative damage to cells and tissues. Antioxidants are found in various foods, particularly fruits, vegetables, nuts, and seeds, and they are also synthesized by the body. Examples of antioxidants include vitamins C and E, beta-carotene, flavonoids, and polyphenols. Consuming antioxidant-rich foods is associated with numerous health benefits, including reduced risk of chronic diseases such as heart disease, cancer, and neurodegenerative disorders. [22]

The antioxidant activity of plant extracts is often attributed to their rich content of phytochemicals such as flavonoids, phenolic compounds, carotenoids, and vitamin C. These compounds can scavenge free radicals, neutralizing their damaging effects on cells and tissues. Various assays, like DPPH (2,2-diphenyl-1-picrylhydrazyl) or ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) assays, are commonly used to measure the antioxidant capacity of plant extracts. [23]

1.8 Determination of Total Phenolic Content

Phenolic compounds or polyphenols are the secondary plant metabolites that are all over present in plants and plant products. Many of them are responsible for the reduction of risk of evolving chronic diseases (cardiovascular disease, cancer, diabetes, etc.), due to their antioxidant activities. Phenolic compounds pay to the overall antioxidant activities of plants mainly for their redox activities. Generally, the mechanisms of phenolic compounds for antioxidant activity are neutralizing lipid free radicals and preventing decomposition of hydroperoxides into free radicals. [24]

In the present study, the total phenolic content of ethanol extract of Ampelocissus barbata was determined by using Folin-Ciocalteu (FC) reagent with analytical grade Gallic acid as the standard (Marinova et al., 2005). Extract or standard solution (15.62-500 mg/L) of 1 ml was added to distilled water (9 mL). Then 1 ml FC reagent (10 times diluted with distilled water) was added. After 5 minutes; 10 mL 7% Na2CO3 was added to the mixture. Then it is kept for 30 minutes at room temperature. Then absorbance was measured against blank at 750 nm using UV spectrophotometer. Total phenolic content of the extract was determined from the standard curve and expressed as mg Gallic acid equivalent (GAE)/100 g dried plant extract. (Javanmardi et al., 2003). [25]

1.9 Determination of Total Tannin Content

Tannin is natural organic biomolecule. It is an astringent, bitter plant polyphenolic compound that binds to and precipitates proteins and various other organic compounds including amino acids and alkaloids. The anticarcinogenic and antimutagenic potentials of tannins may be related to their antioxidative property, which is important in protecting cellular oxidative damage, including lipid peroxidation. The generation of superoxide radicals was reported to be inhibited by tannins and related compounds. (Marinova et. al., 2005).

In the present study, the total tannin content of ethanol leaf extract of was determined by *Ampelocissus barbata* using Folin-Ciocalteu (FC) reagent with analytical grade Gallic acid as the standard. Total tannin content of the extract was determined from the standard curve and expressed as mg Gallic acid equivalent (GAE)/100 g dried plant extract. (Vernon et. al., 1999). [26]

1.10 Determination of Total Flavonoid Content

Flavonoids are polyphenolic compounds that are ubiquitous in nature and are categorized, according to chemical structure, into flavanols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones. The flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health-they have been reported to have antiviral, anti-allergic, antiplatelet, anti-inflammatory, and antitumor and antioxidant activities. Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage. Oxidative stress has been linked to cancer, aging, atherosclerosis, ischemic injury, inflammation and neurodegenerative diseases (Parkinson's and Alzheimer's). Flavonoids may help provide protection against these diseases by contributing, along with antioxidant vitamins and enzymes, to the total antioxidant defense system of the human body.

In the present study, the total flavonoid content of ethanol leaf extract of Ampelocissus barbata was determined by analytical grade Quercetin as the standard. Total tannin content of the extract was determined from the standard curve and expressed as mg Quercetin equivalent (QE)/100 g dried plant extract. (Meda et. al., 2005). [27]

1.11 Determination of DPPH Scavenging Assay

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) is a popular, quick, easy, and affordable approach for the measurement of antioxidant properties that includes the use of the free radicals used for assessing the potential of substances to serve as hydrogen providers or free-radical scavengers (FRS).The assay is based on the measurement of the scavenging capacity of antioxidants towards it. The odd electron of nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidants to the corresponding hydrazine (Contreras-Guzman and Srong 1982). [28]

DPPH free radical scavenging is an accepted mechanism for screening the antioxidant activity of plant extracts. In the DPPH assay, based on the measurement of the scavenging capacity of antioxidants towards it. [28]

The ability to scavenge the DPPH radical was expressed as percentage inhibition and calculated using the following equation: The results based on the measurement of the scavenging capacity of antioxidants towards it.where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

The free radical scavenging activities (antioxidant capacity) of the plant extracts on the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were estimated by the method of Brand-Williams et al., 1995. [29]

In the present study, the DPPH of ethanol leaf extract of Ampelocissus barbata was determined by 2.0 ml of a methanol solution of the extract at different concentration were mixed with 3.0 ml of a DPPH methanol solution (20μg/ml). The antioxidant potential was assayed from the bleaching of purple colored methanol solution of DPPH radicalby the plant extract as compared to that of tert-butyl-1-hydroxytoluene (BHT) and ascorbic acid (ASA) by UV spectrophotometer. [29]

1.12 Determination of Nitric Oxide (No) Scavenging Assay

Nitric oxide, also known as nitrogen monoxide, is a molecule with chemical formula No. It is a free radical and is an important intermediate in the chemical industry Nitric oxide is a by-product of combustion of substances in the air, as in automobile engines, fossil fuel power plants, and is produced naturally during the electrical discharges of lightning in thunderstorms. Nitric oxide is a molecule that has a single nitrogen and oxygen atom. This gas is produced when an enzyme group known as Nitric oxide Synthase (NOS), breaks down L-arginine (an amino acid) into L-citrulline (Hou et al., 1999). [30]

Nitric oxide is a reactive gas and cause respiratory damage. Nitric acid and nitrous acid are formed with the presence of moisture in air and in the lungs. This is highly corrosive to the pulmonary system of the body.

NO is an important bio regulatory molecule, which has a number of physiological effects including control of blood pressure. neural signal transduction, platelet function, antimicrobial and antitumor activity. Low concentrations of No are sufficient. in most cases, to effect these beneficial functions. However, during infections and inflammations, formation of NO is elevated and may bring about some undesired deleterious effects (Marcocci et al., 1994 a, b). [30]

The NO does not interact with the bioorganic macromolecules such as the DNA or proteins directly. However, in the aerobic conditions, the NO molecule is very unstable and reacts with the oxygen to produce intermediates such as NO2. N2O4. N3O4 the stable products nitrate and nitrite (Marcocci et al., 1994 a, b) and peroxynitrite when reacted with superoxide (Wink et al., 1991). These products progenitors are highly genotoxic, the deamination of guanine, cytosine and adenine is mediated primarily by the N_2O_3. In addition to the formation of nitrosoamines and deamination of the DNA bases, recent studies indicate that the NO may also act by affecting the enzymatic activities of several thiols rich DNA repair proteins like DNA alkyl transferase, formamopyrimidine-DNA glycosaisse and the DNA ligase that play a critical role in the maintenance of the genetic integrity (Wink et al., 1991). [31]

The formation of carcinogenic N-nitrose compounds, deamination, and oxidation of the DNA bases and inhibition of the critical DNA repair protein leads to mutagenesis and an initiation towards the process of carcinogenesis. There is now increasing evidence to suggest that NO and its derivatives produced by the activated phagocytes may have a genotoxic effect and may contribute in the multistage&inogenesis process (Wink et al., 1991). Direct link between chronic inflammation and induction of chlangiocarcinoma has been found in the infections by the parasites such as opisthrchis viverrini (liver fluke). The continuous exposure to free radicals generated from the chronic inflammation has been found to cause more cancers than environmental chemicals (Ames and Gold, 1990). [31]

Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reaction as described previously. Sodium nitroprusside in aqueous solution at physiological při spontaneously generates nitric oxide (Green et al., 1982; Marcocci ef al., 1994a, b), which interacts with oxygen to produce nitrite ions that can be estimated by use of Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide (Marcocci et al., 1994 a. b). [32]

1.13 Determination of Hydrogen Peroxide Scavenging Assay

Hydrogen peroxide itself is not a free radical as it does not contain any unpaired electrons. However it is a precursor to certain radical species such as peroxyl radical hydroxyl radical, superoxide, Since it is a superb oxidizing agent it can also react with certain other molecules and convert them into free radicals. These hydroxyl radicals in turn readily react with and damage vital cellular components especially those of the mitochondria (Giorgio et al: 2007). [33]

Antioxidants act as peroxide decomposer. Both enzymatic and nonenzymatic antioxidants exist in the intercellular and extracellular environment to decompose hydrogen peroxide (Frie B et al: 1988). The ability of the extracts to scavenge hydrogen peroxide was determined according to the method of Roch. The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. The percentage of hydrogen peroxide scavenging by the extracts and standard compounds was calculated as follows: % Scavenged (H2O2) = [(Ao-A1)/Ao] x 100 where Ao is the absorbance of the control and A, is the absorbance in the presence of the sample of extract and standard. [33]

1.14 Determination of Hydroxyl Radical Scavenging Assay

The hydroxyl radical. HO, is the neutral form of the hydroxide ion (HO), Hydroxyl radicals are highly reactive and consequent. short lived: most notably hydroxyl radicals are produced from the decomposition of hydro peroxides (ROHO) Mechanisms for scavenging peroxyl radicals for the protection of cellular structures includes endogenous antioxidants such as melatonin and glutathione and dietary antioxidants such as mannitol and vitamin E. [34]

The hydroxyl radical scavenging activity can be measured by studying the competition between deoxyribose and the plant extracts for hydroxyl radicals generated with Fe" /ascorbate/EDTA/H_2O: system. This can be determined spectrophotometrically at 530nm. [34]

1. 15 Data Collection

All the relevant data has been collected from two types of sources:

➢ Primary sources: direct personal contact and observations of the experiments carried out in the laboratory.

➢ Secondary sources: various publications like journals, papers, documents and websites.

1.16 Research Protocol