



EXTRACTION, PURIFICATION, CHARACTERIZATION OF BIOACTIVE COMPOUNDS OF POLYSACCHARIDES FROM EDIBLE MUSHROOM

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INTRODUCTION

The edible mushroom, with 105-209 J in 100 g-1 of fresh matter, should be classified as a dietary item due to the substantial water content and low calorific value. *P. Ostreatus* cultivars are distinguished by their medium calorific value, which is 151 J per 100 g of edible component (Manzi et al. 2001). Many cultures around the world have traditionally prized mushrooms as highly nutritious and curative foods. In Asian nations, people use mushrooms as a form of medicine, and numerous scientific studies have been done on their therapeutic properties. In Indian Ayurveda and traditional medicine, mushrooms are employed. India, which relies heavily on agriculture, generates 620 million tonnes of agro-waste annually. By using new technologies, India's current agricultural situation must become a global economic power in terms of agricultural productivity. Any type of mushroom is grown commercially in India; the most popular varieties are white button, oyster, and shiitake mushrooms. Paddy straw, milky, and reishi mushrooms are also grown on a smaller scale. About 95% of total production and exports are made up of button mushrooms. While oyster, milky, and paddy straw mushrooms are grown in tropical and subtropical locations, button mushrooms are grown in Himachal Pradesh, Jammu and Kashmir's temperate regions. The growing of mushrooms is now a commercial endeavour focused on export. India's mushrooms are primarily exported to Canada, the United States, Israel, and Mexico.

There are two types of mushrooms exported: fresh mushrooms and processed or preserved mushrooms. Over the past few years, there has been a noticeable rise in demand for processed mushrooms. They can be used in the culinary business for mushroom pickles and sauces, as well as being canned, dried, or packaged in frozen forms. The goal of India's FDI (Foreign Direct Investment) strategy is to draw capital into the technological advancement and regulated production of vegetables and mushrooms. The Indian government is working to boost mushroom research and development and to motivate mushroom producers to create cutting-edge R&D practises. In order to improve the growing environment for a higher yield and the mushroom breeding process, the R&D and biotechnology laboratories are essential **(Singh and Sidhu, 2014)**.

The Basidiomycetes group of macrofungi includes mushrooms. Epigeous and subterranean mushrooms can both grow in soil (hypogeous). Gourmet cuisine uses mushrooms as a preferred ingredient since they have a distinctive flavour and may be used to great use in cooking. There are 2000 different varieties of mushrooms, 25 of which are considered edible, and only a few are used commercially. Additionally, mushrooms are valued for their nutritive, organoleptic, and therapeutic qualities. Although their therapeutic benefits were known for a long time, they have only recently come to be appreciated. Even thousands of years ago, Chinese traditional medicine used mushrooms for their therapeutic benefits, as it still does today. Essential amino acids, minerals, proteins, and physiologically active polysaccharides are abundant in mushrooms.

New antibacterial compounds, terpenes, steroids, anthraquinones, quinolones, and derivatives of benzoic acid, as well as oxalic acid, peptides, and proteins (primary metabolites) can all be found in mushrooms. The nutritionally significant B1, B2, B12, C, D, and E tannins found in edible mushrooms make them a rich source of several nutraceuticals that exhibit the synergistic effects of many bioactive chemicals.

Since mushrooms' pharmacological potential has increased over the past few decades, they are now recognised and marketed as miniature pharmaceutical factories. Variations in strain, substrate, culture, developmental stage, age, storage conditions, processing, and cooking techniques can all have a significant impact on the concentration of physiologically active compounds. Whatever the case, mushrooms are a rich source of bioactive compounds. Acids, terpenoids, sesquiterpenes, polyphenols, lectins, alkaloids, lactones, sterols, metal chelating agents, nucleotide analogues, vitamins, glycoproteins, ergosterols, volatile organic compounds, and polysaccharides are all included as usual in the full list.

Lovastatin is one of the most significant fungal products. Lovastatin is one of many compounds that basidiomycetes produce that are crucial to the advancement of humanity's efforts to improve health and eradicate some diseases and their effects. Several fungal strains synthesise this compound as secondary metabolites that are created via the polypeptide pathway. Since lovastatin is highly effective in lowering blood cholesterol levels, it shields users from heart disease and the dangers of having high blood cholesterol (Pandey et al., 2019). As a therapy for high cholesterol, the Food and Drug Administration (FDA) originally approved lovastatin C₂₄H₃₆O₅ in 1987 under the trade name Monakolin K. Mellinolin **(Shivakumar & Ravurim, 2018)**. Mevalonate synthesis from 3-hydroxy-3-methylglutaryl CoA (HMGCoA) via 3-hydroxy-3-

methylglutaryl CoA reductase is a critical step in the body's production of cholesterol. When lovastatin binds to this enzyme, it prevents the production of cholesterol.

In addition, it helps to lower the hazardous cholesterol levels of low-density lipoprotein (LDL) and slightly increases the levels of helpful cholesterol in high-density lipoprotein (HDL) (Raj et al., 2019). Recent studies have shown that lovastatin can effectively cure a variety of illnesses, including coronary heart disease, renal disease, Alzheimer's, bone fractures, and many types of cancers due to its ability to inhibit tumour growth in vivo (Praveen et al., 2014). In this work, lovastatin will be extracted from a local fungal isolate from the environment of Iraq and its antioxidant effectiveness will be evaluated.

Edible mushrooms are famous for their wealth of nutrients, including carbohydrates, proteins, vitamins, minerals, distinctive flavour components, and other bioactive components. They are frequently used as food, flavouring agents, or traditional folk medicines. Meanwhile, due to their functional contents, products made from edible wild and farmed mushrooms have drawn significant interest for their biological capabilities, such as enhancing immunity, antioxidant, anti-cancer, and anti-viral activity.

Natural polysaccharides and their conjugates, which have long been utilised in food and medicine, have undergone extensive research into their structure and bioactivity mechanism. Numerous studies have shown that several natural polysaccharides are effective at preventing oxidative damage to human bodies during the growth and development of living organisms. As a result, natural polysaccharides are viewed as a possible source of new antioxidants, and further study into polysaccharide mechanism is required.

Mushrooms have been used as a source of medicine for countless years. The name "Lingzhi" refers to the plant *Ganoderma lucidum*, a member of the Polyporales branch of the Ganodermataceae family. The fruiting body of *G. lucidum*, also known as the "marvellous herb," has been used for centuries in East Asia. Strong anti-oxidant, immune-modulating, and anti-tumor activities have all been linked to *G. lucidum* polysaccharides (Jia et al., 2009; Lin et al., 2006; Li, Fang, & Zhang, 2007).

These biologically important components can be extracted from a variety of sources, including plants, animals, bacteria, and even microorganisms. For solid and semisolid materials, the traditional liquid extraction methods, such as stirring extraction and Soxhlet extraction, are typically time-consuming and labor-intensive. Ultrasound-assisted extraction (UAE) is a technology with higher productivity and minimal impact on the environment. It disrupts the target chemical from cells using high-frequency sound, typically above 16 kHz. According to several reports, the use of UAE has been used to separate a variety of biologically active compounds, including the anti-cancer drug camptothecin from *Nothapodytes foetida* (Fulzele & Satdive, 2005), isoflavones from freeze-dried ground soybean (Rostagno, Palma, & Barroso, 2003), phenolic compounds from alperujo (Priego-Capote, Ruiz-Jimenez, & de Castro, 2004), and astaxanthin from microorganism (Hans, Lee, Jung, & Choi, 2002).

However, there is very little evidence to suggest that UAE is used to separate the polysaccharides from *G. lucidum* are interested in mushrooms because they are a source of key substances such lectins, laccases, proteases, ribonucleases, ribosome inactivating proteins, antibacterial proteins, antifungal proteins, and

polysaccharides (Ng 2004). Numerous mushroom laccases have been documented in the literature, and many of them are members of the white rot fungus. *Pleurotus nebrodensis* has only a few publications to date, the majority of which focus on the taxonomy of the mushroom and the connection between *P. Nebrodensis* and *P. Eryngii*. According to one study, the mushroom is rich in riboflavin and vitamin B12, has a low calorie count, and has a high culinary value. *P. Nebrodensis* has only yielded one protein, a haemolysins. The goal of the current study was to isolate and characterise a laccase from the mushroom *P. Nebrodensis* and to compare its traits to those of other laccases from the genus *Pleurotus* in light of the significance of laccases.

Mushrooms have long been valued as a source of vitamins and medications. Medicinal mushrooms are a particularly diverse subset of the fungi kingdom, most of which are higher basidiomycetes. The biological activities of the secondary metabolites produced by the basidiomycetes belonging to the genera *Agaricus*, *Auricularia*, *Phellinus*, *Ganoderma*, *Pleurotus*, *Trametes*, and *Lentinus* have attracted significant scientific attention.

Mushrooms are regarded as a functional food that offers advantages to health in addition to the conventional nutrients they contain. However, understanding of the composition and nutritional worth of culinary mushrooms was restricted until the last ten years as compared to vegetables and species of medicinal mushrooms. Because gourmet mushrooms have only ever been considered a delicacy, and because their consumption has been relatively low in many industrialised nations, researchers haven't been very interested in them.

The number of original articles published each year is currently far larger than it was 10–15 years ago, therefore things have started to shift noticeably (Kalac et al., 2012). The *Pleurotus* genus produces a lot of unique “mycochemicals” in addition to an abundance of edible mushrooms.

Numerous studies from various parts of the world have demonstrated that the *Pleurotus* mushroom contains a wide range of bioactive substances, including terpenoids, steroids, phenols, alkaloids, lectins, and nucleotides. These substances have been isolated and identified from the fruit body, mycelium, and culture broth of mushrooms and have demonstrated promising biological effects (Lindequist et al. 2005). But the results are largely dispersed. We have outlined the most recent findings in this review with reference to a variety of *Pleurotus* mushroom-related topics.

Pleurotus ostreatus

Pleurotus ostreatus (Jacq.ex.fr) P.kumm is the binomial name. About 40 species of the *Pleurotus* genus are known as “oyster mushrooms” and are widely distributed in tropical and subtropical regions. They are also simple to artificially cultivate. The *Pleurotus* genus includes species such as *P. Ostreatus*, *P. Sajorcaju*, *P. Florida*, *P. Flabellatus*, *P. Highbing 51*, *P. Cystidiosus*, *P. Sapidus*, *P. Eryngii*, *P. Tuberegium*, *P. Ulmarium*, *P. Pulmonarius*, *P. Citrinopileatus*, *P. Geesteranus*, and others, some of which are of particular (Kues and Liu 2000; Chang and Miles 1989). Whereas the *P. Ostreatus* mushroom's scientific categorization was,

Taxonomic Description:

- ❖ Kingdom : Fungi
- ❖ Phylum : Basidiomycetes
- ❖ Class : Agaricomycetes
- ❖ Order : Agaricales
- ❖ Family : Pleurotaceae
- ❖ Genus : *Pleurotus*
- ❖ Species : *P. Ostreatus*

Mycological characteristics:

The fruiting body's form is mentioned in both the scientific and colloquial names. While the Latin *ostreatus* and the English common name oyster refer to the shape of the cap that resembles the bivalve of the same name, *Pleurotus* (sideways) refers to the sideways growth of the stem with regard to the cap. *P. Ostreatus* has a broad, fan-shaped or oyster-shaped cap that is 5 to 25 cm long and can be white, grey, tan, or dark brown in natural specimens. The margin is smooth when young but can sometimes be slightly lobed and wavy. Figure 1 depicts *P. Ostreatus*' fruit bodies.

Flesh: Due to the configuration of the stipes, the flesh is white, hard, and varies in thickness.

Gills: If present, the mushroom's gills, which range in colour from white to cream, descend on the stalk.

Stipe: The stipe has a lateral attachment to wood and is off-center. Spore print: A dark surface is preferable for viewing the mushroom's spore print, which ranges in colour from white to lilac-grey (Hassen et al. 2011).

A and B *Pleurotus ostreatus* fruit bodies are displayed.

**Calorific Value:**

The edible mushroom, with 105-209 J in 100 g-1 of fresh matter, should be classified as a dietary item due to the substantial water content and low calorific value. *P. Ostreatus* cultivars are distinguished by their medium calorific value, which is 151 J per 100 g of edible component (Manzi et al. 2001).

Flavour:

Although they do not have a vital role in nutrition, compounds of fungal scent stimulate the appetite and give dishes with mushrooms their distinctive flavour. Various mushroom species include about 150 aromatic chemicals. Octavalent carbonate alcohols and carbonyl compounds, such as 1-octanol, 3-octanol, 1-caprynyl-3-ol, 1-octynol-3-ol, and 1-caprynyl-3-on, are primarily to blame for the scent of the majority of edible mushrooms (Mau and Hwang 1997). The fructifications of *P. Ostreatus* are characterised by the dominance of the compound 1-octynol-3-ol (Beltran- Garacia et al. 1997). The presence of amino acids, nucleotides, and other substances like nitrogen, phosphorus, potassium, sulphur, iron, and zinc, as well as the autoxidation of unsaturated fatty acids, all have a role in the mushroom's scent (Bernas et al. 2006).

NUTRITIONAL VALUES:

There are portions of the world's population that eat cereal-based foods or reside in areas where the soil has an unbalanced mineral content, which frequently leaves them deficient in important minerals (John and Eyzaguirre 2007). The normal rhythms of numerous systems, like as metabolism, sleep, and body temperature, can be disrupted, though, which results in a number of illnesses (Mcclung 2007). Therefore, the mycelium and mushroom bioaccumulation capability of nutrients by fungi enhanced with necessary components for human health has been studied (Silva et al. 2010). *P. Ostreatus* is the second most significant mushroom that is grown for food reasons globally (Chang 1991). In terms of nutrition, it is thought to be high in protein, fibre, carbohydrates, minerals, and vitamins and low in fat. It also has a distinct flavour and aroma (Herndndez et al. 2003 and Kalmis et al. 2008). Comparing commercially grown mushrooms to their wild counterparts reveals equal nutritional value. The chemical composition of *P. Ostreatus* products varies both qualitatively and quantitatively according on the strain, origin, extraction method, and growth circumstances (Wang et al. 2001).

ACTIVE CONSTITUENTS:

Regarding the chemical components of *P. Ostreatus* and related species, numerous reports have been published. The majority of research have presented the nutritional benefits of mushrooms in the form of dried fruit bodies. Fresh *Pleurotus* mushrooms typically have a moisture content of 85–95%. (Khan 2010). *P. Ostreatus*' fruiting body has over 100 different bioactive substances, most of which are thought to represent potential novel sources of dietary fibre. Contrarily, the cell walls of fungi are abundant in non-starch polysaccharides, the most interesting functional component of which is β -glucan, and phenolic compounds like protocatechuic acid, gallic acid, homogentisic acid, rutin, myricetin, chrysin, naringin, tocopherols like α -tocopherol and γ -tocopherol, ascorbic acid, and β -carotene. (Wang et al., 2001 and Ferreria et al., 2009).

Macronutrients of P. Ostreatus.

| Nutrient | Content (g/100g dried mushroom) |
|-----------------|--|
| Proteins | 17-42 |
| Carbohydrates | 37-48 |
| Lipids | 0.5-5 |
| Fibers | 24-31 |
| Minerals | 4-10 |
| Moisture | 85-87% |

Hence to prove that the present investigation to extract, Purify and characterize the bioactive compounds of po from edible mushroom.

MATERIALS AND METHODOLOGY

COLLECTION OF SAMPLE:

The Fresh mushroom were processed from the local supermarket of Tiruvannamalai District. The bacterial isolates were collected from Arunai medical college, Tiruvannamalai. [E.Coli, Klebsiella, Pseudomonas, Shigella&Salmonella].

IDENTIFICATION OF BACTERIA

The organisms were identified using morphological and biochemical characteristics from Bergey's manual of determinative bacteriology.

EXTRACTION:

50 g of fresh mushroom was cutted into small pieces and grounded finely using ethanol, acetone for 24 hours. The extracts were filtered and then concentrated in rotary evaporator. The extracts were stored and used for further studies.

SCREENING FOR PHYTOCHEMICAL ANALYSIS:

Screening for the antimicrobial analysis of mushroom with phytochemical tests.

SCREENING OF PROTEIN BY MILLION'S TEST:

The few ml of extract added with few drops of million's reagent and heated.

SCREENING OF ALKALOIDS BY DRANGENDORFF'S TEST:

The few ml of mushroom extract added with few drops of drangendorff's reagent.

SCREENING OF CARBOHYDRATE BY BENETIC'S TEST.

Few ml of benetic's reagent with few drops of mushroom extract and heated.

SCREENING OF FLAVONOIDS BY SHINODA'S TEST:

Few ml of extract with 1gm of magnesium and few drops of concentrated HCL was added and mixed well.

SCREENING OF TREPENOID'S BY SALKOWSKI TEST:

Few ml of extract with few drops of concentrated sulphuric acid and chloroform was added.

ANTIOXIDANT ACTIVITY:**SCAVENGING DPPH RADICALS:**

By using 1,1-diphenyl-picryl-hydrazil, mushroom extract's capacity to scavenge free radicals was assessed (DPPH). Because it was changed slightly, the methodology used was nearly identical to that employed by other writers (ibanez et al., 2003; Dorman et al., 2004). 1 ml of extract and 2 ml of a 0.05 mg/ml DPPH radical solution in methanol were put into cuvettes. After vigorously shaking, the mixture was allowed to sit at room temperature for 30 minutes. After that, a spectrophotometer ("Jenway" UK) was used to detect the absorbance at 517 nm. Three substances were utilised as positive controls: ascorbic acid, butylated hydroxyanisole (BHA), and -tocopherol. The following equation was used to determine the DPPH radical concentration:

$$\text{DPPH scavenging effect (\%)} = ((A_0 - A_1) / A_0) \times 100$$

A₀ = Absorbance of the negative control

A₁ = Absorbance of the reaction mixture or standards.

When comparing the radical scavenging activity, the metric employed was the inhibition concentration at 50% inhibition (iC₅₀). Better radical scavenging was associated with a lower iC₅₀.

EXTRACTION AND PURIFICATION OF POLYSACCHARIDE:

According to Yan et al.'s methodology, fruiting body polysaccharides were extracted and purified. Essentially, fruiting bodies powder of mushrooms are suspended in distilled water at the established volume and agitated for extraction at the established temperature and time. After that, the mixture was centrifuged for 20 minutes at 5000 rpm. By evaporating the supernatant at 45 EC, it was reduced to 1/5 of its initial volume. The filtered solution was mixed with three litres of 100% ethanol, which caused polysaccharide precipitate to form. Following centrifugation at 5000 rpm for 20 min, the precipitated components were collected, and the traditional Sevag process was used to purify them. The maximum polysaccharide OUTPUT IN THESE CIRCUMSTANCES WAS 8.45 G/100 G.

Sterility check:

The silver nanoparticle of mushroom were streaked separately on Muller hinton agar and Nutrient agar to check the purity. After 24 hours of incubation the plates were used for bioassay.

PROCEDURE.

Nutrient agar and Muller hinton agar was prepared and poured in petriplates and allowed to get solidify. The silver nanoparticle of mushroom were streaked in the agar plates and incubate at 37°C for 24 hours. After incubation the plates were observed for any contamination.

The contaminated silver nanoparticle of mushroom. The contaminated silver nanoparticle were discarded.

ASSESSMENT OF ANTIOXIDANT ACTIVITY OF POLYSACCHARIDES :**ASSAY OF Fe²⁺- CHELATING ACTIVITY:**

The production of the ferrous iron-ferrozine complex was used to measure the chelating activities of polysaccharides and BHT on Fe²⁺. When combined with 3.7 mL of deionized water, various doses of polysaccharides or BHT (0.2, 0.4, 0.6, 0.8, and 1.0 mg mL⁻¹) were then subjected to a reaction with FeSO₄ (2 mM, 0.1 mL). For 30 seconds, the reaction was permitted to continue. The solution was mixed, allowed to stand for 10 minutes at room temperature, and then the mixture's absorbance was measured at 562 nm after the addition of 0.2 mL of 5 mM ferrozine. The following equation was used to determine the chelating activity of Fe²⁺.

$$\text{Chelating activity (\%)} = \frac{(A_c + A_s)}{A_c} \times 100$$

Where,

A_c = absorbance of the control (deionised water, instead of sample)

A_s = absorbance of the test sample mixed with reaction solution.

ASSAY OF ANTIBACTERIAL ACTIVITY:

Overnight cultures were kept ready for antibacterial activity.

DISC DIFFUSION METHOD:

MHA plates prepared and swabbed with bacterial isolates then sterile filter paper disc prepared from the bioactive polysaccharide of mushroom aseptically transferred into the plates with sterile forceps. The plates were incubated at 37°C for 24 hours and then measuring the diameter in millimetre of zone to which the bioactive polysaccharide inhibited the growth of organism. Chloramphenicol used as the positive control.

RESULT AND DISCUSSION

The mushroom became more popular in Indonesia when people realized their benefits. Over the years, people have consumed several varieties of mushroom, such as white oysters, shiitake, black jelly and straw mushroom, because of their benefits. Today, mushrooms have been processed and consumed in various forms as vegetables, crackers, and herbs for health purposes.

Mushrooms possess high content of qualitative protein, crude fibre, minerals and vitamins. Apart from their nutritional potential, mushrooms are also sources of physiological beneficial bioactive substances that promote good health. They produce a wide range of secondary metabolites.

Health promoting properties Eg: Antioxidant, antimicrobial, anticancer, cholesterol lowering and immunomodulatory effects have been reported, fruiting bodies and Mycelium of mushroom contains compounds with wide ranging of antioxidant & antimicrobial activities.

The present investigation was undertaken to extract, purify & characterisation the bioactive polysaccharide isolated from edible mushroom.

The bacterial samples were collected from Arunai medical college and confirmed by the staining procedure & Biochemical tests. The results were shown in **Table 1** The organisms are inoculated on the selective medium plates for the further tests. The organisms were confirmed as E. Coli, Salmonella, Pseudomonas aeruginosa, Bacillus subtilis and staphylococcus aureus.

The extract yield can be used for the study. The ethanol extract yields up to 8.8% and acetone yield up to 7.02%. The extract yield can be used as a reference to find out the amount of simply needed to make a certain number of thick extracts. Similar to our study (**EGR et al., 2019**) that the highest yield of extract was with the hexane extract, it was less than 1%. The yield on extracting mushrooms with acetate ethyl was 6.02%. The n-hexane solvent has 0.5% yield.

The Mushroom extract were screened for the phytochemical potentials it shows positive results the bioactive components presence were confirmed by the test. The results were shown in **Table 3**

The scavenging activities DPPH radicals were studied. The inhibition concentration at 50% Inhibition (I50) was the parameter used to compare the radical scavenging activity of DPPH radical the results of the reducing power assay of extracts were shown in **Table 4**. Similar to our study (**kosanic et al., 2011**) The scavenging DPPH radicals of the studied extracts was the inhibition concentration at 50% inhibition (IC50) was the parameter used to compare the radical scavenging activity. A lower IC50 meant better radical scavenging activity. Acetone and methanol extract of the tested mushrooms showed a good scavenging activity on DPPH radical. There was statistically significant difference between extracts and control ($P < 0.05$). The IC50 values of all extracts ranged from 86.279 – 262.08 μ g/ml. Acetone extract from *Russula cyanoxantha* showed

largest DPPH radicals scavenging activity ($IC_{50} = 86.272 \mu\text{g/ml}$) than those from other samples and nearly as α -tocopherol. The scavenging activity was also good for the acetone extracts from ($IC_{50} = 99.197 \mu\text{g/ml}$). Methanol extracts from tested mushroom showed better DPPH radical scavenging activities than acetone. IC_{50} for the methanol extracts were $185.70 \mu\text{g/ml}$ for *Amanita rubescens*, $192.57 \mu\text{g/ml}$ for *Cantharellus cibarius*, $172.80 \mu\text{g/ml}$ for *Lactarius piperatus* and $262.08 \mu\text{g/ml}$ for *Russula cyanoxantha*.

The reactive oxygen radicals, hydroxy radical was known as powerful radical. It could induce severe damage to adjacent biomolecules in the body, which results in cell damage that caused ageing, cancer and several other diseases. The removal of hydroxy radical was probably one of the most effective ways to defence oxidative damage of human body. Therefore, hydroxyl radical scavenging activity was the most important antioxidant mechanism. Similar to our study (**Singapore J. Chem et al., 2017**) Hydroxyl radical scavenging activity of *Pleurotus ostreatus* and *Agaricus bisporus*. With the increase of concentration, the scavenging abilities of FB polysaccharides and BHT on hydroxyl radical also increased. Hydroxyl radical; scavenging activity of P1 and P2 ranged 35.14 – 68.32 and 24.02-62.54% at 0.2 – 1.0 mg mL⁻¹, polysaccharide showed stronger scavenging activity nearly equal to BHT. These results suggested that polysaccharides better natural antioxidant than BHT in scavenging hydroxyl radical.

According to the research, some transition metals, such Fe²⁺ and Cu Co²⁺, can start a chain reaction that amplifies cellular damage. Due to its high reactivity, Fe²⁺ is the most potent pro-oxidant of these metal ions and speeds up lipid oxidation by converting hydrogen and lipid peroxidase into reactive free radicals via the Fenton reaction 9. In this experiment, ferrozine and Fe²⁺ might react to generate ferrozine-Fe²⁺ red complexes. The production of ferrozine-Fe²⁺ was disturbed when there was another chelating agent, which led to a decrease in red complexes. By measuring the reaction solution's absorbance at 562 nm, the antioxidant's Fe²⁺-chelating activity can be calculated. *Pleurotus ostreatus* polysaccharides (P1) and *Agaricus bisporus* (P2) polysaccharides are demonstrated to have Fe²⁺-chelating activity. P1 and P2 were both effective at chelating Fe²⁺, with activities ranging from 28.06 to 68.44 and 19.42 to 59.24%, respectively, at 0.2 to 1.0 mg mL⁻¹. In a dose-dependent manner, polysaccharides had greater ($p < 0.05$) Fe²⁺-chelating activity that was approximately equivalent to BHT.

The antibacterial activity of the mushroom by disc diffusion method shows that the ethanolic extract of oyster mushroom shows that highest zone of inhibition towards *Salmonella* with 21mm and Acetone extract shows that 18mm towards *E.coli*. The activity of white button mushroom shows that with ethanol extract is 18mm for *Salmonella* and 16mm for acetone extract. The results were shown in **Table 5** Similar to our study (**EGRA et al., 2019**) The antimicrobial activity of oyster mushroom extract has also conducted against one of the fungus, *C. Albicans*. The results of the assay displayed that oyster mushroom extract indicated the formation of barriers on some level zone concentration of extract. Mushroom extract on the concentration of 25 μg showed the lowest activities of anti-candida with inhibition of 8.53 mm width. Increasing inhibition activity was shown on the concentration of 50 μg with inhibition of 10.5 mm width. The best activities were indicated by mushroom extract on the concentration of 100 μg with inhibition of 10.8 mm width.

The bioactive polysaccharides have the strong reducing power and the antibacterial power. This investigation shows that the edible mushroom has the antioxidant and the antibacterial compounds which can be used as a dietary substance and also in the drug therapies.

Table 1 : Biochemical Test [IMVIC]

| ORGANISM | INDOLE | METHYL RED | VOGES PROSKAUER | CITRATE | TSI | Gram staining | Catalase | Oxidase | Motility |
|-------------------------------|--------|------------|-----------------|---------|----------|---------------|----------|---------|----------|
| <u>Bacillus subtilis</u> | - | - | + | - | A\G | + | - | + | + |
| <u>Pseudomonas aeruginosa</u> | - | - | - | + | - | - | + | + | + |
| <u>E.coli</u> | - | + | - | - | - | - | + | - | + |
| <u>Staphylococcus aureus</u> | - | - | - | + | + | + | + | - | - |
| <u>Salmonella typhimurium</u> | - | + | - | - | K\A G | - | + | - | + |

Table 2: Colony morphology of organisms

| S.No | Medium | Colony morphology | Organism |
|------|--------------------|--|------------------------|
| 1. | PLET medium | Mucoid, opaque, white colour colonies | Bacillus subtilis |
| 2. | EMB agar | Metallis sheen | E.coli |
| 3. | SS agar | Smooth and opaque or colourless | Salmonella typhimurium |
| 4. | Mannitol salt agar | Yellow in colour colonies, cocci, round in shape | Staphylococcus aureus |
| 5. | Cetrimide agar | Yellowish green | Pseudomonas aeruginosa |

Table 3 :

Phytochemical analysis of mushroom extracts

| Test | Ethanollic Extract | Acetone Extract |
|------------------------|--------------------|-----------------|
| Alkaloids | + | + |
| Carbohydrates | + | + |
| Flavonoids | - | - |
| Protein and Amino acid | + | + |
| Trepenoids | + | + |

TABLE 4(A) : Antioxidant activity of Acetone extracts

| S.No. | Sample | Ascorbic acid | BHA | α -tocopherol |
|-------|-----------------|---------------|------|----------------------|
| 1. | Oyster mushroom | 4.32 | 8.42 | 50.4 |
| 2. | Button mushroom | 3.53 | 7.5 | 62.4 |

Table 4 (B): Antioxidant activity of Ethanol extracts

| S.No | Sample | Ascorbic acid | BHA | α - tocopherol |
|------|-----------------|---------------|-----|-----------------------|
| 1. | Oyster mushroom | 5.5 | 8.4 | 62.45 |
| 2. | Button mushroom | 6.5 | 7.5 | 65.45 |

Table 5 : Antibacterial activity of oyster mushroom by Disc Diffusion method

| Organism | Oyster Mushroom (Ethanol extract) | Button mushroom (acetone extract) | Chloramphenicol |
|------------------------|--------------------------------------|--------------------------------------|-----------------|
| Bacillus subtilis | 10mm | 10mm | 20mm |
| Staphylococcus aureus | 17mm | 15mm | 23mm |
| Pseudomonas aeruginosa | 13mm | 12mm | 18mm |
| Salmonella | 21mm | 15mm | 22mm |
| E. coli | 20mm | 18mm | 20mm |

Table 6 : Antibacterial activity of white button mushroom by disc diffusion method

| Organisms | White button mushroom(ethanol extract) | White button mushroom(Acetone extract) |
|-------------------------------|--|--|
| <u>Bacillus subtilis</u> | 10mm | 15mm |
| <u>Staphylococcus aureus</u> | 11mm | 12mm |
| <u>Pseudomonas aeruginosa</u> | 16mm | 14mm |
| <u>Salmonella</u> | 18mm | 16mm |
| <u>E.coli</u> | 14mm | 13mm |

CONCLUSION

The present work is aimed to investigate that the most efficient eco-friendly product edible mushroom has the bioactive compounds which is water soluble and alcohol soluble which have the biopharmaceutical potential as well as antioxidant potential. The extracts exhibited strong ability of hydroxyl radical scavenging activity and antimicrobial activity. All the results implied that it could be a promising new natural antioxidant in drug therapy as well as in food industry. The bioactive polysaccharide have the strong reducing power the antibacterial power. This investigation shows that the edible mushroom has the antioxidant and the antibacterial compounds which can be used as a dietary substances and also in the drug therapies. Further studies are carried out to justify the potential of the bioactive polysaccharides in the food industry as well as drug therapies.

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