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Advances in Bioresearch

ORIGINAL ARTICLE

Evaluation of antimicrobial activity and bioactive compounds from *Pleurotus eous* cultivated on a household waste based substrate

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ABSTRACT

Pleurotus eous or the pink oyster mushroomis an edible oyster mushroom which is delicious, nutrient rich and belongs to the third most importantly cultivated mushroom species as a food source. The present study aimed at the vegetable waste based cultivation and nutrient content analysis of Pleurotus eous, screening and quantification of secondary metabolites and anti-microbial screening. The vegetable waste based cultivation gave a maximum yield of 570g/mushroom bed. The moisture, ash, crude fat and crude fibre ranged between 4.61 to 88.24%.The protein, carbohydrate and lipid content was found to be 65%, 30% and 6.5% respectively. Biochemical screening of the mushroom extract showed the presence of alkaloids, flavonoids and tannins. Quantitative analysis showed that the secondary metabolites of acetone extract had the highest alkaloid content of 9.36mg/g, flavonoid content of 3.5mg/gand tannin content of 10.46mg/g, followed by ethanolic and water extracts. HPLC analysis of the mushroom extracts showed the highest lovastatin content of 16.6µg in acetone extract. The mushroom extracts were screened for antimicrobial activity against bacterial and fungal strains that cause local infections by MIC method. Among all the bacterial strains tested, lowest MIC values were obtained for Pseudomonas aeruginosa and Enterococcus faecalis, followed by Klebsiella pneumoniae and Enterobacter cloacae. Whereas in the anti-fungal screening, the largest Zones of Inhibition where found against Aspergillus flavus, followed by Aspergillus niger. The results show that the vegetable waste cultivation has aided in improving the nutrient content of the mushroom and can be used as a novel nutraceutical in reducing microbial infections.

Keywords: *Oyster mushrooms, Pleurotuseous, Vegetable waste, Secondary metabolites, Lovastatin, Anti-microbial activity, Nutraceutical.*

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INTRODUCTION

Mushrooms have been used as a major food source around the world since centuries due to their high nutrient content. Previous research suggests that edible mushrooms were highly nutritional and compared favourably with meat, egg and milk food sources. Of several thousand mushroom species known worldwide, only around 2000 are considered edible, of which about 20 are cultivated commercially with only 4 to5 under industrial production [1]. Mushrooms grow on a variety of substrates when cultivated within the right condition and they produce various bioactive secondary metabolites such as terpenoids, flavonoids, tannins, alkaloids, and polysaccharides [2].The fruiting bodies and mycelium of mushrooms exhibit health promoting values such as immunostimulatory, antibacterial, and antioxidative properties [3]. The synergistic effect of these substances would give potential therapeutic values.

Food wastage leads to the accumulation of household waste, which is a major reason for most of the microbial infections today. It is the need of the hour to implement strategies that can reduce the accumulation of food waste and thereby control the health hazards. *Pleurotuseous* is endemic to South India and is the third most important cultivated mushroom species for consumption. Nutritionally, it has

a unique flavor and aromatic properties, and it is considered to be rich in protein, fiber, carbohydrates, vitamins and minerals. *Pleurotus* spp. are promising as medicinal mushrooms, exhibiting haematological, antiviral, antitumor, antibiotic, antibacterial, hypercholesteremia and immunomodulating activities[4]. However, this mushroom is not commonly available in the market due to its less shelf life. Its cultivation is and less complicated compared to the other mushroom species. Therefore, it is important to come up with the practice of cultivating *Pleurotus eous* in the households by utilising the easily available eco-friendly substrates.

The extracts and bioactive compounds from *Pleurotus* spp. mushrooms have exhibited notable antimicrobial activity. Some volatile compounds extracted from the fruiting body of *Pleurotus ostreatus* had strong antibacterial effect against *B. cereus, B. subtilis, E. coliand S. typhimurium*[5]. An antifungal peptide called erygin, which inhibits growth of *Fusarium oxyporum* and *Mycosphaerella arachidicola* was isolated from the *Pleurotuseryngii* mushroom [6]. However, there has not been any notable research about the methodologies that can increase the nutrient value, secondary metabolite production and therapeutic properties of *Pleurotus eou*s by adapting to an eco-friendly substrate like household vegetable waste. There is also very scarce literature available about the antimicrobial activity of this mushroom. Thus, the current study aims at the vegetable waste based cultivation of the pink oyster mushroom, *Pleurotus eous,* screening & quantification of the mushroom extracts for the production of secondary metabolites and the screening of its antimicrobial activity.

MATERIALS AND METHODS

Specimen Collection and Mushroom Cultivation

In the current study, the pure culture of *Pleurotus eous* obtained from Centre for Biotechnology (Biocenter), Hulimavu, Bangalore, Karnataka, Indiawas used as the parental strain. Subcultures were made periodically and maintained on potato dextrose agar (PDA) slants at 25±2^{0}C temperature for further investigations. Pure culture was used for the preparation of spawn [7]. Mushroom cultivation was carried out according to standard methods with slight modifications. The mother spawn was prepared on PDA plates. Vegetable waste was boiled (60- 80^{0}C) in water bath for 10-15 min in the proportion of 1:1:1 (Vegetable waste: rice husk: water) and blended with 4% CaCO3 and 2% CaSO4. This was then packed (250g) in polythene bags (200x300 mm) and sterilized in an autoclave at 121ºC for 30 min. Following this, the bags were inoculated with actively growing mycelium of *Pleurotus eous* from PDA slants and incubated for mycelial growth without any light for 10-15 days until the mycelium fully covered the bags. These spawn bags were used for the seeding of mushroom bed. Water was sprayed for maintaining moisture up to the desired level. Five replications were used for each growing trial. The yield parameters were studied in all experiments.

Preparation of mushroom extracts

The Ethanolic , Acetone and Water extracts were prepared according to standard methods[8].

Nutrient value analysis of the harvested mushroom

The fruiting body of *Pleurotus eous* and different extracts were subjected to nutritional analysis. In this study, the moisture and ash content, crude fat content, crude fibre content, presence of carbohydrate, protein and lipids were estimated.

Determination of Moisture and Ash content, Crude Fat and Crude Fibre content

The moisture and ash contents of *Pleurotus eous* fruiting bodies were estimated [8] and the values were recorded. Crude fat was determined using Soxhlet extraction apparatus. Petroleum ether (boiling point 40-600^{0}C) was added to 2g finely ground mushroom sample and placed in the extraction apparatus. Extraction was carried out for 6 hours after which the ether was evaporated to dryness. The amount of fat was obtained from the difference between the initial and the final weight [9].Following this, crude fibre content of *Pleurotus eous* was determined[10].

Determination of Protein, Carbohydrate and Lipid content

The protein content in *Pleurotus eous* was carried out as per standard methods[11]. Carbohydrate content was determined according to an already described method [12,13]. Total lipid content was determined by slight modified method [14]. 5g of ground mushroom was suspended in 50 ml of chloroform: methanol (2: 1 v/v) mixture, then mixed thoroughly and let to stand for 3 days. The solution was filtrated and centrifuged at 1000 RPM by a table centrifuge machine. The upper layer of methanol was removed by Pasteur pipette and chloroform was evaporated by heating. The remaining was the crude lipid. For the determination of total lipid from fresh mushroom, 5g was taken with 50 mlphosphate buffer and homogenized with a tissue homogenizer. 5ml of homogenate was taken with 50ml of chloroform: methanol (2: 1 v/v) mixture and lipid content was determined as mentioned above.

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Biochemical screening for Secondary Metabolites

Ethanolic, Acetone and Water extracts were subjected to preliminary phytochemical screening for the identification of various secondary metabolites using previously described methods[15].

Quantification of Secondary Metabolites

Estimation of Alkaloids

Standard curve was prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1mLof the working standard Caffeine solution (100µg/mL) and the volume was made up to 1mL by adding distilled water. 5mL of bromocresol green solution (heat 69.8 mg bromocresol green with 3mL of 2N NaOH and 5mL distilled water until completely dissolved and the solution was diluted to 1000mL with distilled water) and 5mL of phosphate buffer (pH-4.7) was added. The mixture was shaken with 1, 2, 3 and 4mL chloroform by vigorous shaking and collected in a 10mL volumetric flask and diluted to the volume with chloroform. Samples(10mg/mL) were also prepared in the same manner by taking 100µL of the sample. The absorbance for samples and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer.

Estimation of Flavonoids and Tannins

Flavonoids in methanol and aqueous extract of *Pleurotus eou s*was estimated by the method proposed by [16]. Different aliquots of standard tannic acid (100µg/mL) were taken in different test tubes and 7.5mL of distilled water was added in all the test tubes. Later, 0.5mL of Folin-Calcioteau reagent and 1mL of saturated sodium carbonate was added in all the tubes. The volume in each test tube was made upto 10mL with distilled water and the absorbance was read at 725nm. The samples were also processed in the similar manner by taking 100µL of sample. The standard graph was plotted and the amount of tannin in each sample was calculated [17].

HPLC analysis for the presence of Lovastatin

Statins were isolated according to an already available method [18]. The identification and quantification of Lovastatin were carried out on the mushroom extracts by HPLC, using a Merck LiChrospher 100 RP18 reverse phase column with a diode array detector at a flow rate 2 ml/min and elution with gradient 90:10 water:methanol (v/v).

Antimicrobial screening

The Acetone, Ethanol and Water extracts of *Pleurotus eous* were assessed for antimicrobial screening against different bacterial and fungal pathogens[19,20].

Anti-Bacterial Screening

Micro titer plate method (96 wells) was carried out to check the Minimum Inhibitory Concentration (MIC) against 24h cultured bacterial pathogens – *Pseudomonas aeruginosa, Streptococcus mutans, Klebsiella pneumoniae, Bacillus cereus, Staphylococcus aureus, Escherichia coli, Salmonella typhi, Enterobacter cloacae, Staphylococcus epidermis, Serratia marcescens, Enterococcus faecalis* and *Proteus mirabilis.* 10mg of the extracts (Acetone extract, Ethanol extract and Water extract) dissolved in 1 mL DMSO (Dimethyl sulfoxide), respectively. Luria Bertani (LB) broth was prepared and autoclaved at 121°C for 15mins. 200µL of deionized water was added in the wells of the micro titer plate (A_1-A_{12}, B_{12}-H_{12}, H_{11}-H_1, and G_1-B_1) to prevent the sample from drying. 100µL of sterilized LB broth was added to all the remaining wells. 30µL of 0.1% of resazurin was added to the wells B_2- G_2 in respective plates and named as colour blank. In wells B_3- G_3test organism and 30µL of 0.1% of resazurin was added in respective plates as culture control. 100µL of the mushroom extract (Acetone extract, Ethanol extract and Water extract) was added to respective plates from wells B_4-$G_4$and serially diluted by transferring 100µL of the sample to subsequent wells upto B_{11}-$G_{11}$and 100µL of the excess sample was discarded from B_{11}-G_{11}, 100µL of the test organisms and 30µL of 0.1% of resazurin dye was added to the diluted samples. The plates were incubated at 37° C for 24h.

Antifungal Screening

The Acetone, Ethanol and Water extracts of *Pleurotus eous* mushroom were screened for anti-fungal activity against local infection causing fungi, *Aspergillus niger, Aspergillus flavus and Candida albicans.* Well diffusion method was carried out to check the minimum inhibition concentration (MIC) of the mushroom extracts. 100mg of pink mushroom extracts (Acetone, Ethanol and Water extract) was dissolved in 1mL of DMSO (Dimethyl sulfoxide) in sterilized eppendorf. Aliquots of 100µg, 200µg, 300µg, 400µg of concentration was prepared and the volume was made upto 50µL by adding DMSO **.** Potato Dextrose Agar and autoclaved at 121°C for 15 mins.

Approximately 30mL of media was poured into the sterilized petriplates and allowed it to solidify. 100µL inoculum of *Aspergillus niger, Aspergillus flavus and Candida albicans* added to the respective PDA plates and spread thoroughly using a plate spreader. Five wells are made using the borer in respective plates. The wells are filled with 50µL sample in respective wells and 50µL Control (DMSO) in the middle well.

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The culture plates was incubated at 25°C for 72 hours. The zone of inhibition was recorded in Mm around the wells.

Statistical Analysis

Statistical analyses were performed by one-way analysis of variance (ANOVA) Results were expressed as mean ±S.D for secondary metabolites. P values<0.05 were considered significant.

RESULTS AND DISCUSSION

Mushroom Cultivation

In the current study, the harvested mushroom data were recorded periodically during the growing season, namely 1st flush, 2nd flush and 3rd flush. The fruiting bodies before and after harvest is given in Fig:1. The mushrooms were harvested three times From 12th to 26th day of harvest. The first harvest of the mushrooms was taken on 12th to 14th day, Second and third harvests were taken on 18th to 20th day and 24th to 26th day respectively. The mushroom yield in first second and third harvest are 350g, 120g and 100g, respectively. The total yield of *Pleurotus eous* was 570g for single mushroom bed.

Nutrient value analysis of the harvested mushroom

The results of the nutrient content of *Pleurotus eous* is represented in Fig-2. The moisture content of *Pleurotus eous* was found to be 88.24%, which is similar to the results obtained by [21].The moisture content of some selected mushrooms ranged from 66.7 to 90.7% [22]. The fresh mushrooms contained about 90% moisture and dry mushrooms contained about 90% dry matter and 10% moisture [23]. In the current study, the Ash content of *Pleurotus eous* was found to be 5.81. Previously it was reported that the ash content of fresh mushrooms ranged from 1.1 to 1.28%[24]. *Babita and Narender* (2014) reported that the ash content of three strains of *M. procera* was to be 1.93%*, M. rhacodes* 2.16% and in *M. dolichaula* 7.3% [24,25]

The crude fat content in *Pleurotus eous* was found to 4.61% .According to the literature crude fat content of two different strains of *Hypsizygus ulmarius* ranged between 3.55% to 4.8% [21]. The crude fibre content in *Pleurotus eous* was found to be 19.5%. It was reported that the fibre content inedible mushroom *Pleurotus spp.* ranged from 25.5 to 27% [26]. The fairly high level of fibre in the mushroom was a desirable characteristic since fibre plays an important role in human diet.

Phytochemical analysis is of paramount importance in identifying new source of therapeutically and industrially valuable compounds having medicinal mushrooms have been chemically investigated [27]. *Pleurotus eous* was harvested when attained their full growth. The harvested fruiting bodies were used to prepare the different extracts and dried for nutritional analysis. The methanolic, ethanolic and water extracts were prepared according to standard methods. In the current study, the presence of carbohydrate, protein and lipids were estimated.

In *Pleurotus eous,* the protein content was comparatively higher than the other nutrients . The protein content was found to be 65% (Fig:2). Mushrooms are rapidly becoming recognized as a promising source of novel proteins.They can offer solutions to several medicinal and biotechnological problems such as microbial drug resistance, low crop yield and demands for renewable energy. Whereas, large-scale production and industrial application of some fungal proteins proves their biotechnological potential and establishes higher fungi as a valuable although relatively unexplored, source of unique proteins [28]. Whereas, the content of protein in *Pleurotus spp.* is reported to vary according to strains, physical and chemical parameters, composition of the substrate and harvest time [7]. Protein content of mushroom was reported to vary according to genetic structure of species and physical and chemical differences in growing medium [23, 28]. It was previously reported that the protein content of *Calocybe indica* was 21.60%, *Agaricus bisporus* 41.06%, *Pleurotus florida* 27.83% and *Russula delica* 26.25% [29]. The result of the current study was found to be higher than the other studies conducted on other strains of edible oyster mushrooms [7,21].

In the present study, the carbohydrate content was found to be 30% (Fig:2) . The results seem to be higher than the previously reported [21]. The carbohydrate comprises various compounds-monosaccharide, their derivatives and oligosaccharides (commonly called sugars) and both reserve and construction polysaccharides (Glucans), which are important in the proper functioning of the alimentary tract [30]. A considerable proportion of the carbohydrates occur in the form of polysaccharides with particles of the different size. Fungal polysaccharides are represented by glycogen and such indigestible forms as dietary fibre, cellulose, chitin, mannans and glucans considered important in the proper functioning of the alimentary tract [31,32]. Carbohydrates are one such group of carbon compounds, which are essential to life.

Mushrooms are not considered as a significant source of essential fatty acids for fulfilling the requirements of human body. The nutritional contribution of mushroom lipids is limited due to low total

lipid content and a low proportion of desirable n-3 fatty acids. Nevertheless, linoleic acid is a precursor of the attractive smell of dried mushrooms [30]. The lipid content in *Pleurotus eous* was found to be 6.5%. This is possibly because of the low fat content of mushrooms, which are usually rich in protein and fibre.

Different phytochemicals have been found to possess wide range of medicinal properties, which may help in protection against various diseases. The quantitative estimation of primary and secondary metabolites reveals various chemical constituents present in the mushroom. Secondary metabolites analysis is necessary for extraction, purification, separation, crystallization, identification of various phytocompounds. Several studies have indicated that antioxidants prevent the onset of degenerative illness such as certain cancers, cardiovascular and neurodegenerative diseases, contracts, oxidative stress dysfunctions and aging [27].

Biochemical screening for secondary metabolites

The Acetone, Ethanolic and Water extracts of *Pleurotus eous* was prepared through successive distillation and extraction. The biochemical screening shows that the different extracts of *Pleurotus eous* contain*s* secondary metabolites such Alkaloids, Flavonoidsand Tannins. The results obtained are quite similar to other *Pleurotus and Ganoderma species as* reported earlier [33,34]. The abundant occurrence of alkaloids and flavonoids in the mushroom extract reflects its antioxidant and anti-microbial activity.

Quantification of secondary metabolites

From the quantitative analysis and statistical studies, it was found that the total alkaloid content of *Pleurotus eous* was found to be; 9.36mg/g of acetone extract, 8.63mg/gof ethanol extract and 7.36mg/g of water extract. Alkaloids protect against chronic diseases and earlier recorded that bitter leaf contains an alkaloid that is capable of reducing headaches associated with hypertension. Alkaloids are a diverse group of secondary metabolites found to have antimicrobial activity by inhibiting DNA topoisomerase [35]. The result obtained was found to be higher when compared to other mushroom species that include *Hypsizygus ulmarius CO2 strain (8.57 mg / g) and Hypsizygus ulmarius* IIHR HU1 (6.12 mg / g) [21].

The quantity of flavonoids was found to be 3.5 mg/g in acetone extract, 2.1 mg/g in ethanol extract and 1.06 mg/g in water extract which is higher than that found in and edible mushroom in a recent study [36]. The flavonoid content in *Pleutorus eous* was found to be lower than value obtained in a similar study [6]. Flavanoids are one of the most diverse group of natural compounds that have been shown to possess a broad spectrum of chemical and biological activities including radical scavenging properties, antiallergenic, antiviral, anti-inflammatory and vasodilating actions [37,38]. Past studies suggests that flavonoids in whole foods might decrease the absorption of fat and may increase calorie expenditure.

Tannin content was found to be 10.46mg/g in acetone extract, 3.79mg/g in ethanolic extract and 9.08mg/g in water extract, which is similar to the studies conducted. Tannins have shown potential antibacterial and antiviral effects.The growth of many fungi, yeasts, bacteria and viruses was inhibited by tannins. Apart from these, tannins contribute the property of astringent activity i.e. faster the healing of wounds and inflamed mucous membrane. As a potent antioxidant, tannins provide protection from the oxidation of cells by free radicals .Tannins may also be useful as a dietary aid for weight-loss due to their interference with the conversion of certain nutrients and food substances during digestion [39].

HPLC analysis of Lovastatin

The present study was conducted to isolate and analytically determine the lovastatin content present in the ethanolic, acetone and water extracts of *Pleurotus eous.* The HPLC results of *Pleurotus eous* extracts show that the acetone extracts gave the highest lovastatin level that was 16.6μg /10mg of mushroom (Fig:3). Statins are found to be an inhibitor of the enzyme HMGCoA reductase that catalyzes the reduction of HMG-CoA to mevalonate during synthesis of cholesterol [40,41].

Since this is the first report on the lovastatin analysis of *Pleurotus eous*, much more clarity is required in the variability of the results. The possible reason for the difference in the percentage of lovastatin in ethanolic, acetone and water extracts can because of the difference in the pH in the extracts and also the nutrient content. The values were higher than the lovastatin standard which was 2.46%. The values are comparatively higher than the lovastatin content of *Pleurotus ostreatus* as reported before [18]. It is important to know that the statins are present under two types of structural forms, depending on pH. One of them is the _-hydroxyacid form being in alkaline solution, while the hydroxy acid and _-hydroxylactone form being in equilibrium in acid condition. The HPLC laboratory analysis showed clearly this equilibrium, which contributes to double-fold the lovastatin concentration. These preliminary results from previous studies found in poor nutrient culture conditions, suggest to us that it is possible to increase the yield of statins produced by *Pleurotuseous,* by using eco-friendly substrates for cultivation such as vegetable waste. Futhermore, the fact that lovastatin is present in high proportion in this mushroom, makes it an important food supplement for patients suffering from hypercholesterolemia [42].

Antibacterial activity of *Pleurotus eous* extracts

In the antibacterial study, various extracts showed relatively similar MIC values as given in Graph -1. The results of the micro titer plate method of acetone, ethanol and water extracts are given in Fig- 4,5 & 6 respectively. Among all the bacterial strains tested, lowest MIC values were obtained for*Pseudomonas aeruginosa* and *Enterococcus faecalis,* followed by *Klebsiella pneumoniae* and *Enterobacter cloacae.* The results show that the mushroom extracts have shown promising antimicrobial activities against nine bacterial pathogens, in comparison with the study conducted previously [20]. All the three extracts have resulted in some antimicrobial activity against all the tested organisms. This is the first report on the anti-bacterial activity of *Pleurotus eous* extracts and thus opens the door to elaborate research on its therapeutic properties.

Anti-fungal activity of *Pleurotus eous*

Anti-fungal activity of the various extracts against the test organisms showed high variability. Maximum Zone of Inhibition was found in the water extract, followed by acetone and ethanolic extracts (Fig: 7) . Water extract has resulted in the most powerful antifungal activity against all tested organisms (Table .1). The largest Zones of Inhibition where found against *Aspergillus flavus*, followed by *Aspergillus niger* (Fig:7). However, the various extracts of *Plerotus eous* show commendable antifungal activity. The lower antifungal activities of acetone ad ethanolic extracts can be explained by the absence of potent secondary metabolites due to the effectiveness of the extraction method, the extraction capacity of the solvent, any differences in the solubilities of the various bioactive compounds, the extraction time, etc. Another possible reason can be ability of the tested pathogens to produce similar secondary metabolites as produced by the mushroom, since all fungi are capable of releasing certain class of bioactive compounds. As this is the first report on the anti-fungal property of *Pleurotus eous* extracts more elaborate study can be further conducted to explore its antimicrobial benefits.

Table - 1 : Antifungal screening – Zones of Inhibition in Mm formed by water extract of *Pleurotus eous* against fungal pathogens

Water extract in µg	Fungal zone of inhibition in Mm		
	Aspergillus niger	*Aspergillus flavus*	*Candida albicans*
100µg	17	16	17
200µg	18	18	18
300µg	20	22	19
400µg	20	23	19

Figure1 ; *Pleurotus eous* fruiting bodies before and after harvest

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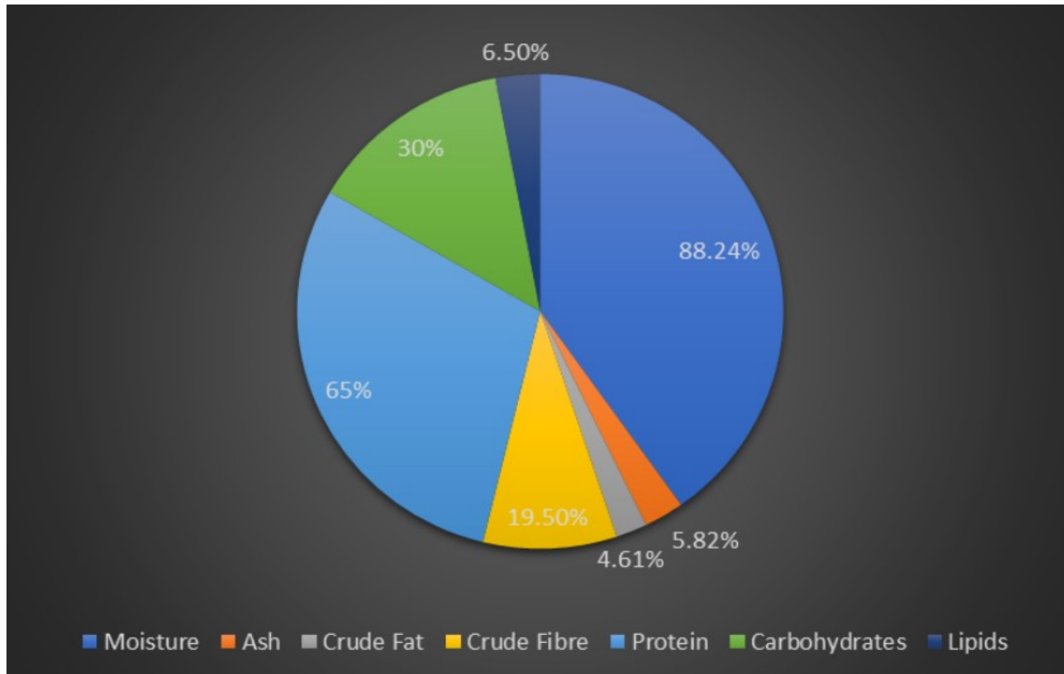
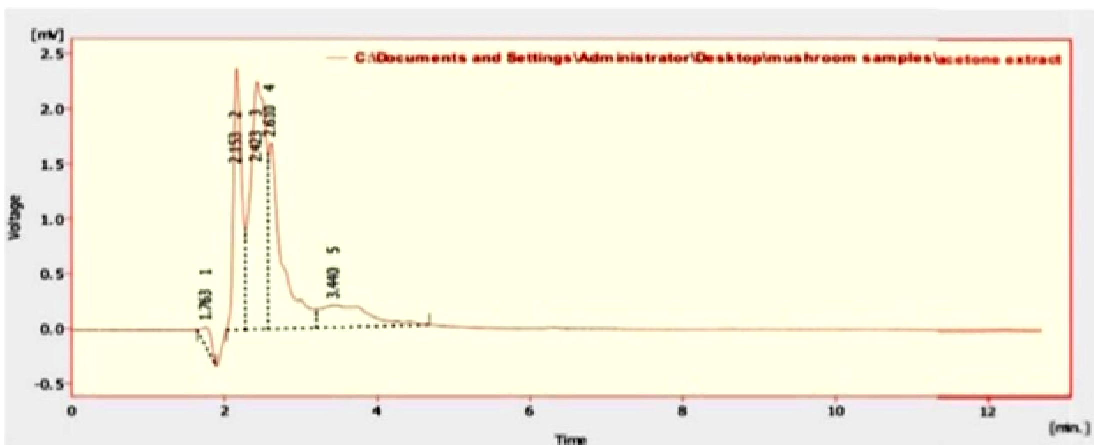


Figure 2 ; Nutrient content analysis of *Pleurotus eous*

Autostop : None
Detector 1 : Signal 1

External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 10 Samp. per Sec.



Result Table (Uncal - C:\Documents and Settings\Administrator\Desktop\mushroom samples\acetone extract)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.763	1.681	0.185	2.1	2.8	0.18
2	2.153	17.470	2.371	21.7	35.4	0.12
3	2.423	31.075	2.248	38.7	33.6	0.25
4	2.610	21.077	1.686	26.2	25.2	0.13
5	3.440	9.041	0.198	11.3	3.0	0.73
	Total	80.344	6.688	100.0	100.0	

Figure 3 ; HPLC analysis of lovastatin content in acetone extract of *Pleurotus eous*

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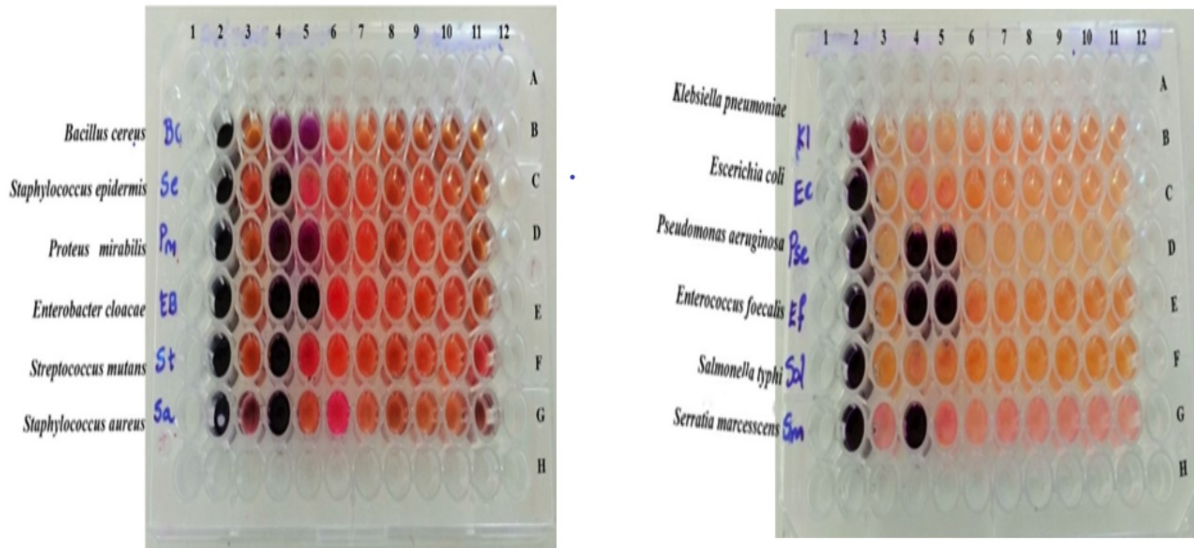


Figure 4: Antibacterial screening of acetone extract - Micro titer plates 1 & 2; Determination of minimum inhibitory concentrations of the acetone extract of *Pleurotus eous* against 24 hr cultured bacterial pathogens. The numbers from 2 to 11 at the top of the microtiter plate indicate the different concentrations of the extract in the wells, i.e., wells B_4-G_4 - 100µL of the mushroom acetone extract; wells up to B_{11}-G_{11} - serially diluted acetone extract by transferring 100µL of the sample to subsequent wells. In the wells A_1-A_{12}, B_{12}-H_{12}, H_{11}-H_1, and G_1-B_1 - 200µL of deionized water. 100µL of the test organisms and 30µL of 0.1% of resazurin dye was added to the diluted samples; wells B_2- G_2 -30µL of 0.1% of resazurin-positive control (colour blank); wells B_3- G_3 - 30µL of 0.1% of resazurin (culture control).

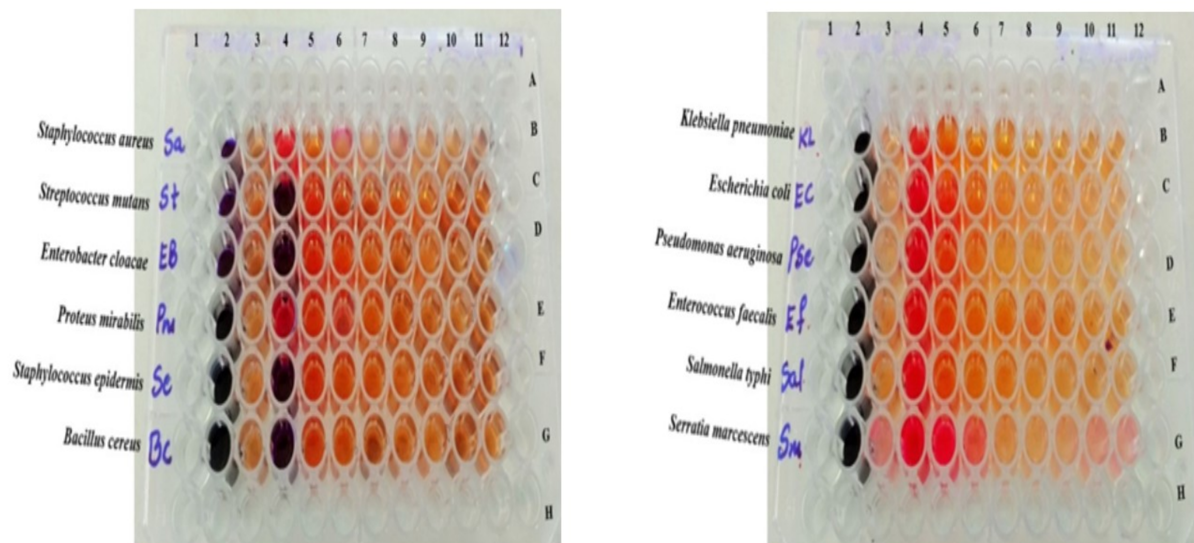


Figure 5*:* Antibacterial screening of ethanol extract - Micro titer plates 1 & 2; Determination of minimum inhibitory concentrations of the acetone extract of *Pleurotus eous* against 24 hr cultured bacterial pathogens. The numbers from 2 to 11 at the top of the microtiter plate indicate the different concentrations of the extract in the wells, i.e., wells B_4-G_4 - 100µL of the mushroom acetone extract; wells up to B_{11}-G_{11} - serially diluted acetone extract by transferring 100µL of the sample to subsequent wells. In the wells A_1-A_{12}, B_{12}-H_{12}, H_{11}-H_1, and G_1-B_1 - 200µL of deionized water. 100µL of the test organisms and 30µL of 0.1% of resazurin dye was added to the diluted samples; wells B_2- G_2 -30µL of 0.1% of resazurin-positive control (colour blank); wells B_3- G_3 - 30µL of 0.1% of resazurin (culture control).

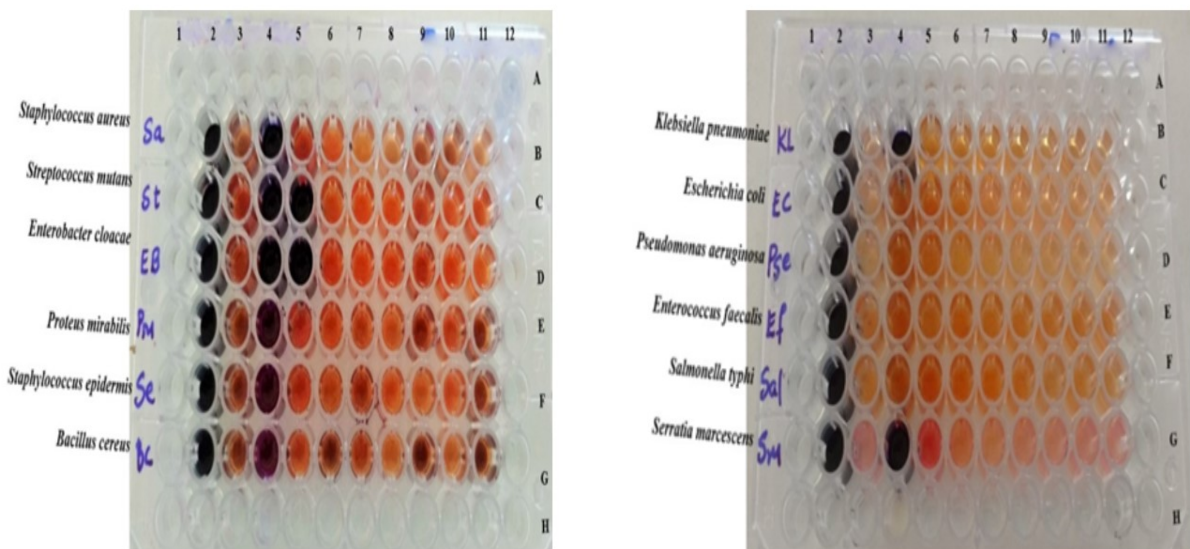


Figure 6*:* Antibacterial screening of water extract - Micro titer plates 1 & 2; Determination of minimum inhibitory concentrations of the acetone extract of *Pleurotus eous* against 24 hr cultured bacterial pathogens. The numbers from 2 to 11 at the top of the microtiter plate indicate the different concentrations of the extract in the wells, i.e., wells B_4-G_4 - 100µL of the mushroom acetone extract; wells up to B_{11}-G_{11}- serially diluted acetone extract by transferring 100µL of the sample to subsequent wells. In the wells A_1-A_{12}, B_{12}-H_{12}, H_{11}-H_1, and G_1-B_1 - 200µL of deionized water. 100µL of the test organisms and 30µL of 0.1% of resazurin dye was added to the diluted samples; wells B_2- G_2 -30µL of 0.1% of resazurin-positive control (colour blank); wells B_3- G_3 - 30µL of 0.1% of resazurin (culture control).

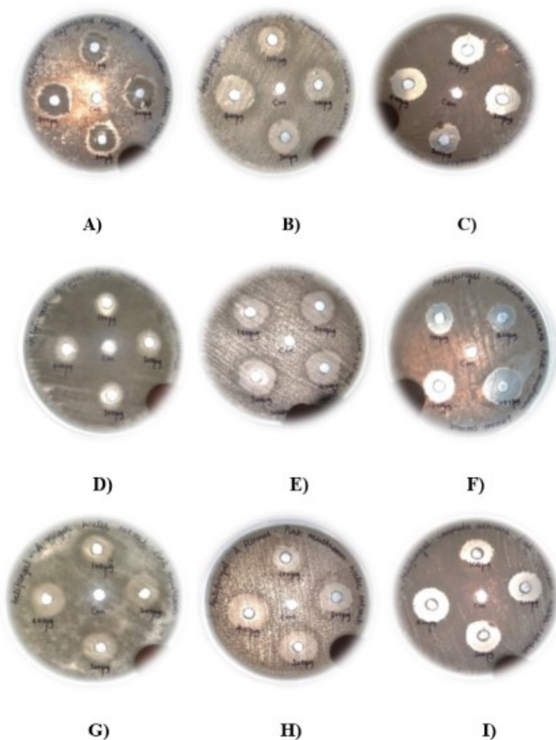
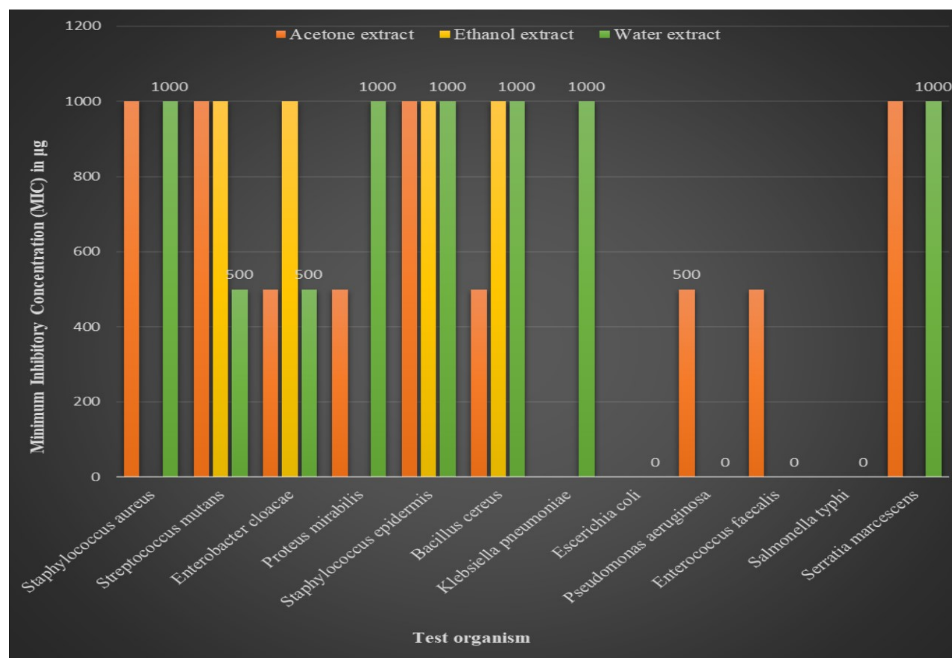


Figure 7 : MIC plates for *Acetone extract* showing the Zones of Inhibition in µg – *A) Aspergillus niger, B) Aspergillus flavus, C) Candida albicans;* MIC plates for *Ethanol extract* showing the Zones of Inhibition in µg – *D) Aspergillus niger ,E) Aspergillus flavus, F) Candida albicans ;* MIC plates for *Water extract* showing the Zones of Inhibition in µg – *G) Aspergillus niger ,H) Aspergillus flavus, I) Candida albicans*



Graph -1 : MIC values of *Pleurotus eous* extracts against bacterial pathogens

CONCLUSION

Mushrooms have received increasing attention from the researchers in food and pharmaceuticals. Nowadays there is an increasing public interest in the secondary metabolites from mushrooms for discovering new drugs or lead compounds. Herbal medicines are free from side effects, adverse effects and they are economical and easily available will be beneficial for the mankind over the centuries. Exploration for bioactive compounds effective in treating pathogenic microorganisms resistant to present-day drugs is very helpful [43]. Currently, there is a growing interest in searching for new antimicrobial agents from natural sources such as bacteria, fungi, and plants [37,44]. The data recorded in the above experiments demonstrate that the qualitative and quantitative screening of the extracts of edible mushroom *Pleurotus eous* showed the presence of bio pharmaceutical bioactive metabolites such as flavanoids, alkaloids, tannins and lovastatin. The vegetable waste based cultivation of the mushroom shows an increase in the nutrient content such as Crude fibre, Proteins, Carbohydrates and Lipids compared to the conventional methods of cultivation. The use of an eco-friendly, vegetable waste based cultivation has resulted in a significant rise in the nutrient value of the mushroom. All extracts of *Pleurotus eous* have shown potent antimicrobial activities against the tested organisms. Further investigation is needed to evaluate and confirm the antimicrobial activities of the extracts against a wider range of human pathogenic microorganisms. Finally, isolation, identification, and explanation of the mode of action of the bioactive compounds responsible for the antimicrobial activities are indispensable steps prior to the development of antibiotics.This study exhibits the first report on the nutrient content antimicrobial activity of the pink oyster mushroom, *Pleurotus eous*. In conclusion, edible oyster mushroom *Pleurotus eous* can potentially be used as a source of nutraceutical based treatment against bacterial and fungal pathogens as it produces higher amounts of secondary metabolites and lovastatin. The high nutrient content, especially Carbohydrates and Proteins gives evidence that it can play a significant role in daily diet. To achieve this, further analysis and quantification of the secondary metabolites has to be conducted to identify their therapeutic properties.

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COMPETING INTERESTS

The authors have declared that no competing interest exists.

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