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ORIGINAL ARTICLE

Antimicrobial Activities and Phytochemical Constituents of Edible Mushrooms *Pleurotus Ostreatus* and *Lentinus sajor-caju*

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ABSTRACT
Mushrooms are known to possess bioactive metabolites with active pharmacological properties. Preliminary phytochemical screening of the mushroom Pleurotus ostreatus and Lentinus sajor- caju was performed. Studies on extracts of Pleurotus ostreatus and Lentinus sajor-caju revealed the presence of some essential phytochemicals which include proteins, carbohydrates, alkaloids, glycosides and saponins. These extracts may also contain bioactive compounds which also serve as potential antibacterial activity of aqueous and methanolic extracts from edible mushrooms. Pleurotus ostreatus and Lentinus sajor-caju evaluated against three model pathogenic bacteria strain S.aureus, B.subtilis, E.coli and yeast C. albicans using agar well diffusion method. The best antibacterial activity was displayed by the methanol extracts of Pleurotus ostreatus against B. subtilis (IZD = 8.00 ± 1.40mm) and S.aureus (IZD = 6.0 ± 0.11 mm) at concentration of 50mg/ml. Conversely, the aqueous extract from the mushrooms exhibited relatively weaker antibacterial activity compared to the methanol extracts. Interestingly, none of the tested extracts displayed any antifungal activity against C. albicans in these studies. These findings underscore the potential bioactive properties of Pleurotus ostreatus and Lentinus sajor-caju mushrooms and highlight their capacity to inhibit the growth of certain pathogenic bacteria. While further research is necessary to elucidate the specific bioactive compounds responsible for these effects and to explore their clinical applications, this study contributes to our understanding of the medicinal potential of these mushrooms, particularly in the context of antibacterial activity.
KEYWORDS: *Pleurotus ostreatus*, *Lentinus sajor-caju,* antimicrobial, phytochemicals, mushroom.

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INTRODUCTION

Despite the huge diversity of antibacterial compounds, bacterial resistance to first-choice antibiotics has been drastically increasing. Moreover, the association between multi-resistant microorganisms and nosocomial infections highlights the problem and the urgent need for solutions. Natural resources have been exploited in the last years and among them, mushrooms could be an alternative source of new antimicrobials. *Pleurotus* spp. possesses high contents of qualitative protein, crude fibre, minerals and vitamins. Apart from their nutritional potential, mushrooms are also sources of physiologically beneficial bioactive substances that promote good health. The search and exploration for bioactive compounds effective in treating pathogenic microorganisms resistant to present-day drugs have become very intense. Presently, there is a growing interest in searching for new antimicrobial agents from natural sources such as bacteria, fungi, and plants [1].

Natural products especially microbial and plant products constitute the major sources of new drug molecules [2]. They produce a wide range of secondary metabolites with high therapeutic value. Health-promoting properties, e.g. antioxidant, antimicrobial, anticancer, cholesterol-lowering, and immune stimulatory effects, have been reported for some *Pleurotus spp*. Both fruiting bodies and the mycelium contain compounds with wide-ranging phytochemical ingredients and antimicrobial activities [3]. Mushroom species are known to produce and release various bioactive compounds which include; alkaloids, terpenoids, flavonoids, tannins, and polysaccharides [1]. Despite the wealth of bioactive compounds possessed by mushrooms, they remain underexplored for useful natural compounds. The

bioactive compounds are found in various cellular components and secondary metabolites, which have been isolated and identified from the fruiting bodies. The fruiting bodies and mycelium of mushrooms exhibit health-promoting values such as immune-stimulatory antibacterial and antioxidative properties [1]. Because of that, the aim of this study is to examine phytochemical and antimicrobial activity of the aqueous and methanol extract of the selected *Pleurotus* spp.

MATERIAL AND METHODS

Cultivation of *Pleurotus* spp. Mushrooms

Preparation of Substrates.

The substrate preparation was achieved from modified method as proposed by Gupta S et. al. 2019 [4]. Finger paddy straw (*Oryza sativa L.*) substrates were collected from districts of Dangs. The substrates were transported to Ganpat University, India and chopped into sections of 2 to 5 cm long. These finger paddy straw, was used as cultivation substrates. The weight of substrates was measured, and they were soaked in a separate clean, freshwater plastic cane for 15 minutes. Excess water is manually removed by squeezing with the hand and is wrapped inside muslin cloth bag and is subjected to autoclave at 121°C for 15-20 minutes at 15 pascal pressure and then allowed to keep cool for 10-15 minutes in open sterilized condition to evaporate with appropriate humidity of 30-40% and allowed to cool simultaneously. The previously sterilized straw is then packed inside the polythene bag (1500 g/bag) with alternate layers of previously prepared spawn in wheat as prepared substrate. The mouths were plugged by inserting water-absorbing cotton with the help of plastic string. These autoclavable bags in Figures 1 show growth substrate processing during practical work.

Spawning

One table spoonful of mother culture grain containing mycelia of *Pleurotus ostreatus* and *Lentinus sajor-caju* was placed aseptically through the hole of each bag separately. The bags were placed in a dark cropping room at 25°C for spawn running and 17–20°C in a BOD incubator for fruiting body formation up to 21 days. Bags were sprinkled with water twice a day to maintain relative humidity. To ensure the uniformity of humidity the spawn bag was kept on a jute bag that could absorbed and retain water content required for maintaining optimum humidity. Figure 1 shows the cultivation of *Pleurotus spp*. including harvesting of fruiting bodies.

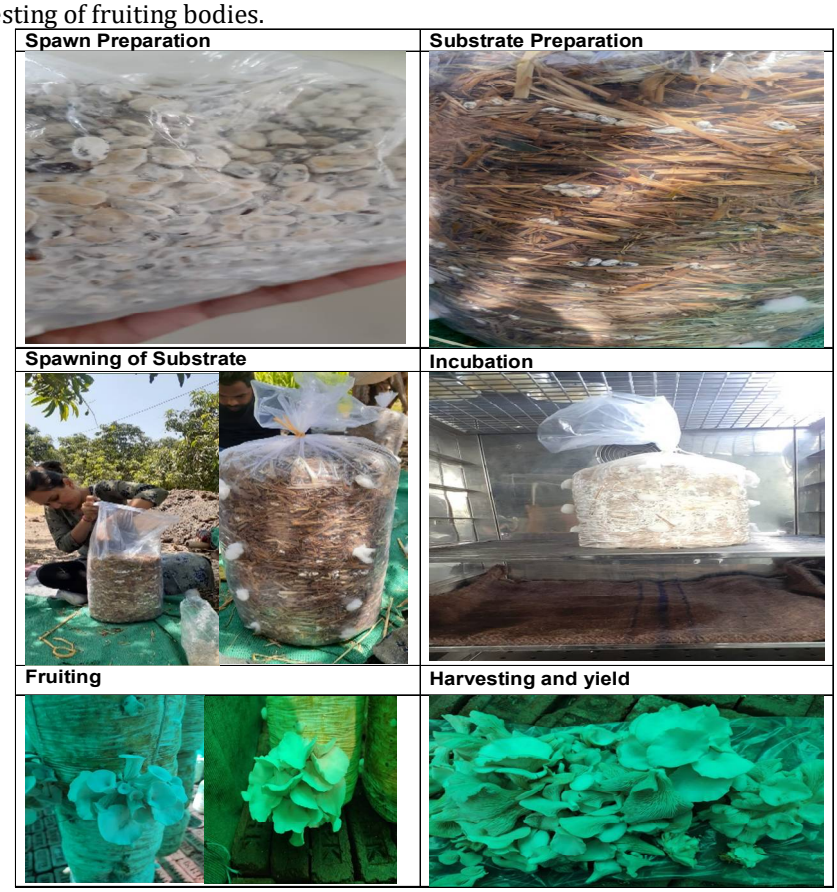

Figure 1: Cultivation of *Pleurotus ostreatus*

Fungal Growth Measurement

Days for the Spawn Running Phase

Colonization of substrates with fungal mycelia was monitored at five-day intervals. The number of days with full mycelia coverage of substrates after the day of spawning was recorded.

Days for the Pinhead Formation

After the bags were partially opened, primordia formation was observed every day, and the number of days taken for the first primordial formation was observed and recorded.

Days for the Fruiting Body Formation

The number of days taken to form the fruiting body of *Pleurotus ostreatus* and *Lentinus sajor-caju* was inspected daily after the formation of effective primordia, and the days were numerically recorded.

Pileus Diameter

The pileus diameter of the caps of *Pleurotus ostreatus* and *Lentinus sajor-caju* was measured by using the measuring ruler and expressed in centimetre (cm).

Harvesting of Mushrooms

Harvesting was performed when gills were well formed. Harvesting was performed by gently pulling or twisting the mushrooms from the substrate.

Biological Efficiency [5]

The total weight of the fruiting bodies harvested from the substrates measures the efficiency of mushrooms. Biological efficiency was calculated by the following formula:

$$\text{B.E.} = \frac{\text{FWM}}{\text{DWS}} X\ 100 \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots Eq.(1)$$

Where, BE is the biological efficiency, FWM is the fresh weight of mushrooms, and DWS is the dry weight of substrates.

Preparation of extracts

Fruiting bodies of mushroom samples were cultivated as above method from pure spores obtained from dang forests and authenticated by the taxonomist of Gujarat State. About one kilogram of harvested mushrooms was cut into small pieces with the help of a knife and sun-dried for 10 days. These dried pieces of mushrooms were then pulverized into a coarse powder with the help of a grinding machine (Boss Gold; model no.: 801) and stored in an airtight container for further use at room temperature in dark condition.

Extraction of Mushroom Crude Extracts Methanol Extracts [6]

In the sample preparation process, a total of 50 grams from each sample was accurately weighed. These samples were then subjected to successive extractions using 500 mL of 70% v/v methanol. The extraction process involved mixing the sample and methanol thoroughly, after which the mixture was allowed to stand at room temperature for duration of three days. During this period, the mixture was agitated at regular intervals.

Following the extraction period, the resulting mixture was processed to obtain the desired extracts. To achieve this, the supernatant (the liquid portion above the sediment) was separated from the mixture. This separation was accomplished through filtration, utilizing Whatman filter paper (No. 1) sourced from Sigma-Aldrich in Michigan, USA. Once the filtration process was complete, the filtrates containing the desired compounds were collected. These filtrates were then concentrated in a rotary evaporator at a controlled temperature of 40°C under reduced pressure. This concentration step helped to remove excess solvent from the filtrates. Subsequently, the concentrated extracts were subjected to lyophilization. This step is essential for preserving the extracts and maintaining their stability over time.

Finally, the processed extracts were appropriately stored in airtight containers, and each container was clearly labeled for identification. These containers were securely stored until the extracts were required for further analysis or use in the study.

Warm aqueous extraction

In the sample preparation process, 50 grams of each sample were accurately weighed and transferred into a 500 mL beaker containing heated warm water at 50°C. The beaker was then placed on a hot plate, maintaining a temperature of 70°C, and allowed to stand for a period of 4 hours to facilitate the extraction process. Following the extraction, the samples were subjected to a filtration step using a sieve cloth to separate the liquid extract from any solid residues. Subsequently, the filtrates were processed further through centrifugation at a speed of 4500 rpm for a duration of 30 minutes. This centrifugation step helped to clarify the extract by separating any particulate matter. The resulting supernatants, which were the clear liquid portions above the sediment, were collected. These supernatants were then conventionally heated to reduce their volume, ultimately reaching a final volume of 5 mL. This

concentration step aimed to condense the extract and enhance its potency. Once the extracts were prepared, they were carefully stored in airtight containers and appropriately labelled for identification. These containers were sealed to prevent any contamination or deterioration of the extracts and were stored until they were needed for subsequent use. The cultivation, including the processes of spawning and harvesting, as well as extract preparation, were conducted separately for *Pleurotus ostreatus* and *Lentinus sajor-caju.* This separation ensured the maintenance of pure strains of the selected mushrooms and helped prevent any cross-contamination between the two strains.

Preliminary phytochemical screening The freshly prepared extracts were subjected to standard phytochemical analysis to ensure the presence of the following phytoconstituents. [7,8]

Tests for Alkaloids:

Mayer's Test: This test is used to detect the presence of alkaloids in the extract. Alkaloids are a class of naturally occurring compounds found in plants, some of which have medicinal properties. In this test, a few milliliters of the extract are mixed with Mayer's reagent. If a white creamy precipitate forms, it indicates the presence of alkaloids.

Wagner's Test: Similar to Mayer's test, this test also detects alkaloids but uses Wagner's reagent. A reddish-brown precipitate that forms when the reagent is added to the extract confirms the presence of alkaloids.

Dragendorff's Test: Another test for alkaloids, Dragendorff's reagent is added to the extract. A prominent yellow/orange precipitate indicates the presence of alkaloids.

Tests for Carbohydrates:

Molisch's Test: This test is used to detect carbohydrates in the extract. Carbohydrates are essential nutrients and include sugars and starches. In Molisch's test, a few milliliters of the extract are mixed with alcoholic α-naphthol and concentrated sulfuric acid. The presence of a violet ring after standing indicates the presence of carbohydrates.

Fehling's Test: This test specifically detects reducing sugars. The extract is boiled and treated with Fehling's solutions A and B. The formation of a red precipitate indicates the presence of reducing sugars.

Benedict's Test: Like Fehling's test, Benedict's test is used to detect reducing sugars. A characteristic color precipitate is formed when the extract is heated with Benedict's reagent.

Barfoed's Test: This test is used to differentiate between monosaccharides and disaccharides. Heating the extract with Barfoed's Reagent produces a red precipitate in the presence of monosaccharides.

Detection of Glycosides:

Keller-Killiani Test: This test is used to detect cardiac glycosides, which have cardiovascular effects. Glacial acetic acid and FeCl3 are added to the extract, followed by concentrated sulfuric acid. The appearance of a reddish-brown ring at the junction of the two liquid layers indicates the presence of cardiac glycosides.

Legal's Test: Legal's test is employed to detect glycosides. The extract is made alkaline with NaOH and then treated with sodium nitroprusside. The presence of a blue coloration indicates the presence of glycosides.

Detection of Proteins and Amino Acids:

Millon's Test: This test detects proteins. Millon's reagent is added to the extract, and the formation of a white precipitate indicates the presence of proteins.

Biuret Test: The Biuret test is used to detect proteins. An aliquot of the extract is heated with CuSO4 solution and ethanol, followed by KOH pellets. A pink color in the ethanolic layers indicates the presence of proteins.

Test for Detection of Flavonoids:

Shinoda Test (Magnesium Hydrochloride Reduction Test): This test is used to detect flavonoids. Magnesium ribbon and concentrated HCl are added to the test solution. The appearance of various color changes indicates the presence of flavonoids.

Alkaline Reagent Test: In this test, NaOH is added to the test solution. The formation of an intense yellow color, which turns colorless upon the addition of dilute acid, indicates the presence of flavonoids.

Detection of Triterpenoids and Steroids:

Libermann Burchard's Test: This test is used to identify the presence of steroids and triterpenoids in the mushroom extract. First, a few drops of acetic anhydride are added to the extract, and the mixture is boiled and cooled. Then, concentrated sulfuric acid is added to the mixture from the sides of the test tube. The appearance of a brown ring at the junction of two layers and the upper layer turning green indicate the presence of steroids, while the formation of a deep red color indicates the presence of triterpenoids.

Salkowski Test: This test is also used to detect steroids and triterpenoids. The extract is treated with a few drops of concentrated sulfuric acid, shaken well, and allowed to stand for some time. A red color

appearing in the lower layer indicates the presence of steroids, and the formation of a yellow-colored lower layer indicates the presence of triterpenoids.

Test for Detection of Phenolic Compounds and Tannins:

Ferric Chloride Test: This test is employed to identify phenolic compounds in the mushroom extract. A few drops of a neutral 5% ferric chloride solution are added to 5 mL of the extract. The appearance of a dark green color indicates the presence of phenolic compounds.

Lead Acetate Test: This test is used to detect phenolic compounds as well. To 5 mL of the extract, 4 mL of a 10% lead acetate solution is added. The formation of a bulky white precipitate indicates the presence of phenolic compounds.

Test for Saponins by Froth Test:

This test is utilized to determine the presence of saponins in the extract. 5 mL of the extract is vigorously shaken with 8 mL of distilled water in a test tube for 30 seconds and then left undisturbed for 20 minutes. The persistence of froth (bubbles and foam) on the surface of the liquid indicates the presence of saponins.

Test for Anthraquinones:

Anthraquinones are identified using this test. 2 mL of the extract is shaken with 10 mL of benzene. The solution is then filtered, and 5 mL of a 10% NH4OH solution is added to the filtrate. The appearance of a pink, red, or violet color in the ammoniacal (lower) phase indicates the presence of anthraquinones.

Estimation of Total Phenolic Content [8]**:**

The quantification of total phenolic compounds in the mushroom extracts was determined using a colorimetric assay, which was based on the procedures originally described by Singleton and Rossi in 1965 [9], with some modifications. Phenolic compounds are known for their antioxidant properties. To measure the phenolic content, 1 mL of the mushroom extract, prepared at a concentration of 1000 µg/mL in aqueous extract, is mixed with 5 mL of Folin-Ciocalteu reagent. After a 5-minute reaction period, 4 mL of a 7.5% sodium carbonate (Na_2CO_3) solution is added. The reaction proceeds for 2 hours at room temperature. After this, the absorbance of the solution is measured at a wavelength of 765 nm using a spectrophotometer. A blank sample, containing all reagents except the mushroom extract, is used as a reference. This entire process is performed in triplicate and repeated three times for each extract. The quantification of total phenolic content is determined using a standard curve constructed with Gallic acid. The results are expressed as milligrams of Gallic acid equivalents (GAE) per gram of dry extract.

The formula $C = C_1 \times V/m$, is used to calculate the concentration of total phenolic compounds in the mushroom extracts based on the results of the colorimetric assay. This quantification is valuable for assessing the potential health benefits of the mushroom extracts due to their antioxidant properties.

These tests are essential tools for researchers and pharmacologists to identify specific classes of compounds in plant or mushroom extracts, which can help in understanding their potential medicinal properties. The detailed procedures ensure accurate results in phytochemical screening. Additionally, the estimation of total phenolic content provides valuable information about the antioxidant potential of the extracts, which is crucial in evaluating their health benefits.

Quantitative estimation of Nutraceutical components using KjelTRON 6M:

The bioactive extracts obtained from the mushrooms were subjected to a screening process to determine the presence of various biochemical components, including proteins, carbohydrates, fibers, and lipids. This biochemical profiling of the selected mushroom species was carried out using a KjelTRON-KjelDIST EA instrument, specifically Model No.: KDIGB 6M.

The following steps were performed during the analysis:

1. Preparation of Catalysis Mixture: A catalysis mixture was prepared by combining $CuSO_4$ and K_2SO_4 in a proportion of 1:5, typically ranging from 0.1 to 0.5 grams of this mixture.
2. Acid Addition: In each clean and dry tube, 10 mL of concentrated H_2SO_4 (sulfuric acid) was added. One of these tubes was designated as the blank for reference.
3. Activation: The instrument was switched on, and the catalysis mixture was loaded into the tubes containing H_2SO_4.
4. Scrubber System: The scrubber system was immediately activated to ensure the homogeneity and prevent stickiness.
5. Instrument Operation: The power button was pressed, followed by the run button to initiate the analysis process.
6. Completion of Process: Once the process was completed, the display on the instrument indicated "process over," and the instrument was switched off.
7. Waiting Period: The tubes were left for one hour to allow the color of the sample to turn bluish green.

8. Cooling: After an hour, the tubes were removed from the instrument and placed in a cooling stand.

Before initiating the analysis, it was ensured that the bottles of the scrubber system were filled with distilled water (1 litre) and a 15% NaOH (sodium hydroxide) solution (1 litre), respectively.

Estimation of total % Nitrogen and % protein content

Ensured that the top aspirator was filled with distilled water and aspirator was in on condition before switching on the main switch of protein distillation system. Opened the raw tap water for condensation and switched on the main switch of protein distillation system. When steam was ready after 10-15 minutes (showed in display), put 40% NaOH containing flask at right side of the system. Continuous pressed alkali button for 4 to 5 seconds and removed the tube. First loaded the blank tube in the distillation system by using the slider mechanism. Took a flask and added 25ml 4% boric acid and 5 drops each (methyl red & bromocresol green). Selected the sample mode 1 and started protein distillation by pressing the run button.

After run, the sequence program started as follow:

Table 1: Sequence program RUN command in KjelTRON 6M

Steps in Seq No.	Program No.	Sample dilution command
1	01	Sample dilution- 01
2	01	Sample dilution -02
3	01	Alkali (NaOH) -14
4	01	Neutralizing – 02
5	01	Process/steam injection - 06 or 07
6	01	Sample settle time – 02
7	01	Process Over

After the operation process was completed, an intimation display arrived. The flask was titrated as distillate sample with 0.1N HCl. After the process was finished, the tube were taken out and loaded in another digestion tube, repeating the process for all digestion tubes. Took a 500ml beaker with water, using the manual addition button for alkali, rinsed thoroughly the reagent in the hoses for 30 seconds. Unloaded the distillation tube and disposed of the collected residue. Took 100ml of distilled water and loaded it into the distillation tube by pressing the manual addition button to initiate steam distillation and let it run for 3 minutes. Pressed the same button again to switch off the steam. Finally, switched off the distillation system. Calculated total nitrogen % content and % protein content using following formulae:

$$\% \text{ of Nitrogen} = \frac{14 \times \text{Normality of Acid} \times \text{Actual titrant value} \times 100}{\text{Sample weight} \times 1000}$$

$$\% \text{ of Protein} = \% \text{ of Nitrogen} \times \text{Power Factor (6.25)}$$

Fat estimation using FibroTRON system:

For using FibroTRON system the sample must be de-fated. Weighed the mushroom sample accurately and noted down the weight (W). Weight of the dry mushroom must be 0.50 to 2.5 gm. Took 4 piece of clean cloth and added the sample into it. Then stapled the cloth properly to transfer mushroom samples in different crucibles. Placed the crucible into rubber adaptors of FibroTRON extraction unit and ensured proper sealing of crucible against the adaptor rubber.

Acid Wash

100 ml of 1.25% H_2SO_4 was poured into the extractors from the top of the system. Pressed the power button and run button to start the process. After 30-35 minutes, when the display showed "process over," drained the acid by rotating the knob to the ON side while confirming that the pressure knob was set to ON. Once the acid was completely drained, turned OFF all the knobs and added distilled water to all tubes for 3-4 minutes. Drained the distilled water by rotating the knob to the ON side while keeping the pressure knob ON. For the alkali wash, poured 100 ml of 1.25% NaOH into the extractors from the top of the system and switched on the instrument and pressed the run button to start the process. After 30-35 minutes, when the display showed "process over," drained the alkali by rotating the knob to the ON side while simultaneously turning the pressure knob. Once the alkali was completely drained, all knobs were turned OFF and distilled water was added to all tubes after 3-4 minutes. Drained the distilled water by rotating the knob to the ON side along with the pressure knob. After the alkali wash, took out the crucibles and dried them in a hot air oven until they became free from moisture. The hot crucibles were cooled to room temperature using a desiccator and weighed all the crucibles and recorded the readings (W1). Then placed all the crucibles in the muffle furnace at 450˚C for 5-6 hours. After ashing, cooled the hot crucibles to room temperature using desiccators. Finally, weighed the crucibles again and recorded the readings (W2):

$$\% \text{ of Crude Fiber} = (W1 - W2 / W) \times 100 \quad \text{Eq. (3)}$$

Where, Initial sample weight = W After hot air oven sample weight =W1
After muffle furnace sample weight = W2

Antibacterial activity of Mushrooms

A Total of three test bacterial species and yeast were tested to evaluate antimicrobial activity of selected mushrooms. The Gram-positive species were *Staphylococcus aureus* MTCC-2408 and *Bacillus subtilis* MTCC-736 while the Gram-negative species was *Escherichia coli* MTCC-1650 in addition, the study also include yeast was *Candida albucans* ATCC-10231.

Agar well diffusion assay [10]

Agar well diffusion techniques, as described by Gashaw G. *et. al.,* [10] were adopted for the study. Mueller Hinton agar plates were inoculated with 0.1 ml of an overnight broth culture of each bacterial isolate (equivalent to 3 x 10^7 CFU/ml according to the McFarland standard) in a sterile Petri dish. The seeded plates were gently rocked for uniform distribution of the isolates and allowed to set. Wells with an 8 mm diameter were created on the plates using a standard sterile cork borer. Equal volumes (100 µl) of the test extracts were transferred into the wells using a micropipette. The experiments were carried out in triplicate. The plates were then incubated at 37°C for 24 hours, and observations were made until a marked decline in the potency of the extracts to inhibit the growth of the test isolates was observed. The zones of inhibition were measured in millimeters (mm), and the average values were calculated and recorded. For control purposes, Chloramphenicol and Streptomycin were used as standard antibiotics against selected Gram-positive and Gram-negative bacteria cultures, respectively. Ketoconazole was used as a control for the antifungal assay.

Statistical analysis

Experimental values are represented as means ± standard deviation (SD). Statistical significance was determined by one-way variance analysis (ANOVA), with significant difference considered at $P<0.05$. The Microsoft Excel (2016) and SPSS version 20 software were used for analysis. All experiments were conducted in triplicates

RESULT AND DISCUSSION

Fungal Growth Measurement

Pleurotus ostreatus and *Lentinus sajor-caju* were grown on various crop residues as substrates. The growth performance of *Pleurotus ostreatus* and *Lentinus sajor-caju* on substrate alone is depicted in table 2 and figure 2.

Spawn Running Phase

The days for full spawn run ranged from 24-28 days and 21-34 days for *Pleurotus ostreatus* and *Lentinus sajor-caju* respectively. The lowest growth period (21 days) for primordia initiation was recorded on paddy straw alone, statistically significant with the longer duration (34 days) for primordia development recorded on paddy straw as substrate (Figure 2a).

Pinhead Formation

The maximum number of days taken from pinhead formation to fruiting bodies' formation exhibited a significant difference of 3 days between *Pleurotus ostreatus* and *Lentinus sajor-caju* species (Figure 2b).

Fruiting Body Formation

The bags of treatments took four to twelve days after primordial formation to maturation of fruiting bodies. There are no statistically significant variations between days taken for fruiting body formation among different treatments of substrates (Figure 2c).

Pileus Diameter

In our experiment, the pileus diameter was the highest (4.58 cm) on paddy straw which was significantly different from the lowest (2.95 cm) diameter observed on *Pleurotus ostreatus.* For *Lentinus sajor-caju* the pileus diameter was found maximum of 3.62cm and minimum of 2.49 cm on paddy straw as a substrate.

Fresh Weight of Fruiting Bodies

The highest (253.07 g) fresh weight of harvested mushrooms was recorded on paddy straw alone, and the lowest (101.48 g) was recorded for *Pleurotus ostreatus* and for *Lentinus sajor-caju* higher fresh weight harvested was recorded as 216 grams harvested per fixed volume of taken substrate (1500 gm).

Figure 2: Cultivation of *Pleurotus ostreatus*
a. Spawning growth (Mycelial); b. Pin Head c. Fruiting bodies

Biological Efficiency

Biological efficiency varied significantly among the substrates used. The highest percentage of biological efficiency (50.20%) was reported from paddy straw which was significantly different from the least (20.80%) observed in *Pleurotus ostreatus*. Similarly for *L sajor-caju* highest percentage of biological efficiency (70.32%) was reported from paddy straw which was significantly different from the least (24.18%) observed.

Table:2. Fungal Growth Measurement of *Pleurotus ostreatus* and *Lentinus sajor-caju*

Parameters of Growth	**Mushroom species**	
	Pleurotus ostreatus	*Lentinus sajor-caju*
Spawn running time	24-28 days	21-34 days
Appearance of pinheads	19 days	22 days
Fruiting body formation	24-28 days	21-34 days
Pileus Size in diameter	2.95-4.58 cm	2.49-3.62 cm
Fresh weight of fruiting bodies	101.48-253.07/1500 gm substrate	216 grams/1500 gm substrate
Biological Efficiency	20.80-50.20 %	24.18-70.32 %

Preliminary qualitative phytoconstituents analysis

The qualitative phytochemical analysis of *Pleurotus ostreatus* and *Lentinus sajor-caju* indicates that these mushrooms are rich sources of various phytoconstituents, including carbohydrates, glycosides, triterpenoids, and phenolic compounds. These findings are consistent with previously reported literature, suggesting that these mushrooms have a known history of containing these chemical components. This information highlights the potential nutritional and bioactive properties of *Pleurotus ostreatus* and *Lentinus sajor-caju*, which can be valuable for various applications, including in the food and pharmaceutical industries.

Table.3: Phytochemical analysis of edible mushrooms *P. ostreatus* & *Lentinus sajor-caju*

Phytochemical Tests	***Pleurotus ostreatus***		***Lentinus sajor-caju***	
	(Methanol)	**(Aqueous)**	**(Methanol)**	**(Aqueous)**
Alkaloids				
- Mayer's Test	+	+	+	+
- Wagner's Test	+	+	+	+
- Dragendorff's Test	-	+	-	-
Carbohydrates				
- Molisch's Test	+	+	+	+
- Fehling's Test	-	-	-	-
- Benedict's Test	+	-	-	-
- Barfoed's Test	+	+	+	+
Glycosides				
- Legal's Test	+	+	+	+
- Keller-Killiani Test	+	-	-	-
Proteins and Amino Acids				
- Millon's Test	+	-	+	-

- Biuret Test	+	+	-	-
Flavonoids				
- Shinoda Test	-	-	-	-
- Alkaline Reagent Test	+	+	+	+
Phytosterols				
- Libermann-Burchard's Test	-	-	-	-
Triterpenoids				
- Libermann-Burchard's Test	+	+	+	+
- Salkowski Test	+	+	+	+
Phenolic Compounds and Tannins				
- $FeCl_3$ Test	+	+	+	+
- Lead Acetate Test	+	+	+	+
Saponins				
- Frothing Test	+	+	+	+
- Emulsion Test	+	+	+	+
Anthraquinones				
- NH_4OH Test	-	-	-	-

*The symbols (+) indicate a positive test result, (-) indicates a negative result

The table 3 provides a clear overview of the results of phytochemical tests for each type of extract, including methanol and aqueous, for both *Pleurotus ostreatus* and *Lentinus sajor-caju*. The phytochemical analysis of edible mushrooms, *Pleurotus ostreatus,* and *Lentinus sajor-caju,* revealed the presence of several major phytoconstituents. These include alkaloids, saponins, steroids, phenols, glycosides, terpenoids, and flavonoids. The analysis was conducted using two different solvents for extraction: methanol and aqueous. It was observed that the methanol extract contained a greater number of phytoconstituents compared to the aqueous extract. Figure 3 demonstrates the results of biochemical tests that were performed to confirm the presence of these phytochemicals in *Pleurotus ostreatus* and *Lentinus sajor-caju*. These tests are important for characterizing the chemical composition of these mushrooms, as the presence of these phytoconstituents can have various implications for their potential use in food, medicine, or other applications. The results provide valuable information about the bioactive compounds present in these mushrooms, which may contribute to their nutritional and medicinal properties.

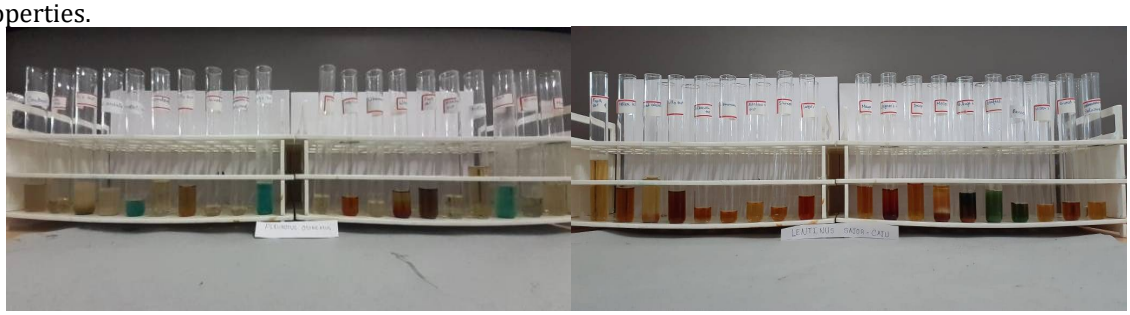

Figure 3: Phytochemicals presence in *Pleurotus ostreatus* and *Lentinus sajor-caju*.

Quantitative estimation of Total Phenolic Content

The Folin–Ciocalteu reagent is a used to measure the amount of phenolic compounds in a sample [11]. When added to a sample containing phenols (like those found in plant extracts), the reagent causes a color change. This reaction occurs under alkaline conditions provided by sodium carbonate. The intensity of the resulting blue color is directly related to the amount of phenolic compounds in the sample. A spectrophotometer is used to measure color change and calculate the phenolic content, often expressed in milligrams of Gallic acid equivalents (GAE) per gram of the sample. This method helps assess the antioxidant potential of natural products.

Table.4: Total Phenolic Content of edible mushrooms *P. ostreatus* & *Lentinus sajor-caju*

Mushroom	Unknown Absorbance (OD at 620 nm)	Gallic acid equivalent (µg/ml)	Total Phenolic Content (µg/g)
Pleurotus ostreatus	0.381	179.017	179.017
Lentinus sajor-caju	0.576	269.057	269.057

The results, as presented in Table 4, indicate the mean total phenolic content of the extracts. This content was measured using the Gallic Acid Equivalent (GAE) equation, which is expressed as $y = 0.0021x + 0.016$, with a coefficient of determination (R^2) of 0.9846. The quantitative determination of the total phenolic content is expressed as milligrams of Gallic Acid equivalent per gram of dry weight of the sample (mg GAE/g dry extract). Specifically, for *P. ostreatus*, the phenolic content was found to be 179.01 mg GAE/g dry extract, while for *Lentinus sajor-caju*, the phenolic content was significantly higher at 393.72 mg GAE/g dry extract. These findings provide valuable information about the concentration of phenolic compounds in the studied mushroom extracts. Phenolic compounds are known for their antioxidant properties, so a higher phenolic content suggests that *Lentinus sajor-caju* may have a greater potential for antioxidant activity compared to *P. ostreatus*. These results can be important for various applications, including the development of functional foods or natural products with health benefits.

Quantitative estimation of Nutraceutical components using KjelTRON 6M:

Table 5: Values of Nutraceutical components using KjelTRON 6M

Nutraceutical Ingredient	*Pleurotus ostreatus*	*Lentinus sajor-caju*
Total Protein (gm%)	25.20± 1.251	22.84± 1.059
Nitrogen content (gm%)	4.032± 2.052	3.256±0.267
Total Fat (gm%)	0.01± 0.003	0.01±0.001
Fibers (gm%)	50±2.057	44±2.438

The result (Table 5) shows the presence of consistent number of macromolecules of nutraceutical importance. Despite of variation in the gm% composition, both the selected mushrooms serve to have enough amount of fibre content along with total protein content which signifies the nutraceutical importance. The selected mushrooms contain lesser amount of fat approx. 0.01gm% which could be focused for healthy diet consumption in patients with elevated cholesterol levels.

Antimicrobial Activity

The development of our understanding of drugs has progressed alongside scientific and social advancements. Drugs derived from macro fungi, such as mushrooms, have gained attention for their effectiveness, ready availability, affordability, and relatively low occurrence of side effects. In the search for potential antibacterial and antifungal compounds from mushrooms, initial screening is often conducted using crude extracts [12]. One of the most commonly employed methods for assessing the antimicrobial potential of these extracts is the disc or agar well diffusion assay. This method involves creating zones of inhibition on agar plates, which helps researchers determine the susceptibility of microorganisms to the compounds present in the mushroom extracts.

The antibacterial activity of various extracts from *Pleurotus ostreatus* and *Lentinus sajor-caju* was tested against four human bacterial pathogens such as *Candida albicans* ATCC-10231, *E. coli* MTCC-1650, *Bacillus subtilis* MTCC-736, and *Staphylococcus aureus* MTCC-2408.

The results, as shown in Table 6, indicate that the methanolic extract was the most effective against these bacteria. Specifically, the maximum antibacterial activity of the methanolic extract from *Pleurotus ostreatus* was observed against *Bacillus subtilis* MTCC-736, with a zone of inhibition measuring 4.8 mm. On the other hand, the minimum antibacterial activity (4.0 mm) was observed against *Candida albicans*. Interestingly, the methanolic extract showed a similar antibacterial activity (4.5 mm) against *E. coli*, which aligns with the findings of a previous study [13,14] that reported the ethanol extracts of *P. eryngii* exhibited activity against different bacteria. This suggests that the mushrooms may contain a broad spectrum of antibiotic compounds, and the choice of solvents and test organisms can influence the results. Notably, the inhibition activity against *Staphylococcus aureus* MTCC-2408 remained consistent whether methanol or aqueous extracts were used. This information provides insights into the potential antimicrobial properties of these mushroom extracts, which could have applications in various fields, including medicine and food preservation.

Table 6: Inhibition zone Diameter (mm) at different concentration of mushrooms

Mushroom	Extract	Test Organism	Inhibition zone Diameter *IZD (mm) of different concentration		
			100 µl	50 µl	25 µl
Pleurotus ostreatus	*Methanol*	*E. coli* MTCC-1650	4.5	4.0	4.0
		Bacillus subtilis MTCC-736	4.8	4.0	4.0
		Staphylococcus aureus MTCC-2408	4.1	4.0	4.0
		Candida albicans ATCC-10231	4.0	4.0	4.0
	Aqu	*E. coli* MTCC-1650	4.0	4.0	4.0
		Bacillus subtilis MTCC-736	4.0	4.0	4.0
		Staphylococcus aureus MTCC-2408	4.1	4.0	4.0
		Candida albicans ATCC-10231	4.2	4.0	4.0
Lentinus sajor-caju	*Meth*	*E. coli* MTCC-1650	4.3	4.0	4.0
		Bacillus subtilis MTCC-736	4.2	4.0	4.0
		Staphylococcus aureus MTCC-2408	4.0	4.0	4.0
		Candida albicans ATCC-10231	4.2	4.0	4.0
	Aqu	*E. coli* MTCC-1650	4.1	4.0	4.0
		Bacillus subtilis MTCC-736	4.0	4.0	4.0
		Staphylococcus aureus MTCC-2408	4.0	4.0	4.0
		Candida albicans ATCC-10231	4.2	4.0	4.0

Note: *Chloramphenicol and Streptomycin (10 µg/ml) showed 22 mm IZD and 21 mm IZD as standard antibacterial agent and ketoconazole (50 µg/ml) as standard antifungal showed 13 mm IZD which served as reference antimicrobial agents against test organism.*

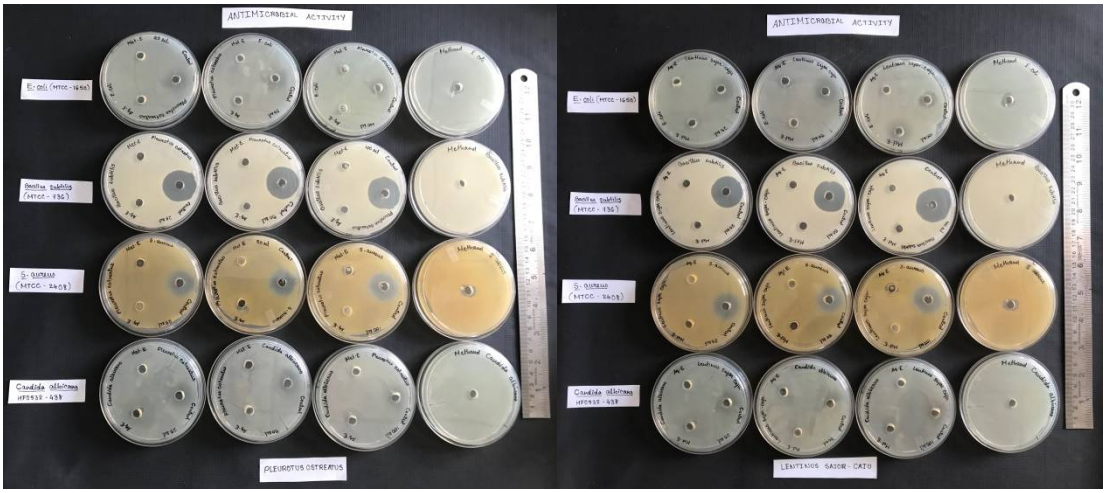

Figure 4: Antimicrobial activity of *Pleurotus ostreatus and Lentinus sajor-caju*

The antibacterial activity of the mushroom samples showed variations depending on the solvents used, as illustrated in Figure 4. The data obtained in this study also supports the observation that, similar to plant extracts, mushroom extracts tend to be more effective against Gram-positive bacteria compared to Gram-negative bacteria. This pattern has been consistently reported in numerous previous studies.

In summary, the choice of solvents for extracting bioactive compounds from mushrooms can impact their antibacterial activity. Additionally, the preference for inhibiting Gram-positive bacteria over Gram-negative bacteria, as observed with mushroom extracts, aligns with existing scientific literature and is an important consideration in the evaluation of the antimicrobial potential of these natural products.

CONCLUSION

Mushrooms can be cultivated in laboratory on an optimized medium to achieve consistently better yield. A phytochemical study reveals the presence of alkaloids, carbohydrates, glycosides, proteins, flavonoids, phenolic compounds, saponins and tanins in both *P. ostreatus* and *L. sajor-caju.* However the results are negative in terms of presence of phytosterols and anthraquinones. Quantitative assays supports the presence of 25.20±1.251 gm% and 22.84±1.059 gm% of total protein; 4.032±2.052 gm% and

3.256±0.267 gm% of total nitrogen content; 0.01±0.003 gm% and 0.01±0.001 gm% of fat with 50±2.057 gm% and 44±2.438 gm% in *P. ostreatus* and *L. sajor-caju* respectively. The extracts of *P. ostreatus* and *L. sajor-caju* prepared with methanol revealed potential antimicrobial activities against Gram positive bacteria (*S. aureus* and *B. subtilis*), with the best activity against *B. subtilis*. However, 75% of the extracts studied had no antagonistic effect against any of the test bacteria or yeast used in this study. This study justifies the antibacterial claims and usage of mushrooms as herbal treatment. Nevertheless, there is need to further fractionate, identify and characterize the bioactive compounds embedded in the mushrooms, to maximize their pharmaceutical potentials and applications.

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