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QUANTITATIVE PHYTOCONSTITUENTS, ANTIBACTERIAL ASSAY AND ANTIOXIDANT ACTIVITY OF EDIBLE MUSHROOM *PLEUROTUS EOUS* CULTIVATED FROM DIFFERENT SUBSTRATES

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ABSTRACT

Objective: The present investigation was circumspectly carried out on cultivation of *Pleurotus eous* using agricultural wastes such as paddy straw and sugar cane leaves. **Methods:** The fastest spawn running was observed in paddy straw when compared to sugar cane straw. Harvesting of *Pleurotus eous* with different substrates of three flushes and the maximum total yields were observed in paddy straw (2 kg) compared sugar cane (1 kg). In biochemical composition, the highest percentage was observed in protein compared to carbohydrates in both the substrates. **Results:** The preliminary phytochemicals showed the presence of six bioactive compounds like alkaloids, flavonoids, terpenoids, glycosides, saponins and proteins from ethanolic extract of *Pleurotus eous*. HPLC, five peaks corresponds to five bioactive compounds like Ergosterol, Phenyl, β- glucan, Pectin and Alpha-hydroxylase. Free radical scavenging activity of total antioxidant capacity and Nitric Oxide (NO) was observed from ethanolic extract of *Pleurotus eous*. Both the antioxidant activities were compared with standard ascorbic acid. The percentage of inhibition in both the antioxidant assays were higher in increased concentrations. Ethanolic extract of *Pleurotus eous* was against human pathogens such as *Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsilla pneumoniae*. The better range of inhibition was shown against *Staphylococcus aureus,* followed by *Klebsilla pneumoniae* and *Pseudomonas aeruginosa*. Hence, **Conclusion:** The present study was carried for *Pleurotus eous* can be used as treatment for several disease caused by human pathogenic bacteria.

KEYWORDS

Biochemical, HPLC, Phytochemical, Antibacterial activity and Antioxidant.

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INTRODUCTON

All over the world and especially in developing countries, there is a problem of shortage of protein. Producing cultured mushrooms can be one suitable solution to this problem. Because of rapid industrialization, the amount of waste materials has increased and utilization of these wastes is very important for government economy and natural balance[1]. Fungi are eukaryotic, achlorophyllous, nucleated, shade loving, gametophytes, haploid, heterotrophic, nonvascular, spore producing,

cryptogenic, thallophytic plants which are surrounded by cell wall containing chitin. They do not use carbon-dioxide as their carbon source and hence are dependent on external sources for organic carbon. Fungi range from simple forms like thread fungi to complex forms like mushrooms[2]. Cultivation of edible mushroom might be the only current process that combines the production of protein rich food with the reduction of environmental pollution[3]. It represents one of the most efficient biotechnological processes of lignocellulosic organic waste recycling[4]. Mushrooms are now considered as an important source of food and income both in the developing and developed countries as well[5]. The substrate used, following the harvesting of the mushrooms, is valuable as a fertilizer and a soil conditioner for the growth of the plants[6]. Mushroom have been a major focus of investigations for novel biologically active compounds from natural resources and in recent years pharmaceutical companies have spent a lot of time developing these natural products to produce more affordable and cost effective remedies[7]. Mushrooms have anti-diabetic, cardiovascular and immune modulating effect in addition to preventing the risk of cancer and controlling blood sugar level[8], with substantive antioxidant activity recorded in both wild and cultivated species[9]. These agro lingo cellulosic wastes from rice field are underutilized and disposed of in the environment without any proper treatments. Sometimes, they are even burnt as fuel leading to serious environmental pollution problems. However, these agricultural wastes can be potentially bio-converted into value added products such as food protein from mushroom, pulp, animal feed, and biofuel as well as bio-fertilizer through the action of lignin-degrading enzymes secreted by fungi[10]. Pleurotus spp. are promising as medicinal mushrooms, exhibiting hematological, antiviral, antitumor, antibiotic, antibacterial, hypocholesterolic and immunomodulation activities[11]. Free radicals are constantly formed in the human body during energy production, in the mitochondrial electron transport chain, phagocytosis, arachidonic acid metabolism, ovulation, fertilization and in xenobiotic metabolism[12] Living organisms are endowed with endogenous and exogenous antioxidant defense systems capable of countering the adverse reactions of free radicals[13]. The generation of free radicals in the body beyond its antioxidant capacity actually leads to oxidative stress and this has been implicated in the etiology of a number of disorders[14]. The *Pleurotus eous* is selected for the present study, which is commonly known as pink oyster mushroom and the species can be easily identified by the pinkish tints of the pileus and the small narrow spores. In the recent years, much attention has been paid to the investigation of nutraceuticals from various edible mushrooms[15]. The present study evaluates the quantitative phytoconstituents, antibacterial assay and antioxidant activity of oyster mushroom *pleurotus eous* cultivated from different substrates.

MATERIAL AND METHODS

Collection of Organism

The organism *Pleurotus eous* APK1 was obtained from Department of Plant Pathology, Tamil Nadu Agriculture College, Madurai. This was maintained and sub-cultured on Potato Dextrose Agar (PDA) media (Hi Media, India) plates at room temperature. The oyster mushroom cultivation was conducted in a thatched shed in Biodiversity Garden of Periyar University.

Spawn Preparation

Sorghum grains were used as a substrate for mother spawn preparation. The grains were pre-cooked for about 30minutes. The excess water was drained completely and dried under shade. At 50 per cent moisture level the substrate was mixed thoroughly with calcium carbonate at 20 g/kg and filled in wide mouth glass bottles of 1liter volume up to its 3/4th capacity. Then, the mouth was tightly plugged with non-absorbent cotton and sterilized at 15 lbs pressure cooker for two hours. The substrate was allowed to cool after autoclave sterilization and inoculated with cultures, separately. The sterile sorghum grains mixture was aseptically inoculated with 10 mm mycelial disc from *Pleurotus eous* APK1 stock culture and incubated at laboratory

temperature (25 ± 3°C) for 15 days. Instead of glass bottles, 5" × 12" heat resistant polypropylene bags were used. After sterilization and cooling, as described earlier, mushroom cultures from their respective mother spawns were transferred, aseptically and incubated at laboratory temperature (25 ± 3°C) for 15 days.

Bed Preparation

The well matured paddy straw and sugarcane leaves were cut into 3- 5 cm in length and soaked in water overnight. The drained paddy straw was washed for removal of unwanted particles and steam sterilized for 45 min and shade dried in a dust free place until the moisture content is around 50 - 60 %. The shade dried paddy straw was filled in polypropylene bags. The bags were perforated with 1cm diameter holes before packing. A layer of paddy straw is followed by sprinkling of one hand full of spawn (30g) over the first layer. Likewise, five layers of spawn and seven layers of paddy straw were filled and the bag was tied with a nylon thread and hanged in the mushroom cultivation chamber for spawn running. The chamber was maintained at temperature range of 23 - 25°C and humidity should be 60-70 % for the growth of mushrooms.

Spawn Run and Harvesting

Mushroom beds were stored in thatched shed using a new method viz hanging rope system. Observations on number of beds accommodated per unit area, total yield were recorded. In this study, only one shed was utilized, instead of separate shed for spawn running and cropping. The mycelial cover in the beds was observed and the number of days taken to cover the entire bed was recorded in days. The total duration and days to first harvest were also record of in days. Watering was withheld a day before harvesting. Harvesting of sporophores was done before spore shedding. Three flushes of the sporophores were harvested from all the beds. Data recorded included the spawn run period i.e. the number of days from spawning to complete colonization of the cultivation substrate by the mycelia, number of days from spawning till appearance of pinheads, the days from spawning to first flush and fresh yield of sporophores.

Estimation of Total Protein

To 0.1ml of protein sample, 5ml CBB G-250 reagent was added and thoroughly mixed. The absorbance was read at 595 nm in a spectrophotometer against a reagent blank. The amount of total protein was calculated using a standard graph prepared with Bovine Serum Albumin (BSA) ranging from 10 to 100µg/ml. (Bradford, 1976).

Estimation of Total Carbohydrate

To one ml of sample, about 4ml of anthrone reagent was added, the mixture was thoroughly mixed by stirring. The solution was incubated in boiling water bath for 10 minutes. The optical density was read at 620nm. Standard graph was prepared with different concentrations of glucose ranging from 10 to 100µg/ml. (Anthrone method).

Phytochemical Screening

Ethanol extract of *Pleurotus eous* was subjected to phytochemical screening following the method of Trease and Evans[16] with certain modifications.

HPLC Analysis

A waters HPLC system, consisting in two module pumps, model 1525, a manual injector (Breeze 7725i, Rheodyne) and UV-Vis detector (Waters 2487) at 277 nm, was used. The analyses were carried out on an Eclipse Plus Zorbax C18 Agilent (150 mm×4.6 mm i.d., 5 µm particle size) column as a stationary phase. The mobile phase was a mixture of 5% acetic acid aqueous solution and methanol (80:20, v/v) used in mode isocratic elution. Twenty µL of sample was injected into the HPLC system. The overall run time was 70 min and the flow rate was 1.0 mL/min. All the analyses were carried out at room temperature. Results were acquired and processed by internal software.

Antibacterial Activity of *Pleurotous eous*

Test Microorganisms

Totally three bacterial strains (*Staphylococcus aureus, Klebsiella pneumonia* and *Pseudomonas aeruginosa*) were used throughout investigation. All the bacterial cultures were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. The young bacterial broth cultures were prepared before the screening procedure.

Assay for antibacterial activity

Bacterial strains and growth conditions

Bacterial Strains

The test microorganisms include Gram's positive bacteria and Gram's negative bacteria.

- Gram's positive bacteria: *Staphylococcus aureus*.
- Gram's negative bacteria: *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa*

All the microbial cultures were maintained at 4°C on nutrient agar slants (for bacteria).

Preparation of inoculums

Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures of experiment were prepared by transferring a loop ful of cells from the stock cultures to test tube of Muller-Hinton broth (MHB) for bacteria that were incubated without agitation for 24 hrs at 37°C and 25°C respectively. The cultures were diluted with fresh Muller-Hinton broth to achieve optical densities corresponding to 2.0 X 10^6 colony forming units (CFU/ml) for bacteria.

Antibacterial susceptibility test

The disc diffusion method (Bauer *et al.,* 1966) was used to screen the antibacterial activity. *In vitro* antibacterial activity was screened by using Muller Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 minutes and 0.1% inoculums suspension was swabbed uniformly and the inoculums were allowed to dry for 5 minutes. The concentration of extracts is 40 mg/disc was loaded on 6 mm sterile disc. The loaded disc was placed on the surface of medium and the extract was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37°C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. The standard disc is chloramphenicol 50.

Antioxidant Assay

Nitric oxide scavenging assay

Different concentrations (10µl, 20µl, 30 µl, 40µl and 50µl) of sample were taken and added 50µl of 10mM sodium nitroprusside dissolved in 0.5M phosphate buffer (pH 7.4). The tubes were incubated under fluorescent light at room temperature for 15 minutes. After incubation period 125 µl of Griess reagent was added. [Griess reagent: 0.1% of N-1-Naphthyl-ethylene diamine dissolved in water, 1% Sulphanilic acid dissolved in 5% Orthophosphoric acid]. The tubes were incubated again at room temperature for 10 minutes. The absorbance values were recorded at 546nm using shimadzu UV 1800.

Total antioxidant capacity assay

Different concentrations (10µl, 20µl, 30 µl, 40µl and 50µl) of extracts were taken and 1ml of reagent solution was added [Reagent solution: 0.6M sulphuric acid, 28mM sodium phosphate and 4Mm Ammonium molybdate]. The tubes were capped and incubated in thermal block at 95ºC for 90 minutes. After the time interval the tubes were cool down at room temperature the absorbance was recorded at 695 nm using shimadzu UV 1800 spectrophotometer.

RESULTS

The present study was carried out cultivation of Oyster mushroom of *Pleurotus eous* using agricultural wastes of paddy straw and sugar cane. Biochemicals analysed from different substrates of *Pleurotus eous*. Screening of bioactive compounds such as alkaloids, flavonoids, terpenoids, phenols, amino acids, glycosides, saponins, tannins, carbohydrates and proteins from ethanolic extract of *Pleurotus eous*. HPLC analysis of ethanolic extract *Pleurotus eous* for characterization of bioactive compounds. Ethanolic extract of *Pleurotus eous* was evaluated an antioxidant activity like Total antioxidant capacity (TAC) and Nitric oxide scavenging activities. Antibacterial activity was observed strongly inhibition of the growth of both gram positive (*Staphylococcus aureus*) and gram negative bacteria (*Pseudomonas aeruginosa* and *Klebsilla pneumonia*).

Cultivation and Harvesting of *Pleurotus eous*

Pleurotus eous was cultivated on two different substrates. *Pleurotus eous* spawn rapidly was running among paddy straw and sugar cane straw.

The mycelium of *Pleurotus eous* was covered in whitely of both substrates the sorghum grains. The fastest spawn running was observed in paddy straw compared sugar cane straw and their spawn running days were 12 days and 14 days. Harvesting of *Pleurotus eous*, number of flushes, flush wise yield and total yield were record with their data from different substrates. The different substrates of three flushes and the maximum total yields were showed in paddy straw (2 kg) compared sugar cane (1 kg) (Table No.1; Figure No.1).

Biochemical composition of *Pleurotus eous* using different substrates

Different substrates of *Pleurotus eous* was found out the Biochemicals such as protein and carbohydrates. The highest percentage of Protein was observed in both substrates compared carbohydrates. Significantly, maximum percentage of protein was paddy straw (30.2%) compared sugar cane (28%). Whereas the maximum percentage of carbohydrates was observed in paddy straw (12%) compared sugar cane (11%) (Table No.2; Figure No.2).

Phytochemical screening of ethanolic extract of *Pleurotus eous*

The present study was carried out in preliminary phytochemical analysis of *Pleurotus eous*. The ethanolic extract of *Pleurotus eous* revealed the presence of medicinally important bioactive ingredients. Ethanolic extract of *Pleurotus eous* showed the presence of six bioactive compounds like alkaloids, flavonoids, terpenoids, glycosides, saponins and proteins. Phenols, amino acids, tannins and carbohydrates were absent in ethanolic extract of *Pleurotus eous*. Phytochemicals of ethanolic extract of *Pleurotus eous* was recorded in (Table No.3).

HPLC analysis of ethanolic extract of *Pleurotus eous*

The results from HPLC ethanolic extract of *Pleurotus eous* showed five peaks at the retention time between 0 to 25 minutes. Five peaks were showed five bioactive compounds and their rention time (RT) like Ergosterol (RT: 04.04), following by Phenyl (RT: 05.14), β- glucan (RT: 10.07), Pectin (RT 11.03) and Alpha-hydroxylase (RT: 16.06). Peak assignment was confirmed by injection of standard (Figure No.3).

Antioxidant activity of ethanolic extract of *Pleurotus eous*

Total antioxidant capacity radical scavenging

Total antioxidant capacity radical scavenging activity of *Pleurotus eous* was evaluated with ethanol extracts and standard ascorbic acid. The Total antioxidant capacity was absorption at 695nm.The percentage inhibition (% inhibition) at various concentration (10, 20, 30, 40 and 50µg/ml) of extract as well as standard IC_{50} ethanol extract (10, 20, 30, 40 and 50µg/ml) were calculated and plotted. The increase of the concentration of the extract was showed to increase the values. The ethanolic extract of Total antioxidant capacity was the highest concentration was 50µg/ml (78.41%) and followed by 40µg/ml (69.33%), 30µg/ml (55.07%), 20 µg/ml (45.31%) and lowest concentration 10µg/ml of 39.64%. Totally, IC_{50} value was 31.88% compared standard ascorbic acid (Table No.4; Figure No.4).

Nitric Oxide (NO) radical scavenging activity

The free radical Nitric Oxide (NO) was showed the absorption at 546 nm. Significantly, increase concentrations on exposure to radical-scavengers. The highest inhibition nitric oxide value was found in 50µg/ml (70.60%) and followed by 40µg/ml (64.05%), 30µg/ml (56.95%), 20 µg/ml (52.00%) and lowest concentration 10µg/ml of 47.72% compared standard ascorbic acid. Graduately, increase the concentrations were increased the % of inhibition of NO activity. Finally, IC_{50} value was 34.30% (Table No.5 and Figure No.5).

Antibacterial activity form ethanolic extracts of *Pleurotus eous*

The antibacterial activity, ethanolic extracts of *Pleurotus eous* was against human pathogens of three bacterial strains such as one Gram positive bacteria (*Staphylococcus aureus*) and two Gram negative bacteria (*Pseudomonas aeruginosa* and *Klebsilla pneumoniae*). The highest growth of inhibition was observed in *Staphylococcus aureus,* followed by *Klebsilla pneumoniae* and *Pseudomonas aeruginosa* (14mm, 10.5mm and

9mm) respectively compared to control (Table No.6; Figure No.6 and Figure No.7, 8).

DISCUSSION

The present study was carried out cultivation of Oyster mushroom of *Pleurotus eous* using agricultural wastes of paddy straw and sugar cane. Biochemicals analysed from different substrates of *Pleurotus eous.* Screening of bioactive compounds such as alkaloids, flavonoids, terpenoids, phenols, amino acids, glycosides, saponins, tannins, carbohydrates and proteins from ethanolic extract of *Pleurotus eous*.

HPLC analysis of ethanolic extract *Pleurotus eous* for characterization of bioactive compounds. Ethanolic extract of *Pleurotus eous* was evaluated an antioxidant activity like Total antioxidant capacity (TAC) and Nitric oxide scavenging activities. Antibacterial activity was observed strongly inhibition of the growth of both gram positive (*Staphylococcus aureus*) and gram negative bacteria (*Pseudomonas aeruginosa* and *Klebsilla pneumonia*).

Table No.1: Yield of *Pleurotus eous* in paddy straw and sugarcane leaves

S.No	Substrate	Days taken for spawn run	Days taken for pin head formation	Ist harvest (days)	IInd harvest (days)	IIIrd harvest (days)	Total yield (kg)
1	Paddy straw	12	16	18	23	28	2kg
2	Sugarcane leaves	14	18	20	25	30	1kg

Table No.2: Biochemical compositions of *Pleurotus eous* using paddy straw and sugarcane leaves as substrate

S.No	Components	% of Compounds in Mushroom Grown from Paddy Straw	% of Compounds in Mushroom Grown from Sugarcane Leaves
1	Protein	30.2%	28%
2	Carbohydrate	12%	11%

Table No.3: Phytochemical Screening of *Pleurotus eous*

S.No	PHYTOCHEMICALS	ETHANOL EXTRACT
1	Glycosides	+
2	Alkaloids	+
3	Terpenoids	+
4	Phenolics	_
5	Flavonoids	+
6	Amino acids	_
7	Saponins	+
8	Tannins	_
9	Carbohydrates	_
10	Proteins	+

(+) Present (-) absent

Table No.4: Total antioxidant capacity radical scavenging assay of *Pleurotus eous*

S.No	Sample Marking	Extract Concentration (µg/mL)	OD at 695nm% Inhibition of TAC Antioxidant Activity	
			TAC(µg/ml)	IC50 Value
1	***Pleurotus eous***	10	39.64	31.88
2		20	45.31	
3		30	55.07	
4		40	69.33	
5		50	78.41	

Table No.5: Nitric oxide scavenging assay of *Pleurotus eous*

S.No	Sample Marking	Extract Concentration (µg/mL)	OD at 546nm% Inhibition of DPPH Antioxidant Activity	
			NO(µg/ml)	IC50 Value
1	***Pleurotus eous***	10	47.72	35.41
2		20	52.00	
3		30	56.95	
4		40	64.05	
5		50	70.60	

Table No.6: Antibacterial activity of ethanolic extract

S.No	Name of Bacteria	Control	Positive control (AB)	Ethanol extract (PE)
1	*Staphylococcus aureus*	12.5	17	14
2	*Pseudomonas* aeruginosa	12	14.3	9
3	*Klebsiella pneumoniae*	8.5	12.8	10.5

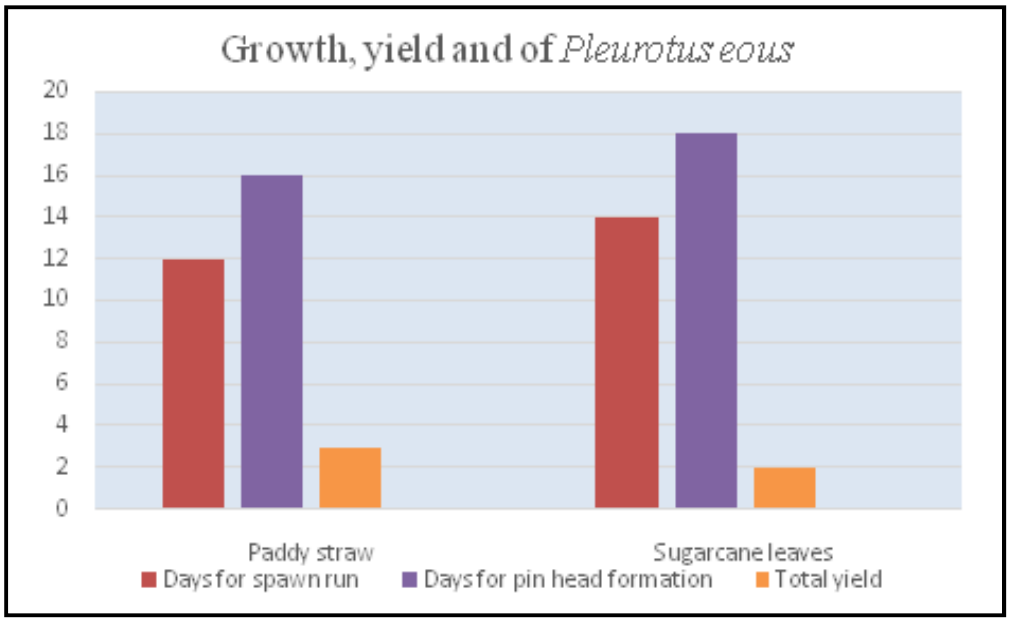

Figure No.1: Growth and Yield of *Pleurotus eous*

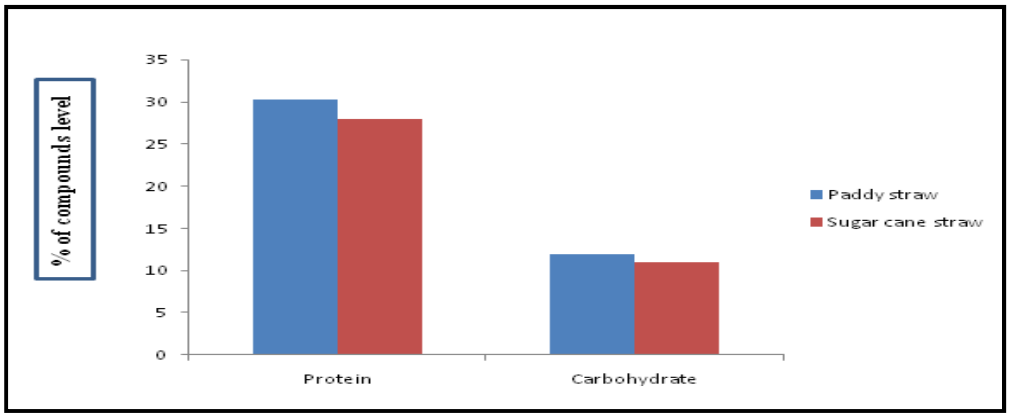

Figure No.2: Biochemical compositions of *Pleurotus eous* using different substrates

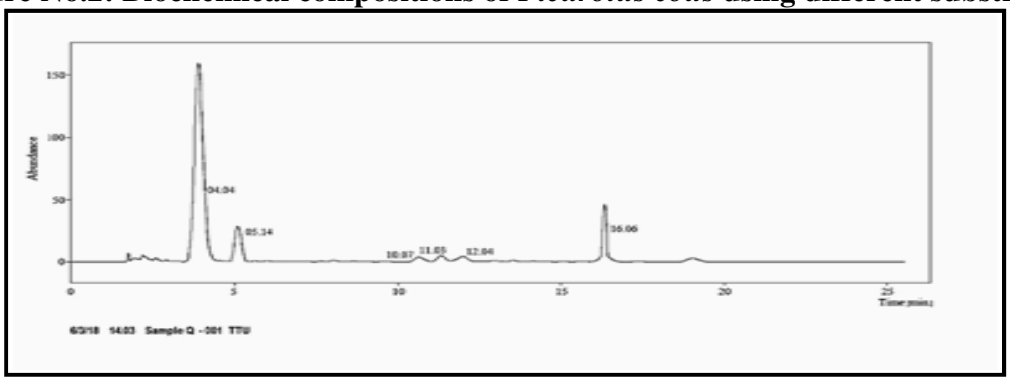

Figure No.3: HPLC Analysis

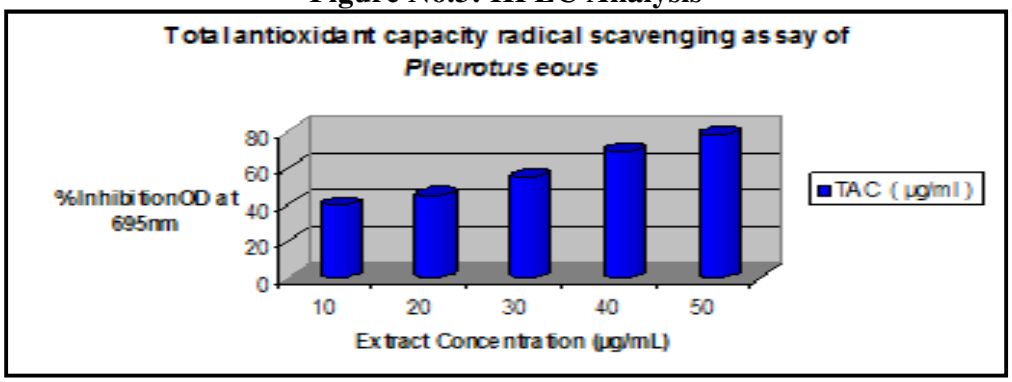

Figure No.4: TAC scavenging activities of *Pleurotus eous*

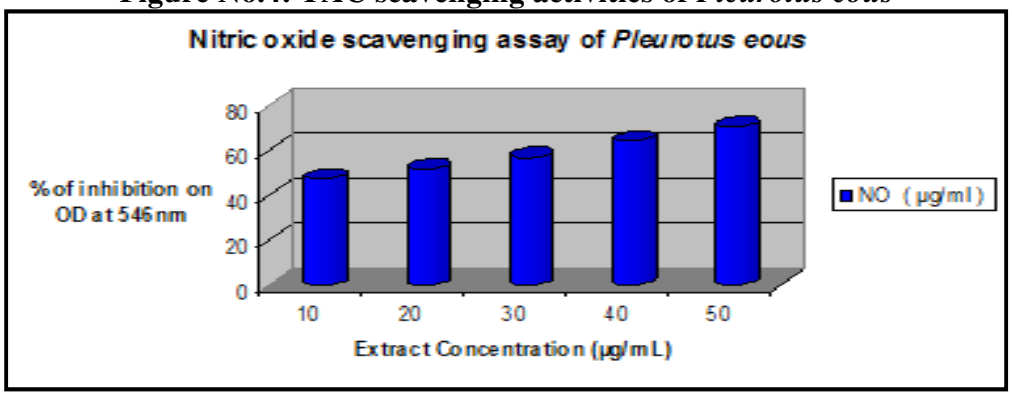

Figure No.5: NO scavenging activities of *Pleurotus eous*

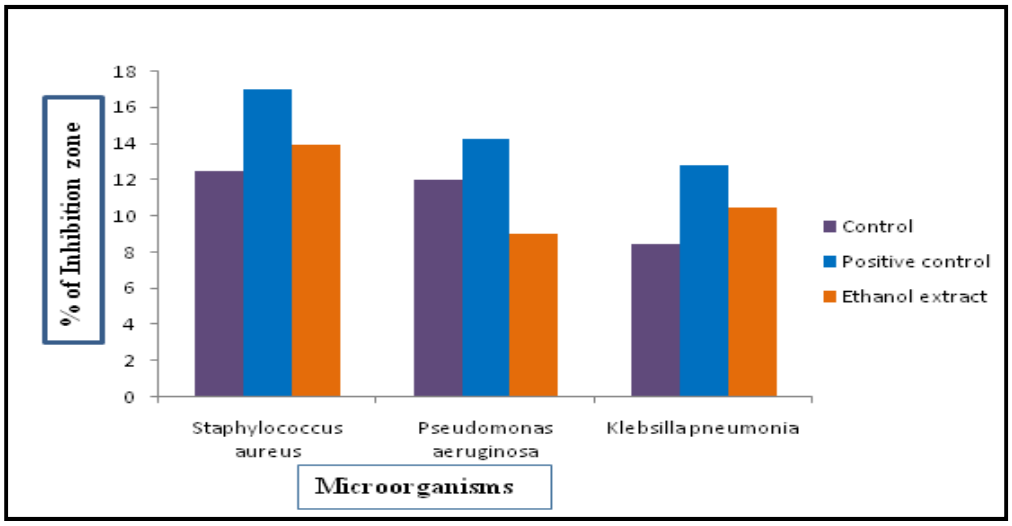

Figure No.6: Antibacterial activity of ethanolic extract of *Pleurotus eous*

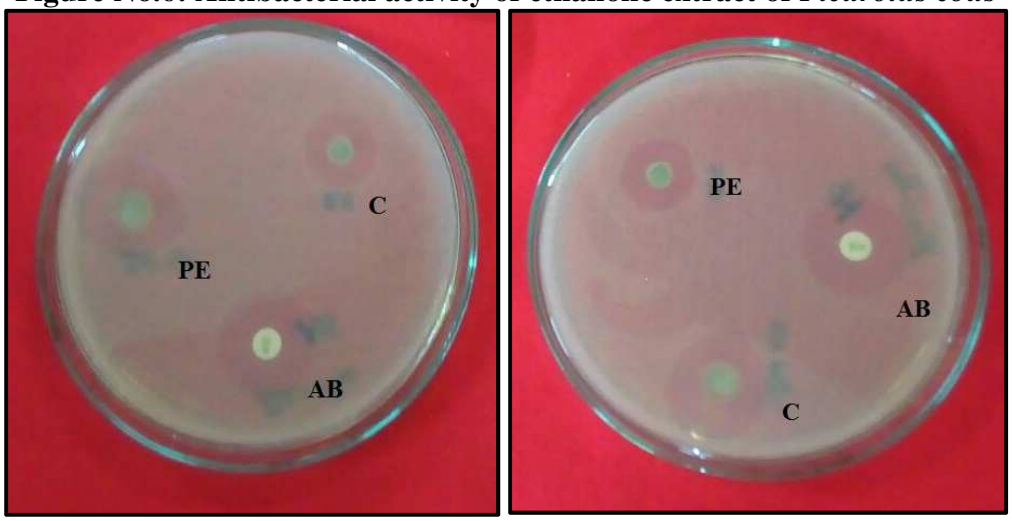

Figure No.7: *Staphylococcus aureus Klebsilla pneumonia*

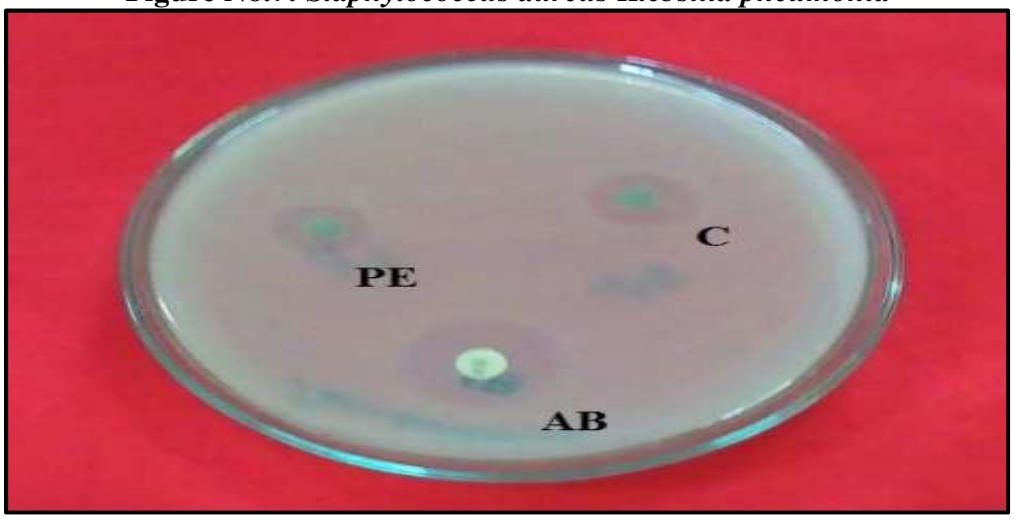

Figure No.8: *Pseudomonas aeruginosa*

Note: C - Control; AB - Positive control and PE – *Pleurotus eous*

CONCLUSION

The present investigation was circumspectly carried out on cultivation of *Pleurotus eous* using agricultural wastes of paddy straw and sugar cane straw. The fastest spawn running was observed in paddy straw compared sugar cane straw. Harvesting of *Pleurotus eous* with different substrates of three flushes and the maximum total yields were observed in paddy straw (2 kg) compared sugar cane (1 kg). The highest percentage of biochemical composition was showed in protein compared carbohydrates in both substrates. The preliminary phytochemicals showed the presence of six bioactive compounds like alkaloids, flavonoids, terpenoids, glycosides, saponins and proteins from ethanolic extract of *Pleurotus eous*. HPLC, five peaks were showed five bioactive compounds like Ergosterol, Phenyl, β- glucan, Pectin and Alpha-hydroxylase. Free radical scavenging activity of total antioxidant capacity and NO was observed from ethanolic extract of *Pleurotus eous*. Both antioxidant activities were compared with standard ascorbic acid. The highest inhibition of both antioxidant values were found in increase concentration. Ethanolic extract of *Pleurotus eous* was against human pathogens such as *Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsilla pneumonia*. The better growth of inhibition was showed against *Staphylococcus aureus,* followed by *Klebsilla pneumoniae* and *Pseudomonas aeruginosa*. *Pleurotus eous* used as treatment of several disease caused by human pathogenic bacteria.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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