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Comparative Studies of Some *Pleurotus* spp. with Special Reference to Their Biochemical, Antioxidant and Antimicrobial Activities

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Abstract

Oyster mushroom (*Pleurotus* spp.) cultivation is a very simple low cost technology. Four *Pleurotus* spp. viz. *P. ostreatus*, *P. florida*, *P. pulmonarius* and *P. flabellatus* were grown in 30 ± 2^0C in non-sterilzed rice straw. All the biochemical studies were done using 70% methanolic extract of dried powder of mushroom fruiting bodies. Flavonoids, total phenol, orthodihydric phenol content of all the species were measured in addition to their protein and carbohydrate contents. *P. ostreatus* showed highest protein and carbohydrate. *P. flabellatus* showed highest flavonoid. *P. florida* showed highest phenol content whereas *P. florida and P. pulmonarius* showed highest orthodihydric phenol contents. Antioxidant activities of the mushroom extract in terms of ABTS and DPPH oxidation and reducing power was estimated in all the species. Though ABTS scavenging activities are more or less similar but DPPH scavenging activities differ markedly in different *Pleurotus* spp. and *P. ostreatus* showed the highest result (74+2.2%). The highest reducing power was found in *P. florida*. The fruiting body as well as mycelial extract of *Pleurotus* species showed antibacterial activities in as low as 36-45 mg fresh fruiting body equivalent which is comparable to standard commercial antibiotic erythromycin. The present study does not establish the superiority of any single species but in general all the studied *Pleurotus* species contained different nutraceuticals which may be utilized for the benefit of nutritional as well as health status of people from third world countries.

Keywords:

Antioxidant;
Antimicrobial activity;
Neutraceuticals;
Oyster mushroom;
Pleurotus spp.

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1. Introduction

Pleutorus spp., commonly known as oyster mushroom grow in the wild in tropical, sub tropical and temperate regions and are commercially cultivated throughout the world (Li, 2017). They are most popular edible mushrooms in India due to their easy cultivation procedures within a broad range of temparatures (15 – 30^{0}C) on different varieties of substrates like agro forest residues, weeds and wastes and produce food, feed, enzymes and a number of pharmaceuticals (Das & Mukherjee, 2007; Gregory et al. 2007). According to Sanchez (2010) oyster mushroom positioned second in the world in respect to their consumption which is just after the button mushroom (*Agaricus* spp.).

They are designated as healthy foods, low in calories, rich in protein, fibre, chitin, vitamins and minerals (Das et al, 2015, Jayakumar et al, 2009; Akindahunsi & Oyetayo 2006; Manzi et al, 2004). A number of workers reported that *Pleurotus* spp. produce delicious fruiting bodies which are the sources of a large no. of nutraceuticals that can be used for prevention and treatment of different diseases (Weinheim, 2006, Khatun et al, 2015). Both fruiting body and the mycelium of oyster mushrooms (*Pleurotus* spp.) contain compounds with wider ranging antimicrobial activity. They are affluent sources of natural antibiotics. One of the cell wall component glucan are well known for their immunomodulatory properties. They produce different secondary metabolites which combat against bacteria, fungi and viruses (Benedict and Brady 1972; Suzuki et al, 1990).
In the present investigation four *Pleurotus* spp. (i.e. *P. florida, P. flabellatus, P. pulmonarius and P. ostreatus*) were grown in higher temperature (30 ± 2^{0}C). A comparative studies of biochemical parameters and antioxidant properties of fruiting bodies and antimicrobial properties of both fruiting bodies and mycelium grown in this condition are carried out.

2. Research Method

Mushroom strains
Pleurotus flabellatus (MTCC 1799), *Pleurotus ostreatus* (MTCC 1802) and *Pleurotus pulmonarius* (MTCC 1805) were obtained from Microbial Type Culture Collection, IMTECH, Chandigarh, India. *Pleurotus florida* (ITCC 3308) was obtained from Society for Rural Industrialization, Ranchi, India. All the mushroom strains were maintained in Potato dextrose agar (PDA) medium (Das et al., 2015).

Bacterial species
Bacillus cereus MTCC 430, *B.* thuringiensis MTCC 869, *Pseudomonas putida* MTCC 2445 were obtained Microbial Type Culture Collection, IMTECH, Chandigarh, India. *Bacillus cereus* NCIM 5557, *B. safensis* NCIM 5558 were isolated and identified in this Laboratory and later deposited in National Centre for Industrial Microorganisms, NCL, Pune. *E. coli* DH5α was collected from University of Calcutta at Kolkata. All the bacterial strains are maintained in Nutrient Agar (NA) medium.

Preparation of mushroom spawn
Mushroom spawns were prepared using wheat grains at 30 ± 2^{0}C according to Das et al (2010).

Substrate preparation
Dried chopped rice straw (5 – 6) cm was collected from a local farm at Barasat, West Bengal, India and substrates for mushroom cultivation was prepared at 30 ± 2^{0}C according to Das et al. (2015).

Preparation of fruiting body extract
Fresh fruiting bodies of mushroom were kept in oven at 60 ˚C for overnight until the constant weight was gained. Oven dried mushroom powder (1gm) was crushed with 10 ml of 70% methanol. It was centrifuged at 10,000 rpm for 10 min and supernatant was collected.

Protein Determination
Protein concentration was determined by the method of Lowry et al. (1951) using BSA as standard.

Carbohydrate Determination
Total carbohydrate was determined by anthrone reagent according to Pons et al. (1981) using glucose as standard.

Determination of Total Phenol
Total phenolic content was determined using gallic acid as standard by the method of Mukhia et al (2014) with slight modifications using Folin-Ciocalteu reagent.

Determination of Total Flavonoid
Flavonoid content was determined according to Sultana et al. (2009) using quercetin as standard.

Determination of Total Orthodihydric phenol
Total orthodihydric phenol present in the mushroom samples was estimated as described by Mahadevan and Sridhar (1986) using catechol as standard.

Determination of Antioxidant activity

DPPH reduction
Reduction of the DPPH (2, 2 – diphenyl – 1 picrylhydrazyl) radical was measured according to Sharma and Bhat (2009).
Scavenging activity of the sample was calculated based on percentage decolorization of the sample according to following equation:
% inhibition of DPPH activity = $[(A_0 – A_1)/A_0] \times 100$ where, A_0 is the absorbance value of the control reaction or blank sample and A_1 is the absorbance value of tested sample.

2, 2´ -azinobis (3 – ethylbenzthiazoline–6–sulphonic acid)/ ($ABTS^+$) scavenging antioxidant assay
$ABTS^+$ radical scavenging activity of extract was determined spectrotometrically by the method of Lee et al. (2007)
Scavenging activity of the sample was calculated based on percentage inhibition of absorbance at 734 nm against the reagent blank by the following formula .
Inhibition % = $[(A_0 – A_1)/A_0] \times 100$
A_0 = Absorbance of control
A_1 = Absorbance of sample

Reducing power assay
Iron reducing capability of methanolic mushroom extracts were determined using the method of Gulcin (2009).

Antimicrobial testing of *Pleurotus* extract
70% methanolic extracts of dried fruiting body/ mycelial powder was tested for antimicrobial activity by agar-well diffusion technique (Kunjadia et al., 2014). 1gm dried powder was extracted in 10 ml of 70% ethanol and 30µl extract was used in each well. Inhibition of growth was measured as diameters of inhibitory zones in the nearest 0.1 mm. Erythromycin (30 µg) was used as standard.

Statistical Analysis
All the experiments were done in nine replicates (3 sets x 3 batches) and the parameters were given as mean ± standard deviation. Both mean and standard deviation were performed using the statistical package of Microsoft® Excel Version 2007.

3. Results

The 70% methanolic extract showed maximum protein content in *Pleurotus ostreatus* followed by *P. florida* and *P. flabellatus* whereas lowest in *P. pulmonarius*. *P. ostreatus* also showed highest sugar content (362 µg/gm) whereas *P. pulmonarius* showed lowest sugar content (308 µg/gm) (Table 1).

Table 1. Protein and carbohydrate contents of four *Pleurotus* strains in 70% methanolic extract

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Strain no.	Protein (mg/gm dry wt.)	Carbohydrate (mg/gm dry wt.)
1799	193±4.0	316 ±4.0
1802	373±13.0	362±5.0
1805	167±8.0	308±8.0
3308	293±1.0	328±6.0

Maximum amount of flavonoid was present in *P. flabellatus* (78 ± 4.8µg/g dry wt) followed by *P. florida* whereas least amount of flavonoid was present in *P. pulmonarius* (46.8 ± 2.4 µg/g dry wt.) (Fig. 1). Total phenol content was maximum in *P. florida* (536 ± 8.3 µg/g dry wt) whereas lowest in *P. ostreatus* (376 ± 4.0 µg/g dry wt.) (Fig. 2). Orthodihydric phenol content was maximum (272 ±3.5 µg/g dry wt.) in *P. florida* and minimum (208±2.6 µg/g dry wt.) in *P. pulmonarius* (Fig. 3).

All the *Pleurotus* spp. showed good amount of antioxidant activities. ABTS scavenging activities were more or less similar in all the four species which ranged from (88±2.8)% - (83±2.4)% (Fig. 4). The result of DPPH oxidation activities however, varied widely in four species. Highest activity was found in *P. ostreatus* (74±2.2)% followed by *P. pulmonarius*, *P. flabellatus* and the least activity was in *P. florida* (57± 4.3)% (Fig. 5). The highest amount of reducing power was found in methanolic extract of *P. florida* followed by *P. pulmonarius and P. ostreatus* whereas lowest amount was found in *P. flabellatus* (Fig. 6).

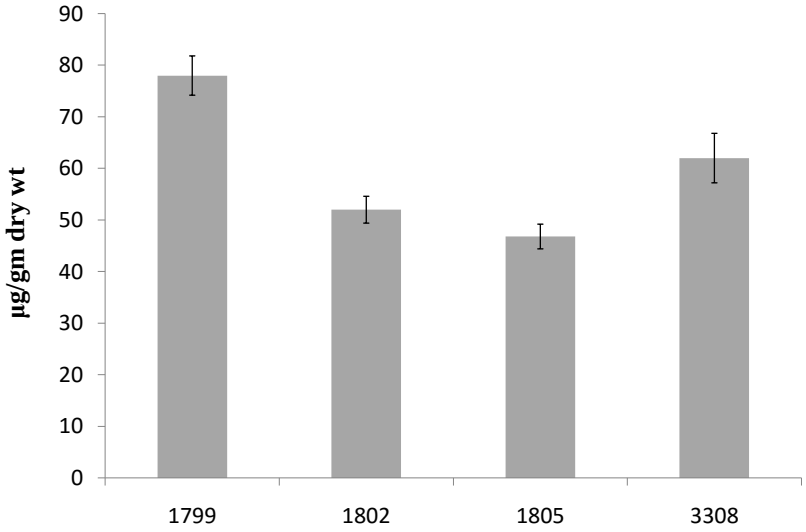

Figure 1. Estimation of total flavonoid in different *Pleurotus* strains grown in 30± 2ºC. Bars= Standard deviations.

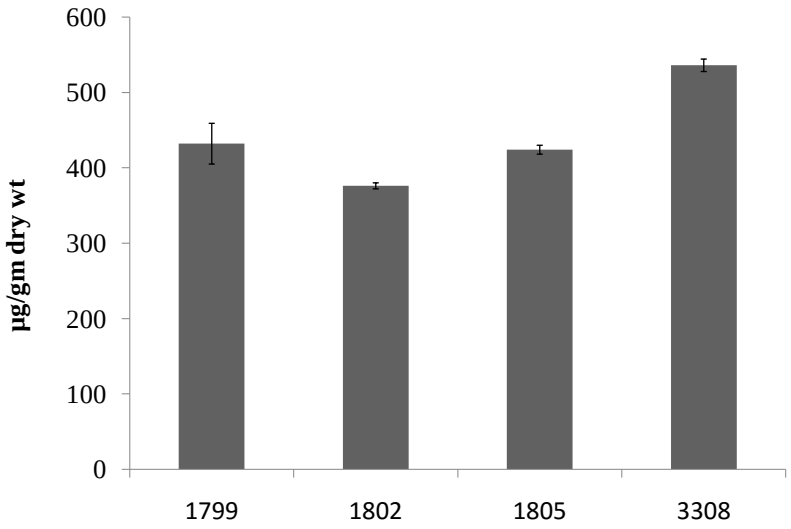

Figure 2. Estimation of total phenol in different *Pleurotus* strains grown in 30± 2ºC. Bars= Standard deviations.

The fruiting body extract and mycelial extract of all the four mushroom strains showed antimicrobial activities against the bacterial strains (Table 2). Fruiting body extract showed more inhibition than the mycelial extracts. Mycelial extract of mushroom strains inhibited the growth of all the tested bacteria whereas fruiting body extract of mushroom strains showed differential inhibition pattern on tested bacteria (Table-2). Though in general the diameter of inhibition zones of fruiting body extracts were more than the zones inhibited by mycelial extracts except *P. pulmonarius* and *P. ostreatus* against *B. cereus.* Gram negative bacteria *E. coli* showed resistance against all the fruiting body extracts except *P. ostreatus*. Growth of the *B. safensis* inhibited by all the mycelial extracts but the bacteria showed resistance against all the fruiting body extracts except *P. florida*.

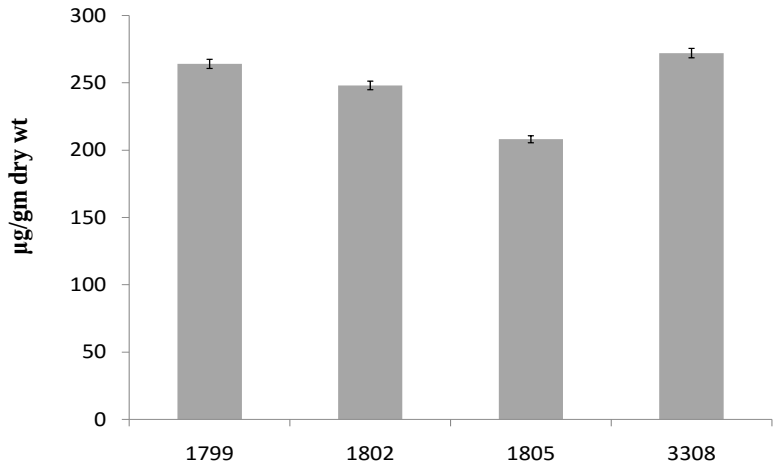

Figure 3. Estimation of orthodihydric phenol in different *Pleurotus* strains grown in 30± 2ºC. Bars= Standard deviations.

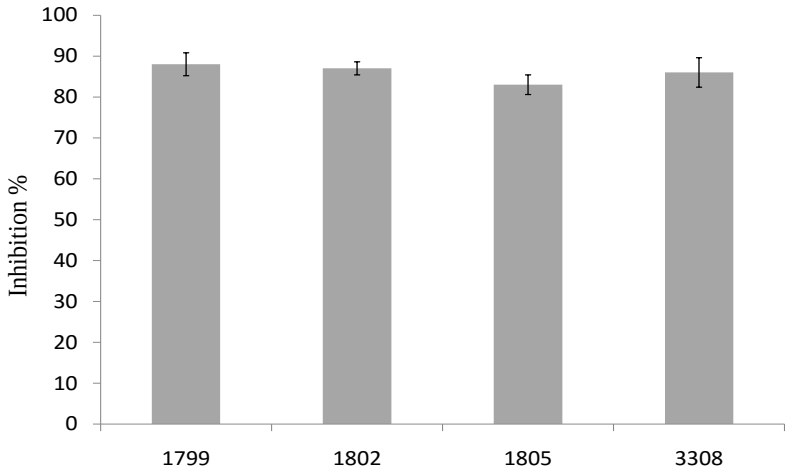

Figure 4. Estimation of ABTS radical scavenging antioxidant in different *Pleurotus* strains grown in 30± 2ºC. Bars= Standard deviations.

4. Discussion

Cultivation of oyster mushroom (*Pleurotus* spp.) is very simple and the agroclimatic condition of India is very much advantageous for their growth. In the present investigation fruiting of mushroom has been carried out at 30±2° C which is slightly higher than the mostly adopted temperature ranges (22-27°C) for oyster mushroom cultivation (Ashraf et al, 2013). In addition to higher temperature, non-pasteurized rice straw are also used for cultivation of oyster mushroom. In our previous communication we have evaluated the biological efficiency, fruiting, morphometric data and chemical composition of different oyster mushroom (Das et al., 2015).

Protein is one of the most significant parameter for analysis of mushroom nutrients. The methanolic extract showed the highest protein content in *P. ostreatus* (37.3±1.3% dry wt.) followed by *P. florida* and *P. flabellatus*. The least amount of protein was found in *P. pulmonarius* (16.7±0.8% dry wt.) among these four strains (Table 1). Khatun et al (2015) also reported that *P. pulmonarius* showed (16.8± 1.3% Dry wt) protein content whereas *P. florida* showed slightly less 23.8±1.3% protein content in methanolic extract of dry mushroom samples. In our previous study we found highest amount of protein content in aqueous extract of fresh *P. ostreatus* fruiting bodies and the amount is 856±29 µg/g fresh tissue (Das et al. 2015). The sugar content of all the four tested mushroom varied from 308-362 mg/g dry wt (Table 1). The highest amount of sugar was also found in *P. ostreatus* (36.2±0.5% dry wt) but lowest in *P. pulmonarius* (30.8±0.8). Dundar et al. (2008) showed the carbohydrate contents of *P. ostreatus*, *P. sajorcaju* and *P. eryngii* as 37.87, 37.72 and 39.85 g/ 100 g dry material.

The genus *Pleurotus* is considered as an medicinal mushroom due the presence of a number of nutraceuticals (Gregori et al., 2007; Paul et al., 2017). Flavonoids are an important nutraceutical found in different mushroom including *Pleurotus*. They can reduce the risk of tumor formation, coronary heart diseases, menopausal symptoms and other diseases in human which are associated with oxidative damages of nucleic acids, proteins or membranes (Ferguson, 2001; Rice Evans et al.,

1996). In the present investigation all the *Pleurotus* spp. showed high flavonoid contents within which *P. flabellatus* showed highest amount.

Antioxidants are the possible defender against oxidative damage by free radicals unless otherwise causes a number of diseases like ageing, diabetis, atherosclerosis, cirrhosis even cancer (Paul et al., 2017). According to Hatano et al. (1989) phenolic compounds played a major role in scavenging activity due to the presence of hydroxyl group whereas Duh et al (1999) considered them as key antioxidants. Mushrooms are very rich in phenolic compounds which are the source of their major antioxidant machineries than other antioxidant components like ascorbic acid, tocopherol, β-carotene, lycopene etc. (Khatun et al. 2015; Chirinang and Intarapichet, 2009). In the present investigation Orthodihydric phenol and total phenol contents were measured. *P. florida* showed the highest contents of both orthodihydric phenol and total phenol but *P. pulmonarius* showed the lowest orthodihydric phenol content whereas *P. ostreatus* showed the lowest total phenol content (Fig. 2 & 3). A number of researchers showed that high phenolic content food consumption can reduce the development of atherosclerosis and thus minimize the risk of heart disease (Elmastas et al. 2007).
ABTS and DPPH oxidation are two major methods to estimate free radical scavenging activity of antioxidants. Antioxidant activity was found in all the *Pleurotus* spp. ABTS scavenging activities are more or less similar in *P. flabellatus*, *P. ostreatus* and *P. florida* whereas slightly less in *P. pulmonarius* (Fig. 4). The result of DPPH oxidation activities however, varies in four species. Highest activity is found in *P. ostreatus* followed by *P. pulmonarius* and *P. flabellatus*. *P. florida* showed minimum DPPH antioxidant activities (Fig. 5).

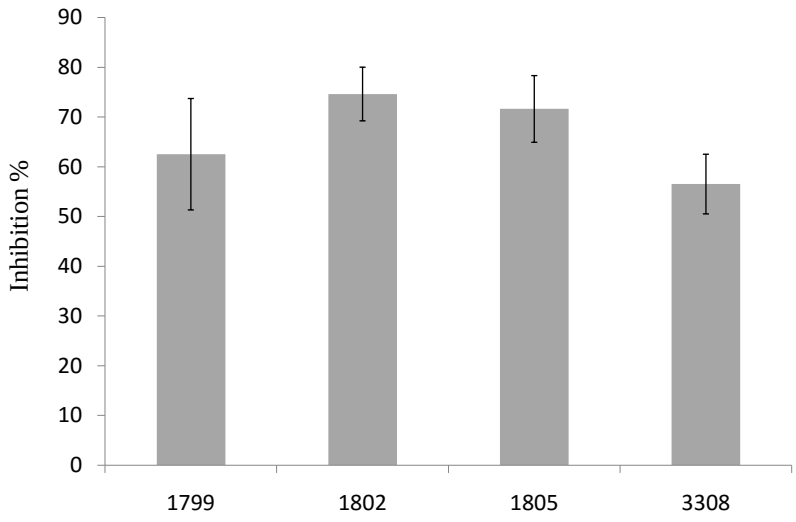

Figure 5. Estimation of DPPH radical scavenging antioxidant in different *Pleurotus* strains grown in 30± 2ºC. Bars= Standard deviations.

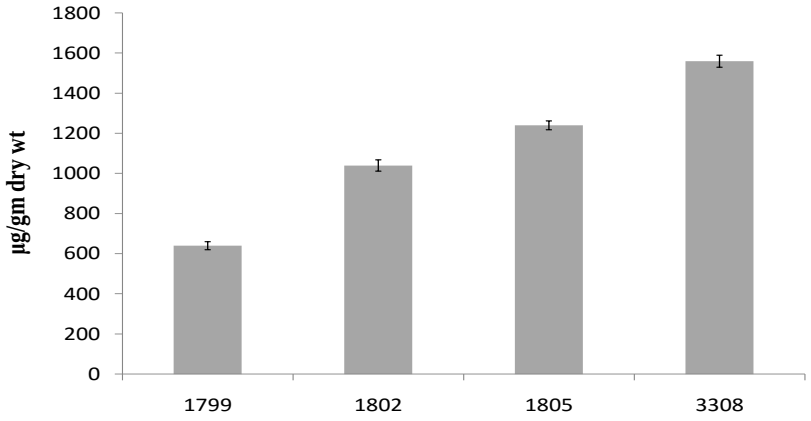

Figure 6. Estimation of reducing power in different *Pleurotus* strains grown in 30± 2ºC. Bars= Standard deviations.

Reducing power of a compound is also associated with its antioxidant activity (Mier et al. 1995). According to Wong et al. (2006) a substance exercises their reducing function by breaking the free radical chain by donating single electron or a hydrogen atom. In the present study *P. florida* showed the highest reducing power (Fig. 6).

In the present study fruiting body extract as well as mycelial extract (both in 70% methanol) of the tested mushroom show antibacterial activities against some gram positive and gram negative bacteria (Table 2). The fruiting body extracts show comparatively larger inhibition zones than the mycelial extracts though all the mycelial extracts are effective against the tested bacteria. Antimicrobial activity of *P. ostreatus* crude extracts have been reported against some gram positive, gram negative bacteria and the fungus *Aspergillus niger* (Gerasimenya et al., 2002). *P. sajor-caju* inhibits the growth of *Pseudomonas aeruginosa and S. aureus* (Nagi and Ng, 2004) whereas *P. eryngii* shows growth inhibition of *Bacillus spp.* (Nagi and Ng, 2006). We reported earlier that fresh mycelia and fruiting body extract (both aqueous and 70% ethanol) of *P. floridanus* showed antibacterial activities but only *P. florida* fruiting body extract inhibited bacterial growth (Das et al., 2012). In the present study 30 µg dry powder which is more or less equivalent to 36-45 mg fresh fruiting body [the moisture content varied 85-88% (Das et al., 2015)] are competent to inhibit the bacterial growth.

Table 2. Zone of inhibition of mushroom (*Pleurotus* spp.) extract using 70% methanol.

Name of bacteria	Fruitbody extract[1] (cm)				Mycelial extract[1] (cm)				Erythromycin[2]
	3308	1802	1805	1799	3308	1802	1805	1799	
B. cereus	1.2-1.5	1.3-1.8	1.2-1.3	1-1.2	0.6	1.7-1.9	1.6-1.9	0.7	1.3-1.4
E. coli	-	0.5-1.0	-	-	0.5	0.4	0.5	0.6	1.0-1.2
S. auerus	1.0-1.5	1.5-1.8	1.2-1.4	1.2	0.6	0.6	0.5	0.6	1.5-1.6
B. thuringiensis	1.5	1.7-1.8	1.2-1.3	1.1-1.2	0.8	0.6	0.4	0.7	0.7-0.8
B. safensis	1.2	-	-	-	0.7	0.5	0.8	0.5	1.7-1.8

[1]30 µl extract
[2]50µg/30µl were used in each well

5. Conclusion

Pleurotus spp. are a group of edible oyster mushroom. They can be cultivated in tropical conditions and fruiting bodies are still enriched with nutraceuticals. They possess high protein, carbohydrate phenol, flavonoid and antioxidants which varies from species to species.. All the species showed antibacterial activities in very low quantity. From the present study it is not possible to select the best species of *Pleurotus* but it can be suggested that all the studied species might be included in the diet system to improve the nutritional and health scenario of common people particularly in the third world countries.

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