EXTRACTION AND ANALYSIS OF SECONDARY METABOLITES FROM *PLEUROTUS OSTREATUS* FOR ITS BIOMEDICAL APPLICATIONS

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ABSTRACT

This study investigates the medicinal and nutritional properties of *Pleurotus ostreatus (P. ostreatus)*, a mushroom species known for its significant health benefits. The research focuses on the quantification of primary and secondary metabolites, including carbohydrates, proteins, amino acids, alkaloids, flavonoids, phenols, tannins, and vitamins, which contribute to *P. ostreatus's* therapeutic potential. The study also explores the mushroom's antibacterial and antioxidant activities, revealing its high antioxidant capacity and effective antibacterial action against various microorganisms. The findings underscore *P. ostreatus's* potential as a functional food and a source of bioactive compounds for treating various human ailments. This research contributes to the understanding of the medicinal mushrooms' role in health and nutrition, emphasizing the value of *P. ostreatus* in developing natural therapeutic agents.

Key Words: *Pleurotus ostreatus*, secondary metabolites, antioxidant, mushroom

1. INTRODUCTION

Fungi, especially mushrooms, have long been valued for their medicinal properties, with over 97,330 species identified, including various forms like yeasts and filamentous fungi. Among these, *Pleurotus ostreatus*, known as oyster mushrooms, stands out for its nutritional and medicinal value. Pleurotus species, ranked as the third most significant commercially, grow in diverse climates, from temperate to tropical regions.

Pleurotus ostreatus is notable for its low fat and high vitamin and mineral content. It's chemical composition varies depending on various factors. This mushroom has been associated with several health benefits, including anti-inflammatory, immunostimulatory, and anti-cancer properties. Its bioactive compounds, such as polysaccharides, peptides, and glycoproteins, contribute to these medicinal qualities).

Mushrooms are functional foods that, in addition to the typical nutrients they contain, can help with health improvement. Due to the widespread perception that green medicine is safe, readily available, and has fewer adverse effects than conventional medicine, herbal remedies have grown in popularity as a means of treating numerous disorders (Savithramma, 2011).

Phenolic compounds, flavonoid and in smaller amounts peptides, carotenoids, ascorbic acid to cop (Ruiz et al., 2009; Valverde et al., 2015). For species like Pleurotus species, Agaricus species, *Ganoderma lucidum* and *Lentinula edodes*, recognized for their powerful antioxidant activity, the radical scavenging properties of mushrooms have been thoroughly explored and recorded. Having natural antioxidants and nutritious commercial preparations, *Phellinus erinaceus, Hericium rimosus* and *Cordyceps sinensis* can be employed as nutraceuticals (Rathore et al., 2017).

An increase in CAT (catalase) gene expression was seen in the liver and kidneys of rats when they were given an ethanol extract of P. ostreatus, along with a concurrent decrease in protein carbonylation in these organs (Jayakumar et al., 2010). Reactive oxygen species-induced oxidative stress contributes significantly to the etiology of many diseases, including arteriosclerosis, carcinogenesis, neurodegenerative disorders, and diabetes and its consequences. Antioxidants are therefore viewed as substances that not only stop the progression of disease but also have promise for use in its treatment (Hajhashemi et al. 2010). Since natural antioxidants are thought to be safe and have high consumer acceptance, the food industry and consumers are growing more and more interested in replacing these artificial antioxidants. As a result, the use of natural antioxidants has increased globally

AIMS AND OBJECTIVES

Aims and objectives of this research are;

1. To investigate the metabolites present in the mushroom *Pleurotus ostreatus*.
2. To investigate the antibacterial activities of *Pleurotus ostreatus*.
3. To investigate the antioxidant potential of *Pleurotus ostreatus.*

2. Existing Research

Pleurotus ostreatus, found on all continents except Antarctica, is rich in essential nutrients like vitamins C, niacin, riboflavin, thiamin, and B12. It also contains oleic acid and linoleic acid, which help lower serum cholesterol levels. Given the growing interest in natural remedies with fewer side effects, mushrooms like *Pleurotus ostreatus* are increasingly recognized for their role in treating various diseases, backed by numerous in vitro and animal studies (Savithramma, 2011). This research aims to delve deeper into the specific medicinal properties and potential applications of *Pleurotus ostreatus*, contributing to the expanding field of functional foods and natural therapeutics.

Water and alcoholic extracts of *P. ostreatus* mycelium have been employed in studies on antibacterial activity against several types of microorganisms. Aqueous extract was the most efficient against the germs *Staphylococcus aureus* and *Escherichia coli*, as well as the fungi *Candida albicans*, *Cryptococcus humicola*, and *Trichosporon cutaneum*. The active substance in the extract was discovered to be 3-(2-aminophenyl-1-thio)-3 hydroxypropanoic acid, having MICs of 30 gmL-1 and 20 gmL-1 against fungus and bacteria, respectively (Younis et al., 2015). P. ostreatus mycelium methanolic extract exhibited effectiveness against gramme positive and gramme negative bacteria with a MIC of 4 to 8 g mL-1 (Chowdhury et al., 2015). Ethanol extract hindered the growth of Salmonella typhi, *Staphylococcus aereus, Bacillus subtilis, Bacillus atrophaeus, and Klebsiella pneumoniae*.

Foods naturally contain antioxidants, but only in very small amounts. As a result, additional amounts are given through a process called free radical termination to decrease oxidation and enhance overall quality and lifespan. Oxygen-centered free radicals and ribosome-inactivating proteins (ROS), which enhance oxidative damage to biomolecules like carbohydrates, proteins, lipids, and DNA, are produced by a number of biochemical and physiological processes in the human body. Various chronic disorders may therefore arise (Cai et al., 2004).

According to Yuxiwang et al., 2014 twenty widely consumed edible mushrooms' total phenolic content (TPC) and distribution of antioxidant characteristics in four extraction solvents were studied. The findings demonstrated that compared to organic solvent extracts, aqueous extracts (water extracts and hot water extracts) had substantially better antioxidant activities (acetone extracts and

ethanol extracts). The *Agaricus subrufescens* water extract had the greatest antioxidant activity of all the extracts.

By Okwulehie et al. (2013), four edible and non-edible species of mushrooms were chosen for the study to examine their phytochemical features, mineral analysis, proximate analysis, and heavy metal compositions (*Cheimonophyllum candidissimus*, Pleurotus sp., Russula sp., and Auricularia sp.). The bioactive components found in mushrooms include alkaloids (0.1200.021.0103 percent), tannins (0.44±0.09-1.38±0.6 percent), phenols (0.13±0.01-0.26±0.00 percent), saponins (0.14±0.03-0.32±0.04 percent), and flavonoids (0.08±0.02-0.34±0.02 percent). According to the proximate composition analysis, the mushroom had a protein content of 5.17±0.06–12.28±0.16 percent, a fat content of 0.16±0.02-0.67±0.02 percent, a fiber content of 1.06±0.03–8.49±0.03 percent, and a carbohydrate content of 62.06±0.52–80.01±4.71 percent. The findings show that mushrooms can be used in the production of medicines and are a good source of phytochemicals, proximate components, and minerals required for maintaining good health. According to Kamil et al., 2016 a widely used and grown fungus having medicinal value is *Pleurotus ostreatus*. It has a wide range of biological functions and the ability to prevent and treat illnesses

According to Piska *et al*., 2017, with the exception of Antarctica, all continents are home to the oyster mushroom, *Pleurotus ostreatus* (Basidiomycota). Fresh fruiting bodies have a vitamin C content of 15%, niacin, riboflavin, and thiamin content of 40%, and a vitamin B12 content of 0.5 mg per 100 gram. In addition, this species has a high concentration of oleic acid (40%) and linoleic acid (55%) as well as compounds that lower serum cholesterol levels.

3. MATERIALS AND METHODS

3.1 COLLECTION OF MUSHROOM

The *Pleurotus ostreatus* mushroom was collected from Kharak Mirpur, Azad Kashmir. These mushrooms were washed thoroughly by tap water and shade dried for 7 days.

3.2 MUSHROOM POWDER

The shade-dried mushrooms were ground in a grinder to make powder. The mushroom powder was then saved in an airtight jar for further use.

3.3 PREPARATION OF EXTRACTS

For mushroom extract preparation, three solvents (distilled water, ethanol, and methanol) were used. 10g of mushroom powder was put in 100 mL of each solvent in a conical flask. Place it in a shaking incubator for 48 hours. Filter the mixture, the filtrate obtained with methanol and ethanol was spread on the large petri plates and the filtrate with water was centrifuged. The crude extract obtained after evaporation of methanol and ethanol was preserved for further use after

centrifugation. Then weigh each extract and dissolve it in a solvent that is a multiple of ten.

3.4.1 Flavonoids

3.4.1.1 Alkaline reagent test

Evans et al. (1989) applied 1 mL of 2N NaOH solution to 1 mL of extracted materials. The presence of flavonoids in the sample was revealed by its yellow hue.

3.4.2 Phenols

3.4.2.1 Ellagic acid test

To 1 mL of mushroom extract samples, a few drops of 5% glacial acetic acid were added, followed by a few drops of 5% $NaNO_2$ solution. The presence of phenols in the test samples was detected by the creation of a muddy brown hue (Archana et al., 2012).

3.4.3 Tannins Detection

3.4.3.1 *Alkaline reagent test*

volume of 2 mL of 1N NaOH was added to 2mL of mushroom extract samples. The appearance of yellow to red color revealed the existence of tannins (Archana et al., 2012)

3.4.4 Saponins

3.4.4.1 Foam test:

A little sample was placed in a test tube together with a small amount of water and violently shaken. Saponin is present if foam appears and lasts for 10 minutes (Subbulakshmi *et al*., 2016).

3.4.5 Alkaloids

3.4.5.1 Hager's test

Hager's reagent, a saturated picric acid solution, was administered to 2 mL of the appropriate mushroom extract for this test. The appearance of brilliant yellow precipitates indicated the presence of alkaloids (Archana et al., 2012).

3.5 PRIMARY METABOLITIES IDENTIFICATION

3.5.2 Proteins Identification:

3.5.2.1 Ninhydrin test:

Ninhydrin was added to the sample in small amounts. The presence of blue suggests a favorable outcome (Subbulakshmi et al., 2016).

3.5.3 Carbohydrates

3.5.3.1 Molisch's test

The sample was mixed with Molisch's reagent, and Conc.H2SO4 was applied to the sidewalls of the test tube. The interference's reddish violet ring indicates the presence of carbs (Subbulakshmi et al., 2016).

3.6 QUANTITATIVE ANALYSIS OF METABOLITES

3.6.1 Flavonoids

The total flavonoids were determined using the aluminum chloride colorimetric method. According to Tibuhwa, distilled water (4 mL) was used to dilute each extract, which was 1 mL. At this point, add 1ml of the 10% Al $(NO_3)_3$ solution. It was held for six minutes. Incorporate a 5% $NaNO_3$ solution and incubate at room temperature. At this point, add 10 mL of a 4% NaOH solution. Keep it for 15 minutes, and then check the optical absorption at 510 nm.

Rutin was used as a standard. A working dilution of rutin stock solution was used as a standard and was used for the calibration curve. Rutin solution was also used in the same aliquot concentration as the sample extract. Using a freshly prepared rutin solution's calibration curve, the total flavonoids content of the extract was expressed in rutin equivalents (mg/mL) for flavonoids.

3.6.2 Total Phenolic Content

The 50µL of each water extract, methanol extract, and ethanol extract, blank solution, and standard solution were combined with 3 mL of distilled water, 250 µL of Folin-Ciocalteu reagent, and 750 µL of a 7% Na2CO3 solution to measure the concentration of mushroom extracts. The mixes were then vortexed to ensure that the components were evenly mixed.

After 8 minutes at room temperature, each combination got 950 L of distilled water. The mixes were then allowed to stand at room temperature for an additional hour (Wang et al., 2014). Using distilled water as a blank, the absorbance of each combination was measured at 510 nm with a UV-Visible spectrophotometer.

A freshly prepared Gallic acid solution was used as the standard for the calibration curve. Gallic acid solution is also used in the same aliquot concentration as the sample extract. The total phenolic compounds of the extract were articulated in Gallic acid equivalents (mg/mL), utilizing a calibration curve of Gallic acid solution, which is freshly prepared.

3.6.3 Saponins

The approach was used to determine the presence of saponin. A water bath was used to boil 1 g of the sample for 1.30 min while it was still hot with 20ml of 20 % ethanol. After collecting the filtrate, heating it for 30 minutes in 8 mL of ether, and then pouring it into a separating funnel, the lower portion was collected, 12.5 mL of n-butanol was added, and the upper layer/part was collected while the bottom portion was discarded (Okwulehie *et al*., 2013). The filtrate was heated to a

steam bath temperature of 70 °C in an oven, evaporated to dryness, chilled, and weighed.

3.6.4 Tannins

Tannins in aqueous extract were extracted using the method described by Bray and Thorpe in 1954. After combining 1 mL of the sample with 5 mL of the vanillin hydrochloride reagent, the mixture was allowed to sit at room temperature for 20 minutes. At 500 nm, absorbance was measured against a blank for the reagent. The analysis was carried out three times, and readings were noted (Devi *et al*., 2015).

A freshly prepared tannic acid solution was used as a standard for the calibration curve. Tannic acid solution was also used in the same aliquot concentration as the sample extract. The tannins compounds of the extract were articulated into tannic acid equivalents (mg/mL), utilizing a calibration curve of tannic acid solution which is freshly prepared.

3.6.5 Alkaloids

The alkaloids were quantified following the methods described by (Harborne et al., 1973). After weighing 1 g of the substance in a 250 mL beaker, 150 mL of 10% acetic acid generated in ethanol were added. For four hours, the solution combination was covered and let to stand.

When the proper period of time had elapsed, the mixture was filtered, and the resultant filtrate was concentrated in a water bath to decrease its volume to roughly one-fourth of what it had been initially. Conc.NH4OH was administered to the extract sample drop by drop until complete precipitation occurred. The filtrate was filtered after allowing the solution to settle, collecting the precipitate, and washing with dilute NH4OH. The residue was properly dried and weighed to ascertain the proportion of alkaloids in the test sample.

3.7 PRIMARY METABOLITES

3.7.1 Carbohydrates

The total soluble carbs were estimated using the following method. 1.0 mL of sample, 1.0 mL of phenol solution, and 5.0 mL of 96% sulphuric acid were added to each tube. The tubes were then thoroughly shaken. After 20 minutes in a boiling water bath, the absorbance at 490 nm was measured in contrast to a blank reagent. The analysis was performed in triplicate, and the findings were represented as mg/mL samples.

As the calibration curve's standard, a newly produced glucose solution was employed. The same aliquot concentration of glucose solution as the sample extract was also utilized. The term "calibration" refers to the process of calculating the amount of time it takes for a certain piece of equipment to do a specific task.

3.7.2 Proteins Determination

F Lowry's (Lowry et al., 1957) approach was used to determine protein content. To 1 mL of sample, 0.5 mL of 0.1 N NaOH and 5 mL of alkaline copper reagent were added, and the mixture was incubated at room temperature for 30 minutes. After adding the Folin-Ciocalteu reagent, the mixture was incubated for another 10 minutes at room temperature. The absorbance of the reagent was measured at 660 nm against a blank. The analysis was repeated three times, and the findings were expressed in miligramme per millilitre of material.

Freshly prepared bovine serum solution was used as the standard for the calibration curve. Bovine serum solution is also used at the same aliquot concentration as the sample extract. The protein content of the extract was articulated in bovine serum equivalents (mg/mL), utilizing a calibration curve of bovine serum solution, which is freshly prepared.

3.8 ANTIBACTERIAL ACTIVITY:

To test the antibacterial activity of this mushroom extract, four distinct gram-positive and gram-negative bacterial strains were utilized. Positive control was given by commercially available rifampicin.

3.8.1 Test Organisms

For the evaluation of the antibacterial activity of particular mushroom extracts, *Escherichia coli, Staphylococcus aureus, Bacillus pumilus,* and *Pseudomonas aeruginosa* were utilized as test organisms.

3.8.2 Media Preparation

Nutrient broth and nutrient agar media were used to check the antibacterial activity.

3.8.3 Preparation of Nutrient Broth

In a flask, dissolve 1.6 g of nutrient broth in 100 mL of distilled water, then autoclave at 121°C for 15 minutes.

3.8.4 Preparation of Nutrient Agar

Take 1.6 g of nutrient broth and 1.5 g of agar in a flask and dissolve well in 100 mL of distilled water, then autoclave at 121°C for 15 minutes.

3.8.5 Dried Filter Paper Discs preparation

Pleurotus ostreatus extracts were tested for their antibacterial activity using three solvents: methanol, ethanol, and water. The 6 mm discs were prepared with Whattman No. 1 and autoclaved for sterilization after that.

3.8.6 Preparation of bacterial culture

Four falcon tubes were used and the names of the bacterial strains were written on them. Each falcon tube received 20 mL of nutritional broth medium before receiving 20 µL of bacterial growth stock solution. For 24 hours at 37°C, all falcon tubes were kept in a shaking incubator.

3.8.7 Disc diffusion method

By using the disc diffusion approach, antibacterial activities were conducted. To guarantee the growth of that specific strain on the plate, the test strain inoculum was evenly spread on the petri plates by swabbing with sterile cotton swabs in three dimensions. Each test sample was impregnated with 20 µL on sterile filter paper discs (6 mm in diameter), dried, and then placed on top of the media.

3.8.8 Incubation:

The plates with the bacterial inoculum were incubated at 37 °C for 24 hours. Each bacterial strain was subjected to the experiments three times.

3.8.9 Zone of inhibition

To determine the activity of test samples against various strains after the designated amount of time, the widths of the inhibition zones that formed around the discs were measured in millimeters (mm).

3.9 ANTIOXIDANT ACTIVITY

3.9.1 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH) Radical Scavenging Activity

The method of Chu, Chang, and Hsu (2000) was modified to assess the scavenging activity of the methanol and water extracts from mushrooms on DPPH radicals. A test tube containing 1 mL of varying doses of mushroom methanol or water extract (ranging from 1.5 to 9 mg/mL) was filled with an aliquot of 0.5 mL of 0.1 mM DPPH radical in methanol.

3.9.2 Antioxidant Assessment

Amarowicz *et al* study was employed to assess the free radical scavenging capacity of leaf extracts using DPPH (2, 2-diphenyl-1-picrylhydrazyl, Sigma, Aldrich UK). 0.0012 mg/mL of DPPH was produced as a control, methanol, ethanol or water was utilized in place of the mushroom sample. At room temperature, the reaction mixture was vortex, and the absorbance (Abs), which was measured using a spectrophotometer at 520 nm, was calculated right away and was used to determine the scavenging activity (percent) (SA) of DPPH radicals. The DPPH solution and extract were combined in the cuvette, thoroughly mixed, and left at room temperature for 30 minutes. During the incubation period at room temperature, the color of the solution changed. The absorbance was measured at 517 nm against a blank, and DPPH free radical scavenging activity was assessed using a UV visible spectrophotometer.

Ascorbic acid served as the standard. Each sample was examined three times. The percentage of extracts that were scavenged was determined using the following equation:

$$\text{Inhibition percent} = \left[\frac{\text{A blank} - \text{B extract}}{\text{A blank}}\right] \times 100$$

Where A blank represents the absorbance of the control reaction, where B extract is the absorbance of the test compound and A blank is the absorbance of the

control reaction, which contains all reagents but the test compound. The graph of the inhibition percentage versus extract concentration was used to compute the extract concentration providing 50% inhibition.

RESULTS AND DISCUSSION

4.1 PREPRATION OF EXTRACT OF MUSHROOM *PLEUROTUS OSTREATUS*

The extraction yield of mushrooms with aqueous was 2.66 g in 26.6 mL of distilled water, with ethanol 4.5 g in 45 mL of ethanol, and with methanol 1.74 g in 17 mL of methanol.

4.2 STUDY OF METABOLITES OF PLEUROTUS OSTREATUS

Medicinally, mushrooms play a vital role in the health sector, and medicinally they are very important. That's why mushrooms are known as neutraceuticals in the modern world because of their high-quality proteins, vitamins, and fibers as well as their numerous therapeutic characteristics. Compared to other medicinal mushrooms, Pleurotus is gaining more attention as a health enhancer and environmental restorer. In order to update the current situation and predict future prospects for Pleurotus for their biomedical potential, databases were exhaustively searched, gathered, and analyzed for this research. The facts will provide a fresh perspective.

4.2.1 Study of Secondary Metabolite

Secondary metabolites play a significant role in the ecological interactions between mushrooms and their surroundings. But medicinally they are very important and they were studied by two ways.

4.2.1.1 Qualitative study of secondary metabolites

Qualitative analysis indicates the presence of some secondary metabolites that are medicinally very important. The presence of various metabolites, such as flavonoids, phenols, tannin, alkaloids, and saponin, was confirmed through testing.

The qualitative analysis of secondary metabolites' results of *Pleurotus ostreatus* extracts have been presented in Table-3.1. The presence of flavonoids, phenolic, tannin, saponin, and alkaloid was found in mushroom extracts.

Moreover, the aqueous extract of *Pleurotus ostreatus* conferred the presence of flavonoids, alkaloids, and tannin in a higher amount, while saponin and phenolic acid are present in a lesser amount. Ethanol extract had a higher concentration of phenolic, saponin and proteins but a lower concentration of flavonoid and alkaloid. Alkaloid and flavonoid were found in higher concentrations in the methanol extract, while phenols, saponin, and tannin were found in lower concentrations.

Table 4.1: Qualitative analysis of secondary metabolites from *Pleurotus ostreatus*

S. No.	Sample	Flavonoids	Phenolic compounds	Tannins	Alkaloids	Saponins
01	Aqueous	+++	++	+++	+++	++
02	Ethanol	+++	++	+	++	+++
03	Methanol	++	+	+	++	+

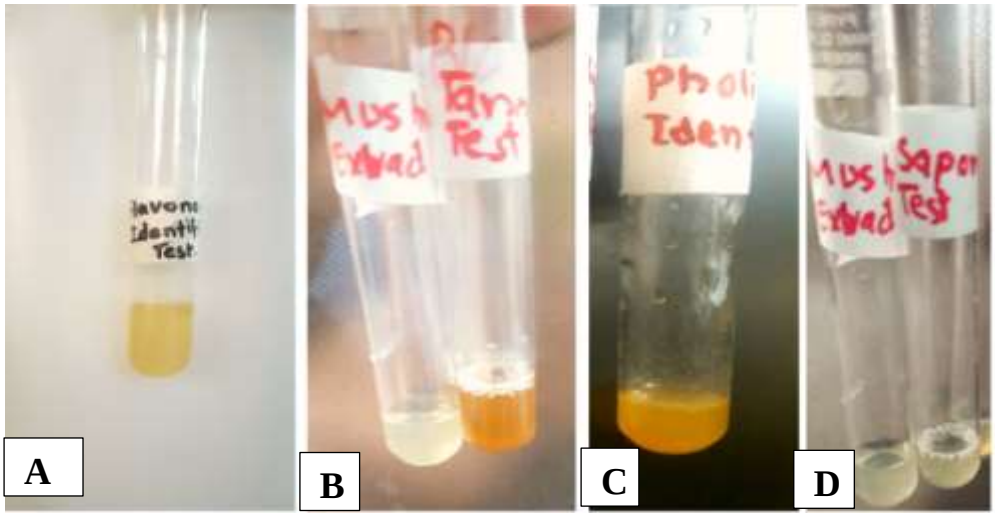

Figure 4.1: Qualitative estimation of secondary metabolites (A) Flavonoids, (B) Tannins (C) Phenols (D) Saponins

4.2.1.2 Quantitative analysis of secondary metabolites

4.2.1.2.1 Flavonoids

Using the method of Jaita *et al*, *Pleurotus ostreatus* extract was used to calculate the total flavonoid concentration. The total flavonoid content of the extract was determined as rutin equivalents (mg/mL) using a calibration curve of a freshly made rutin. There were variations in the flavonoid content across all samples table 4.2 and Fig-4.1 shows the results of flavonoid.

Inhibition of the transcription factor activator, quenching of free radicals, inhibition of lipid peroxidation, and modification of carcinogen detoxifying enzymes are just a few of the many biochemical and pharmacological properties that flavonoids are known to have, including the ability to inhibit cancer (Havsteen, 2002).

Table 4.2: Quantitative analysis of flavonoids of *Pleurotus ostreatus*

S. No.	Sample	Flavonoids Conc. (mg/mL)
01	Aqueous	200
02	Ethanol	182
03	Methanol	170

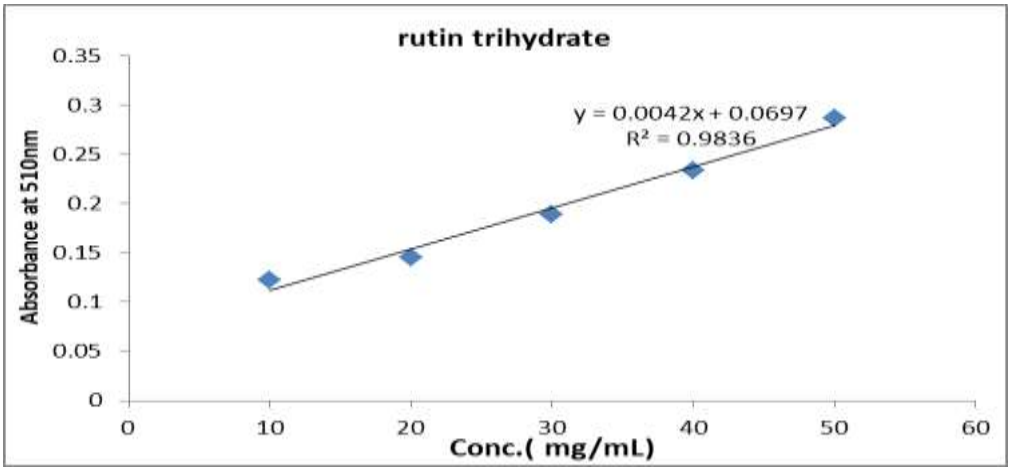

Figure 4.2: Calibration curve of standard with flavonoid

4.2.1.2.2 Total phenolic compounds

Using the Folin-Ciocalteu reagent, (Wang *et al*., 2014) estimated the complete calibration curve of a freshly generated gallic acid solution. The total phenolic content of the extract was calculated as gallic acid equivalents (mg/mL), as shown in Fig-4.2. The greatest value ever measured was in the aqueous extract. It is shown in Table-4.3 that *Pleurotus ostreatus* total phenolic content ranged in concentration from 8.87 to10 mg/mL. The methanol extract had the lowest value, while the ethanol extract had the highest value.

Table 4.3: Quantitative analysis of total phenolic compounds of *Pleurotus ostreatus*

S. No.	Sample	Total Phenolics Conc. (mg/mL)
01	Aqueous	10
02	Ethanol	8.87
03	Methanol	9.72

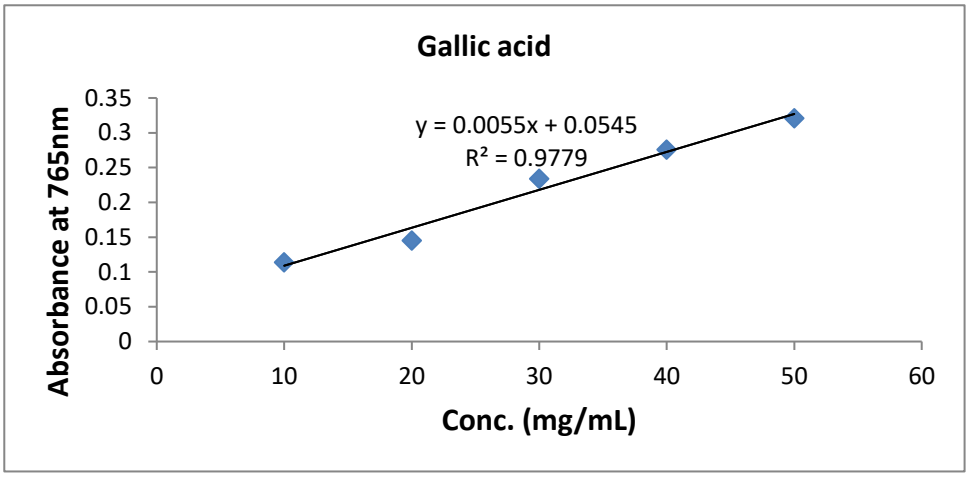

Figure 4.3: Calibration curve of standard with total phenolics

4.2.1.2.3 Tannins

The Bray and Thorpe method was used for qualitative estimation of tannin. By applying this method to *P. ostreatus* extract, it was discovered that methanol extract has a high tannin value, which was 3.5 mg/mL, and a greater overall tannin concentration, while aqueous extract has the lowest value, which was 1.03 mg/mL and methanol extract has a 1.77 mg/mL value of tannin. Okwu *et al*. (2006) performed an experiment for the estimation of tannin. It was found that the total content of tannins was higher in *P. sajor caju* (6.840±12 mg/g).

Tannins have been shown to impede the growth of numerous fungi, yeasts, bacteria, and viruses. of tannins of pleurotous ostreatus. In addition to having this astringent activity, tannins also aid in the speedy healing of wounds and inflamed mucous membranes Tannins may potentially have antiviral and antibacterial properties.

Table 4.4: Quantitative analysis of tannins of *Pleurotus ostreatus*

S. No.	Sample	Tannis Conc. (mg/mL)
01	Aqueous	1.03
02	ethanol	1.77
03	methanol	3.5

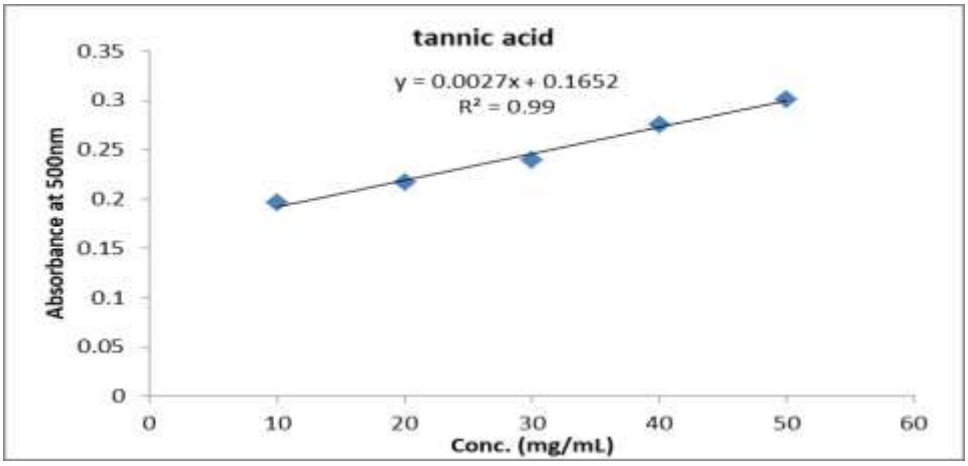

Figure 4.4: Calibration curve of standard with tannin

4.2.1.2.4 Alkaloids

For alkaloid estimation, (Harborne *et al*., 1973) methodology was utilized. By this method, the highest quantity was in ethanol extract, which was 0.382 mg, the lowest value was in aqueous extract, which was 0.267 mg and in methanol extract, which was 0.321 mg.

Devi *et al*., 2015 reported the result of alkaloids in which the level of alkaloids was (2.81±0.61 mg/mL). A prior study found that bitter leaf contains an alkaloid that can reduce headaches brought on by hypertension. Alkaloids defend against chronic disorders. A wide range of secondary metabolites known as alkaloids have been reported to have antibacterial effects via preventing DNA topoisomerase.

Table 4.5: Quantitative analysis of alkaloid of *Pleurotus ostreatus*

S. No.	Sample	Alkaloids Conc. (mg /mL)
01	Aqueous	0.267
02	Ethanol	0.382
03	Methanol	0.321

4.2.1.2.5 Saponins

Saponin determination was performed by the method of Harborne 1973 by which the highest quantity was obtained in ethanol extract at 0.36 mg/g and the lowest value was 0.296 mg/g in methanol extract. The quantity obtained in the aqueous extract was 0.32 mg/g.

Because saponin has a high foaming capacity and creates a frothy effect in the food sector, it is valuable in the pharmaceutical and medical industries. Okwulehie et al., 2013 quantify saponin in four mushrooms *C. candidissimus*,

Pleurotus species, Russula species and Auricularia species. And their values are 0.32±0.04, 0.24±0.02, 0.14±0.03 and 0.15±0.02 respectively.

Table 4.6: Quantitative analysis of saponin of *Pleurotus ostreatus*

S. No.	Sample	mg /mL
01	Aqueous	0.32
02	Ethanol	0.36
03	Methanol	0.296

4.3 PRIMARY METABOLITES

Primary metabolites are created during the growth phase as a result of energy metabolism and are thought to be necessary for optimal growth. Primary metabolites are directly involved in growth and development, whereas secondary metabolites are not directly involved but have been used as biocatalysts. Primary metabolites are critical for mushroom growth and are essentially necessary. The importance of many primary metabolites lies in their role as precursors or pharmacologically active metabolites of pharmaceutical substances such as antipsychotic medications. During this study, only two primary metabolites were studied, which are the most important metabolites, and medicinally they are very useful.

4.3.1 Qualitative Estimation of Primary Metabolites

The presence of some primary metabolites that are medicinally significant is indicated by qualitative analysis. Testing confirmed the presence of two metabolites such as carbohydrates and proteins. According to fig 4.5 the results of extracts for proteins and carbohydrates for qualitative estimation were same.

Table 4.7: Qualitative analysis of primary metabolites from *Pleurotus ostreatus*

S. No.	Sample	Carbohydrates	Proteins
01	Aqueous	+++	+++
02	Ethanol	+++	+++
03	Methanol	+++	+++

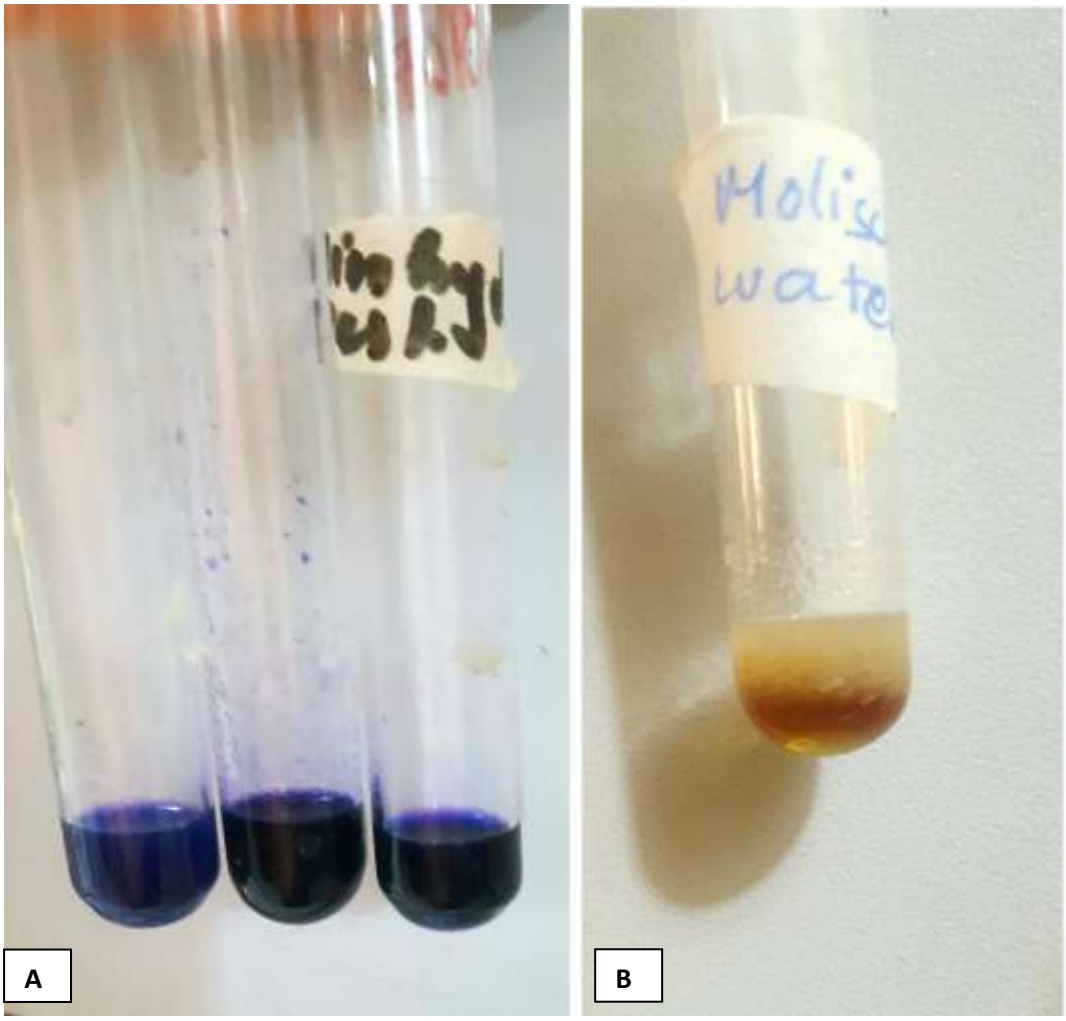

Figure 4.5: Qualitative estimation of primary metabolites, (A) protein (B) carbohydrates

4.3.2 Quantitative Estimation of Primary Metabolites

4.3.2.1 Carbohydrates

For carbohydrates quantification Dubosis *et al*., 1956 technique was employed by which in ethanol extract has the highest value 8.04 mg/mL and in methanol extract has the lowest value that was 5.2 mg/mL. In aqueous extract it was 6.93 mg/mL.

Carbohydrates were quantified by Dubois et al., 1956. In *P sajor caju it was* 2.53±0.40 mg/g. One such class of carbon molecules that are necessary for life is carbohydrates. Almost all living things employ carbohydrates as the building blocks for their cells and really make use of their abundant potential energy.

Table 4.8: Quantitative analysis of total carbohydrates of *Pleurotus ostreatus*

S. No.	Sample	mg /mL
01	Aqueous	6.93
02	Ethanol	8.04
03	Methanol	5.2

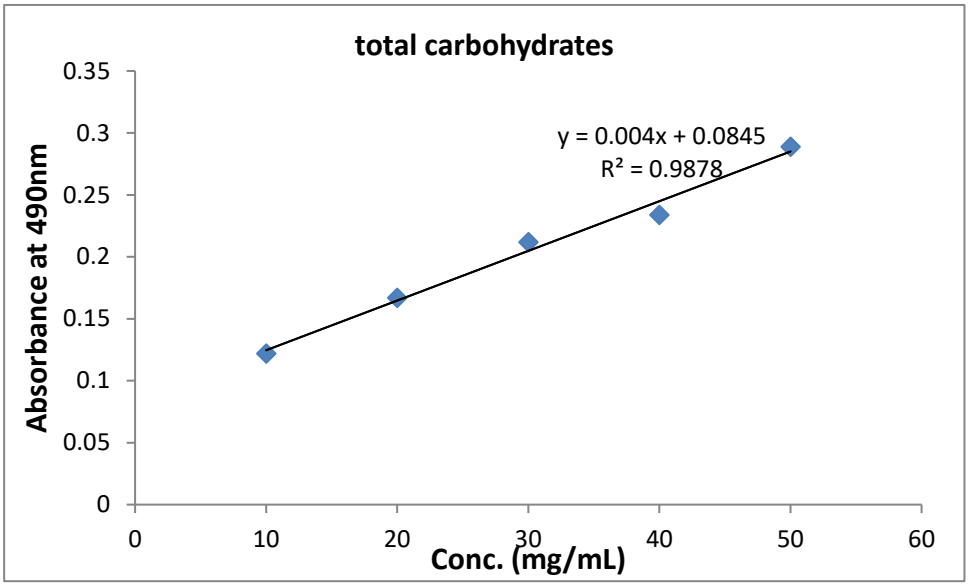

Figure 4.6: Carbohydrates content of *Pleurotus ostreatus*

4.3.2.2 Proteins

Proteins were quantified by methods of Lowry et al by which it was found that aqueous extract has the highest value that was 1.84 mg/mL while ethanol extract has lowest value that was 1.13 mg/mL and methanol extract has 1.38 mg/mL value. Devi *et al*., 2015 quantify proteins from *P. sajor caju*.the highest value obtained was 7.593±0.238 mg/g.

Table 4.9: Quantitative analysis of total protein of *Pleurotus ostreatus*

S. No.	Sample	mg /mL
01	Aqueous	1.84
02	Ethanol	1.13
03	Methanol	1.38

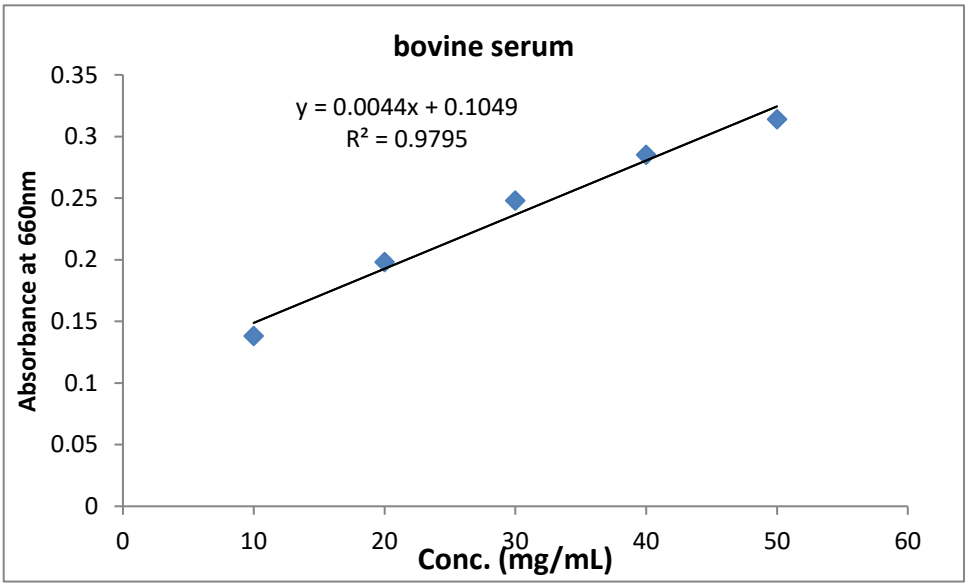

Figure 4.7: Calibration curve of standard with protein

4.4 ANTIBACTERIAL ACTIVITY

By using the disc diffusion method, the antibacterial effects of various extracts of *Pleurotus ostreatus* were evaluated against gram positive and gram negative bacteria. The results are shown in table 4.9. The findings of our investigation showed that aqueous extracts have the strongest antibacterial effects against *S.aureus* that was 21±1 mm, but for all other bacterial strains it shows no result. In the case of ethanol extract of *Pleurotus ostreatus*, the highest activity was against *B. pumilus* that was 25±1mm and the minimum activity against S.aureus that was 13±1. The activity against *P. aeruginosa* was 17±1. The antibacterial activity in the case of methanol extract was highest in the case of *S.aureus* that was 14±1 and the minimum antibacterial activity was against *P.* aeruginosa that was 10.33±0.577 mm. In the case of *B.pumilus* the activity was 12.66±1 mm. All three *Pleurotus ostreatus* extracts show zero activity against *E. coli*.

Rifampicin was utilized as a reference antibiotic, and the zones of inhibition of several *Pleurotus ostreatus* extracts were compared to those of rifampicin *P.aeruginosa* displayed the lowest zone of inhibition, while *B.pumilus* displayed the greatest. All bacterial strains, including *B. pumilus, S.aureus, P. aeruginosa*, and *E. coli*, exhibited zones of inhibition against antibiotics in *Pleurotus ostreatus* that were, respectively, 35 mm, 27 mm, 20.33 mm and 16 mm in size.

According to the results of the extraction procedure, ethanol overall responded better to antimicrobial testing. This is mostly caused by the solvent's capacity to dissolve endogenous substances. The presence of specific metabolites that may help to boost its antibacterial activity was confirmed in the current study, which demonstrated that mushroom extract had greater potential against all tested bacterial strains than those previously investigated by using (Deka *et al.,* 2017) ethanol as a solvent. The antibacterial qualities of a species are influenced by the secondary metabolites of macrofungi. Similar microbial suppression caused by saponin presence has been documented in the past. According to some researchers, the investigated species' antibacterial activity may be caused by the presence of steroids. Flavonoids have highest antibacterial activity. *Trametes elegans* has also been linked to antimicrobial activity (Sahelian, 2014). The current study came to the conclusion that *Pleurotus ostreatus* extract could be used as an antibiotic alternative to treat *B.pumilus*, *S.aureus* and *P. aeruginosa* infections.

Table 4.9: Antibacterial activity of extract of *Pleurotus ostreatus*

S. No.	Solvents Used	*S. aureus*	*P. aeruginosa*	*B. pumilus*
01	Rifampicin	27±1	20.33±0.577	35±1
02	Aqueous	21±1	0±0	0±0
03	Ethanol	13±1	17±1	25±1
04	Methanol	14±1	10.33±0.577	12.66±1.154

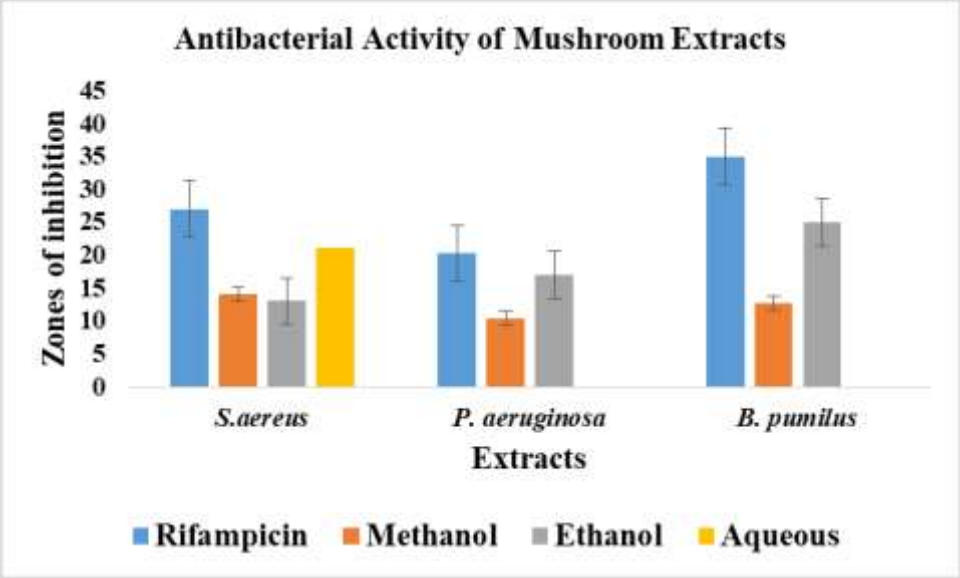

Figure 4.8: Antibacterial activity of extracts *Pleurotus ostreatus*

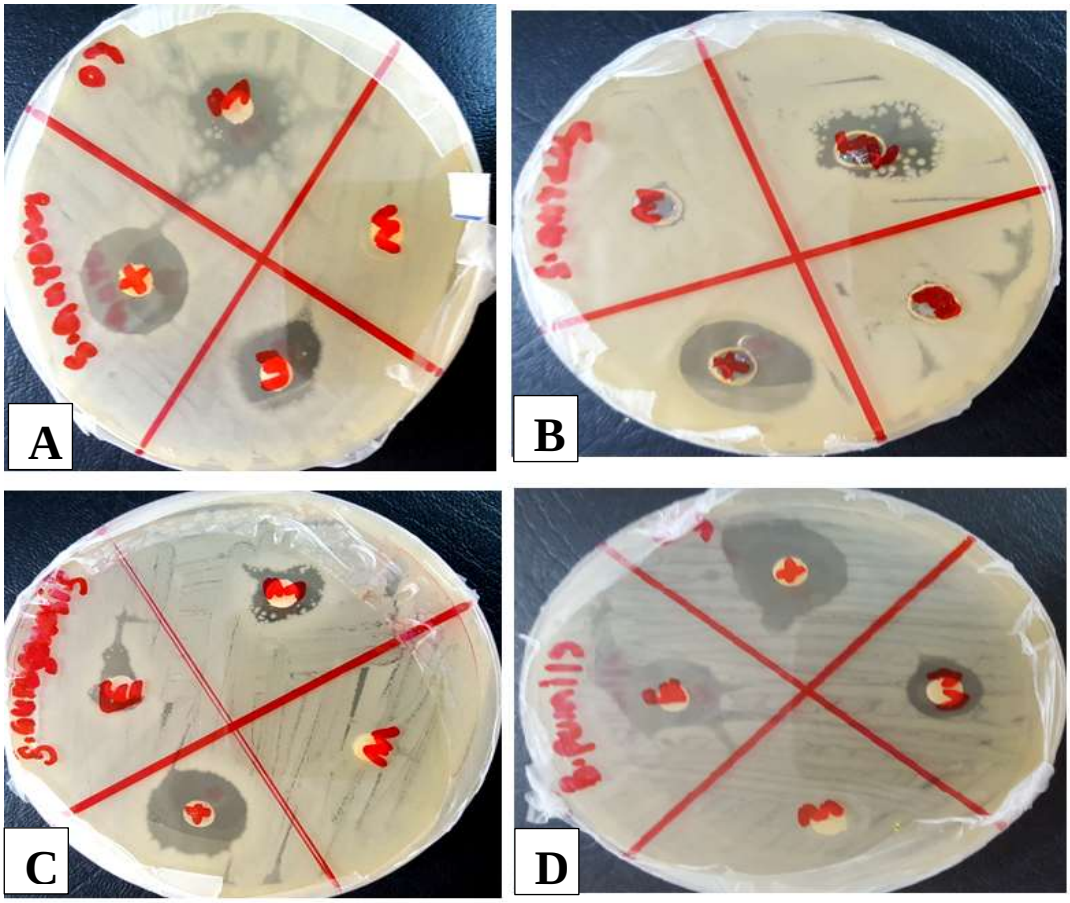

Figure 4.9: Antibacterial activity of three extracts of *P. ostreatus* against (A) *S.aureus*, (B) *S.aureus*, (C) *P. aeruginosa*, (D) *B.pumilus*

4.5 ANTIOXIDANT ACTIVITY

In Table 4.10 the results of all the samples demonstrating antioxidant activity were displayed. Each one of their antioxidant capacities varies. Methanol extract of *Pleurotus ostreatus* had the best antioxidant activity. Methanol extract demonstrated the least amount of antioxidant activity, with an IC50 value of 3. Ethanol extract had IC50 values of 8.63 while aqueous extract had 5.86 IC50 values.

Phenols and flavonoid compounds have high antioxidant activity. Because of their hydroxyl groups, phenolic compounds have been shown to be potent chain-breaking antioxidants. The total phenolic content of the mushroom *P. ostreatus* was determined in the current investigation that various mushroom extract to scavenge free radicals has been extensively tested using the DPPH free radical complex; the antioxidant present neutralizes the DPPH by transferring an electron or hydrogen atom. The color shifts from purple to yellow, and the decline in DPPH's absorbance at 517 nm reveals how much it can be reduced. The efficacy of the *P. ostreatus* aqueous extract to scavenge DPPH was assessed in relation to ascorbic acid.

Ascorbic acid is a powerful antioxidant, and therefore it was expected to have a high DPPH scavenging ability. (Du *et al*., 2009) also reported a highly significant linear association between vitamin C and DPPH scavenging ability in Actinidia fruit. Despite being much less effective than ascorbic acid (60.2%), (Jayakumar *et al*., 2009) showed a 56.2% hydroxyl radical-scavenging efficacy in *P. ostreatus* extract at a dosage of 10 mg/ml.

Due to the production of reactive oxygen species and oxidative stress, both of which are substantial contributors to the development of many diseases; natural antioxidants have become increasingly important in recent years. Through a variety of mechanisms, antioxidants counteract the effects of free radicals and shield the body from a wide range of illnesses. Since synthetic antioxidants like butylated hydroxytoluene and butylated hydroxyanisole have recently been demonstrated to be harmful to humans, antioxidants can be either natural or synthetic. As a result, natural antioxidants have drawn a lot of interest for their antioxidant properties (Gupta et al., 2006).

Table 4.10: IC50 value of extract of *Pleurotus ostreatus*

S. No.	Sample	IC50
01	Ascorbic acid	1.29± 0.017
02	Aqueous extract	5.86 ±0.060
03	Ethanol extract	8.63 ±0.034
04	Methanol extract	3 ±0.76

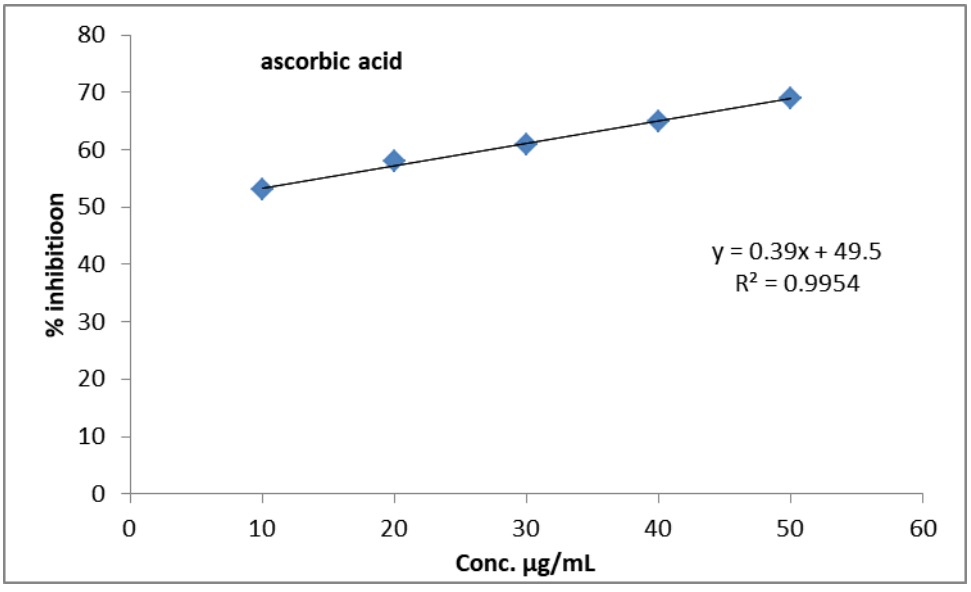

Figure 4.10: Dosage (µL) effect and antioxidant activity of standard ascorbic acid

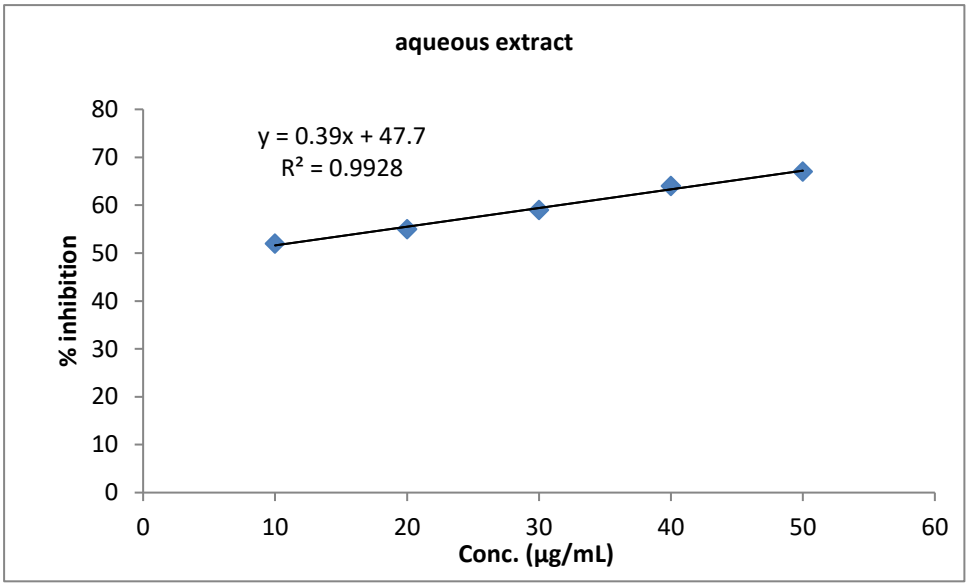

Figure 4.11: Dosage (µL) effect and antioxidant activity of aqueous extract of *Pleurotus ostreatus*

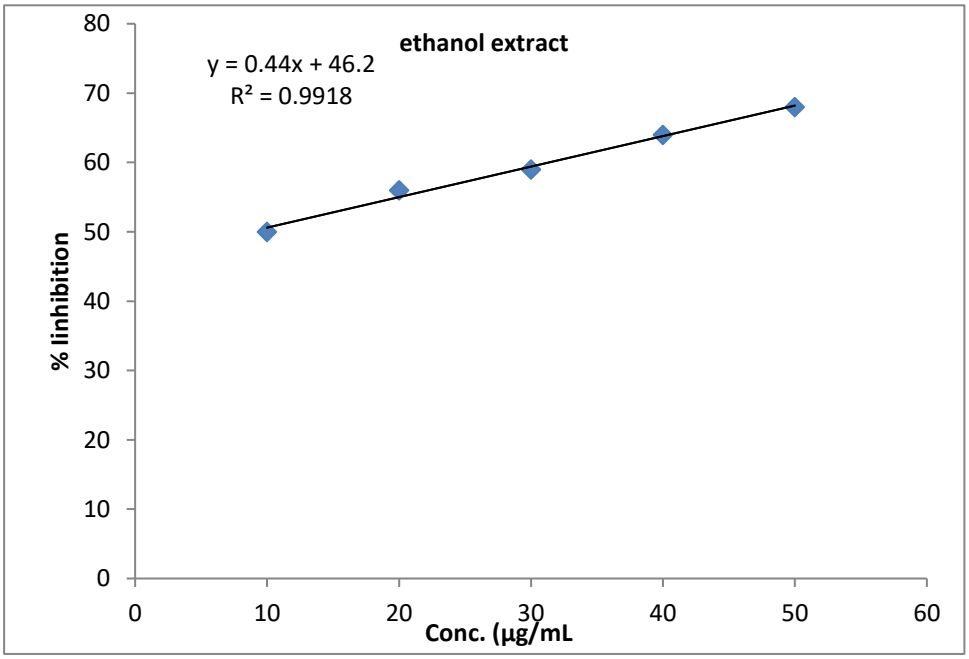

Figure 4.12: Dosage (µL) effect and antioxidant activity of ethanol extract of *Pleurotus ostreatus*

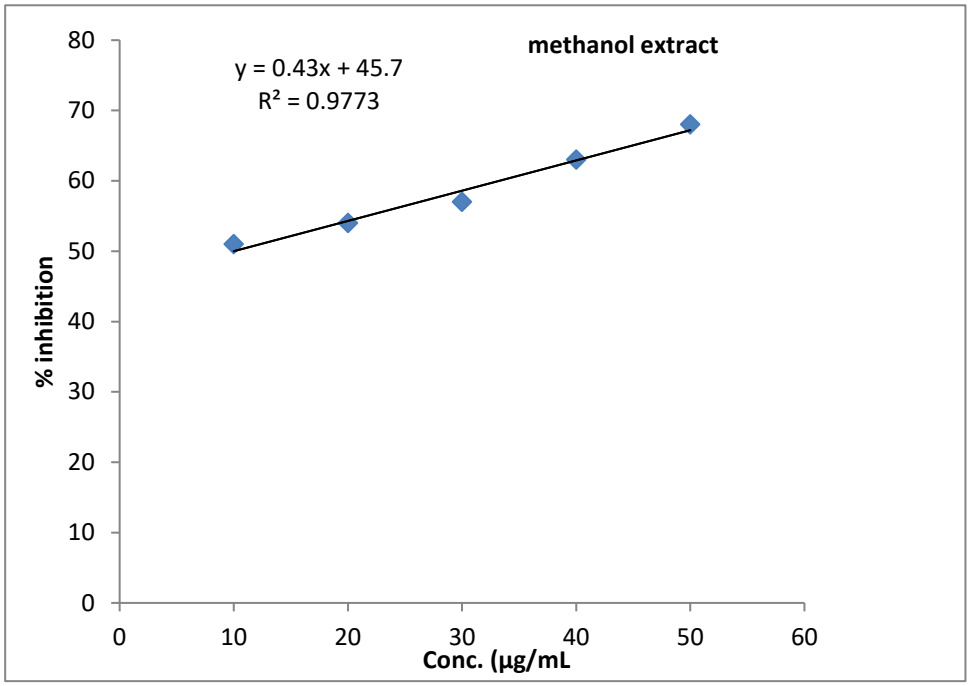

Figure 4.13 Dosage (µL) effect and antioxidant activity of methanol extract of *Pleurotus ostreatus*

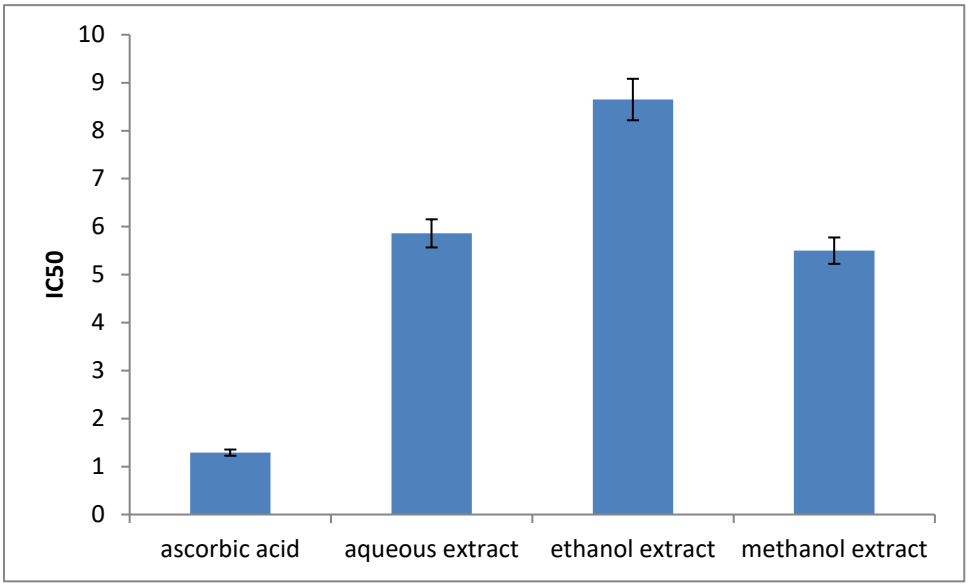

Figure 4.14: IC50 value of different mushroom extracts

CONCLUSIONS

Pleurotus ostreatus is a novel edible mushroom with significant nutritional and biological relevance. Because it includes a lot of bioactive components, it has developed a wide range of therapeutic functions. Furthermore, these mushrooms may offer important protection against malnutrition sickness due to their high nutritional properties. As a result, there have been fewer studies on extracts from farmed fungi, with the majority of research focusing on extracts from the fruiting bodies. The fact that herbal medications have no negative side effects, are inexpensive and are readily available will benefit humanity. Protein, glucose phenols, flavonoids, tannins, alkaloids, and saponin are all secondary and primary metabolites found in mushrooms. The chemicals, in mushrooms give them their antibacterial, anticancer, antipyretic, astringent, and antiviral properties, which are essential in the treatment of many disorders. The findings imply that both main and secondary bioactive chemicals have significant commercial and medicinal value.

The nutritional potential and therapeutic significance of secondary metabolites requires analysis of the basic metabolites of mushrooms. *Pleurotus ostreatus* are thought to have extract that has a wide range of changes in phytochemical content, antibacterial and antioxidant potential. The goal of the current study was to determine how valuable mushrooms are. According to the research, these plants offer a wide spectrum of antibacterial and antioxidant capabilities because of the large concentration of phenolic and flavonoids they contain. Additional investigation will result in the discovery of bioactive substances that can be employed instead of the possibility of antibiotic resistance will be reduced by the identification of bioactive substances that can be used as alternatives to manufactured antibiotics. Numerous illnesses, including Parkinson's disease and cardiovascular disease, are caused by free radicals. These extracts have great promise for treating illnesses brought on by excessive free radical production. These mushrooms can be employed in many pharmaceutical industries for the creation of medications and should be researched for a wide range of therapeutical and pharmacological properties. A thorough molecular level investigation is necessary to determine the potential of particular mushrooms for usage as a treatment for deadly diseases.

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