# Phytochemical Screening and In-Silico Studies in *Pleurotus ostreatus* and *Agaricus bisporus*

A project report submitted in partial fulfilment of the requirements for the degree of

"Bachelor of Technology" in Biotechnology

**By**

**Akshita katti (160116805002)**

**Brinda Dacha (160116805005)**

**Sanjana Gorlla (160116805026)**

Under the Guidance of

**Dr.G.Vijaya laxmi**


Department of Biotechnology

CHAITANYA BHARATHI INSTITUTE OF TECHNOLOGY (Autonomous)

(Affiliated to Osmania University) Hyderabad-500075

**April 2020**

CHAITANYA BHARATHI INSTITUTE OF TECHNOLOGY (A)
Kokapet(Village), Gandipet, Hyderabad, Telangana-500075. www.cbit.ac.in

COMMITTED TO RESEARCH, INNOVATION AND EDUCATION 41 years

# CERTIFICATE

This is to certify that the project entitled **"Phytochemical Screening and *Insilico* Studies in *Pleurotus ostreatus* and *Agaricus bisporus*"** submitted by **Ms. Akshita Katti (160116805002)**, **Ms. Brinda Dacha (160116805005)** and **Ms. Sanjana Gorlla (160116805026)** in partial fulfilment for the degree of "Bachelor of Technology" in Biotechnology, Osmania University, is a bonafide record of work carried out under the supervision of Dr**. G. Vijaya Laxmi, Assistant Professor, Department of Biotechnology** and has not been submitted to any other university or institute for the award of degree or diploma.

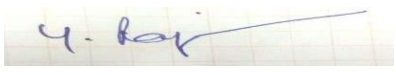
**Internal guide**

Name : Dr.G.Vijaya laxmi

Signature :  31/5/2020

**I/C Head of the Department**

Name : Dr. Y. Rajashri

Signature: 

# DECLARATION

This is to certify that the project entitled **"Phytochemical Screening And *Insilico* Studies in *Pleurotus ostreatus* and *Agaricus bisporus*"**submitted by **Ms. Akshita Katti (160116805002), Ms. Brinda Dacha (160116805005) and Ms. Sanjana Gorlla (160116805026)** in partial fulfilment for the degree of "Bachelor of Technology" in Biotechnology, Osmania University, is a bonafide record of work carried out by us under the supervision of **Dr. G. Vijaya Laxmi, Assistant Professor, Department of Biotechnology**, Chaitanya Bharathi Institute Of Technology, Hyderabad, and have not submitted to any other university or institute for the award of degree or diploma.

Student Name : Akshita katti

Signature : 

Student Name : Brinda Dacha

Signature : 

Student Name : Sanjana Gorlla

Signature : 

Date of Submission : 02/06/2020

# ACKNOWLEDGEMENT

Apart from the efforts we have put into this project, we would like to convey my sincere thanks to several individuals as this desertion would not have been possible without the guidance and help of several individuals who, in one way or the other, contributed and extended their valuable assistance in preparation and completion of this study.

Firstly, we would like to thank **Dr. P. Ravinder Reddy**, Principal, CBIT, for giving us the opportunity to do the project.

We would like to thank **Dr. Y. Rajashri**, Head, Department of Biotechnology, Chaitanya Bharathi Institute of Technology, for the valuable support given by her during our project work.

We would like to gratefully acknowledge the supervision of our guide managing **Dr. G. Vijaya Laxmi** and **Dr. V. Parvati Sai Arun**, Assistant Professor, Department of Biotechnology, CBIT, for their support, knowledge, encouragement, and invaluable assistance for completion of our project.

We would also like to acknowledge the non-teaching staff at Department of Biotechnology, Chaitanya Bharathi Institute of Technology, for their valuable support and helping us during the practical lab sessions throughout our project work.

We would also like to acknowledge all teaching staff at Department of Biotechnology, Chaitanya Bharathi Institute of Technology, for supporting and helping throughout our project.

# CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# ABSTRACT

Mushroom are fleshy, spore-bearing fruiting bodies of fungi, typically these are produced above the ground, on soil or in its food. There are several different types of mushrooms. But, edible mushrooms gained importance due to its nutritional and medicinal activities which play a dual role both in food and medicine. There are various species of edible mushrooms. However, in India *Agaricus bisporus* and *Pleurotus ostreatus* are widely cultivated on a commercial scale as these species not only exhibit various therapeutic activities but also these are rich in nutrients. The presence of various phytochemicals is the main reason for its potential health benefits. The present study is mainly focused on the phytochemical screening and in-silico studies of flavonoids present in *Pleurotus ostreatus* and *Agaricus bisporus*. The presence of alkaloids, tannins, saponins, flavonoids, terpenes and terpenoids was confirmed by biochemical tests. The detailed screening of phytochemicals was performed using simple mass spectrometry. A total of 22 flavonoids were detected in aqueous extracts of cultivated *P.* ostreatus, commercial *P.* ostreatus and *A. bisporus*. Catechin, daidzein and chrysin were found to be common in both the species. Furthermore, *insilico* analysis of these common flavonoids against human Topoisomerases I & II revealed the daidzein to be the potent compound. It can also be inferred that compounds like daidzein which acts as a dual inhibitor can be considered for the design of specific inhibitors of human topoisomerases. Hence, it can be concluded that compounds extracted from both the species can be used in the preparation of topoisomerases inhibitory drugs. Furthermore, a novel drug can be developed using daidzein which possesses dual inhibitory activity.

# CHAPTER 1

# **INTRODUCTION**

# 1. INTRODUCTION

Mushrooms are macro fungi with distinctive fruiting bodies which are either epigenous or hypogenous and sufficiently conspicuous to the naked eye to be hand-picked. They are considered as the most widely used food ingredient across the globe. There are about 2,000 species of mushrooms. Among which 25 species are widely accepted as food and few are commercially cultivated. As these have high nutritional and functional value, they are also accepted as nutraceutical foods. These also possess various medicinal properties and serves as functional foods. However, most widely eaten edible mushrooms are considered to have many therapeutic properties [1-4].

Mushrooms play an important role in human diet as they are highly rich in nutritional content. Mushroom constitutes of various nutrients like carbohydrates, fibers, proteins, vitamins, minerals. The mushrooms are rich in protein and carbohydrate content, whereas low in lipid content. Apart from this, mushrooms have enormous functional activities such as antibacterial, antiviral, antioxidant, anticancerous, and hypocholesterolemic. Mushrooms are also rich in linoleic and oleic acids, which are essential unsaturated fatty acids and are necessary for the proper functioning of the body. Apart from this, they contain many essential minerals, which play an important role in proper metabolism of many pathways. Mushrooms grow on decayed matter so they are rich in lignin, cellulose, and other important carbohydrates. It is economical, possess various pharmacological properties, easy to cultivate, requires low resources and area, and can be grown all over the world. Secondary metabolites present in mushrooms are responsible for various medicinal properties. Compounds which are produced in response to stress that help in its survival by signalling and defence but are generally not required by the fungi for their normal activities like growth and reproduction are known as secondary metabolites.

These secondary metabolites are bioactive and have low molecular weight. Polysaccharides are the most responsible secondary metabolite which gives mushroom medicinal properties. These polysaccharides belong to 1, 3-β-glucans family, having anti-tumour activities which are achieved by enhancing and blocking cellular immunity pathways. High molecular weight glucans are more effective than low molecular weight glucans. Other secondary metabolites like include lectins, lactones, terpenoids, alkaloids, antibiotics, and metal-chelating agents are responsible for other pharmacological activities [5, 6].

*Agaricus bisporus* is widely cultivated mushroom worldwide followed by *Lentinus edodes, Pleurotus spp*., and *Flammulina velutipes*. China is the biggest producer of mushrooms around the world. *Agaricus bisporus, Pleurotus sajorcaju* and *Volveriell* are widely cultivated in India. Of the three cultivated species, the white button mushrooms (*Agaricus bisporus*) are cultivated all parts of the world and accounts for nearly 90 per cent of total mushroom production as these have high consumer demand. The oyster mushroom (*Pleurotus spp.*) grows during only winter months and therefore, it requires proper preservation techniques so as to promote their consumption and excess mushroom is processed into food products acceptable to consumers [2, 7-9].

*Pleurotus, Ganoderma, Agaricus, Tricholoma* and *Phellinus* species exhibit various therapeutic properties and are widely cultivated all over the world (Table1). They have various therapeutic applications like Anti cancer activity, anti arthritic activity, prevention of cardiovascular diseases, anti- neurodegenerative capabilities, anti diabetic activity, anti- obesity and many more (Figure 1) [10].

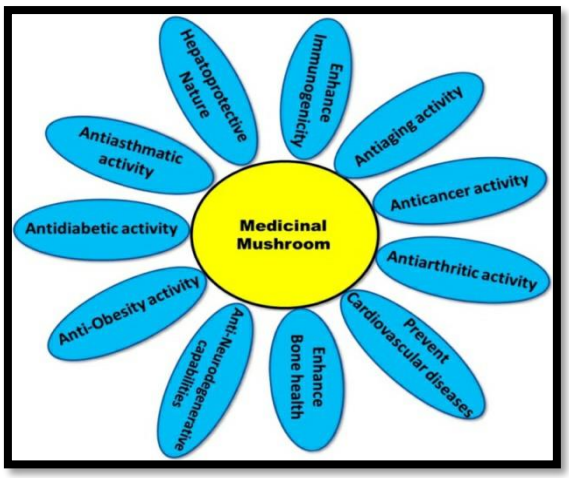



**Figure 1**. Schematic illustration of therapeutic applications of edible mushroom

The secondary metabolites are not only responsible for stimulating immune system but also help in modulating specific cellular responses by interfering with particular transduction pathways. *Pleurotus ostreatus* is an edible mushroom having the ability to block the cell proliferation of breast and colon cancer, by up-regulation of the cell cycle regulatory protein. The extract of *Pleurotus pulmonaris* suppresses liver cancer development by inhibiting PI3k/AKT signalling pathway. Extracted proteoglycans from *Ganoderma lucidum* have a protective effect against liver injury. The methanolic extracts of white button mushroom, *Agaricus bisporus*, exhibit anti proliferative and pro apoptotic activities, these inhibit prostate cancer by the regulation of extracellular regulated kinase (ERK/AKT) and NF-kappa. A fucomannogalactan (FMG-Am) and a (1→3), (1→6)-linked d-glucan (GLC-Am) isolated from *G. lucidum* fruiting bodies activate the immune response(cell and humoral immunity) by activating NK cell and inducing gene expression of nitrous oxide in macrophages and interferon-γ (INF-γ) transcription regulation by up regulation. Ethanolic and proteoglycan extracts from *Pleurotuslinteus* illustrate anti-inflammatory effect in collagen-induced arthritis [11].

**Table 1.** Medicinal value of some edible mushrooms

| S.no | Mushroom | Medicinal value |
|---|---|---|
| 1. | *Pleurotus* | Anticancer, antioxidant, antitumor, antiviral, antibacterial, antidiabetic, anti-hypercholesterolemic, eye health, anti arthritic, immunomodulatory, hepatoprotective, anti-obesity. |
| 2. | *Ganoderma* | Antiviral, hepatoprotective, antioxidant, antiallergenic, anticancer, hypoglycemic, immunomodulatory, hypotensive, antithrombotic, anti-inflammatory, hypocholesterolemic, antibacterial, antimutagenic, anti-osteoporotic, anti-ageing. |
| 3. | *Agaricus* | Anticancer, antidiabetic, antihypercholesterolemic, immunomodulatory, hepatoprotective, antiviral, antimutagenic. |
| 4. | *Tricholoma* | Anti-hypercholesterolemic, anti- ageing. |
| 5. | *Phellinus* | Anti-inflammatory, antidiabetic, Hepatoprotective |

There are about 40 different species of genus *Pleurotus* that are commonly referred to as “Oyster mushroom”. Among these *Pleurotus ostreatus* (*P. ostreatus*) is known for its unique taste, flavor, high nutritional values and medicinal properties and is consumed all over the world. Because of the presence of numerous nutritional compositions and various active ingredients in *P.ostreatus*, have been reported to have anti-diabetic, anti-bacterial, anti-cholestrolic, anti-arthritic, antioxidant, anticancer, eye health and antiviral activities. In this review, we particularly expose the high nutritional values of *P. ostreatus*, in relation to their potential medicinal usage which suggest that the *P. ostreatus* mushrooms are the most important nutraceutical functional foods. *Agaricus bisporus* is considered to have high biological activity, low toxicity and has significance folklore and

ethanopharmacological significance. Apart from food and food beverages it has a role in perfumery, cosmetic industries and pharmaceutical industries.It has been reported lots of primary and secondary metabolites responsible for the therapeutic activity for the prevention and treatment of many diseases such as cancer, hyperlipidemia, microbial diseases, cardiovascular problems, liver diseases, and immune problems. *A. bisporus* is a litter degrading basidiomycete commonly found in humic-rich environments which are useful as a model organism and cultivated in large scale for the food industry. Due to its ecological niche, it produces a variety of enzymes for detoxification and degradation of humified plant litter [12, 13].

Applied mushroom biology deals with the complete application of mushroom biology. It consists of three main components: mushroom science, mushroom biotechnology and mushroom bioremediation. Mushroom science is concerned with the cultivation and production of mushrooms. Mushroom biotechnology is concerned with mushroom products (mushroom derivatives) and encompasses the principles of mushroom microbiology, fermentation technology, and bioprocess. Mushroom biotechnology, both as a technology and as the basis for new mushroom products, requires industrial development. It, like many bioscience industries, operates at the cutting edge of science and involves numerous regulatory issues. The third component of applied mushroom biology has been developed in recent years. This is mushroom bioremediation and is concerned with the beneficial impacts of mushrooms on the environment (from mushroom mycelia) and encompasses principles of mushroom biology/microbiology, ecology, and bioconversion technology (Figure 2) [14].

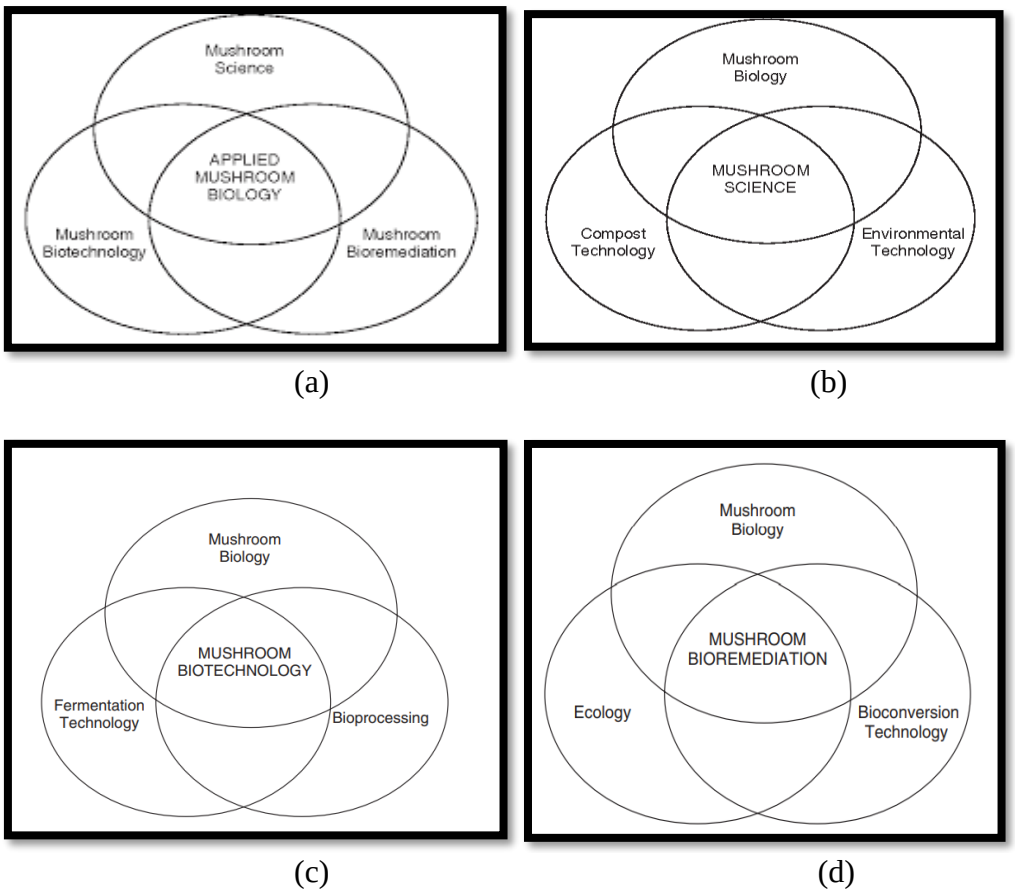



**Figure 2**. Classification of Mushroom Biology

(a) Applied mushroom biology consists of three components: mushroom science, mushroom biotechnology, and mushroom bioremediation

(b) Mushroom science: concerned with mushroom cultivation and production

(c) Mushroom biotechnology: concerned with mushroom products (mushroom nutriceuticals/dietary supplements

(d) Mushroom bioremediation: concerned with beneficial impacts of mushrooms on environment

Of the 1.5 million estimated fungi, it has been estimated that 140,000 species produce fruiting bodies of sufficient size and suitable structure to be considered macro fungi, which can be called mushrooms. Therefore so far we know 14,000 mushroom species. Of these, about 50%, or 7000 species, are considered to possess varying degrees of edibility, and more than 3000 species from 31 genera are regarded as prime

edible mushrooms. To date, only 200 of them are experimentally grown, 100 economically cultivated, approximately 60 commercially cultivated, and about 10 have reached an industrial scale of production in many countries. About 2000 are medicinal mushrooms which have different health benefits. And still about 126,000 species are unknown. Therefore, only 10% are well known [15-17].

Accordingly mushrooms can be grouped into four categories [18-20]:

- Edible mushroom category,
- Medicinal mushrooms,
- Poisonous mushrooms,
- Miscellaneous category, which includes a large number of mushrooms whose properties are less well defined.

Medicinal mushrooms are considered as a prominent source of nutraceuticals. *Agaricus*, *Lentinula* and *Pleurotus* are the most cultivated mushroom species globally. Out of which *Agaricus bisporus* and *Pleurotus ostreatus* are widely cultivated and accounts for the 38% of worlds cultivated mushrooms. Oyster mushrooms are known to have several bioactive compounds. Amongst which several species are known to have high nutritional, therapeutic and economic significance, and are widely cultivated and consumed by several countries across the world [21-25].

### 1.1 Oyster mushrooms

There are about 40 species categorized into *Pleurotus* genus. Oyster mushrooms are great functional foods because of their nutritional and medicinal importance. These are rich in proteins with essential amino acids, polysaccharides, essential fatty acids, dietary fibers, important minerals (Ca, P, Fe, K and Na), and some vitamins (B1, B2, C and D). These contain high molecular weight compounds (polysaccharides, peptides and proteins) and low molecular weight compounds

(terpenoids, fatty acid esters and polyphenols). Due to the presences of these bioactive substances, these exhibit different pharmacological activities such as antimicrobial, antioxidant, anticancer, anti-inflammation, antihypercholesterolemia, antigenotoxic, antihypertensive, antiplatelet-aggregating, antihyperglycaemic, anti-hypertensive, anti-diabetic, immunomodulatory, anti-neoplastic, anti-atherosclerotic, anti-diabetic, hepato-protective and anti-allergic activities.These species are ubiquitous i.e., these are found both in temperate as well as the tropical parts of the world and can be easily cultivated artificially. Usually these are grown on dead and decaying wooden logs or sometimes on the outer bark of living trees. These are also useful for utilizing lignocellulosic biomass [23, 26-31].

**Figure 3**. *Pleurotus ostreatus* (Source: Mushroom Farm, ECIL)

Oyster mushrooms have many significant advantages over the other types of mushrooms as listed below [27]:

I. Easy to grow and cultivate.

II. Posses the Capability to grow on lingocellulosic wastes, and easily degrade lignins and leaving behind cellulose and hemicelluloses.

III. Helpful in biodegradations.

IV. Have high nutritional and pharmacological value.

Some of the species of *Pleurotus* genus *are sajor caju, sapidus, ostreatus, florida, citrinopileatus, flabellatus, pulmonarius, geesteranus, ulmarius, tuberregium, cystidiosus, eryngii*.Out of which *Pleurotus ostreatus* and *pleurotus pulmonarius* have high economic significance [23,32].

### 1.1.1 Structure

These have three distinct parts- a fleshy shell or spatula shaped cap (*pileus*) which is normally shell-like (about 5-20 cm in diameter; 1.9-7.8 inches), a short or long lateral or middle stalk called *stipe* and underneath the pileus there are gills or *lamellae*. The gills stretch from the edge of the cap down to the stalk and bear the spores; and their color can be white, cream, yellow, pink, brownish, or dark gray.

**1.1.2 Taxonomy**: Oyster mushrooms are generally classified as follow;

Phylum - *Basidiomycotina*;

Class - *Basidiomycetes*;

Sub class - *Holobasidiomycetidae*;

Family - *Polyporaceae*;

Genus - *Pleurotus*;

Species - *sajor caju sapidus, ostreatus, florida, citrinopileatus, flabellatus, pulmonarius, geesteranus, ulmarius, tuberregium, cystidiosus, eryngii* and others. [32]

### 1.1.3 Life cycle of *Pleurotus ostreatus*

*Pleurotus* have the life cycle of basidiomycetes. This typical life cycle start with germination of a spore in a suitable substrate. This gives rise to form a mycelium which is monokaryotic (n). The fertile dikaryon (n+n) is formed when two compatible

monokaryotic mycelia are in a close contact and undergo hyphal fusion or plasmogamy. The dikaryotic mycelium, under appropriate environmental conditions (temperature, light, relative humidity) will differentiate into fruit bodies having specialized structures called basidia, which are formed in the lamellae, furthermore, karyogamy and meiosis take place.The four new basidiospores are formed on the basidium when the haploid nuclei move to sterigmata on the basidium. When the fruit bodies are mature, basidiospores are discharged, starting the sexual life cycle again.*Pleurotus ostreatus* is an oyster mushroom with nutritional and medicinal properties [32-35] (Figure 4).

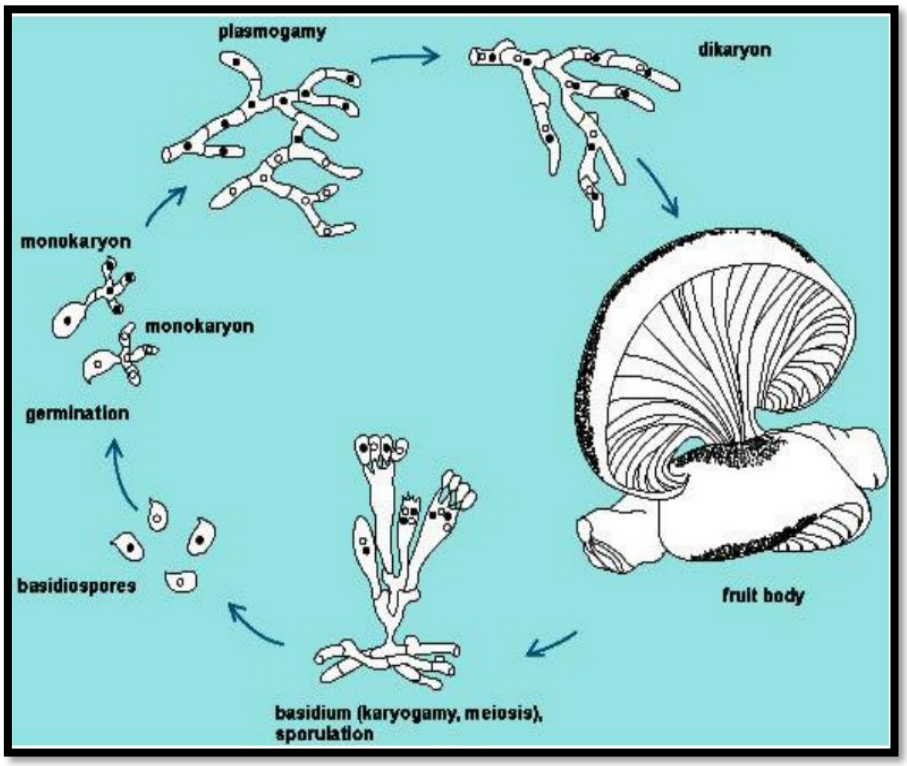



**Figure 4**. Life cycle of the oyster mushroom *Pleurotus ostreatus*

### 1.1.4 Cultivation

The major steps involved in mushroom cultivation are selecting suitable mushroom species, development of the spawn, preparation of suitable substrate for

growth, care of mycelia (spawn) running, management of fruiting and mushroom development, and careful harvesting of mushrooms [14].

The following steps are involved in cultivation according to Sonali. D. *et.al* [9]:

1. **Substrate preparation:** Oyster mushroom was previously successfully grown on various substrates like paddy straw, Wheat straw, vegetable plant residues etc. Of all the substrates paddy straw is cheap and easily available. Hence, mushrooms are widely cultivated on paddy straw. But, it is extremely important to use the fresh and well dried substrate.
2. **Soaking:** After selecting and procuring suitable substrate to grow mushrooms, the substrate is usually chopped into 3-5 cm pieces and soaked in fresh water for about 8-16 hours. The excess water from the straw is drained off by spreading it on filter paper.
3. **Heat Treatment:** To remove the contaminants from the substrate, the substrate is subjected to heat treatment. Removal of contaminants also leads to higher yields. General acceptable treatment is pasteurization.
4. **Pasteurization:** In pasteurization the water is boiled in a container. The wet substrate is filled in gunny bags. The filled bag was dipped in hot water of 80-85$^0$C for about 10-15 minutes. To avoid floating, it is then pressed with some heavy material.
5. **Spawning:** After the cooling of pasteurized substrate to room temperature, it is then filled along with the spawn. Generally, for the cultivation Polythene bags (35 x 50 cm, 150 gauges) or polypropylene bags (35 x 50 cm, 80 gauges) are used. Approximately One 500 ml bottle spawn (200-250 g) can be used for 10-12 kg wt straw (3 bags). Spawning is done in layer spawning or through spawning. In layer spawning, the bag filled by layering the spawn

and the substrate simultaneously one upon the other. 2-3 layers of the spawn and the substrate is layered. After the spawning the bags are properly sealed. These bags are then stacked on racks in neat and clean place, in closed position by maintaining proper temperature and humidity. Temperature at 25-35$^0$C and humidity at 70-85% was maintained by spraying water twice a day on walls and floor. It takes 15- 20days when bags were fully covered with white and pink mycelium respectively.

6. **Cropping and harvest:** After 20-22 days, when bags were fully filled with white mycelium, they are then transferred into cropping room. As soon as the small pinhead structures appear water is sprayed lightly. Once the pinheads grow in size a heavier watering is done to stop them to grow. To maintain the quality they are plucked before they shed spores. After each flush of harvest the outer layer is scrapped off to help to initiate next flush which appears after 10 days of harvesting.
7. **Maintenance:** To maintain the freshly harvested mushrooms they are packed in perforated (5-6 small holes) polythene bags. This helps to keep them fresh. Generally, it loses freshness after about 6 hours. So to enhance them we can refrigerate them. In case of Oyster mushroom they are shed dried for 2 days and dried product marketed in polythene bags. Dried mushrooms were soaked in water for 10minutes before use.

### 1.1.5 Nutritional aspects of *Pleurotus ostreatus*

*Pleurotus ostreatus* is known for its unique flavour, taste and aromatic properties. It is also rich in various nutrients like protein, fiber, carbohydrates, minerals and vitamins. It is less in fat content. Wild mushrooms and commercially

cultivated mushrooms have the similar nutritional components. However, there are qualitative and quantitative differences in the chemical composition of *P. ostreatus* products highly depend upon various factors like the strain, origin, extraction process and cultivation conditions [35].

According to Adebayo EA *et al*. and Krishnamoorthy *et al*. [31, 35], the following nutritional value and aspects are discussed below.

1. **Proteins:** In *Pleurotus ostreatus* many factors like strains, physical and chemical differences in growing medium, composition of the substrate, size of the pileus, and harvest time effect the protein content. Numerous studies have suggested that protein content ranges from 17 to 42 g per 100 g dried fruit bodies. These species contains lectin which is a dimeric protein with a molecular weight of 40 kDa and 41 kDa and known to have antihepatoma and antisarcoma properties. It was reported that approximately 7mg of amino acids are present in 100 g of edible part of these mushrooms. The 20 amino acids have been obtained from of *P. Ostreatus*, which are rich in asparagine; aspartic acid and glutamic acid but poor in proline; glycine and methionine.
2. **Carbohydrates:** In *P. Ostreatus* polysaccharides are the main carbohydrates present. There are different polysaccharides like glycogen and such indigestible forms as dietary fibers, cellulose, chitin, α- and β- glucans and other hemicelluloses like mannans, Xylans and galactans.The glucans are present with different types of glycosidic linkages, such as branched (1→3), (1→6)-β-glucans and linear (1→3)-α- glucans. *P. ostreatus* contains a specific β-glucan called pleuran, which serves as a source of antitumor polysaccharides. The composition of these polysaccharides in the fruit bodies vary with the strains, ranging from 37 to 48 g/100 g dry fruit bodies.

3. **Fibers:** Mushrooms are a potential source of dietary fibers due to the presence of non-starch polysaccharides. Whereas, the stem part of the mushroom contained more insoluble dietary fibers than the pilei in all the cases. However, the total dietary fiber (TDF) in mushrooms is the sum of intrinsic non-digestible carbohydrates mainly chitin. Mushroom glucans are also components of soluble (SDF) or insoluble (IDF) dietary fiber. Their solubility in water strongly depends on the molecular structure and conformation. It was also reported that about 4-9% and 22-30% for soluble and insoluble fiber, respectively. It is evident that mushroom contains other structural polysaccharide in addition to chitin. It was also reported that the content of dietary fiber in 100g of edible parts ranges from 4.1 g in *P. ostreatus* mushroom.
4. **Fatty acids:** The essential fatty acids (EFA), linoleic and linolenic acids are two long-chain fatty acids that are essential to human diets. Total fat or lipid content production varied from one species of Pleurotus to the other. So also, distribution of fatty acids varied amongst different species of the Pleurotus. *P. ostreatus* was reported to produce fatty acids such as palmitic, stearic, oleic, linoleic, and lauric.
5. **Vitamins:** Edible mushrooms have been reported to be a good source for several vitamins including thiamine (vitamin B1), riboflavin (vitamin B2), niacin, biotin, and ascorbic acid (vitamin C). Out of all the species *P. Ostreatus* produce rich amount of vitamins E, A and C. It contains more folacine, vitamin B1, vitamin B3 but less vitamin B12 than other mushroom species.

6. **Essential macro and micro minerals:** *P. Ostreatus* consists of essential macro and micro minerals like zinc, phosphorus, Potassium, manganese, iron, sodium, calcium and copper. These have greater content of copper, iron, potassium, magnesium, phosphorous and zinc and the stipes of the pilei of *P. ostreatus* have greater content of sodium.

### 1.1.6 Enzymes

Mushrooms exhibit different enzymatic activity. *P. ostreatus* has higher enzymatic activity of polyphenoloxidase. This catalyzes the oxidation of phenol, causing a rapid darkening of harvested mushrooms, which in turn reduces their sensory and nutritive properties. Conversely, the darkness of product decreases their keeping quality and hence their market value [35].

## 1.2 *Agaricus bisporus*

*A. bisporus* is an edible basidiomycete mushroom native to grasslands in Europe and North America. It is commonly known as white button mushroom (WBM). It is widely cultivated in most of the countries across the globe. Historical evidence indicates that it was first cultivated in France and that cultivar strains originated in Western Europe [36].

**Figure 5**. *Agaricus bisporus* (Source: Sugam mushroom farms)

### 1.2.1 Taxonomy

Kingdom - Fungi

Divison - *Basidomycota*

Class - *Agaricomycetes*

Order - *Agaricales*

Family - *Agaricaceae*

Genus - *Agaricus*

Species - *Agaricus bisporus*

The mushrooms harvested are differentiated into five stages based on distinguishing features.

1. Egg-shaped (pin-head)
2. Ruptured volva
3. Emergence of pileus (mushrooms are harvested commercially before the veil was broken)

4. Veil opened
5. Fully expanded pileus

It contains rich nutrients like carbohydrates, proteins, lipids, fibers, minerals, and vitamins. Moreover, because of the presence of some active ingredients, such as polysaccharides, lipopolysaccharides, essential amino acids, peptides, glycoproteins, nucleosides, triterpenoids, lectins, fatty acids and their derivatives, these mushrooms have been reported to have anti-microbial, anti-cancer, anti-diabetic, anti-hypercholesterolemic, anti-hypertensive, hepatoprotective and antioxidant activities. [37, 38]

### 1.2.2 Life cycle of *Agaricus bisporus*

Basidiomycetes produce basidiospores that are produced on a specialized structure called basidium. Although haploid, basidiospores may be either uninucleate or binucleate at maturity. After these basidiospores are released from mature fruiting bodies, they germinate to form hyphae. Collectively, hyphae make up mycelium. Hyphae derived from the spores of two different mating types then fuse to form secondary mycelium which has two nuclei and is ultimately responsible for the formation of fruiting bodies (Figure 6) [39].

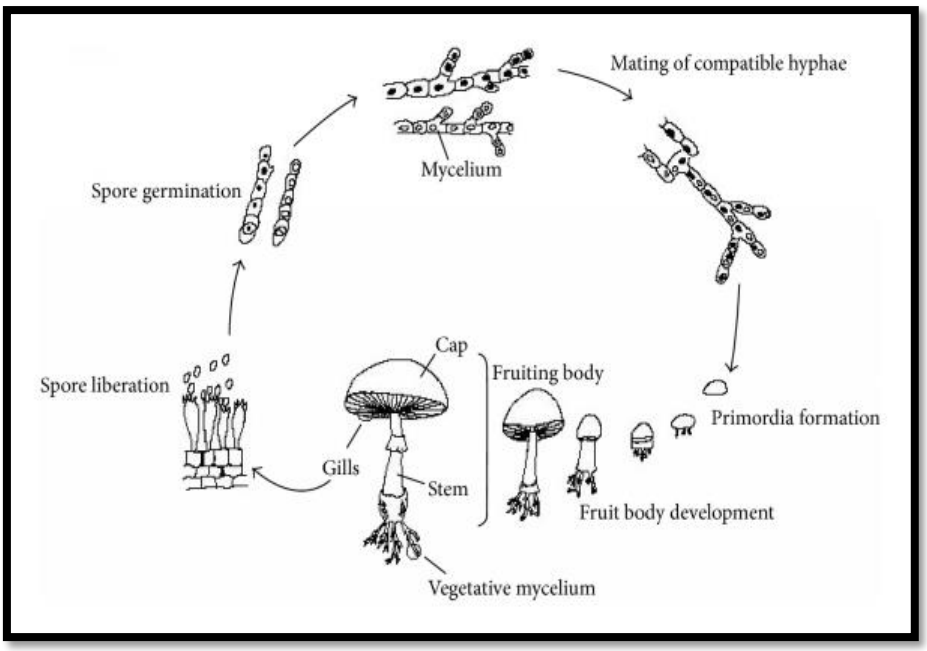



**Figure 6**. Life cycle of *Agaricus bisporus*

It comes under the category of a food which is beneficial for humane health with excellent levels of dietary fibers and antioxidants including vitamins namely; thiamine, ascorbic acid, vitamin D2 etc as well as minerals like folates, ergothioneine (ET) and polyphenols which may provide favorable effects on cardiovascular diseases and diabetes. Around half of the fungal cell wall mass is constituted by β-glucans along with ergosterol, tocopherols, linoleic acid, and lectins. Fungus contains 1-6 mg of phenolics/g of dried mushroom and flavonoid concentrations ranged between 0.9 and 3.0 mg/g of dried matter; as myricetin and catechin. Agaritine and its derivatives which chemically belong to hydrazines is the main aromatic compound of mushrooms. Hydrazines are present in mushroom species like *A. bisporus*, etc., Agaritine was found to contribute to the formation of toxic aryl diazonium ions. Gamma-glutaminyl-4-hydroxybenzene is the principal phenolic compound present in mushrooms [36].

Therefore, *Pleurotus ostreatus* and *Agaricus bisporus* have many significant therapeutic properties and can be used in designing of drugs. However, it is important to prove the molecular mechanism and efficacy of these mushrooms on various activities.

*In-silico* tools made it easier to find out molecular target mechanism of drug leads. Molecular docking is an essential tool in drug discovery in order to know the detail interactions between molecules and the drug targets. The main aim of molecular docking is to predict the ligand-receptor (protein) complex. The goal of ligand-protein docking is to predict the potential binding mode of a ligand (compound) with that of a protein whose three-dimensional structure is already known [40]. In the present study, human DNA topoisomerases (i&ii) was taken as anti-cancer drug targets.

### 1.3 DNA topoisomerases

DNA topoisomerases are enzymes which plays important role in topology of DNA by cleaving and rejoining DNA strands. They regulate physiological function of the genome. Also, DNA processes such as replication, transcription, recombination, repair, and chromosome-decondensation and sister chromatid are controlled by these enzymes [41]. These are important anti-cancer drug targets because of the crucial role of topoisomerases; cells become highly vulnerable when these functions are lost. Therefore, these acts as anti-cancer drug targets [42]. When cleaved DNA strand that has not been bound by the topoisomerase could be released, creating a permanent breakage in the DNA leading to cell death. Due to the activity of these enzymes cancer cells become highly immortal [41]. Therefore, the compounds which inhibit the activity of the topoisomerases are known to play an important role in treatement of cancer.

Hence in this study, the water extracts of *Pleurotus ostreatus* and *Agaricus bisporus* was prepared and Simple Mass spectrometry analysis was carried out for analyzing various phytochemicals. The aim of the present study is to screen the phytochemicals present in both the mushrooms and to study the role of flavonoids as anti-cancer agents using molecular docking studies against human topoisomerase (i & ii).

Here is the list of objectives of the present study:

1. To optimize the growth of *Pleurotus ostreatus* by usingvarious substrates.
2. Phytochemicals analysis of *Pleurotus ostreatus* and *Agaricus bisporus* using various solvents.
3. To detect and analyze the compounds by using simple mass spectroscopy.
4. To perform *in silico* studies of flavonoids from *Pleurotus ostreatus* and *Agaricus bisporus.*

CHAPTER 2

# REVIEW OF LITERATURE

# 2. LITERATURE REVIEW

The present study is focused on analysis of various phytochemicals present in the *P. ostreatus*and *A. bisporus*. So, the review of literature gives us the information about previously work done on various compounds present in mushroom with their functional activities like anti bacterial, anti cancerous, anti oxidant, anti tumor and anti viral,etc. Also, it gives the information about various methods followed for the screening of these phytochemicals.

## 2.1 Phytochemicals present in *Pleurotus ostreatus*

It was reported that Phytochemical screening of ethanolic and aqueous extracts showed the presence of secondary metabolites such as alkaloid, glycosides, saponin, tannin, flavonoid, reducing compound, polyphenol. Saponin, polyphenol and reducing compound on quantification were much higher than the rest of the bases with values 4.02, 3.16 and 4.59%, respectively The GC-MS analysis of the methanol extract of *Pleurotus ostreatus* revealed presence of 30 different acids, alcohols, aldehydes, heterocyclic compounds and certain esters, ketones and aldehydes [43,44]. The fruiting body of *P. ostreatus* contains approximately 100 different bioactive compounds, which mainly considered as a potential new source of dietary fiber. Whereas, fungal cell wall are rich in non-starch polysaccharides, of which β-glucan are most interesting functional components and phenolic compounds such as protocatechuic acid, gallic acid, homogentisic acid, rutin, myrictin, chrysin, naringin, tocopherol like α-tocopherol and γ- tocopherol, ascorbic acid and β-carotene of each having their own outstanding medical effects. Moreover, they are healthy foods, rich in protein, lipids, carbohydrates, vitamin and minerals content but low in calories and

fat content [35]. Different phytochemicals were reported in the *Pleurotus ostreatus* using different methods and various organic solvents (Table 2) [44-47].

**Table 2**. Phytochemicals identified in *Pleurotus ostreatus*

| S.no. | Compounds detected | Solvent | Analysis |
|---|---|---|---|
| 1. | Pyrogallol, gallic acid, homogentisic acid, protocatechuic acid, p-hydroxybenzoic acid, p-coumaric acid, gentisic acid, ferulic acid and myricetin | Methanol | HPLC |
| 2. | Vanillic acid, myricetin, naringin, homogentic acid, 5- ocaffeoylquinic acid, chrysin, routine, gentisic acid, gallic acid, protocatechuic acid, caffeic acid, tannic acid, syringic acid, cinnamic acid and p-coumaric acid. | Ethanol | Not mentioned |
| 3. | Formamide, 1,4-pentanediol, glycerin, 2(5H)-furanone, 3-methyl-, 3-hexyn-2-ol, 4-heptanone, 3-methyl-, 2-pyrrolidinone, butanedioic acid, monomethyl ester, cyclopropanecarboxylic acid, 1-amino-, pentanal, 2,3-dimethyl-, succinimide, cyclobutanone, 2-methyl-4-hydroxy-, heptanoic acid, 2-methyl-2-butyl ester, 5-methoxypyrrolidin-2-one, 1,1,3-trimethyl-3,8,9-trioxa-bicyclo[4,1,4 dioxaspiro[4,4]nonane-7-carboxy,niacin, propanedioic acid, phenyl-, 2-dodecanone, 2-heptanone, 5-methyl-, 2-nonanol, 2-undecene, 4,5-dimethyl-, 2-butyn-1-ol, 4-methoxy, DL-proline, 5-oxo-, methyl ester, acetic acid, 2-propyltetrahydropyran-3, 3-tetradecene, 4-heptanone, 3-methyl, | Methanol | GC-MS |

| | | | |
|---|---|---|---|
| | niacinamide, acetic acid, 2-(1-buten-3-yl)-2-nitro, 9-eicosene | | |
| 4. | Urea, N-methyl-N-nitroso, -amino-gamma –butyrolactone, 3-methyl-2-butenoic acid, 2,2-dimethylpropyl ester,4H-1-2,4-Triazole, 4-methyl, D-lysine,1-undecanol,11-mercapto-,N-guanidino-spermidine, pentadecanoic acid, ethyl ester, 9,1-octadecadienoic acid , methyl ester, 14,17-octadecadienoic acid, methyl ester, methanamine,n-pentylidene, dicarbododecaborane-c,c bis-9-propanenitrile,cholestane3,7,12,25-tetrol,tetraacetate, (3a,5a,7a,12a)- | Hydro-alcholic extract | GC-MS |

### 2.1.1 Bioactive compounds and medicinal properties of *Pleurotus ostreatus*

It was reported that dried fruit bodies of mushrooms contain high nutritional value. Generally, fresh *Pleurotus* mushroom contain 85-95% moisture and therefore dried fruits contains high nutritional value. *P. ostreatus* have been reported to have anti-cancer, anti-tumor, and immunomodulatory, anti-hypercholesterolemic, anti-atherogenic, anti-viral, anti-bacterial, anti-fungal, anti-oxidative, eye health, hypoglycemic, anti-inflammatory, anti- arthritic, anti-atopic dermatitis and anti-cataractogenic activities (Figure 7) (Table 3) [23, 38, 45].

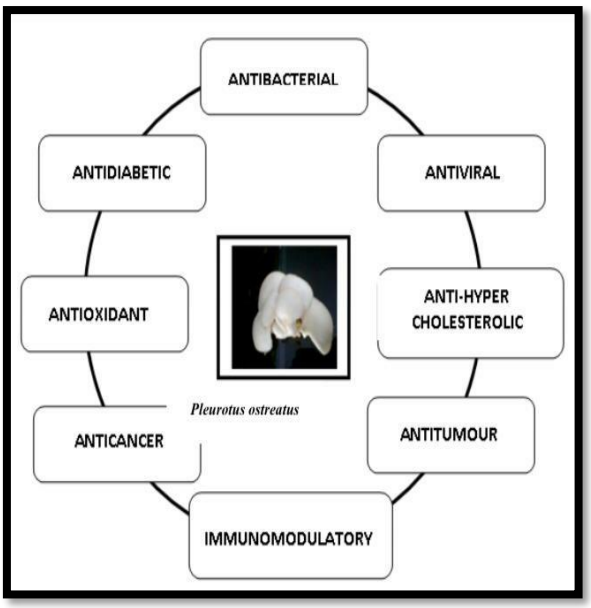



**Figure 7**. Pharmocological properties of *Pleurotus ostreatus*

**Table 3**. Medicinal properties of *Pleurotus ostreatus*

| S.no. | Activity | Bioactive compounds |
|---|---|---|
| 1. | Anti-cancer | β-glucans,α-glucan, proteins |
| 2. | Anti-tumor | Polysaccharides, proteoglycans, lectin |
| 3. | Immunomodulatory | Polysaccharides, heteroglycan |
| 4. | Anti-hypercholesterolemic | Lovastatin, ergosterol |
| 5. | Anti-atherogenic | Chrysin, lovastatin |
| 6. | Anti-viral | Proteins |
| 7. | Anti-bacterial | β-glucans |
| 8. | Anti-fungal | Proteins |
| 9. | Anti-oxidative | Polysaccharides |
| 10. | Hypoglycemic | Unspecified |
| 11. | Anti-inflammatory | Polysaccharides |
| 12. | Anti- arthritic | β-glucans |
| 13. | Anti-atopic dermatitis | pleuran |
| 14. | Anti-cataractogenic | Unspecified |
| 15. | Eye health bioactive | Unspecified |

The various activities like anti-microbial, anti-inflammatory, anti-cancer, anti-diabetic, anti- hypercholesterolemic, immunomodulatory of *Pleurotus* species are discussed below.

### 2.1.2 Anti-microbial activity

Several species of genus *Pleurotus* such as *P. ostreatus, P. Pulmonary, P. Cornucopia, P. eryngii and P. Cystidiosus* show anti-microbial activity. There are about 70 species of *Pleurotus* and many of these species show antimicrobial properties [49, 50]. The complete report on the anti-microbial activity of *Pleurotus ostreatus* is mentioned in Table-4. Many bioactive substances were responsible for antimicrobial activity in *P. Ostreatus* [51-57].

**Table 4**. Antimicrobial activity of *Pleurotus ostreatus*

| S.no. | Bioactive substance | Target organism |
|---|---|---|
| 1. | Petroleum ether extract, terpenoids, tanins, steroidal glycosides. | *S. aureus, S. epidermidis, B.subtillis, B.licheniformis, S. typhi, E. coli, Proteus sp. K.pneumonia, P. aeruginosa, H. influenza, C.albicans Saccharomyces cerevisiae*. |
| 2. | Flavonoid, vitamin-c | *Vibrio cholera, Bacillus spp.* |
| 3. | Ethanolic extract | *L. innocua, B. Cereus.* |
| 4. | Phenolics, flavonoids | *S. aureus, E. Coli.* |
| 5. | Ascorbic acid, lycopene, β-carotene, α-tocopherol | *P. aeruginosa, C.albicans, Candida sp.* |
| 6. | Gemmotherapic extract | *B. subtilis, B. cereus, P. aeruginosa, S. Marcescens.* |
| 7. | Cold water, hot water extracts, acetone Ethanol, chloroform | *S. aureus and E. Coli.* |

It was reported that Anti-viral activity of *P. ostreatus* is confirmed due to the Laccase activity. It was first isolated from this species and inhibited hepatitis C virus entry into peripheral blood cells and hepatoma HepG2 cells. It also inhibits the replication of the virus.*Pleurotus ostreatus* also possess anti-fungal activity. The ethanolic extracts of this species showed the maximum inhibition against *Penicillium chrysogenum* [58, 59].

### 2.1.3 Anti-inflammatory activity

Mushrooms consist of several anti-inflammatory components, such as polysaccharides, phenolic and indolic compounds, mycosteroids, fatty acids, carotenoids, vitamins, and biometals.It was reported that the aqueous extracts of *P. ostreatus* suppressed LPS-induced secretion of tumor necrosis factor-α, interleukin-6, and IL-12p40 from RAW264.7 macrophages. Furthermore a study shows the anti-inflammatory activity of extract from *P. ostreatus* against an ear acute inflammation stimulated by xylol on white underbred rat-male.Similarly; Pleuran protein present in the *P. Ostreatus* has been reported to possess anti-inflammatory activity [60-64].

### 2.1.4 Anticancer effects

Different types of extract from *P. ostreatus* have been demonstrated as potential anticancer agents in different cancer cell lines and experimental animals. Many bioactive compounds are responsible for anti cancer property in *P. Ostreatus* (Table 5) (Figure 8) [10, 65-70].

**Table 5**. Properties and mechanisms of bioactive compounds from *Pleurotus ostreatus* extract against human cancers cell lines

| S.no. | Bioactive extract | Cancer | Mechanism |
|---|---|---|---|
| 1. | Flavonoids | Blood | It showed Cytotoxic effect on human cancer cell lines (HL-60) under in-vitro conditions. |
| 2. | Polysaccharide β-glucan | Colon | It induces the anti-proliferative and pro-apoptotic effects on HT-29 cancer cells. |
| 3. | Hot water extract | Breast | It showed anti proliferative activity of MCF-7 human breast cancer cells. |
| 4. | Methanol extracts | Breast<br><br>Colon | • The Suppression of the growth of highly invasive breast cancer MDA-MB-231 cells.<br>• These inhibited the proliferation of highly-invasive colon cancer HCT-116 cells. |
| 5. | Water- extract | Prostate | These produced the most significant cytoxicity and induced apoptosis in PC-3 cells as dose dependent manner. |
| 6. | Protein extract | Colorectal | These exhibited therapeutic efficacy against human colorectal adenocarcinoma cell line (SW 480 cells) and a human monocytic leukemia cell line (THP-1cells) by induced apoptosis in SW 450 cells partially through reactive oxygen species (ROS) production. |

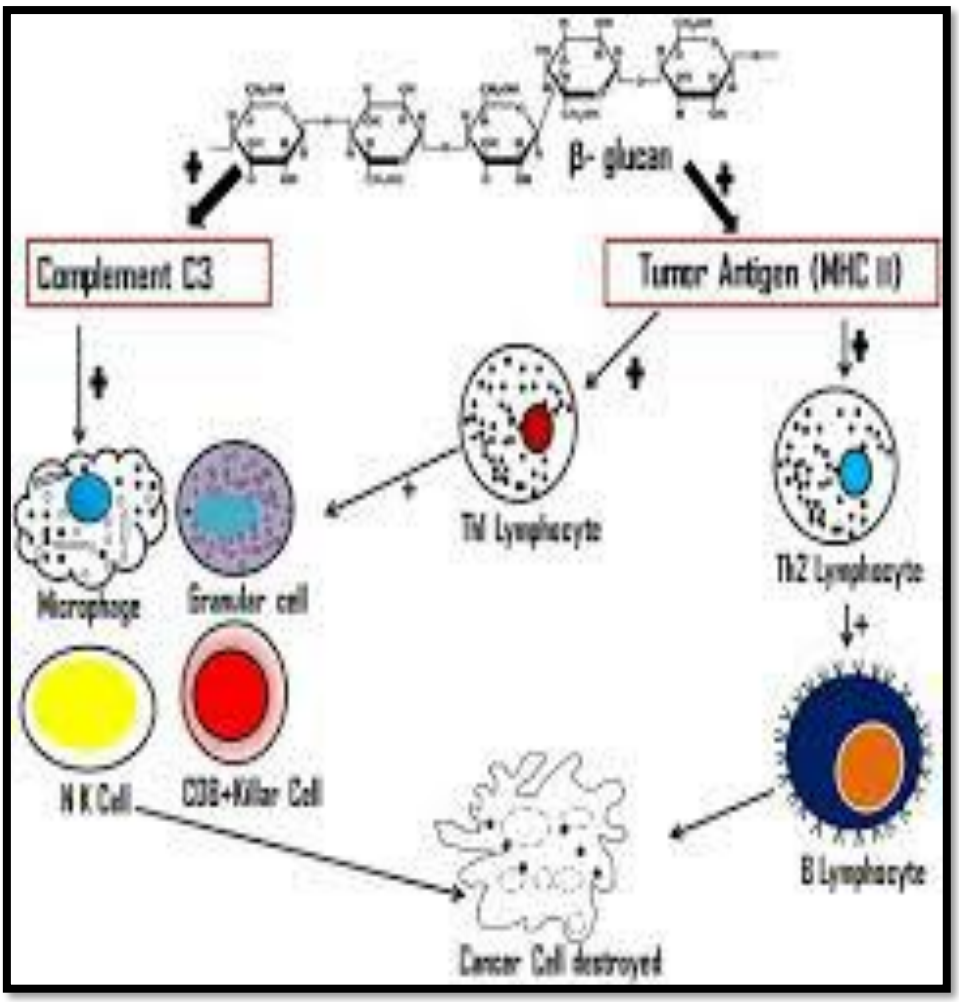



**Figure 8**. Mechanism of anti-cancerous activity of bioactive compound (β-glucan)

### 2.1.5 Anti-tumor activity

There are many compounds of*Pleurotus ostreatus* which are responsible for the anti-tumor activities (Table 6) [71-73].

**Table 6**. Bioactive compounds of *Pleurotus ostreatus* against tumor

| S.no. | Compound | Activities |
|---|---|---|
| 1. | Polysaccharide | In 1972, it was first reported that polysaccharide extracts of *P. Ostreatus* shows anti-tumor activities. |
| 2. | Proteoglycans | The proteoglycans elevated mouse natural killer (NK) cell cytotoxicity and stimulated macrophages to produce nitric oxide. |
| 3. | Glucan (Hetroglucan) | Hetroglucan folded into a triple helical conformation and exhibited enhanced immune cell activation and anti-tumor potential in tumor bearing mice model. |

### 2.1.6 Antioxidant activity

Oxidative stress is the main factor in the progression of many degenerative diseases However; different antioxidants such as phenolic and flavonoid compounds are delaying and inhibiting oxidative processes [20].

Generally, *Pleurotus* mushrooms are rich in vitamin and selenium content which are the natural antioxidants. It was reported that, in aged rats the Catalase gene expression was enhanced and the incidence of free radical-induced protein oxidation was decreased by an extract of *P. ostreatus*, thereby protecting the occurrence of age-associated disorders that involve free radicals. The ethanolic extract exhibit in vitro antioxidant activity by virtue of its scavenging hydroxyl and superoxide radicals, inhibiting lipid peroxidation, reducing power on ferric ions, chelating ferrous ions and quenching 2,3-diazabicyclo[2,2,2]oct-2-ene (DBO). It also exhibits as a good in-vivo antioxidant activity by reducing the intensity of lipid peroxidation and by enhancing the activities of enzymatic and non-enzymatic antioxidants. Therefore, the extracts

show both in-vitro and in-vivo anti-oxidant properties. A study showed that the two polysaccharide fraction (PSPO-1a and PSPO-4a) isolated from the fruiting bodies of *P. ostreatus* exhibited the stronger DPPH and superoxide anion radical scavenging activity with increased concentration, but less effective on scavenging hydroxyl radical. Among these two polysaccharides PSPO-1a, possess more effective free-radical scavenger than PSPO-4a [74-76].

Whereas, free radical scavenging and NOS activation properties of water soluble crude polysaccharide from *P. ostreatus* showed superior antioxidant property which might be due to presence of carbohydrate component mostly β-glucan seemed to be responsible for the antioxidant activity. Therefore, *P. ostreatus* act as good source of antioxidant [76, 77].

### 2.1.7 Antihypercholesterolic activity

In a study conducted on the ethanolic extract and dried fruit of *P. Ostreatus*. The dried fruit of *P. Ostreatus* showed an effective evidence for the anti-hyperlipidaemic activity to the diet of normal wistar male rat and a stain with hereditary hypercholesterolaemia. In contrast, the ethanolic extract did not significantly change TAG level. It wasreported that by feeding the hypercholesterolemic rats with powder of *P. ostreatus* and *P. sajor-caju* reduced the plasma total cholesterol (TC) level and triglycerides (TG) level due to presence of active substance lovastatin [78, 79].

### 2.1.8 Anti-diabetic activity

Hyperglycemia is the key feature of diabetes mellitus. The combination of *P. ostreatus*, *Murraya koenigii* and *Aegle marmelos* was oral administration to diabetic rats, and it revealed that the combination produced synergistic effects have been showed blood glucose-lowering effect in both insulin dependent and insulin-

independent diabetic conditions. However, to evaluate the antihyperglycemic action of oyster mushroom (*P. ostreatus*) and its effect on potential DNA damage, chromosome aberration and sperm abnormalities in Streptozotocin induced diabetic rats. The results showed that the treatment with high level of *P. ostreatus* extract could decrease the high blood glucose level in hyperglycemic rats but less than amaryl treatment. However, the mushroom treatments were more effective for decreasing the genetic alterations and sperm abnormalities in diabetes conditions than amaryl treatment. It waspostulated that antidiabetic potential of oyster mushroom *P. ostreatus* in alloxan-induced diabetic mice. This study showed that the *P. ostreatus* produced a significant hypoglycemic effect in diabetic mice and it is capable of improving hyperlipidemia and the impared kidney functions. Therefore, these findings suggest that *Pleurotus* mushroom are promising as an anti-diabetic nutraceuticals [80-82].

### 2.1.9 Immunomodulatory properties

They also exhibit Immunomodulatory properties alone with low cytotoxicity raise the possibility that it could be effective in the cancer patients receiving conventional chemotherapy and radiational treatment, to build up immune resistance and decreased toxicity. A large number of compounds like lectins, polysaccharides, polysaccharides-peptides, and polysaccharide-protein complex have been isolated from mushroom and many of these compounds have been found to have immunomodulatory effects [20]. These are suggested to enhance cellular components of the immune system. It has been reported that water extract of *P. ostreatus* has a role in increasing the production of reactive oxygen species (ROS) from neutrophil and has immune modulatory properties involving all immune compentent cells. However, the bioactivities of the polysaccharides depend on the binding on the lectin-like surface receptor of the

immune cells. However, it was postulated that Fraction I and ion exchange – passed fraction (crude) strongly interacted with glucose/ mannose- specific lectin Con a, indicating the presence of large number of terminal sugars with glucose/ mannose. These three fractions are not structurally similar but their bioactivities are comparable, which may be due to their differential binding to the immune receptors [83-85].

### 2.2 *Agaricus bisporus*

*Agaricus bisporus* has many medicinal benefits and many bioactive ingredients were isolated from it. It has a very good history of using in many traditional therapies. The use of *A. bisporus* extracts and/or its bioactive compounds as antioxidant, anti-cancer and anti-inflammation is increasing in the world against many human diseases such as coronary heart diseases, diabetes mellitus, bacterial and fungal infections, disorders of the human immune system and cancers. Although there have been relatively few direct intervention trials of mushroom consumption in humans, those that have been completed to date indicate that mushrooms and their extracts are generally well-tolerated with few, if any, side effects [38].

There are medicinal metabolites such as β-glucan and G-glucan; polysaccharide K or PSK “protein bound polysaccharide”; phenols; polyketides, triterpenoids and sterols, triterpenoids, lectins glycoprotein's; ergothioneine, selenium, pyran derivative; essential fatty acids and eicosanoids. The identified sterols in *A. bisporus* are ergosta-7, 22-dienol, ergosta-5,7-dienol, and ergosta-7- enol (fungisterol) [86].

Different bioactive compounds are responsible for different activities (Table 7)[36, 38, 86].

Different phytochemicals were reported in the *A.bisporus* using different methods and various organic solvents (Table 8) [41, 87, and 88].

**Table 7**. Medicinal properties of *Agaricus bisporus*

| S.no. | Activity | Bioactive compounds |
|---|---|---|
| 1. | anti-cancer | • α- glucan, β-glucan, galactomanna, lipopolysaccharide, lovastatin, linoleic acid, linolenic acid, and CLA.<br>• N, N, Ntris 'hydrazine carbonyl" phosphoric triamide, selenium and vaccenic acid. |
| 2. | Immunomodulatory | • α- glucan, β-glucan.<br>• Fatty acids, glycoproteins, polysaccharides and sterols. |
| 3. | Anti-hypercholesterolemic | • Fatty acids, glycoproteins, sterols and vaccenic acid. |
| 4. | Anti-cardiovascular disease | • Vitamin C, D, and B12; folates and polyphenols.<br>• Fatty acids, sterols and pyran derivative. |
| 5. | Hepatoprotective agent | • Triterpenoids |
| 6. | Antihyperlipidemic | • Sterols, lovastatin |
| 7. | Anti-microbial | • Agaritine and alcohols. |
| 8. | Anti-oxidative | • Chitosan,tocopherols,phenolics, seratonin<br>• Polysaccharides |
| 9. | Anti-inflammatory | • Polysaccharides |

**Table 8**. Phytochemicals identified in *Agaricus bisporus*

| S.NO | Compounds detected | Solvent | Analysis |
|---|---|---|---|
| 1. | Gallic acid, protocatechuic acid, catechin, caffeic acid, ferulic acid and myricetin | Ethanol | LC-MS |
| 2. | Caffeic acid , catechin, chlorogenic acid, Pyrogallol, gallic acid, homogentisic acid, protocatechuic acid, p-hydroxybenzoic acid, p-coumaric acid, gentisic acid, ferulic acid and myricetin. | Methanol | HPLC |
| 3. | Acetic, citric, formic, fumaric, lactic, malic malonic, oxalic and succinic acid | Ethanol | HPLC |
| 4. | Gallic acid, protocatechuic acid, p-hydroxybenzoic, ferulic, chlorogenic, caffeic acid, trans-cinamic, syringic acid and p-coumaric acid | Ethanol | UPLC |

## 2.3 Molecular Docking

Molecular docking is one of the most essential tools for the drug discovery. Docking enables us to predict the ligand-receptor complex computationally. It helps us to know the potential drug lead. Generally, receptor is the protein and the drug target. Before performing molecular dockig the structures of ligand and receptors are to be retrieved.

The two main steps involved in docking are [40]:

- To determine the conformation of ligand along with the position and orientation (structural confirmation).
- To assess the affinity of the bind (binding score).

### 2.3.1 Human DNA topoisomerases (i&ii) as anti-cancer drug targets

DNA topoisomerses play an important role in cancer research and treatement. Humans possess 4 types of topoisomerases, i.e. topoisomerase I, II, III and V. However, topoisomerases i and topoisomerase ii are major anti-cancer drug targets [42].

**Topoisomerases I**: These are ATP-independent DNA single strand endonuclease and ligase that functions mainly during transcription but also during DNA replication.

**Topoisomerases II**: Contrary to topoisomerase I, the topoisomerases II are ATP-dependent double strand endonucleases and ligases.

### 2.3.2 Mechanism of topoisomerase inhibitors

The majority of topoisomerase inhibitors interfere with the re-ligation step in the normal action of the enzymes, which leads to a stabilisation of the so-called cleavable complex. This results in DNA single-strand breaks in the case of topoisomerase I or double-strand breaks in the case of topoisomerase II [42]**.**

# CHAPTER 3

# MATERIALS AND METHODS

# 3. MATERIALS AND METHODS

As per literature review, the following protocol was performed with some modifications.The growth and cultivation of the mushrooms was carried out at Mushroom farm, ECIL, Hyderabad.

The methodology involves mainly four steps;

I. Growth and cultivation
II. Extract Preparation
III. Phytochemical Screening (biochemical tests and Simple Mass Spectroscopy)
IV. Molecular docking

## 3.1 Growth and cultivation

- Materials used: Spawn (Source - Pepper Agro Mushroom Spawn), paddy straw, vegetable wastes and paper wastes as raw materials and plastic bags.
- Equipment used: Water bath and hot air oven.

### 3.1.1 Procedure:

The methodology mentioned by Sonali. D. *et al* [9] was followed in cultivation procedure for the growth of *P. ostreatus.*

The growth and cultivation of oyster mushrooms includes mainly of five steps:

1. **Substrate preparation**: Paddy straw was used as the main substrate. The fresh and well dried paddy straw was used.

2. **Soaking**: Paddy straw and wheat straw were chopped into 3-5 cm pieces (Figure 9(a)) and soaked in fresh water for 12 hours (Figure 9(b)). Excess water from raw materials was drained off by spreading it on blotting paper.

**Figure 9a**. Paddy straw chopped into pieces

**Figure 9b**. Paddy straw soaked in fresh water

3. **Heat Treatment:** Heat treatment was done by pasteurization. It is done to minimize the contamination problem. Water was boiled in a wide container. Prepared wet substrates were filled into poly bags. These bags filled with substrate are dipped into hot water ($80\pm5^0$C) for 15 minutes. After pasteurization the excess hot water was drained off the container.

4. **Spawning**: When the pasteurized substrate had cooled down to room temperature, it was ready for filling and spawning. Spawning was done in layer spawning. In layer spawning, substrate was filled in bag, pressed to a depth and spreaded over with a handful of spawn above it. Similarly, the next two layers of substrate were put and simultaneously after spawning, the bags were closed. After spawning, sterilized straw was mixed with 2% spawn and filled in bags. After that it was gently pressed, and the bags were sealed for the development (figure 10).Spawned bags were stacked on racks in neat and clean place, in closed position. Temperature at $25\pm3^0$ C and humidity at 80% was maintained by spraying water twice a day on walls and floor.

**Figure 10**. Spawn inoculation

5. **Cropping and harvesting:** When bags were observed with white mycelium growth, they were transferred into cropping room and the polythene covers were removed. Mushrooms were grown in a temperature range of $20\pm3\ ^0$ C. Relative humidity was maintained by spraying water twice a day on the walls

and floor of the room. As the pin heads appeared light spray of watering was done. Mushrooms were plucked before they shed spores to maintain quality.

The vegetable wastes and paper wastes were also layered along with the spawn in ploythene bags separately.

## 3.2 Extract preparation

Materials used: Blotting paper, inlet and outlet pipes.

Equipment used: Heating mantle.

Glassware used**:** Round bottom flask, short necked bottles, and soxhlet apparatus.

Chemicals used: Distilled water

### 3.2.1 Procedure:

- Mushrooms are harvested and cleaned, cut into slices and shade dried for 4 day, dried mushroom sample is grounded separately to obtain fine powders. (Figure 11, 12).
- Mushroom extracts were prepared using soxhlet extraction by taking 12g of mushroom powder and 100ml water as solvent (Figure 14).
- Mushroom extracts of oyster commercial, oyster cultivated and *Agaricus bisporus* were prepared and stored in short necked bottles (Figure15).

**Figure 11**. Dried oyster mushroom

**Figure 12**. Dried white button mushrooms


**Figure 13**. Commercial dried oyster mushroom powder

**Figure 14**. Soxhlet extraction


**Figure 15**. Aqueous extracts

After the extraction phytochemical screening was performed.

### 3.3 Phytochemical screening

Glassware used: Test tubes, glass rods.

Chemicals used: Ferric chloride, distilled water, sodium hydroxide, aqueous hydrochloric acid, mayer's reagent, potassium ferricyanide, sulphuric acid.

#### 3.3.1 Procedure:

The phytochemical screening was peformed by confirming the presence of the compounds using biochemical tests followed by the simple mass spectrometry analyisis to detect the compounds.

**3.3.2 Biochemical tests:** The extracts were screened for the presence of various phytochemicals (tannins, saponins, flavnoids, alkaloids and terpenoids) as previously described by Ebana *et al.* [89, 90], Preveena *et al*. [91].

- **Test for tannins (phenols):** To 1 ml of the mushroom extracts, 2 ml of 5% ferric chloride was added. The formation of a dark blue or greenish black colouration was taken as positive.
- **Test for saponins**: To 2 ml of mushroom extract 2 ml of distilled water was added and shaken for about 5 to 10 minutes. Formation of about 1 cm layer of permanent froth was regarded as positive.
- **Test for flavonoids**: About 1 ml of 2N sodium hydroxide was added to 2 ml of the extracts. The formation of a yellow colour was regarded as positive.
- **Test for alkaloids:** About 2 ml of each extracts were stirred with 5 ml of 1% aqueous hydrochloric acid on a steam bath and 1 ml of the filtrates then treated with a few drops of Mayer's reagent. The presence of a precipitate was taken as positive.

- **Test for terpenoids (**Salkowski test) : About 0.5 g of hexane extract was placed in a clean test tube. A few drops of concentrated sulphuric (VI) acid were added to the extract in the test tube and shaken. On standing, the lower layer turned yellow at first then read later to indicate the presence of triterpenoids.

After performing the biochemical tests the extracts were analyzed using the simple mass spectrometry analysis to detect the total compounds at Monvi Labs.

### 3.3.3 Simple mass spectrometry method

The simple-MS analysis if water extracts of *Agaricus bisporus*, *Pleurotus ostreatus* (cultivated), *Pleurotus ostreatus* (commercial) was carried out using column oven method with module type: G7130A and order: 1. The instrument was operated with flow conditions at 0.200 mL/min, pressure limit ranged from 0.00 bars (low pressure limit) to 600.00 (high pressure limit), flow gradient was maintained at 100.00 mL/min, about 20.00 µl sample injection was used, temperature was maintained at 25ºC.

## 3.4 Molecular docking

After performing the simple MS analysis the molecular docking was performed.

1. Receptors: Human DNA topoisomerases (i&ii) were selected as the receptors. The crystal structures were sleceted from Data Bank (RCSB) with the URL: https://www.rcsb.org/. The PDB id selected for crystalline protein targets in this studies are

- Human Topoisomerase I - 1K4T (https://www.rcsb.org/structure/1K4T)
- HumanTopoisomerase II -1ZXN- (https://www.rcsb.org/structure/1ZXN)

2. Ligands: After performing the simple MS analysis the common flavonoids present in both the species were chrysin, catechin and daidzein and were used as the ligands. The information about the ligands was obtained from Ligandbook with URL: https://ligandbook.org/ , using this structural information of the selected ligands was downloaded in PDB file format.
    - Chrysin - https://ligandbook.org/search/chrysin/results/1/
    - Catechin- https://ligandbook.org/search/catechin/results/1/
    - Daidzein- https://ligandbook.org/package/1550
3. Docking: The docking studies were conducted in online scoring software called PATCHDOCK accessed using the URL: https://bioinfo3d.cs.tau.ac.il/PatchDock/php.php. The target receptor and the ligand were loaded into PATCHDOCK and the software was run. PATCHDOCK displayed the results of the docking studies. The above procedure was repeated for all the other ligands. The results were downloaded. The docking simulation with the highest score was selected.
4. Visulalization: The result of the docking simulation with highest docking score was loaded into a protein visualization software- JENA 3D viewer which was accessed using the URL: http://jena3d.leibniz-fli.de/. The resulting 3D structure was viewed.

# CHAPTER 4

# RESULTS AND DISCUSSION

# 4. RESULTS AND DISCUSSION

In initial stages the mycelium growth was observed only in the bags layered with paddy straw. The growth was not observed in the bags layered with vegetable and paper waste.

## 4.1 Results of growth and cultivation

In case of paddy straw as substrate, after the substrate preparation, the fresh spawn of *Pleurotus ostreatus* was layered with the substrate and were kept in a clean place under proper conditions. After the inoculation of the spawn on day 22, mycelium growth was observed (figure 16). Later with continuous spraying of water on day 25, pinhead like structures were observed (figure 17). Finally on day 28 full bloom growth (final growth stage) was observed (figure 18). After 28 days of cultivation, freshly cultivated oyster mushrooms were harvested (figure 19).

**Figure 16**. Mycelial growth

**Figure 17**. Pinhead structure

**Figure 18**. Full bloom stage

**Figure 19**. Freshly harvested mushrooms

The freshly cultivated mushrooms were shade dried to eliminate the water content and then powdered. The water extracts of cultivated *Pleurotus ostreatus* powder and the commercially available *Pleurotus ostreatus* powder was prepared.Qualitative phytochemical analysis of these water extracts showed the presence of alkaloids, tannins (phenols), saponins, flavonoids, and terpenes andterpenoids. Qualitative phytochemical analysis of *A. bisporus* water extracts showed the presence of same compounds (Table 9).

**4.2 Results of biochemical tests**

All the three extracts showed the presence of alkaloids, tannins, saponins, flavonoinds, terpens and terpenoids (Table 9).

**Table 9**. Presence of bioactive compounds ('+' indicates presence, '–' indicates absence)

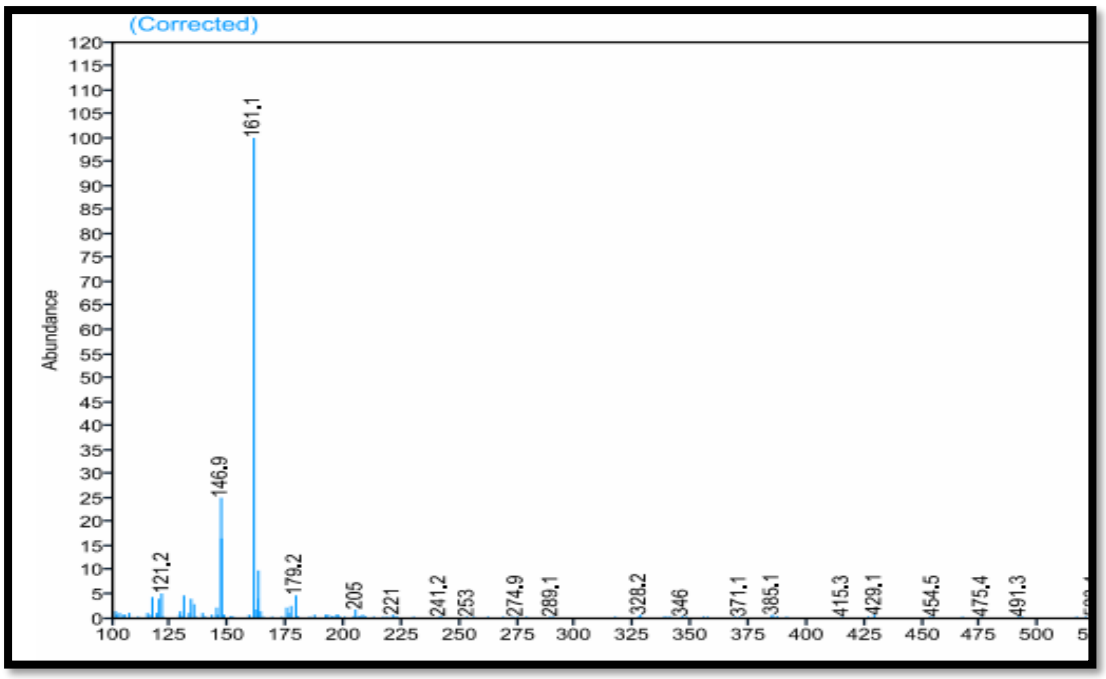
| Mushroom Aqueous Extracts | Alkaloids | Tannins | Saponins | Flavonoids | Terpenes and Terpenoids |
|---|---|---|---|---|---|
| *Pleurotus ostreatus* (cultivated) | + | + | – | + | + |
| *Pleurotus ostreatus* (commericial) | + | + | – | + | + |
| *Agaricus bisporus* | + | + | – | + | + |

### 4.3 Results of phytochemical analysis

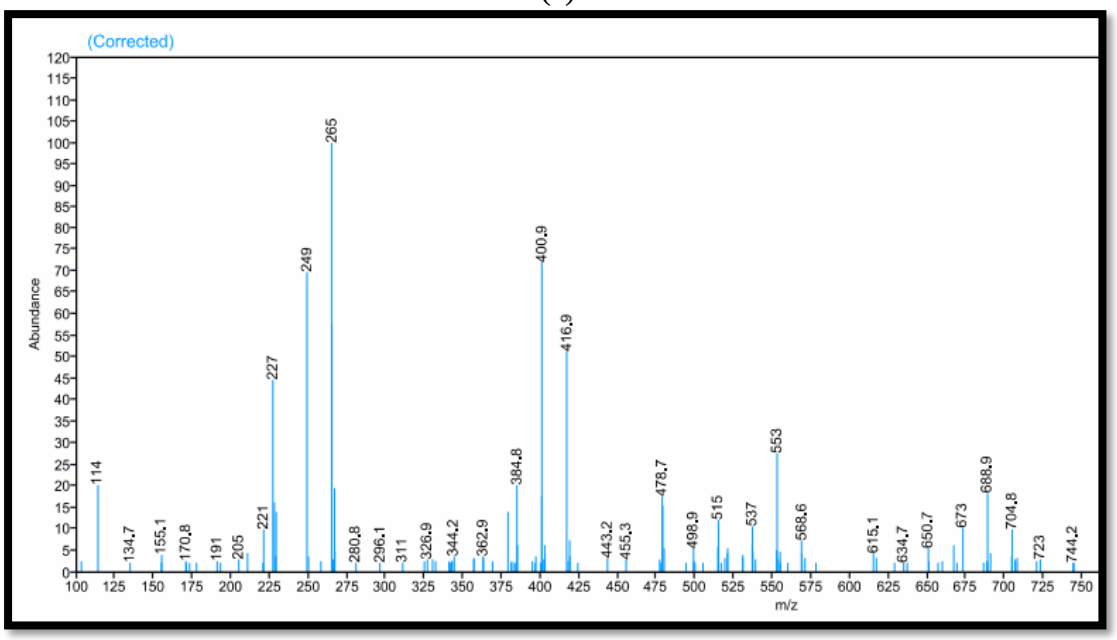
The water extracts of *Pleurotus ostreatus* (cultivated), *Pleurotus ostreatus* (commercial), *Agaricus bisporus* were analysed using simple mass spectrometry. Numerous novel compounds were detected in these extracts using this method.

#### 4.3.1 Phytochemicals screened in *Pleurotus ostreatus* (cultivated)

The compound analysis of *Pleurotus ostreatus* (cultivated) water extracts using SIMPLE-MS detection method was done (Table 10 and figure20). A total of 13 compounds were detected, of which 7 compounds are flavonoids, 2 are phenols, 1 is terpenes and terpenoids and 3 are alkaloids. The peaks of these compounds are represented in the SIMPLE-MS graph (Figure 20). In the signal: MS1 +TIC SCAN ESI FRAG=75V, catechin, chrysin, daidzein, ononin, tangeretin, tricin-5-glucoside, caffeicacid, huperzine A, matrine and N-methyl lystisine were detected (Figure 20a). Whereas, in the signal: MS1 -TIC SCAN ESI FRAG=75V, catechin gallate, gallic acid and atractylenodide III were detected (Figure 20b).




(a)




(b)

**Figure 20**. Simple-MS analysis report of *Pleurotus ostreatus* (cultivated)
(a) MS1 +TIC SCAN ESI FRAG=75V
(b) MS1 -TIC SCAN ESI FRAG=75V

**Table 10**. Compounds detected through simple MS method in *Pleurotus ostreatus* (cultivated)

| **Flavonoids** | **Mol.weight** | **Peak weight** | |
|---|---|---|---|
| | | **+VE** | **-VE** |
| Catechin | 290.3 | 290.1 | |
| Catechin gallate | 442.4 | | 442.2 |
| Chrysin | 254.2 | 254 | |
| Daidzein | 254.2 | 254 | |
| Ononin | 430.4 | 430.1 | |
| Tangeretin | 372.4 | 372.1 | |
| Tricin-5-glucoside | 492.4 | 492.3 | |

| **Phenols** | **Mol.weight** | **Peak weight** | |
|---|---|---|---|
| | | **+VE** | **-VE** |
| Caffeicacid | 180.2 | 180.2 | |
| Gallic acid | 170.1 | | 169.8 |

| **Terpenes And Terpenoids** | **Mol.Weight** | **Peak Weight** | |
|---|---|---|---|
| | | **+VE** | **-VE** |
| Atractylenodide III | 248.3 | | 248 |

| **Alkaloids** | **Mol.weight** | **Peak weight** | |
|---|---|---|---|
| | | **+VE** | **-VE** |
| Huperzine A | 242.3 | 242.2 | |
| Matrine | 248.4 | 248 | |
| N-methyl lystisine | 204.3 | 204 | |

**Table 11**. Functional activities of compounds in *Pleurotus ostreatus* (cultivated)

| Compounds | Functions |
|---|---|
| Catechin gallate | Antioxidant, antimicrobial. |
| Chrysin | Anti-cancer, anti-diabetic, anti infammatory, anti-obesity, hepatoprotective, neuroprotective |
| Daidzein | Anti-cancer, anti-cardiovascular diseases, anti-osteoporosis, anti-diabetic activity, anti-aging activity, anti-inflammatoryactivity, neuroprotective activity. |
| Ononin | Antiinflammatory, antimycotic and radical scavenging. |
| Tangeretin | Anti-tumor, neuro protection |
| Gallic acid | Anti neoplastic, anti inflammatory |
| Caffeic acid | Anti cancer, anti viral. |
| Huperzine A | Anti oxidant, neuroprotective. |
| Matrine | Anti-tumor therapy, anti-metastatic effects of matrine on hepatocellular carcinoma (HCC) |
| Atractylenodide III | Anti inflammatory, gastro protective, neuro protective |

Numerous studies showed that the compounds detected in *Pleurotus ostreatus* (cultivated) have various medicinal activities (Table 11). Therefore, *Pleurotus ostreatus* (cultivated) shows different activities like anti-cancer (daidzein, ononin, tangeretin, caffeic acid, gallic acid, matrine, atractylenodide III), anti-tumor (materine), anti-bacterial (catechin gallate, gallic acid), anti-inflammatory (catechin gallate, chrysin, ononin, tangeretin), anti-oxidant (catechin gallate, chrysin, daidzein,

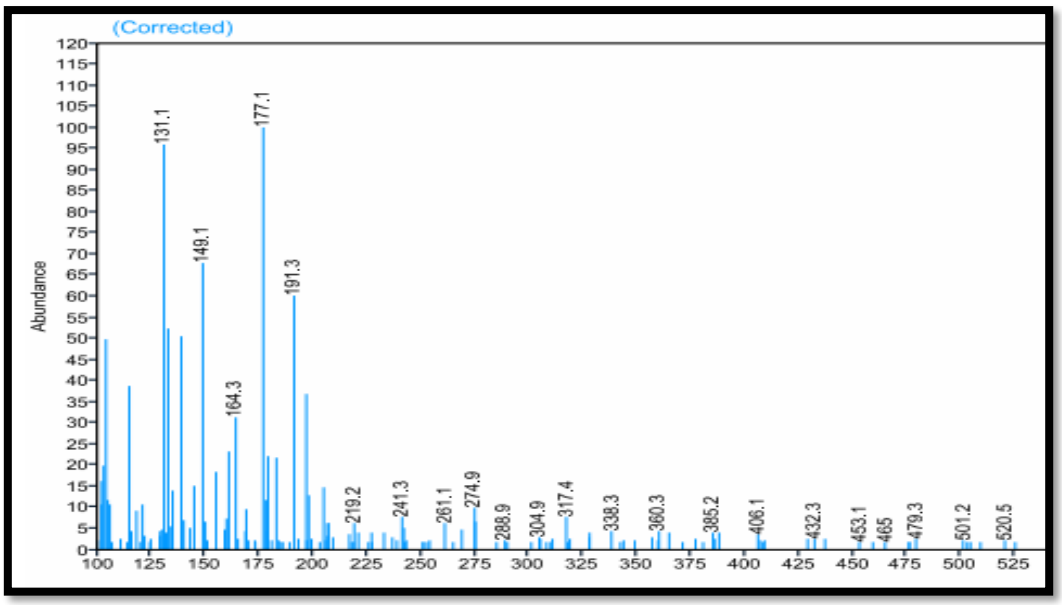
caffeic acid, gallic acid, huperzine A, atractylenodide III), anti-diabetic (daidzein, tangeretin),neuroprotective (daidzein, huperzine A). [92-102]

### 4.3.2 Phytochemicals screened in *Pleurotus ostreatus* (commercial)

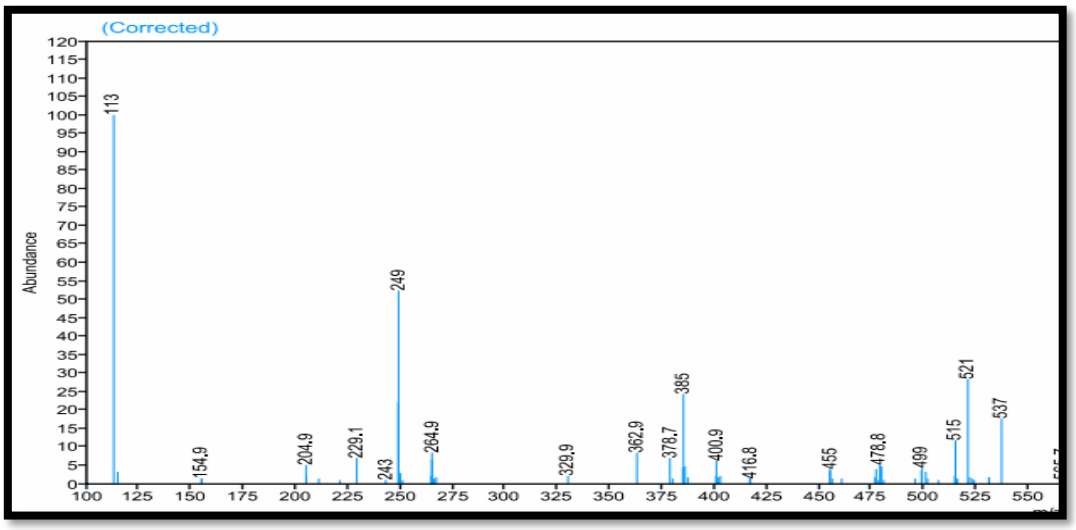
The compound analysis of *Pleurotus ostreatus* (commercial) water extracts using SIMPLE-MS detection method was done (Table 12 and figure 21).

A total of 26 compounds were detected, of which 7 are flavonoids, 3 are phenols, 9 are terpenes and terpenoids, and 7 are alkaloids. The peaks of these compounds are represented in the SIMPLE-MS graph (Figure 21).

In the signal: MS1 +TIC SCAN ESI FRAG=75V, catechin, catechin hydrate, epicatechin, myricetin, veronicoside, epigallocatechin, nimbolide, paeoriflorin, tanshinonel, 5-carboxy strictosidine, hordenine and huperzine a (figure 21a).
Whereas, in the signal: MS1 -TIC SCAN ESI FRAG=75V, diadzin, atractylenolide, eucalyptal, alpha –ne colovene, parthenolide, verproside, vulgarin, vincosamide, berberine hemisulfate, chelerythrine, matrine were detected (Figure 21b).




(a)




(b)

**Figure 21**. Simple- MS analysis report of *Pleurotus ostreatus* (commericial)
(a) MS1 +TIC SCAN ESI FRAG=75V
(b) MS1 -TIC SCAN ESI FRAG=75V

**Table 12**. Compounds detected through simple MS method in *Pleurotus ostreatus* (commercial)

| Flavonoids | Mol.weight | Peak weight | |
|---|---|---|---|
| | | +VE | -VE |
| Catechin | 290.3 | 289.9 | |
| Catechin hydrate | 290.3 | 289.9 | |
| Epicatechin | 290.3 | 289.9 | |
| Myricetin | 318.2 | 318.4 | |
| Veronicoside | 466.4 | 466.0 | |
| Epigallocatechin | 306.3 | 305.9 | |
| Diadzin | 416.4 | | 415.8 |

| Phenols | Mol.weight | Peak weight | |
|---|---|---|---|
| | | +VE | -VE |
| 3-Hydroxy tyrosol | 154.2 | | 153.9 |
| Resveratorl | 228.2 | | 228.3 |
| Shagaol | 276.4 | 275.9 | |

| Terpenes And Terpenoids | Mol.Weight | Peak Weight | |
|---|---|---|---|
| | | +VE | -VE |
| Nimbolide | 466.5 | 466 | |
| Paeoriflorin | 480.5 | 480.3 | |
| Tanshinonel | 276.3 | 275.9 | |
| Atractylenolide | 248.3 | | 248.0 |
| Eucalyptal | 154.3 | | 153.9 |
| Alpha –Ne colovene | 204.4 | | 203.9 |
| Parthenolide | 248.3 | | 248.0 |
| Verproside | 498.4 | | 498.0 |
| Vulgarin | 264.3 | | 263.9 |

| Alkaloids | Mol.weight | Peak weight | |
|---|---|---|---|
| | | +VE | -VE |
| 5-Carboxy strictosidine | 574.6 | 574.3 | |
| Hordenine | 165.3 | 165.2 | |
| Huperzine A | 242.3 | 242.3 | |
| Vincosamide | 498.5 | | 498.0 |
| Berberine hemisulfate | 384.4 | | 384.0 |
| Chelerythrine | 383.8 | | 384.0 |
| Matrine | 248.4 | | 248.0 |

**Table 13**. Functional activities of compounds in *Pleurotus ostreatus* (commericial)

| Compounds | Functions |
|---|---|
| Epicatechin | Anti oxidant, anti angiogenic |
| Veronicoside | Anti oxidant, inhibitory activity on Hep G2 cell proliferation |
| Daidzein | Anti-cancer, anti-cardiovascular diseases, anti-osteoporosis, anti-diabetic activity, anti-aging activity, anti-inflammatoryactivity, neuroprotective activity. |
| 3-Hydroxy tyrosol | Anti oxidant, anti cancer, anti inflammatory, neuro protective, show potentiality for development of dietary supplememts |
| Resveratrol | antioxidant, anti tumor, anti inflammatory, anti carcinogenic, cardio protective, vasorelaxant, neuro protective |
| Shagaol | Anti cancer, anti oxidant, anti microbial, anti inflammatory, anti allergic |
| Nimbolide | Anti proliferative, induction of apoptosis, inhibition of metastatisis, angiogenesis. |
| Tanshinonel | Anti oxidant, anti inflammatory, anti tumor, phyto estrogenic, vasodilation, neuroprotection |
| Atractylenolide | Anti inflammatory, gastro protective, neuro protective |
| Alpha neoclovene | Anti microbial, anti oxidant |
| Parthenolide | Pain relieving, anti inflammatory, treatment of migrane. |
| Verproside | Anti asthamic |
| 5-Carboxy strictosidine | Anti plasmodial,cytotoxic, antibacterial, antifungal, spasmodic, hypotensive, anti-inflammatory |
| Huperzine A | neurodegenarative diseases |
| Vincosamide | cardio protective |
| Berberine hemisulfate | Antioxidant, immunomodulatory effects protective action on the cardiovascular system, liver and kidney, endothelial relaxation, regulator on glucose metabolism and atherosclerosis, anti inflammatory |
| Matrine | Anti-tumor therapy, anti-metastatic effects of matrine on hepatocellular carcinoma (HCC) |

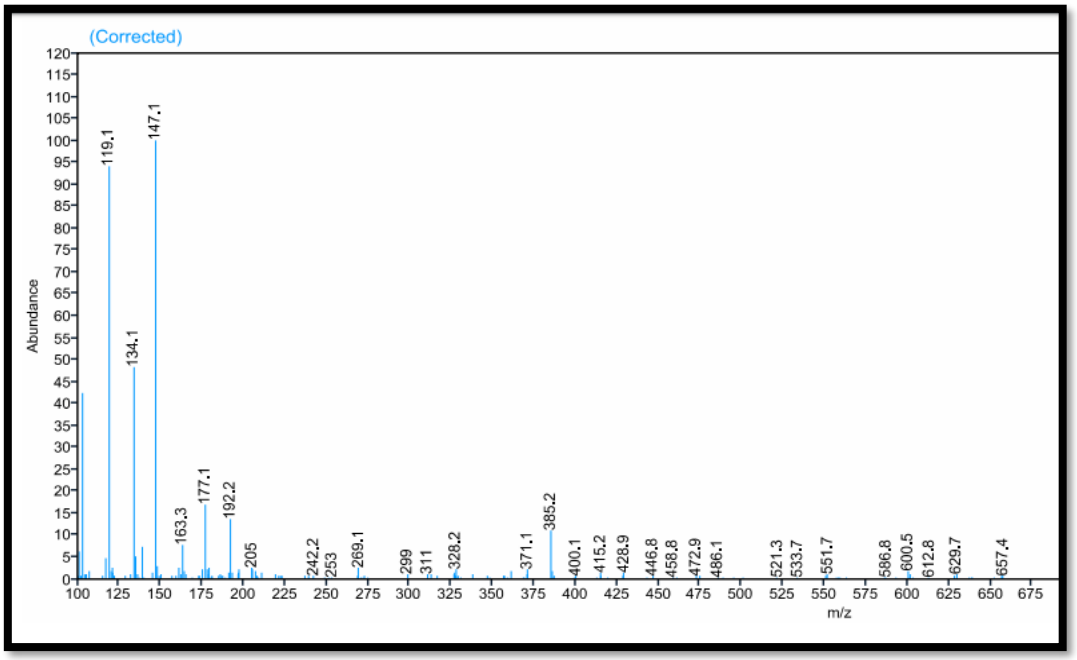
Numerous studies showed that the compounds detected in *Pleurotus ostreatus* (commercial) have various medicinal activities (Table 13).

Therefore, *Pleurotus ostreatus*(commericial )shows different activities like anti-cancer (daidzein, 3-hydroxy tyrosol, resveratrol, shagaol, nimbolide, tanshinonel), anti-tumor (resveratrol, tanshinonel, materine), anti-bacterial (shagaol, alpha neoclovene, 5-carboxy strictosidine), anti-inflammatory (3-hydroxy tyrosol, resveratrol, shagaol, tanshinonel, atractylenolide, parthenolide, 5-carboxy strictosidine, berberine hemisulfate), anti-oxidant (epicatechin, veronicoside, 3-hydroxy tyrosol, resveratrol, shagaol, tanshinonel, alpha neoclovene, berberine hemisulfate), anti-diabetic (daidzein),neuroprotective (daidzein, 3-hydroxy tyrosol, resveratrol, tanshinonel, atractylenolide, huperzine A) [103-115].

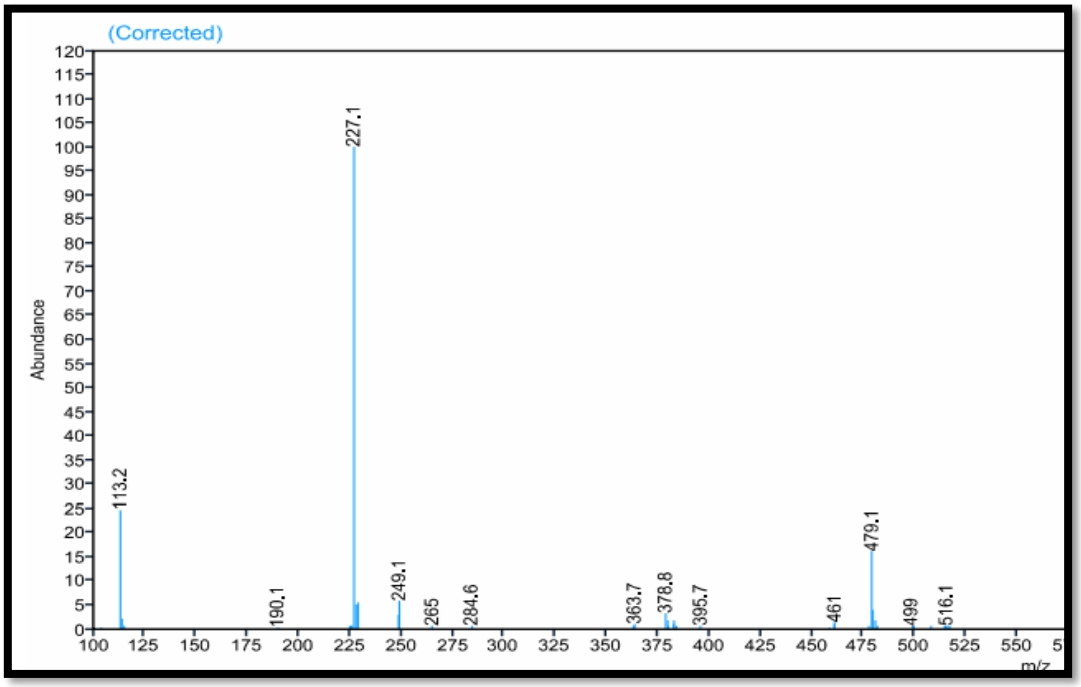
### 4.3.3 Phytochemicals screened in *Agaricus bisporus*

The compound analysis of *Agaricus bisporus* water extracts using SIMPLE-MS detection method was done (Table 14 and Figure 22). A total of 20 compounds were detected, of which 12 are flavonoids, 3 are phenols, and 4 are terpenes and terpenoids and 1 alkaloid. The peaks of these compounds are represented in the SIMPLE-MS graph (Figure 22).

In the signal: MS1 +TIC SCAN ESI FRAG=75V, baicalein, chrysin, daidzein, diosmetin, genistein, isokaempferide, tangeretin, daidzin, galangin, genistin, p-coumaric acid, trans ferulic acid, trans cinamic acid, guggulsterone and dehydroabietic acid were detected (Figure 22a). Whereas, in the signal: MS1 -TIC SCAN ESI FRAG=75V, mearnsitrin, wogonside, Parthenolide and vulgarin were detected (Figure 22b).




(a)




(b)

**Figure 22**. Simple-MS analysis report of *Agaricus bisporus*
(a) MS1 +TIC SCAN ESI FRAG=75V
(b) MS1 -TIC SCAN ESI FRAG=75V

**Table 14**. Compounds detected through simple MS method in *Agaricus bisporus*

| Flavonoids | Mol.weight | Peak weight | |
|---|---|---|---|
| | | +VE | -VE |
| Baicalein | 270.2 | 270.1 | |
| Chrysin | 254.2 | 254 | |
| Daidzein | 254.2 | 254 | |
| Diosmetin | 300.3 | 300 | |
| Genistein | 270.2 | 270.1 | |
| Isokaempferide | 300.3 | 300 | |
| Mearnsitrin | 478.4 | | 478.1 |
| Tangeretin | 372.4 | 372.1 | |
| Wogonside | 460.4 | | 460 |
| Daidzin | 416.4 | 416.2 | |
| Galangin | 270.2 | 270.1 | |
| Genistin | 270.2 | 270.1 | |

| Phenols | Mol.weight | Peak weight | |
|---|---|---|---|
| | | +VE | -VE |
| P-Coumaric acid | 164.2 | 164.1 | |
| Trans ferulic acid | 193.2 | 193.2 | |
| Trans cinamic acid | 148.1 | 148.1 | |

| Terpenes And Terpenoids | Mol.Weight | Peak Weight | |
|---|---|---|---|
| | | +VE | -VE |
| Guggulsterone | 312.5 | 312 | |
| Dehydroabietic acid | 300.4 | 300 | |
| Parthenolide | 248.3 | | 248.1 |
| Vulgarin | 264.3 | | 264 |

| Alkaloids | Mol.weight | Peak weight | |
|---|---|---|---|
| | | +VE | -VE |
| Octopamine hydrochloride | 189.6 | | 189.1 |

**Table 15**. Functional activities of compounds in *Agaricus bisporus*

| Compounds | Functions |
|---|---|
| Baicalein | Anti-tumor, antibacterial, antiviral, and anti-inflammatory |
| Chrysin | Anti-cancer, anti-diabetic, anti infammatory, anti-obesity, hepatoprotective, neuroprotective. |
| Daidzein | Anti-cancer, anti-cardiovascular diseases, anti-osteoporosis, anti-diabetic activity, anti-aging activity, anti-inflammatoryactivity, neuroprotective activity. |
| Diosmetin | Anticancer, antimicrobial, antioxidant, oestrogenic and anti-inflamatory activities, antidiabetic |
| Genistein | Anti-cancer, anti-diabetic, anti-obesity, anti-cardiovascular diseases, antidepressant, anxiolytic effects and anti-osteoporosis |
| Isokaempferide | Hepatoprotective, antiproliferative, and anti-inflammatory effects |
| Mearnsitrin | Anti-cancer, hepatoprotective, anti-viral, anti-oxidant |
| Tangeretin | Anti-tumor, neuroprotection |
| Wogonside | Detoxification, anti-inflammation and nourishing gallbladder, lowering blood pressure, dieresis, anti-allergic reactions, anti-cancer |
| Daidzin | Antidipsotropic |
| Galangin | Antiviral, antimicrobial, anticancer properties, antidiabetic, antigenotoxic effects antioxidant, antiinflammatory effects, hepatoprotective Effects, effects on bone health. |
| P-Coumaric Acid | Antioxidant, anti-cancer, antimicrobial, antivirus, anti-inflammatory, antiplatelet aggregation, anxiolytic, antipyretic, analgesic, and anti-arthritis activities, anti-diabetes, obesity, hyperlipaemia |
| Trans ferulic acid | Anti-viral activity |
| Octopamine hydrochloride | Biogenic amine neurotransmitter, stimulates lipolysis in mammalian adipocytes |
| Guggulsterone | Anti-cancer, hypolipidemic, antioxidant, andanti-inflammatory activities |
| Dehydroabietic acid | Anti-inflammatory |
| Parthenolide | Antiapoptic, anti inflammatory and anti-hyperalgesic effects |

Numerous studies showed that the compounds detected in *Agaricus bisporus* have various medicinal activities (Table 15).

Therefore, *Agaricus bisporus* shows different activities like anti-cancer (chrysin, daidzein, diosmetin, genistein, mearnistrin, wognoside, galangin, p-coumaric acid and guggulsterone), anti-tumor (biacalein and tangeretin), anti-bacterial (biaclein, glangin, p-coumaric acid, diosmetic, dehroabetic acid), anti-viral (baicalein, mearnsitrin,p-coumaric acid, galangin, trans-ferulic acid) , anti-inflammatory ( biaclein, chrysin, daidzein, diosmetric, Isokaempferide, guggulsterone, wognoside,dehydroabietic acid, parthenolide, galangin, p-coumaric acid), anti-oxidant (diosmetin, mearnsitrin, galangin, p-coumaric acid, guggulsterone, dehyroabetic acid), anti-diabetic (chrysin, daidzein, diosmetin, genistein, galangin, p-coumaric acid), hepatoprotective (chrysin, Isokaempferide, mearnistrin, galangin), neuroprotective (chrysin, daidzein, tangeretin) [116-131].

In commercial *Pleurotus ostreatus* 26 compounds were screened where as in cultivated *Pleurotus ostreatus* only 13 compounds were detected. Hence, the results have shown that commercial *Pleurotus ostreatus* are rich in health-promoting phytochemical compounds than cultivated.

After comparing the simple MS results of *Pleurotus ostreatus* (cultivated and commercial) with the literature survey gallic acid and caffeic acid (Table 2) are the common compounds identified. Whereas, the novel compounds which are detected as shown in Table 10 and Table 12 are catechin gallate, chrysin, daidzein, ononin, tangeretin, huperzine A, matrine, atractylenodide III, epicatechin, veronicoside, daidzein, 3-hydroxy tyrosol, resveratrol, shagaol, nimbolide, tanshinonel,

atractylenolide, alpha neoclovene, parthenolide, verproside, 5-carboxy strictosidine, huperzine A, vincosamide, berberine hemisulfate and matrine.

In case of *Agaricus bisporus,* trans-ferulic acid and p-coumaric Acid (Table 8) are the common compounds identified.

Whereas, the novel compounds which are detected as shown in Table 14 are baicalein, chrysin, daidzein, diosmetin, genistein, isokaempferide, mearnsitrin, tangeretin, wogonside, daidzin, galangin, octopamine hydrochloride, guggulsterone, dehydroabietic acid (and its derivatives) and parthenolide.

Chrysin (Flavone), daidzein (Isoflavone) and catechin (Falvonol) are the flavonoids present in both the species. Therefore, numerous compounds were identified by using water as the solvent. Therefore, water is a good solvent for phytochemical screening. The phytochemical analysis of water extracts of *Pleurotus ostreatus* cultivated, *Pleurotus ostreatus* commerical and *Agaricus bisporus* using Simple mass spectrometryrevealed the presence of many compounds which were not identified previously by different methods like HPLC and GC-MS.Therefore, Simple mass spectrometry is one of the easiest, less time consuming and less expensive methods for phytochemical screening.

### 4.4 Results of molecular docking

After conducting ligand-receptor docking in online scoring software called PATCHDOCK, the following results were obtained.

#### 4.4.1 Human topoisomerase I

Upon conducting the docking studies, we observed the scores of the various ligands when docked to the human topoisomerase I receptor. Daidzein, which is a isoflavone showed the highest score, indicating that it can very effectively inhibit the

activity of human topoisomerase I. Catechin has the least binding score among chrysin, catechin and daidzein in terms of score obtained from PATCHDOCK (Table 16). The structures of all the docking studies were visualized using JENA-3D viewer. From the above results obtained by docking study, we conclude that daidzein is the effective inhibitor of humanTopoisomerase I (Figure 23).

**Table 16**. Binding scores of ligands docked against human topoisomerase I

| Ligand | Binding Score | Source |
|---|---|---|
| Chrysin | 4142 | http://bioinfo3d.cs.tau.ac.il/PatchDock/runs/1k4t.pdb_57D.pdb_18_29_15_29_4_120/ |
| Catechin | 2436 | http://bioinfo3d.cs.tau.ac.il/PatchDock/runs/1k4t.pdb_CAQ.pdb_26_50_15_29_4_120/ |
| Daidzein | 4592 | http://bioinfo3d.cs.tau.ac.il/PatchDock/runs/1k4t.pdb_HMO.pdb_9_29_17_29_4_120/ |

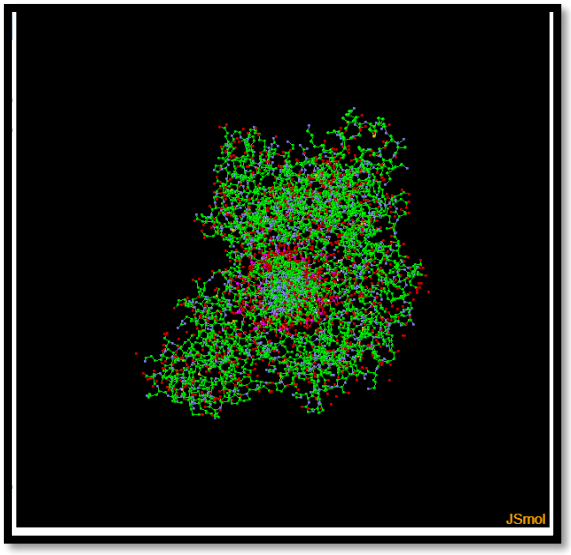



**Figure 23**. Image showing human topoisomerase I docked to daidzein

### 4.4.2 Human topoisomerase II

Upon conducting the docking studies, we observed the scores of the various ligands when docked to the human topoisomerase II receptor. Daidzein, which is a isoflavone showed the highest score, indicating that it can very effectively inhibit the activity of human topoisomerase I. Catechin has the least binding score among chrysin, catechin and daidzein in terms of score obtained from PATCHDOCK (Table 17). The structures of all the docking studies were visualized using JENA-3D viewer (Figure 24).

**Table 17.** Binding scores of ligands docked against human topoisomerase II

| Ligand | Binding Score | |
|---|---|---|
| Chrysin | 4452 | http://bioinfo3d.cs.tau.ac.il/PatchDock/runs/1k4t.pdb_57D.pdb_18_29_15_29_4_120/ |
| Catechin | 2498 | http://bioinfo3d.cs.tau.ac.il/PatchDock/runs/1k4t.pdb_CAQ.pdb_26_50_15_29_4_120/ |
| Daidzein | 4590 | http://bioinfo3d.cs.tau.ac.il/PatchDock/runs/1k4t.pdb_HMO.pdb_9_29_17_29_4_120/ |

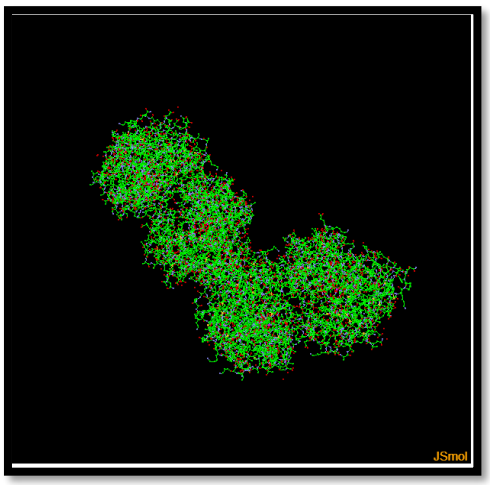



**Figure 24**. Image showing human topoisomerase II docked to daidzein

From the above results obtained by docking study, we conclude that diadzein is the effective inhibitor of human topoisomerase II.

Both human topoisomerase I & II are promising drug targets in treatment design for cancer. Compounds like daidzein, chrysin and catechin can be considered for the design of specific inhibitors of these enzymes. Higher the scoring of the compounds, greater is the inhibitory activity. It can be also be concluded that daidzein is the good inhibitor of both human topoisomerase I & II and can act as dual inhibitor.

# CHAPTER 5

# SUMMARY AND CONCLUSION

# 5. SUMMARY AND CONCLUSION

The present study aims to perform phytochemical screening of the compounds present in *Pleurotus ostreatus* and *Agaricus bisporus* using the simple technique and to conduct molecular docking studies of the common flavonoids present in both the species against the human topoisomerases ( i & ii ). Firstly, oyster mushrooms were cultivated using suitable substrate. The growth was only observed in bags innoculated with paddy straw as substrate. Later, aqueous extracts were prepared. Numerous bioactive compounds were detected in aqueous extracts of both the species using simple mass spectrometry. In present study, presence of various bioactive compounds justifies the use of the nutraceuticals and mushrooms for various ailments. Before attempting wet lab we have tested out the inhibitory effects and control of cancer by daidzein, chrysin and catechin using *insilico* docking. The human topoisomerases are important anti-cancer drug targets because when cleaved DNA strand that has not been bound by the topoisomerase could be released, creating a permanent breakage in the DNA leading to cell death of cancer cells. We were able to inhibit the activity of these enzymes using daidzein and other flavonoids using *insilico* docking technique. Daidzein was found to be the most effective inhibitor based on the scoring results obtained from PATCHDOCK. So, we conclude that flavonoids are effective inhibitors of human topoisomerases and insilico molecular docking studies is a great way of predicting results. Further studies will help the researchers to understand the metabolites and find more metabolites which can be used for potential development of drugs to treat various life-threatening diseases.

# 6. REFERENCES


[1] Chang, S. T. and Miles, P. G, Mushroom biology—a new discipline, The Mycologist 6 (1992) 64–65. doi:10.1016/S0269-915X(09)80449-7.

[2] S.-T. Chang and P. G. Miles. Mushrooms: cultivation, nutritional value, medicinal effect, and environmental impact. CRC Press, Boca Raton. 2004.

[3] P. G. Ergonul, I. Akata, F. Kalyoncu, and B. Erg on ul, Fatty acid compositions of six wild edible mushroom species, The Scientific World Journal 3 (2013) 45-49. doi:10.1155/2013/163964.

[4] E. Guillamon, A. Garcıa-Lafuente, M. Lozano, Edible mushrooms: role in the prevention of cardiovascular diseases, Fitoterapia 81 (2010). 715–723. doi: 10.1016/j.fitote.2010.06.005.

[5] Singh VK, Singh MP, Bioremediation of vegetable and agro wastes by *Pleurotusostreatus*: a novel strategy to produce edible mushroom with enhanced yield and nutrition. Cell Molecular Biology (2014) 60 2–6. doi : 10.14715/cmb/2014.60.5.2.

[6] Vaseem H, Singh VK, Singh MP, Heavy metal pollution dueto coal washery effluent and its decontamination using a macrofungus, *Pleurotusostreatus*", Ecotoxicology Environment Safety. 145 (2017) 42–49. doi: 10.1016/j.ecoenv.2017.07.001.

[7] F. M. N. A. Aida, M. Shuhaimi, M. Yazid, and A. G. Maaruf, Mushroom as a potential source of prebiotics: a review, Trends in Food Science & Technology 20 (2009) 567–575.doi: 10.1016/j.tifs.2009.07.007.

[8] S. Patel and A. Goyal, Recent developments in mushrooms as anticancer therapeutics: a review.3 Biotechnology 2 (2012) 1– 15. doi: 10.1007/s13205-011-0036-2.

[9] Sonali D. Randive, Cultivation and study of growth of oyster mushroom on different agriculturalwaste substrate and its nutrient analysis, Advances in Applied Science Research 3(2012) 1938-1949. ISSN: 0976-8610.

[10] Vivek Kumar Chaturvedi, SonamAgarwal, Krishna Kumar Gupta, Pramod W, Ramteke M, P. Singh, Medicinal mushroom: boon for therapeutic applications, Biotech 8 (2018) 334. doi: 10.1007/s13205-018-1358-0.

[11] Wachtel-Galor S, Yuen J, Buswell JA, Benzie IFF,*Ganodermalucidum* (Lingzhi or Reishi): A medicinal mushroom, Herbal Medicine: Biomolecular and Clinical Aspects. 2nd ed. Boca Raton (FL): CRC Press/Taylor & Francis; 2011.

[12] Krishnamoorthy, Deepalakshmi, SankaranMirunalini, *Pleurotusostreatus*: an oyster mushroom with nutritional and medicinal properties, Journal of Biochemistry Tech 5(2014) 718-726. ISSN: 0974-2328.

[13] Bhushan A, Kulshreshtha M, The medicinal mushroom *Agaricusbisporus*: review of phytopharmacology and potential role in the treatment of various diseases, Journal of Nature Science and Medicine 1 (2018) 4-9. doi**:** 10.4103/JNSM.JNSM_1_18.

[14] Chang, Shu-Ting, Overview of mushroom cultivation and utilization as functional foods, Mushrooms as Functional Foods 1 (2009) 1-33.doi:10.1002/9780470367285.ch1.

[15] Singh, M. P., Srivastva A. K., Vishwakarma, S. K., Singh, V. K. and Pandey, V. K, Mushroom Biotechnology, Recent Trends in biotechnology 1 (2012) 77- 86.ISBN 978-1-60876-148-7.

[16] Patel, Y., Naraian, R. and Singh, V. K, Medicinal properties of *Pleurotus* species (oyster mushroom): a review, World Journal of Fungal and Plant Biology 3 (2012) 01-12. doi: 10.5829/idosi.wjfpb.2012.3.1.303.

[17] Lindequist U, Niedermeyer TH, Jülich WD, The pharmacological potential of mushrooms, Evid Based Complement Alternative Medicine 2(2005) 285-299. doi: 10.1093/ecam/neh107.

[18] Emilia Bernas, GrazynaJaworska and ZofiaLisiewska. Edible mushrooms as a source of valuable nutritive constituents. *Acta* ScientiarumPolonorumTechnologiaAlimentaria 5 (2006) 5-20.

[19] Borchers, A. T, Krishnamurthy, A Keen, C. L. Meyers, F. J. Gershwin, M. E, The immunobiology of mushroom, Experimental Biology and Medicine 233 (2008) 259–76. doi: 10.3181/0708-MR-227.

[20] Eren SH, Demirel Y, UgurluS, Korkmaz I, Aktas C, Güven FM, Mushroom poisoning: retrospective analysis of 294 cases. Clinics (Sao Paulo) 65 (2010) 491–496. doi: 10.1590/S1807-59322010000500006

[21] Chang ST and Buswell JA, Medicinal mushrooms as prominent source of nutriceuticals for the 21st century, Current Topics in Nutraceutical Research 1 (2003) 257-280.

[22] Mohsen HashimRisan, Sulaf. H. Taemor, Athraa H. Muhsin and ShudHussan, Antibacterial activity of *Agaricusbisporus* and *Pleurotusostreatus* extracts against some gram negative and positive

bacteria, European Journal of Biomedical and Pharmaceutical sciences 49 (2017) 09-15.

[23] IwonaGolak-Siwulska, AlinaKaluzewicz, Tomasz Spizewski, MarekSiwulski and Krzysztof Sobieralski, Bioactive compounds and medicinal properties of Oyster mushrooms (*Pleurotus* sp.), Folia Horticulturae 30(2018) 191-201. doi: 10.2478/fhort-2018-0012

[24] Chang ST, World production of cultivated edible and medicinal mushrooms in 1997 with emphasis on *Lentinusedodes* (Berk.) Sing in China, International Journal of Medicinal Mushroom 1(1999) 291-300.doi: 10.1615/IntJMedMushr.v1.i4.10

[25] Adebayo, E.A., Oloke, J.K., Ayandele, A.A., Adegunlola, C.O.Phytochemical, antioxidant and antimicrobial assay of mushroom metabolite from *Pleurotuspulmonarius*, Journal of Microbiology Biotech Research 2 (2012) 366-374.ISSN : 2231 –3168

[26] KuesU and Liu Y, Fruiting body production in basidiomycetes, Applied Microbiology Biotechnology 54 (2000) 141-152.https://doi.org/10.1007/s002530000396.

[27] K. K. Gupta, S. Agarwal, A. Kushwaha, S. Maurya, V. K. Chaturvedi, R. K. Pathak, V. Verma and M. P. Singh, Oyster mushroom: a rich source of anti-oxidant, Nova Science Publishers 5 (2017) 1-11. ISBN: 978-1-53611-097-5.

[28] Eliajh Adebayo and Julius Oloke, Oyster mushroom (*Pleurotus* species); a natural functional food, Journal of microblogy biotechnology and food sciences 7 (2017) 254-264, doi: 10.15414/jmbfs.2017/18.7.3.254-264.

[29] Jose, N. and Janardhanan, K. K, Antioxidant and antitumour activity of *Pleurotusflorida*, Current Science 79 (2000): 941–943.

[30] Pandey, V. K. and Singh, M. P., Biodegradation of wheat straw by *Pleurotusostreatus*, Cellular Molecular Biology 80 (2014) 29-34. PMID: 25535709.

[31] Adebayo EA, Martínez-Carrera D., Oyster mushrooms (*Pleurotus*) are useful for utilizing lignocellulosic biomass, African Journal of Biotech 1 (2014) 1-3. doi: 10.5897/AJB2014.14249

[32] Oloke, J.K., Adebayo, E.A., Effectiveness of immunotherapies from oyster mushroom (*Pleurotus* species) in the management of immunocompromised patients, International Journal of Immunology 38 (2015) 8-20. doi: 10.11648/j.iji.s.2015030201.12

[33] Martínez-Carrera D., Oyster mushrooms, McGraw-Hill Yearbook of Science and Technology(1999) 242-245. ISBN 0-07-052625-7.

[34] Adebayo EA, Oloke JK, Yadav A, Barooah A, Bora TC., Improving yield performance of *Pleurotuspulmonarius* through hyphalanastomosis fusion of dikaryons, World Journal of MicrobiolBiotechnol 29 (2013) 1029–1037.doi: 10.1007/s11274-013-1266-8.

[35] Krishnamoorthy,Deepalakshmi and SankaranMirunalini, *Pleurotusostreatus*: an oyster mushroom with nutritional and medicinal properties, Journal of Biochem Tech 5(2014) 718-726.ISSN: 0974-2328

[36] ApoorvaBhushan and MayankKulshreshtha, The medicinal mushroom *Agaricusbisporus*: Review of Phytopharmacology and Potential Role in the Treatment of Various Diseases, Journal of Nature and Science of Medicine 1 (2018) 4-9. doi: 10.4103/JNSM.JNSM_1_18

[37] Shu-Yao Tsai, Tsai-ping Wu, Shih-Jeng Huang, Jeng-Leun, Non-volatile taste compounds of *Agaricusbisporus* harvested at different stages of maturity, Food chemistry 103 (2007) 1457-1464.doi: 10.1016/j.foodchem.2006.10.073.

[38] Funda Atila1, Mustafa NadhimOwaid and Mohammad Ali Shariati, The nutritional and medical benefits of *Agaricusbisporus*: a review, Journal of Microbiology, Biotechnology and Food science 7(2017) 281-286. doi:10.15414/jmbfs.2017/18.7.3.281-286.

[39] Deepak K. Rahi and Deepika Malik, Diversity of Mushrooms and Their Metabolites of Nutraceutical and Therapeutic Significance, Journal of Mycology 16 (2016) 1-18. https://doi.org/10.1155/2016/7654123

[40] Meng, X. Y., Zhang, H. X., Mezei, M., & Cui, M, Molecular docking: a powerful approach for structure-based drug discovery, Current computer-aided drug design 7(2011), 146–157. doi: 10.2174/157340911795677602.

[41] Kathiravan, M. K., Khilare, M. M., Nikoomanesh, K., Chothe, A. S., & Jain, K. S., Topoisomerase as target for antibacterial and anticancer drug discovery, Journal of Enzyme Inhibition and Medicinal Chemistry, 28 (2012) 419–435. doi: 10.3109/14756366.2012.658785.

[42] Kellner, U., Rudolph, P., &Parwaresch, R, Human dna-topoisomerases – diagnostic and therapeutic implications for cancer, Oncology Research and Treatment, 23(2000) 424–430.doi: 10.1159/000027205.

[43] U. O. Edet, R.U.B.Ebana, C.A. Etok and V.O. Udoidiong, Nutrient profile and phytochemical analysis of commercially cultivated oyster mushroom in Calabar, South-South Nigeria, Advances in research 7 (2016) 1-6.doi: 10.9734/AIR/2016/26196

[44] G F Ramos, Juliet OLUCHI Umejiego and J JRapeles, Gc-ms analysis of bioactive phytochemicals present in methanol extract of *Pleurotusostreatus,* World Journal of Pharmacy and Pharmaceutical Sciences 7 (2018) 143-150. doi: 10.20959/wjpps201811-12458

[45] Irene Palacios, Manuel Lozano, Carlos Moro and MatildeD'Arrigo, Anti-oxidant properties of phenolic compounds occurring in edible mushrooms, Food chemistry 128 (2011) 674-678.doi: 10.1016/j.foodchem.2011.03.085.

[46] Rahimah, S. B., Djunaedi, D. D., Soeroto, A. Y., &Bisri, T, The phytochemical screening, total phenolic contents and antioxidant activities *in vitro* of white oyster mushroom (*PleurotusOstreatus*) preparations, Open access Macedonian journal of medical sciences 7(2019) 2404–2412. doi: 10.3889/oamjms.2019.741

[47] G. F. Ramos, J. O. Umejiego, J. J. Rapales, G. A. Awemu, G. I. Tejanoand E. Faller,Gc/ms determination of bioactive components of *Pleurotusostreatus*, World journal of Pharmacy and Pharmaceutical Sciences 7(2018) 143-150.

[48] Khan MA, Nutritional composition and Hypocholesterolemic effect of mushroom: *Pleurotussajor-caju* and *Pleurotusflorida*, Lambert Academic publishing Gmbh&co, 2010.

[49] Kuznetsov O. I. U; Milkova E. V.; Sosnina A. E.; Sotnikova N. I. U, Antimicrobial action of*Lentinusedodes* juice on human microflora, MikrobiolEpidemiol.Immunobiol 1(2005) 80–82. PMID: 15773410.

[50] Borchers A.; Keen C. L.; Gershwin M. E, Mushrooms, tumors, and immunity: an update, Experimental Biology and Medicine 229 (2004) 393-406.doi: 10.1177/153537020422900507.

[51] Iwalokun B.A., Usen U.A., Otunba A.A., Olukoya D.K, Comparative phytochemical evaluation, antimicrobial and antioxidant properties of

*Pleurotusostreatus*, African Journal of Biotechnology 6 (2007) 1732-1739.ISSN 1684–5315

[52] Mondal, T., Some, R., Dutta, S, Studies on antioxidant and antimicrobial properties of some common mushrooms, Journal of Today's Biological Science Research 2(2013) 60- 67.ISSN 2320-1444

[53] Vamanu E, In vitro antimicrobial and antioxidant activities of ethanolic extract of lyophilized mycelium of *Pleurotusostreatus*, Molecular 17 (2012) 3653-3671. doi: 10.3390/molecules17043653.

[54] Vamanu E, Studies on the Antioxidant and Antimicrobial Activities of Pleurotusostreatus psi101109 Mycelium, Pakistan Journal of Botany 45(2013) 311-317. doi: 10.3390/molecules17043653.

[55] Schillaci, D., Arizza, V., Gargano, M.L., Venturella, G, Antibacterial activity of mediterranean oyster mushrooms, species of genus *Pleurotus* (Higher Basidiomycetes), International Journal of Medicinal Mushroom 15 (2013) 591–594. doi: 10.1615/intjmedmushr.v15.i6.70

[56] Pauliuc, I., Botău, D, Antibacterial activity of *Pleurotusostreatus*gemmotherapic extract, Journal of Horticulture Forest Biotechnology 17(2013) 242-245.

[57] Mohsen HashimRisan, Sulaf. H. Taemor, Athraa H, Muhsin and ShudHussan. Antibacterial activity of *Agaricusbisporus* and *Pleurotusostreatus* extracts against some gram negative and positive bacteria,European Journal of Biomedical and Pharmaceutical Sciences 4 (2017) 09-15.

[58] El-Fakharany, E.M., Haroun, B.M., Ng, T.B., Redwan, E.R, Oyster mushroom laccase inhibits hepatitis C virus entry into peripheral blood cells and hepatoma cells, Protein PeptLett 17 (2010) 1031–1039. doi: 10.2174/092986610791498948.

[59] Vinay Kumar and UditYadav, Screening of antifungal activity of *Pleurotus ostreatus* and *Agaricus bisporus*, Biolifesciences 2 (2014) 918-923. ISSN (online): 2320-4257

[60] Muszynska B, Grzywacz-Kisielewska A, Kala K and Gdula-Argasinka J. Anti-inflammatory properties of edible mushrooms: a review. Food chemistry 243 (2017) 373-381.doi: 10.1016/j.foodchem.2017.09.149.

[61] Jedinak A, Dudhgaonkar S, Wu QL, Simon J and Sliva D. Anti-inflammatory activity of edible oyster mushroom is mediated through the inhibition of NF-κB and AP-1 signaling. Nutritional Journal 52 (2011) 1-10.doi:10.1186/1475-2891-10-52.

[62] Avagyan, I.A., Nanagulyan, S.G., Minasbekyan and L.A. Increasing of fermentative and anti-inflamatory activity of the *Pleurotus ostreatus* (Jacq.Fr.) Kumm. Culture by modification of growth conditions by MM-waves. PIERS Proceedings (2013). ISSN (online): 12-15.

[63] Bobek, P. and Galbavy, S. Effect of pleuran (β-glucan from *Pleurotus ostreatus*) on the antioxidant status of the organism and on dimethylhydrazine induced precancerous lesions in rat colon. Brit Journal of Biomedical Science 58 (2001) 164-168.

[64] Nosál'ová, V., Bobek, P., Cerná, S., Galbavý, S. And Stvrtina, S. Effects of pleuran (β-glucan isolated from *Pleurotus ostreatus*) on experimental colitis in rats. Physiology Research 50 (2001) 575-581. ISSN 0862-8408

[65] Maiti, S., Mallick, S.K., Bhutia, S.K., Behera, B., Mandal, M and Maiti, T.K. Antitumor effect of culinary-medicinal oyster mushroom, *Pleurotus ostreatus* (Jacq. Fr.) P. Kumm., derived protein fraction on tumor-bearing mice models.

International Journal of Medicinal Mushroom 13 (2011) 427-440. doi: 10.1615/intjmedmushr.v13.i5.20.

[66] Lavi, I., Friesem, D., Geresh, S., Hadar, Y. and Schwartz, B. An aqueous polysaccharide extract from the edible mushroom *Pleurotus ostreatus* induces anti-proliferative and proapoptotic effects on HT-29 colon cancer cells. Cancer Lett 244 (2006) 61–70. doi: 10.1016/j.canlet.2005.12.007.

[67] Martin, K.R., Brophy and S.K. commonly consumed and specialty dietary mushrooms reduce cellular proliferation in MCF-7 human breast cancer cells. Experimental Biology and Medicine 235 (2010) 1306–1314. doi: 10.1258/ebm.2010.010113.

[68] Jedinak, A. and Sliva, D. *Pleurotus ostreatus* inhibits proliferation of human breast and colon cancer cells through p53-dependent as well as p53-independent pathway. International Journal of Oncology 33 (2008)1307–1313.

[69] Gu, Y.H., Sivam, G. Cytotoxic effect of oyster mushroom *Pleurotus ostreatus* on human androgen-independent prostate cancer PC-3 cells. Journal of Medicine and Food 9 (2006) 196–204. doi: 10.1089/jmf.2006.9.196.

[70] Wu, J.Y., Chen, C.H., Chang, W.H., Chung, K.T., Liu, Y.W., Lu, F.J., Chen and C.H. Anti-Cancer Effects of Protein Extracts from C*alvatia lilacina, Pleurotus ostreatus* and *Volvariella volvacea*. Evidence-Based Complement Alternative Medicine (2011) 1-10. doi: 10.1093/ecam/neq057.

[71] Yoshioka Y, Ikekawa T, Noda M and Fukuoka F. Studies on anti-tumor activity of some fractions from Basidomycetes. I an anti-tumor acidic polysaccharide fraction of *Pleurotus ostreatus* (Fr.). Quel Chem Pharm Bull 20 (1972) 1175-1180. doi: 10.1248/cpb.20.1175.

[72] Sarangi I, Ghosh D, Bhutia SK, Mallick SK and Maiti TK. Antitumor and immunomodulating effects of *Pleurotous ostreatus* mycelia-derived proteoglycans. International Immunopharmacology 6 (2006) 1287-1297. doi: 10.1016/j.intimp.2006.04.002.

[73] Devi KSP, Roy B, Patra P, Sahoo B, Islam SS and Maiti TK. Characterization and lectin microarray of an immunomodulatory heteroglycan from *Pleurotus ostreatus* mycelia. Carbohydrate polymer 94 (2013) 857-865. doi: 10.1016/j.carbpol.2013.02.017.

[74] Chang ST and Miles PG. Edible mushrooms and their cultivation. CRC press (1989) 1-38.

[75] Jayakumar T, Thomas PA, Isai M and Geraldine P. An extract of the oyster mushroom, *Pleurotus ostreatus*, increases catalase gene expression and reduces protein oxidation during aging in rats. Zhong Xi Yi Jie He Xue Bao 8 (2012) 774–780. doi: 10.3736/jcim20100808.

[76] Zhang YX, Dai L, Kong XW, and Chen L. Characterization and in vitro antioxidant activities of polysaccharides from *Pleurotus ostreatus.* International Journal of Biology and Macromolelecules 51 (2012) 259-265. doi: 10.1016/j.ijbiomac.2012.05.003.

[77] Mitra P, Khatua S and Acharya K. Free radical scavenging and NOS activation properties of water soluble crude polysaccharides from *Pleurotus ostreatus*. Asian Journal of Pharmacy and Clinical Research 6 (2013) 67-70. ISSN - 0974-2441

[78] Opletal L, Jahodar L, Chobot V, Zdansky P,Lukes J and Bratova M. Evidence for the anti-hyperlipidaemic activity of the edible fungus *Pleurotus ostreatus*. Br Journal of Biomedical Sciences 54 (1997) 240-243.

[79] Alam N, Amin R, Khan A, Ara I, Shim M and Lee MW. Comparative effects of oyster mushroom on lipid profile, liver and kidney function in hypercholesterolemic rats. Mycobiology 37 (2009) 37-42. doi: 10.4489/MYCO.2009.37.1.037.

[80] Krishna S and Usha PTA. Hyoglycaemic effect of a combination of *Pleurotus ostreatus*, Murray Koenigii and Aegle marmelos in diabetic rats. Indian Journal of Animal Sciences 79 (2009) 986-987.

[81] Shamtsyan MM, Konusova VG, Goloshchev AM, Maksimova YO, Panchenko AV and Petrishchev NN. Immunomodulating and anti-tumor effects of basidiomycetes *Pleurotus ostreatus* (Jacq. Fr) *P. Kumm* and P. conucopiae (Pau. Ex Pers.) Rollan. Journal of Biology and Physical chemistry 4 (2004) 157-61.

[82] Ravi B, Renitta RE, Praba ML, Issac R and Naidu S. Evaluation of antidiabetic potential of oyster mushroom (*Pleurotus ostreatus*) in alloxan-induced diabetic mice. Immunopathology and Toxicology 35 (2010) 101-109.doi: 10.3109/08923973.2012.710635.

[83] Wang H, Gao J and Ng TB. A new lectin with highly potent antiheptoma and antisarcoma activities from the oyster mushroom *Pleurotus ostreatus*. Biochemistry Biophysics Research Communication 275 (2000) 810-816. doi: 10.1006/bbrc.2000.3373.

[84] Sarangi I, Ghosh D, Bhutia SK, Mallick SK and Maiti TK. Antitumor and immunomodulating effects of *Pleurotous ostreatus* mycelia-derived proteoglycans. International Immunopharmacology 6 (2006) 1287-1297. doi: 10.1016/j.intimp.2006.04.002.

[85] Ghaly IS, Ahmed ES, Booles HF, Farang I and Nada SD. Evaluvation of antihyperglycemic action of oyster mushroom (*Pleurotus ostreatus*) and its effect

on DNA damage, chromosome aberrations and sperm abnormalities in streptozotocin- induced diabetic rats. Global Veterinaria 7 (2011) 532-544. ISSN 1992-6197

[86] Mohamed and E.M. Chemical profile, agaritine and selenium content of *Agaricus bisporus* Brazilian. Archives of Biology and Technology 55 (2012) 911-920. doi: http://dx.doi.org/10.1590/S1516-89132012000600015.

[87] Liu, J., Jia, L., Kan, J. & Jin, C. In vitro and in vivo antioxidant activity of ethanolic extract of white button mushroom (*Agaricus bisporus*). Food and Chemical Toxicology 51 (2013) 310–316. doi: 10.1016/j.fct.2012.10.014.

[88] Monika Gąsecka, Zuzanna Magdziak, Marek Siwulski and Mirosław Mleczek. Profile of phenolic and organic acids, antioxidant properties and ergosterol content in cultivated and wild growing species of *Agaricus*. European Food Research and Technology 244 (2018) 259–268. Doi: https://doi.org/10.1007/s00217-017-2952-9

[89] Ebana RUB, Etok CA and Edet UO. Nutritional and microbial analysis of melon (Citrullus colocynthis Linn) cake and its components- a traditional snack in South-South Nigeria. International Journal of Applied Studies 8 (2014) 1612-1617. ISSN 2028-9324

[90] Ebana RUB, Etok CA and Edet UO .Phytochemical screening and antimicrobial activity of Nypa fruticans harvested from Oporo River in the Niger Delta Region of Nigeria. International Journal of Applied Studies 10 (2015) 1120-1125. ISSN 2028-9324

[91] Praveena J and Estherlydia D. Comparative Study of phytochemical screening and antioxidant capacities of vinegar made from peel and fruit of pineapple

(*Ananas comosus L.*). International Journal of Pharmacy and Bio Sciences 5 (2014) 394–403. ISSN 0975-6299.

[92] Takumi Sakakibara, Yoshiharu Sawada, Jilite Wang, Satoshi Nagaoka and Emiko Yanase. Molecular Mechanism by Which Tea Catechins Decrease the Micellar Solubility of Cholesterol. Journal of Agricultural and Food Chemistry *67* (2019) 7128-7135. doi: 10.1021/acs.jafc.9b02265.

[93] Naz, S., Imran, M., Rauf, A., Orhan, I. E., Shariati, M. A. and Iahtisham-Ul-Haq, Heydari. Chrysin: Pharmacological and therapeutic properties. Life Sciences 235 (2019) 116-797. https://doi.org/10.1016/j.lfs.2019.116797.

[94] Meng-Yao Suna, Ying Yeb, Ling Xiaob, Khalid Rahmanc, Wei Xiad, Hong Zhanga. Daidzein: a review of pharmacological effects. African Journal of Traditional, Complementary and Alternative Medicines 13 (2016) 117-132. https://doi.org/10.21010/ajtcam.v13i3.15.

[95] Hanganu, D., Vlase, L., & Olah, N. Phytochemical Analysis of Isoflavons from some Fabaceae Species Extracts. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 38 (2010) 57-60. ISSN 0255-965X.

[96] Bok SH, Lee SH, Park YB, Bae KH, Son KH, Jeong TS and Choi MS. Plasma and hepatic cholesterol and hepatic activities of 3-hydroxy-3-methyl-glutaryl-CoA reductase and acyl CoA: cholesterol transferase is lower in rats fed citrus peel extract or a mixture of citrus bioflavonoids. The journal of nutrition 129 (1999) 1182-5. doi: 10.1093/jn/129.6.1182

[97] Dong Y, Cao A, Shi J, Yin P, Wang L, Ji G, Xie J and Wu D. Tangeretin, a citrus polymethoxy flavonoid, induces apoptosis of human gastri cancer AGS cells

through extrinsic and intrinsic signaling pathways. Oncology reports 31 (2014) 1788-94. doi: 10.3892/or.2014.3034.

[98] Kroes, B. H., van den Berg, A. J., Quarles van Ufford, H. C., van Dijk, H., and Labadie, R. P .Anti inflammatory activity of gallic acid. Planta medica 58 (2012) 499-504. doi: 10.1055/s-2006-961535.

[99] Louie, G. V., Bowman, M. E., Tu, Y., Mouradov, A., Spangenberg, G., & Noel, J. P. Structure-Function Analyses of a Caffeic Acid O-Methyltransferase from Perennial Ryegrass Reveal the Molecular Basis for Substrate Preference. The Plant Cell 22 (2010) 4114–4127. DOI: https://doi.org/10.1105/tpc.110.077578.

[100] Koshiba, T., Yokoshima, S., & Fukuyama, T. Total Synthesis of (−)-Huperzine A. Organic Letters 11 (2009) 5354–5356. https://doi.org/10.1021/ol9022408.

[101] Wang, Y., Zhang, S., Liu, J., Fang, B., Yao, J., & Cheng, B. Matrine inhibits the invasive and migratory properties of human hepatocellular carcinoma by regulating epithelial-mesenchymal transition. Molecular Medicine Reports 18 (2018) 911-919. https://doi.org/10.3892/mmr.2018.9023

[102] Wang, M., Hu, R., Wang, Y., Liu, L., You, H. and Zhang, J Wei, L. Atractylenolide III Attenuates Muscle Wasting in Chronic Kidney Disease via the Oxidative Stress-Mediated PI3K/AKT/mTOR Pathway. Oxidative Medicine and Cellular Longevity (2019)1–16. doi: 10.1155/2019/1875471.

[103] Abdulkhaleq LA, Assi MA, Noor MHM, Abdullah R, Saad MZ and Taufiq-Yap YH. Therapeutic uses of epicatechin in diabetes and cancer. Veterinary World 10 (2017) 869-872. doi: 10.14202/vetworld.2017.869-872.

[104] Li Yin, Qiuxia Lu, Shancai Tan, Lisheng Ding. Bioactivity-guided isolation of antioxidant and anti-hepatocarcinoma constituents from *Veronica ciliate*. Chemistry Central Journal 10 (2016). doi: 10.1186/s13065-016-0172-1.

[105] Ting Hu, Xiao-Wei He and Jian-Guo. Hydroxy tyrosol and its potential therapeutic effects. Journal of Agricultural and Food Chemistry 62 (2014)1449−1455. doi: 10.1021/jf405820v.

[106] Bahare Salehi, Abhay Prakash Mishram and Manisha Nigam. Resveratrol: A Double-Edged Sword in Health Benefits. Biomedicines 6 (2018).

[107] Ruchi Badoni Semwal, Deepak Kumar Semwal and Sandra Combrinck. Gingerols and shogaols: Important nutraceutical principles from ginger. Phytochemistry 117 (2015) 554–568. doi: 10.3390/biomedicines6030091.

[108] Lingzhi Wang, Do Dang Khoa Phan, Jingwen Zhang, Pei-Shi Ong, Win Lwin. Anti cancer properties of nimbolide and pharmacokinetic considerations to accelerate its development. Oncotarget 7 (2016) 44790-44801. doi: 10.18632/oncotarget.8316.

[109] Xiaoying Wang, Yang Yang, Xiao Liu and Xiumei. Pharmacological properties of tanshinones, the natural products from *Salvia miltiorrhiza*. Advances in Pharmacology 87 (2020) 43-70. doi: 10.1016/bs.apha.2019.10.001.

[110] Venkata raman, B. & La, Samuel & M, Pardha & B, Narashimha & Naga Vamsi Krishna, Ambatipudi & Tm, Radhakrishnan. Antibacterial, antioxidant activity and GC-MS analysis of *Eupatorium odoratum*. Asian Journal of Pharmaceutical and Clinical Research 5 (2012) 99-10. ISSN - 0974-2441

[111] Agatonovic-Kustrin, S., & Morton, D. W. The Current and Potential Therapeutic Uses of Parthenolide. Studies in Natural Products Chemistry 58 (2018) 61–91. DOI: 10.1016/B978-0-444-64056-7.00003-9.

[112] Costa, R. A., Pinheiro, M. L. B., Oliveira, K. M. T. de, Barison, A., Salomé, K. S., Iank, J. Costa and E. V. Structural, Vibrational, and Electronic Properties of the

Glucoalkaloid Strictosidine: A Combined Experimental and Theoretical Study. Journal of Chemistry (2016) 1–16. DOI: 10.1155/2016/1752429.

[113] Qian, Z. M., & Ke, Y. Huperzine A: Is it an Effective Disease-Modifying Drug for Alzheimer's disease. Frontiers in aging neuroscience 6 (2014) 216.

[114] Sanjive kumar. Medicinal importance of *Moringa oleifera*: drumstick plant. Indian Journal of Science 16 (2017)129-132. doi: 10.3389/fnagi.2014.00216.

[115] Neag MA, Mocan A, Echeverría J. Berberine: Botanical Occurrence, Traditional Uses, Extraction Methods, and Relevance in Cardiovascular, Metabolic, Hepatic, and Renal Disorders. Front Pharmacology 9 (2018) 557. doi: 10.3389/fphar.2018.00557.

[116] Hsieh, C.-J., Hall, K., Ha, T., Li, C., Krishnaswamy, G., & Chi, D. S. Baicalein inhibits IL-1β- and TNF-α-induced inflammatory cytokine production from human mast cells via regulation of the NF-κB pathway. Clinical and Molecular Allergy 5 (2007) 5. doi: 10.1186/1476-7961-5-5.

[117] Meng-Yao Sun, Ying Ye, Ling Xiaob, Khalid Rahman, Wei Xia, Hong Zhanga. Daidzein: A review of pharmacological effects. African Journal of Traditional, Complementary and Alternative Medicines 13 (2016) 117. DOI: https://doi.org/10.21010/ajtcam.v13i3.15

[118] Dilani D De, Sylvie Rapior, Kevin David Hyde and Ali Bahkali. Medicinal mushrooms in prevention and control of diabetes mellitus. Springer 56 (2012) 1-29. https://doi.org/10.1007/s13225-012-0187-4.

[119] Patel, K., Gadewar, M., Tahilyani, V., & Patel, D. K. A review on pharmacological and analytical aspects of diosmetin: A concise report. Chinese

Journal of Integrative Medicine 19 (2013) 792–800. doi: 10.1007/s11655-013-1595-3.

[120] Thangavel P, Puga-Olguín A, Rodríguez-Landa JF, Zepeda RC. Genistein as Potential Therapeutic Candidate for Menopausal Symptoms and Other Related Diseases. Molecules 24 (2019) 3892. doi: 10.3390/molecules24213892.

[121] Banskota AH, Tezuka Y, Adyana IK, Xiong Q, Hase K, Tran KQ, Tanaka K, saiki I, Kadota S. Hepatoprotective effect of Combretum quadrangulare and its constituents. Biological & Pharmaceutical bulletin 23 (2000) 456-60. doi: 10.1248/bpb.23.456.

[122] Mohammed Hifnawy**,** Nadia Sokkar**,** Shahira Ezzat**,** Mai Raslan**,** Josline Y. Salib and Amany Sleem. Cytotoxicity and Suppressive Effect of Leaves of *Mimusops laurifolia* on Carbon Tetrachloride-induced Liver Injury in Rats and its Bioactive Constituents. Asian Journal of Plant Sciences 11 (2012) 124-130. Doi:10.3923/ajps.2012.124.130.

[123] Braidy N, Behzad S, Habtemariam, Ahmed T, Daglia M, Nabavi SM, Sabarzo-Sanchez E, Nabavi. Neuroprotective Effects of Citrus Fruit-Derived Flavonoids, Nobiletin and Tangeretin in Alzheimer's and Parkinson's disease. CNS & neurological disorders drug targets 16 (2017) 387-397. doi: 10.2174/1871527316666170328113309.

[124] Han, C., Xing, G., Zhang, M., Zhong, M., Han, Z., He, C., & Liu, X. Wogonoside inhibits cell growth and induces mitochondrial-mediated autophagy-related apoptosis in human colon cancer cells through the PI3K/AKT/mTOR/p70S6K signaling pathway. Oncology Letters 15 (2018) 4463-4470. doi: 10.3892/ol.2018.7852.

[125] So, F. V.; Guthrie, N.; Chambers, A. F.; Moussa, M.; Carroll, K. K. Inhibition of human breast cancer cell proliferation and delay of mammary tumorigenesis by flavonoids and citrus juices. Nutrition and Cancer 26 (1996) 167–181. doi: 10.1080/01635589609514473.

[126] Bacanlı, M., Başaran, A. A., & Başaran, N. Galangin as a Plant Phenolic and Usage in Health and Disease. Polyphenols: Prevention and treatement of human disease (2018) 433-438. DOI: 10.1016/B978-0-12-813008-7.00034-5

[127] Pei K, Ou J, Huang J and Ou S. P-Coumaric acid and its conjugates: dietary sources, pharmacokinetic properties and biological activities. Journal of the sciences of food and agriculture 96 (2016) 2952-62. doi: 10.1002/jsfa.7578.

[128] Wang Z, Xie D, Gan X, Zeng, Zhang A, Yin L, Song B, Jin L, Hu D. Synthesis, antiviral activity, and molecular docking study of trans-ferulic acid derivatives containing acylhydrazone moiety. Bioorganic & medicinal chemistry letters 27 (2017) 4096-4100. https://doi.org/10.1016/j.bmcl.2017.07.038.

[129] Bhat, A. A., Prabhu, K. S., Kuttikrishnan, S., Krishnankutty, R., Babu, J., Mohammad, R. M., & Uddin, S. Potential therapeutic targets of Guggulsterone in cancer. Nutrition & metabolism 14 (2017). doi: 10.1186/s12986-017-0180-8

[130] Deng R. Therapeutic effects of guggul and its constituent guggulsterone: cardiovascular benefits. Cardiovascular drug reviews 25 (2007) 375-90. doi: 10.1111/j.1527-3466.2007.00023.x.

[131] Kang MS, Hirai S, Goto T, Kuryanagi K, Lee JY, Uemura, Ezaki Y, Takshashi N, Kawada T. Dehydroabietic acid, a phytochemical, acts as ligand for PPARs in macrophages and adipocytes to regulate inflammation. Biochemical and biophysical research communications 369 (2008) 333-8. doi: 10.1016/j.bbrc.2008.02.002.