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A Comparative Study of Antioxidant and Antimicrobial Activities of *Pleurotus Ostreatus, Pleurotus Eryngii* and *Pleurotus Djamor*

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***Abstract* – This is a comparative study in which antioxidant and antimicrobial activities of three varieties of edible Mushrooms belonging to *Pleurotus* species namely, *Pleurotus ostreatus*, *Pleurotus eryngii* and *Pleurotus djamor* were studied. The antioxidant activity of *P. ostreatus, P. eryngii* and *P. djamor* was studied by evaluation of their scavenging effect by using red Radish extract as standard and evaluation of the ascorbic acid content in each of the mushrooms. The antimicrobial activity was studied by performing agar well-diffusion method against three pathogenic bacteria namely, *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and showed few positive results.**

Keywords* – Antimicrobial Activity, Antioxidant Activity, Gram-Negative, Gram-Positive, Mushrooms, *Pleurotus

I. INTRODUCTION

Mushroom is defined as "a macro fungus" with a distinctive fruiting body which can be either hypogeous or epigeous, large enough to be seen with the naked eye and to be picked by hand. The first growing mushrooms have received worldwide popularity in recent decades with realization to the fact that they are good source of delicious food with high nutritional value (Stamets, Paul, 2005).A mushroom develops from a module, or pinhead, less than two millimeter in diameter, is called a primordium, which is typically found on or near the surface of the substrate. It is formed with in the mycelium, the mass of thread like hyphae that make up of fungus. *Pleurotus ostreatus* is commonly called as 'white oyster mushroom'. *Pleurotus eryngii* is commonly known as 'brown-grey oyster mushroom'; while *Pleurotus djamor* is known commonly as 'pink oyster mushroom'.

An antioxidant is a molecule that inhibits the oxidation of other molecules. (Pamela Manzi *et al*., 1999) Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. The extracts of oyster mushroom help to reduce oxidative damage to the human body (T. Jayakumar, 2011).

Antimicrobial activity can be defined as any biological activity that acts against bacteria, fungi, or virus. The antimicrobial activity of mushroom extracts against Gram-positive and Gram-negative bacteria was seen to be high (Alves Maria Jose *et al*., 2002).

The present study was designed and carried out to achieve the following objectives, (1) to study the antioxidant activity of *P. ostreatus, P. eryngii and P. djamor* by evaluation of the scavenging effect, (2) to evaluate ascorbic acid content in *P. ostreatus, P. eryngii* and *P. djamor* and (3) to study the antimicrobial activity of *P. ostreatus, P. eryngii* and *P. djamor.*

II. MATERIALS AND METHODS

A. Preparation of solvent-based extracts of P. ostreatus, P. eryngii and P. djamor

The mushrooms, *P. ostreatus, P. eryngii* and *P. djamor* were procured by cultivation at Presentation College of Applied Sciences, Puthenvelikkara, Ernakulam. 10gm of each of the three mushroom varieties were taken and subjected to solvent-based extraction using 10ml of the solvents Ethanol, Methanol, Chloroform and Dimethyl ether using the mortar and pestle. After completion of extraction, the extracts were transferred in to sterile autoclaved vials and preserved in refrigerator for furthur use.

B. Study of antioxidant activity of P. ostreatus, P. eryngii and P. djamor by evaluation of the scavenging effect by using red Radish(Raphanus sativus L.)extract as standard

Red radish (*Raphanus sativus* L.) is a known rich source of antioxidants (Rozario Goyenche *et al.,* 2015). It is found to be the richest source of ascorbic acid (Zakia, S.A *et al*., 1993). Red radish extract (10%) was used as the standard anti-oxidant against Potato extract (10%) and a standard graph was drawn using concentration of red radish extract versus optical density (OD) at 450 nm. The concentration of radical present in 10 ml of test sample was determined using the standard graph and the scavenging effect of the tree mushroom varieties was calculated using the following formula,

$$Scavenging\ effect = (Absorbance\ of\ standard\ radish\ extract - Absorbance\ of\ test)/ Absorbance\ of\ standard\ x\ 100$$

C. Evaluation of ascorbic acid content in P. ostreatus, P. eryngii and P. djamor

A standard calorimetric assay for calculating the ascorbic acid content in the three mushroom extracts was adopted. Ascorbic acid is also determined calorimetrically. The dehydro ascorbic acid alone reacts quantitatively and not the other reducing substances present in the sample extract. Thus this method gives an accurate analysis of ascorbic acid content than the dye method. Ascorbic acid is first dehydrogenated by H_2O.The dehydro ascorbic acid than reacted with 2,4-dinitro phenyl hydrazine to form osazone and dissolved in sulphuric acid to give an orange-red colour solution which is measured at 540 nm. A

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standard graph was plotted by taking concentration of ascorbic acid on X-axis and optical density on Y-axis. From the graph the concentration of the 'test' was calculated.

D. Study of antimicrobial activity of P. ostreatus, P. eryngii and P. djamor against Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa by agar well-diffusion method

The pathogens were procured from Life Science laboratory of Presentation College of Applied Sciences, Puthenvelikara, Ernakulam, Kerala. The media used for enriching *E. coli*, *K. pneumoniae* and *P. aeruginosa* was nutrient agar broth. The inoculated broths were kept for overnight incubation at 32ºC in a rotary shaker. Bacterial culture were subjected to bacterial subculture, after which on the third day nutrient agar plates were prepared and the bacteria were inoculated on to the nutrient agar plates using sterile cotton swabs to study the effects of *P. ostreatus*, *P. eryngii, P. djamor* on the three bacteria. Each plate was inoculated with 1ml. of bacterial culture by spread-plating method and inoculated overnight until the lawn culture of the respective test organisms was obtained. These plates were then subjected to well preparation and extract addition. Each plate consisted of 5 wells namely, well 1(Control), well 2(Ethanol extract-E), well 3(Methanol extract-M), well 4(Chloroform extract-C), well 5(Dimethyl ether extract-DM).The antibiotic ampicillin was used as control in all culture plates. 4 control culture plates were maintained by adding each of the solvents at 50,100,150 and 200µl volumes to confirm the antimicrobial activity of the respective solvent-based mushroom extracts on each of the three test organisms. All inoculated plates were subjected to incubation at 27°C and maintained for furthur analyses of the antimicrobial activities. The antimicrobial activity was studied by calculating the net area of zone of inhibition in the plates. Net area of zone of inhibition was calculated in cm^2 using the following formula:

$$Netareaofzoneofinhibition = \pi(A/2)^2 - \pi(B/2)^2$$

Where, A= diameter of Zone of inhibition,
B = diameter of well.

III. Results and Discussion

a. Study of antioxidant activity of P. ostreatus, P. eryngii and P. djamor by evaluation of the scavenging effect by using Red Radish extract as standard

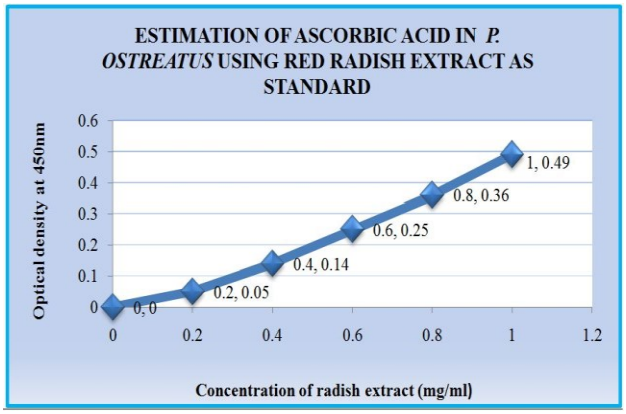

Fig. 1. Graph of estimation of ascorbic acid in *P. ostreatus* using red Radish extract as standard.

The standard graph for radish extract was obtained and the absorbance of test was found to be **0.26** OD units. The concentration of radical was **65 mg/dl** and the scavenging effect was found to be **38.095 %.**

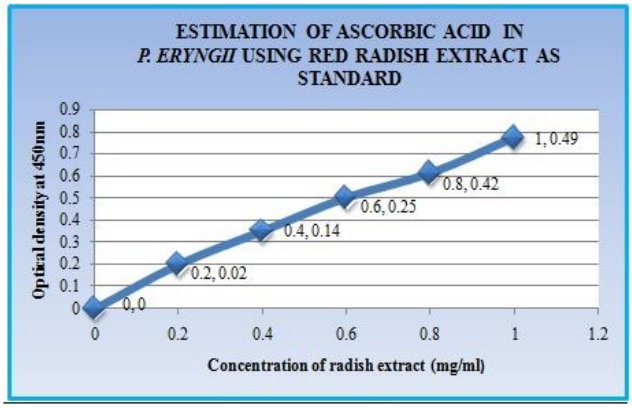

Fig. 2. Graph of estimation of ascorbic acid in *P. eryngii* using red Radish extract as standard

The standard graph for radish extract was obtained and the absorbance of test was found to be **0.07** OD units. The concentration of radical was **38 mg/dl** and the scavenging effect was found to be **50 %.**

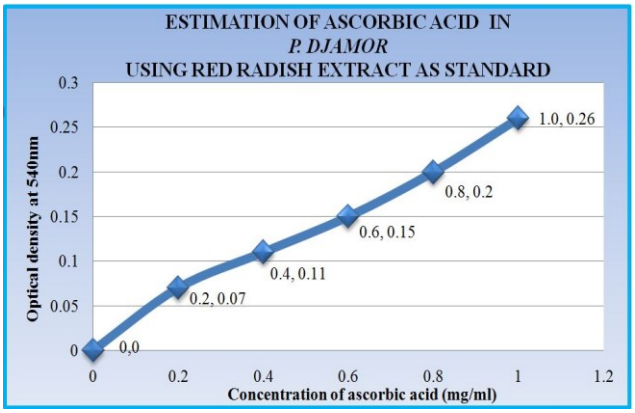

Fig. 3. Graph of estimation of ascorbic acid in *P. djamor* using red Radish extract as standard

The standard graph for radish extract was obtained and the absorbance of test was found to be **0.115** OD units.

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The concentration of radical was **62 mg/dl** and the scavenging effect was found to be **36.11%.**

b. Study of antioxidant activity of P. ostreatus, P. eryngii and P. djamor by evaluation of ascorbic acid content

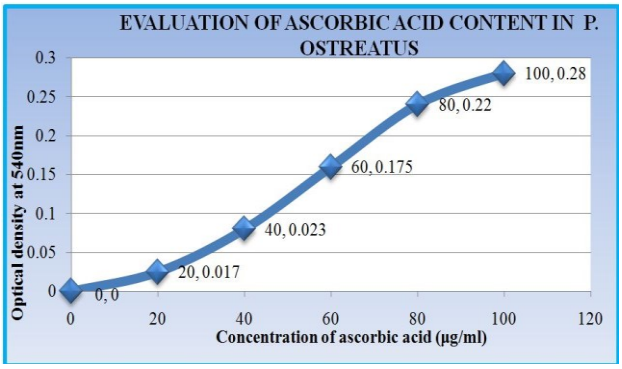

Fig. 4. Graph of evaluation of ascorbic acid content in *P. ostreatus*

The net optical density of test sample after three hours of incubation was found to be **0.26** OD units. The concentration of ascorbic acid content was found to be **98.24 µg/ml**.

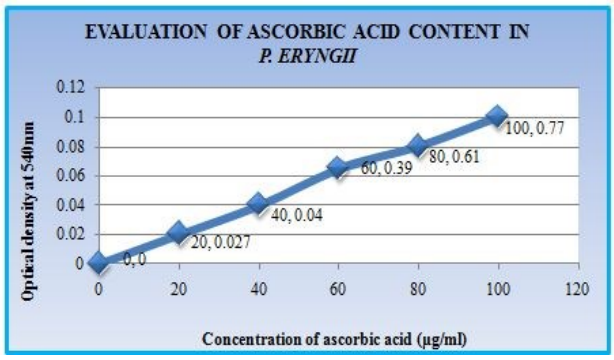

Fig. 5. Graph of evaluation of ascorbic acid content in *P. eryngii*

The net optical density of test sample after three hours of incubation was found to be **0.06** OD units. The concentration of ascorbic acid content was found to be **42.77µg/ml**.

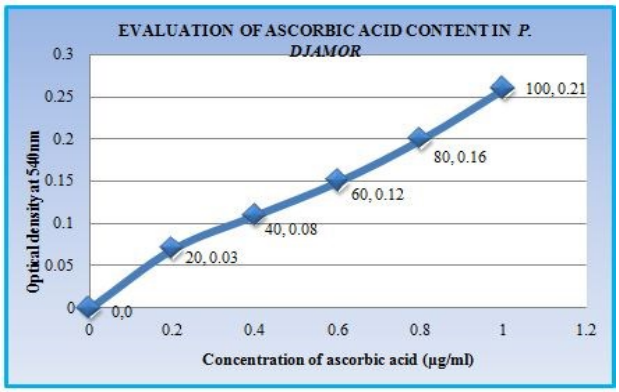

Fig. 6. Graph of evaluation of ascorbic acid content in *P. djamor*

The concentration of ascorbic acid was calculated after three hours of incubation. The net optical density of test sample after three hours of incubation was found to be **0.16** OD units. The concentration of ascorbic acid content was found to be **80.00 µg/ml**.

c. Study of antimicrobial activity of P. ostreatus, P. eryngii and P. djamor

The antimicrobial activity of mushroom, *P. ostreatus* on three pathogenic bacteria, *E. coli, K. pneumoniae* and *P. aeruginosa* was studied by agar well-diffusion method and the following results were observed.

i. Study of antimicrobial activity of P. ostreatus on E. coli

The **dimethyl ether**-based extract of *P. ostreatus* when added in a volume of **200µl** showed the best results on plates inoculated with *E. coli* with a net area of zone of inhibition of **1.22 cm²**; followed by **150µl** of **dimethyl ether**-based extract with a net area of zone of inhibition of **0.345 cm²** .

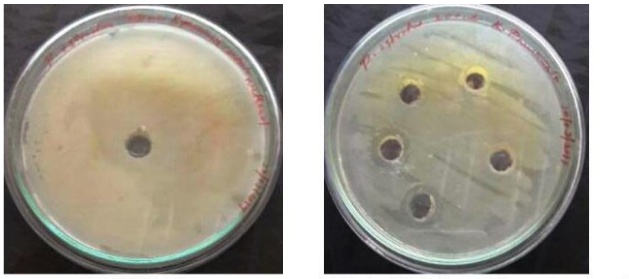

Fig. 7. Study of antimicrobial activity of *P. ostreatus* on *E. coli*

ii. Study of antimicrobial activity of P. ostreatus on K. pneumoniae

The **methanol**-based extract of *P. ostreatus* when added in a volume of **200µl** showed the best results on plates inoculated with *K. pneumoniae* with a net area of zone of inhibition of **1.22 cm²** ; followed by **150µl** of **methanol**-based extract with a net area of zone of inhibition of **0.75 cm²** .

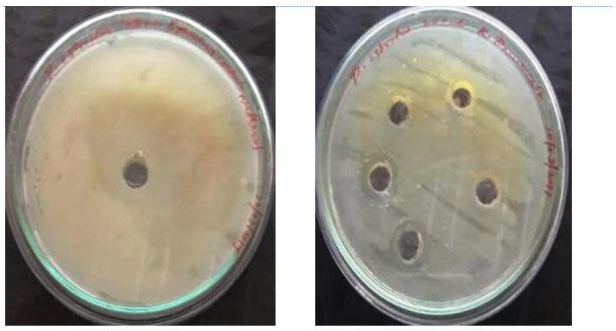

Fig. 8. Study of antimicrobial activity of *P. ostreatus* on *K. pneumoniae*

iii. Study of antimicrobial activity of P. ostreatus on P. aeruginosa

P. ostreatus was found to show no activity against *P. aeruginosa* when different volumes of different solvent-based extracts ranging from 50-200µl were added.

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iv. Study of antimicrobial activity of P. eryngii on E. coli

P. eryngii did not show any activity on *E. coli* when different volumes ranging from 50-200 µl of different solvent-based extracts were added.

v. Study of antimicrobial activity of P. eryngii on K. pneumoniae

P. eryngii did not show any activity on *K. pneumoniae* when different volumes ranging from 50-200 µl of different solvent-based extracts were added.

vi. Study of antimicrobial activity of P. eryngii on P. aeruginosa

Only **chloroform**-based extract of *P. eryngii* was seen to show activity against only *P aeruginosa* when added in a volume of **200µl** with a net area of zone of inhibition of **1.75 cm^2**. *P. eryngii* did not show any activity against *E. coli* and *K. pneumoniae* at these volumes of the different solvent-based extracts other than chloroform-based one.

vii. Study of antimicrobial activity of P. djamor on E. coli

P. djamor did not show any activity on *E. coli* when different volumes ranging from 50-200µl of different solvent-based extracts were added.

viii. Study of antimicrobial activity of P. djamor on K. pneumoniae

P. djamor did not show any activity on *K. pneumoniae* when different volumes ranging from 50-200µl of different solvent-based extracts were added.

ix. Study of antimicrobial activity of P. djamor on P. aeruginosa

The **methanol**-based extract of *P. djamor* when added in a volume of **200µl** showed the best results on plates inoculated with *P. aeruginosa* with a net area of zone of inhibition of **1.22 cm^2**; followed by **150µl** of **methanol**-based extract with a net area of zone of inhibition of **0.54cm^2.**

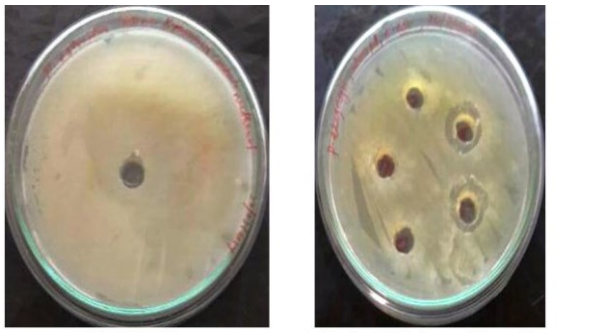

Fig. 9. Study of antimicrobial activity of *P. eryngii* on *P. aeruginosa*

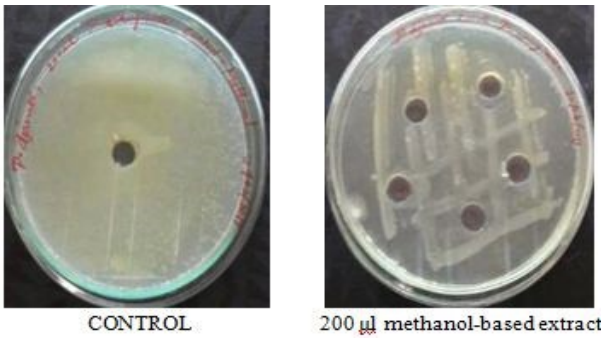

Fig. 10. Study of antimicrobial activity of *P. djamor* on *P. aeruginosa*

Table 1. Summary of antimicrobial activity of *P. ostreatus, P. eryngii* and *P. djamor* against *E. coli, K. pneumoniae* and *P. aeruginosa*

Sl. No.	Mushroom	Volume (µl)	Bacterium	Solvent	Diameter of zone of inhibition of control (cm)	Diameter of zone of inhibition of extract (A) (cm)	Diameter of well (B) (cm)	Area of zone of inhibition $\pi(A/2)^2$- $\pi(B/2)^2$ $(cm)^2$
1	*P. ostreatus*	200	*E. coli*	DM	1.8	1.6	1.0	**1.22**
2	*P. ostreatus*	150	*E. coli*	DM	1.6	1.2	1.0	**0.345**
3	*P. ostreatus*	200	*K. pneumoniae*	M	1.6	1.4	1.0	**1.22**
4	*P. ostreatus*	150	*K. pneumoniae*	M	1.8	1.6	1.0	**0.75**
5	*P. eryngii*	200	*P. aeruginosa*	C	2.0	1.8	1.0	**1.75**
6	*P. djamor*	200	*P. aeruginosa*	M	1.8	1.6	1.0	**1.22**
7	*P. djamor*	150	*P. aeruginosa*	M	1.4	1.3	1.0	**0.54**

In the present study, the antimicrobial activities of *P. ostreatus, P. eryngii* and *P. djamor* was tested against three pathogenic bacteria viz., *E. coli, K. pneumoniae* and *P. aeruginosa* at various volumes of four different solvents (ethanol, methanol, chloroform and dimethyl ether)-based extracts ranging from 50µl-200µl. It was observed from the study that dimethyl ether- based extract of *P. ostreatus* showed the highest activity against *E. coli. P. ostreatus* also showed a minimal activity against *K. pneumoniae.* The chloroform-based extract of *P. eryngii* showed thehighest activity against *P. aeruginosa* and methanol-based extract of *P. djamor* showed the highest activity against *P. aeruginosa.* The diseases caused by *E. coli*, like gastrointestinal diseases can be cured by treatment with medicinal preparations in combination with extract of *P. ostreatus*. Extracts of *P. eryngii* can be used in commercial preparation of medicines against diseases caused by *K. pneumoniae* by using them synergistically with the presently available medicines. *P. aeruginosa*, being a multi-drug resistant bacteria can be controlled to a greater extent by implementing the use of the extracts of *P. eryngii* and *P. djamor* in combination with the antibiotics.

In Asian countries like India, only *P. ostreatus,* commonly known as 'white oyster mushroom' is considered to be palatable and is more in demand. However, *P. eryngii* and *P. djamor* mushrooms are also nutritious as well as rich sources of antioxidants. Promoting of cultivation and commercialization of these mushrooms and their products can be a boost up to the financial and economic status of the farmers, ultimately creating better employment opportunities in the country. Also, the export of these mushrooms will help improve trade-relationships of the nation. Thus, cultivation and commercialization of the mushrooms, *P. ostreatus, P. eryngii* and *P. djamor* is quite advisable.

IV. Conclusion

- *P. ostreatus*, *P. eryngii* and *P. djamor* mushrooms are edible, safe, natural and rich sources of antioxidants.
- They are also suitable for manufacture of drugs and food-supplements.
- The mushroom extracts can be used in manufacture of anti-ageing and amti-wrinkle creams.
- High dosage of these mushroom extracts can be used in medicinal preparations like ointments for wounds and control bacterial infections caused by pathogenic bacteria like *P. aeruginosa*.
- Also, these extracts could be used synergistically with the prevailing medicines against diseases, resulting in lower side-effects.
- Promoting of cultivation and commercialization of these mushrooms and their products can be a boost up to the financial and economic status of the farmers, ultimately creating better employment opportunities in the country.
- Introduction of these mushrooms into global markets will ultimately result in higher economic and financial status of the country folk, creating better employment opportunities.
- The export of these mushrooms will help improve trade-relationships of the nation.

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This dissertation is the result of support, help and blessings of many people. It is a pleasant aspect that I have now got the opportunity to express my gratitude for all of them. First and foremost I praise and thank Almighty God for the unfailing source of support, strength and blessings for the successful completion of this study.

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References

[1] Adejumo, T.O. and Awosanya, D.B., 2005. Proximate and mineral composition of four edible mushroom species from South Western Nigeria. African Journal of Biotechnology, 4: 1084- 1088.

[2] Ahmad, I., 1986. Some studies on oyster mushroom *(Pleurotus spp*.) on waste material of corn industry. M.Sc. thesis. Department of Plant Pathology, Faisalabad, p. 50.

[3] Ahmed, S.A., J.A., Kadam, V.P. Mane, S.S. Patil and M.M.V. Baig, 2009. Biological Efficiency and Nutritional Contents of *Pleurotus florida* (Mont.) Singer cultivated on different Agro-wastes. Nature and Science, 7 (1): 44-48.

[4] Akyuz, M., Yildiz, A., 2008. Evaluation of cellulosic wastes for the cultivation of *Pleurotus eryngii* (DC. ex pr) Quel. Afr. J. Biotechnol., 7: 1494-1499.

[5] Alam, N., A. Khan, M.S. Hossain, S.M.R. Amin and L.A. Khan, 2007. Nutritional Analysis of Dietary mushroom – *Pleurotus florida* 148 Eger *and Pleurotus sajor-caju* (Fr.) Singer. Bangladesh J. Mushroom, 1 (2): 1-7.

[6] Alam, N., R. Amin, A. Khan, I. Ara, M.J. Shim, M.W. Lee and T.S. Lee, 2008. Nutritional analysis of cultivated mushrooms in Bangladesh – *Pleurotus ostreatus, Pleurotus sajor-caju, Pleurotus florida* and *Calocybe indica*. Mycobiology, 36 (4): 228-232.

[7] Alves, Maria Jose, Ferreira, Isabel.C.F.R, Pintado, Manuela, Antimicrobial activity of mushroom extracts and isolated compounds ,2012 pp. 18-20

[8] Bahl, N., 1983. Medicinal value of edible fungi. In: Proceeding of the International Conference on Science and Cultivation Technology of Edible Fungi. Indian Mushroom Science, 11: 203-209.

[9] Chandramohanan, R. and V.K. Moorthy, 1991. Utilization of Areca leaf as a substitute for cultivation of *Pleurotus sajor-caju*. Indian Mushrooms. (Eds. Nair, M.C., S. Balakrishnan and C. Gokulapalan). Proceedings of national Symposium on Mushroom, Kerala Agricultural University, Trissur, pp. 140-142.

[10] Nayana, J. and Janaradhanan, K.K. 2000. Antioxidant and antitumor activity of *Pleurotus florida*. Current Sci. 79 (7): 941 – 943.

[11] Obodai, M., J. Cleland-Okine and K.A. Vowotor, 2003. Comparative study on the growth and yield of *Pleurotus ostreatus* mushcooms on 171 different lignocellulosic by-products. Journal of Industrial Microbiology and Biotechnology, 30 (3): 146-149.

[12] Pamela Manzi (1999) Nutrients in edible mushrooms:an inter-species comparative study, Istituto Nazionale della Nutrizione, Via Ardeatina, 546-00178 Rome, Italy.

[13] Rai, R.D., Saxena, S., Upadhyay, R.C. and Sohi, H.S. 1988. Comparative nutritional value of various *Pleurotus species* grown under identical conditions. Mush. J. Tropics. 8: 93 – 98.

[14] Rosario Goyenche, Sara Roura, Alejandra Ponce, Antonio Vega-Galvez, Issis Quispe-Fuents, Elsa Uribe, Karina DiScala(2015).Chemical characterization and antioxidant capacity of red radish (Raphanus sativus L.)leaves and roots, CEAZA, Cassila, La Serena, Chile

[15] Stamets, P., Chilton, J.S. *The Mushroom Cultivator: A Practical Guide to Growing Mushrooms at Home*. Olympia, Washington. Agarikon Press, 1984.

[16] Stamets, Paul. *Growing Gourmet and Medicinal Mushrooms*. Ten Speed Press. 3rd edition, 2000.

[17] Stamets, Paul. *Mycelium Running: How Mushrooms Can Help Save the World*. Ten Speed Press. 2005.

[18] T. Jayakumar, *In vitro* antioxidant activities of an ethanolic extract of the oyster mushroom, 2005.
[19] Wani, B. A., Bodha, R. H. and Wani, A. H. 2010. Nutritional and medicinal importance of mushrooms. J. Med. Plants Res. 4(24): 2598 – 2604.
[20] Zakia, S.A., Kattan, E.I., Hussein, M.H. and Khaled, A.M. 1993. Chemical composition and processing potential of oyster mushroom, *Pleurotus ostreatus*. Egypt J. Agric. Res.71: 621 - 631. Ahmad, I., 1986. Some studies on oyster mushroom *(Pleurotus spp*.) on waste material of corn industry. M.Sc. thesis. Department of Plant Pathology, Faisalabad, p. 50.
[21] ZakiaBano (2009)*Pleurotus* mushrooms. Part II. Chemical composition, nutritional value, post-harvest physiology, preservation, and role as human food.

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