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A Study of Antioxidant and Antimicrobial Activities of *Pleurotus Ostreatus*

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Abstract **– This is a study in which antioxidant and antimicrobial activities of a widely used variety of edible Mushroom belonging to *Pleurotus* species namely, *Pleurotus ostreatus*, were studied. The antioxidant activity of *P. ostreatus* was studied by evaluation of their scavenging effect by using red Radish extract as standard and evaluation of the ascorbic acid content in it. The antimicrobial activity was studied by performing agar well-diffusion method against three pathogenic bacteria namely, *Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus aureus* and showed few positive results.**

Keywords **– Antimicrobial Activity, Antioxidant Activity, Gram-Negative, Gram-Positive, Mushroom, *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'. 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).

II. Materials and Methods

A. Preparation of Solvent-based Extract of P. ostreatus

The mushroom, *P. ostreatus* was procured by cultivation at Presentation College of Applied Sciences, Puthenvelikara, Ernakulam. 10gm of the mushroom was taken and subjected to solvent-based extraction using 10ml of the solvents Ethanol, Methanol 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 by Evaluation of the Scavenging Effect by using Red Radish (Raphanus sativus) Extract as Standard

Red Radish (*Raphanus sativus*) is a known rich source of antioxidants. 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 mushroom variety was calculated using the following formula,

Scavenging effect = (Absorbance of standard radish extract – Absorbance of test) / *x*100

C. Evaluation of Ascorbic acid Content in P. ostreatus

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 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 against Escherichia coli, Klebsiella pneumoniae and Staphylococcus aureus by Agar Well-Diffusion Method

The pathogens were procured from Life Science laboratory of Presentation College of Applied Sciences, Puthenvelikkara, Ernakulam, Kerala. The media used for enriching *E. coli*, *K. pneumoniae* and *S. aureus* was nutrient agar broth. The inoculated broths were kept for overnight incubation at 32ºC in a rotary shaker. Bacterial cultures 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* on the three bacteria. Each plate was inoculated with 1ml of bacterial

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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 4 wells namely, well 1(Control), well 2(Ethanol extract-E), well 3(Methanol extract-M) and well 4(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 and 150 µl 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 activity of *P. ostreatus*. 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:

$$Net\ area\ of\ zone\ of\ inhibition\ (mm^2) = \pi(A/2)^2 - \pi(B/2)^2$$

Plates prepared were as shown below:

Plate No.	Inoculum added	Wells				Amount added
		1	2	3	4	
1	*E. coli*	C	E	M	DM	50µl
2	*E. coli*(duplicate)	C	E	M	DM	50µl
3	*E. coli*	C	E	M	DM	100µl
4	*E. coli*(duplicate)	C	E	M	DM	100µl
5	*E. coli*	C	E	M	DM	150µl
6	*E. coli*(duplicate)	C	E	M	DM	150µl
7	*E. coli*(control)	C	E	M	DM	100µl
8	*K. pneumoniae*	C	E	M	DM	50µl
9	*K. pneumoniae* (duplicate)	C	E	M	D	50µl
10	*K. pneumoniae*	C	E	M	D	100µl
11	*K. pneumoniae* (duplicate)	C	E	M	D	100µl
12	*K. pneumoniae*	C	E	M	D	150µl
13	*K. pneumoniae* (duplicate)	C	E	M	D	150µl
14	*K. pneumoniae* (control)	C	E	M	D	100µl
15	*S. aureus*	C	E	M	D	50µl
16	*S. aureus* (duplicate)	C	E	M	D	50µl
17	*S. aureus*	C	E	M	D	100µl
18	*S. aureus* (duplicate)	C	E	M	D	100µl
19	*S. aureus*	C	E	M	D	150µl
20	*S. aureus* (duplicate)	C	E	M	D	150µl
21	*S. aureus* (control)	C	E	M	D	100µl

III. Results

A. *Study of Anti-Oxidant Activity :*

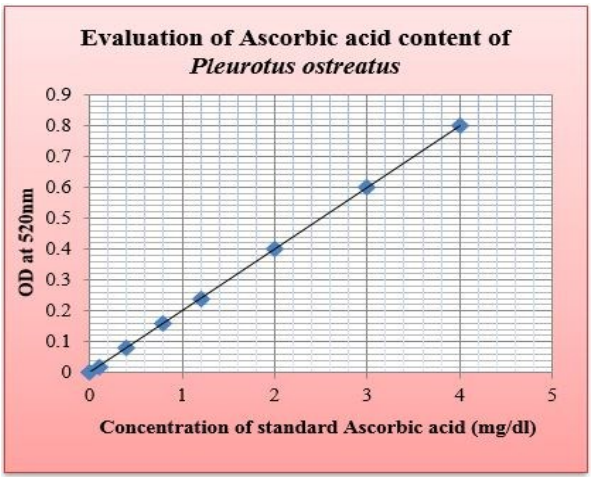
a) *Study of Antioxidant activity of P. ostreatus using Ascorbic acid as Standard:*

Dinitrophenylhydrazine - thiourea - copper sulphate reagent was used to determine the concentration of ascorbic acid of mushroom extract.

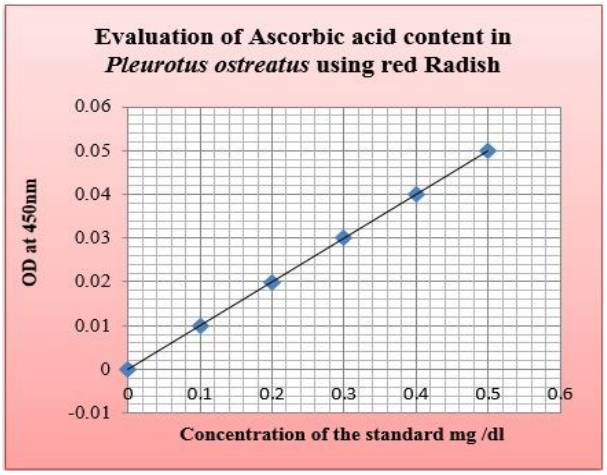
The concentration of ascorbic acid was calculated after three hours of incubation. The net optical density of test sample after three hours of incubation was found to be **0.04.**

The concentration of ascorbic acid content was found to be **52.77mg/dl** and the scavenging effect of test sample was found to be **5%.**

b) *Study of Antioxidant activity of P. ostreatus using Red Radish extract as Standard:*



Graph 1: Evaluation of ascorbic acid content of *P. ostreatus*



Graph 2: Evaluation of radical content in *P. ostreatus* using red Radish extract.

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The standard graph for radish extract was obtained and the absorbance of test was found to be **0.04**. The concentration of radical **14.66mg/dl** and the scavenging effect was found to be **12%.**

B. *Study of Anti-Microbial Activity:*

Anti-microbial activity of *P. ostreatus* was studied using agar well- diffusion method.

Susceptibility of various microorganisms to various solvents and antibiotic as control was done to give the following results:

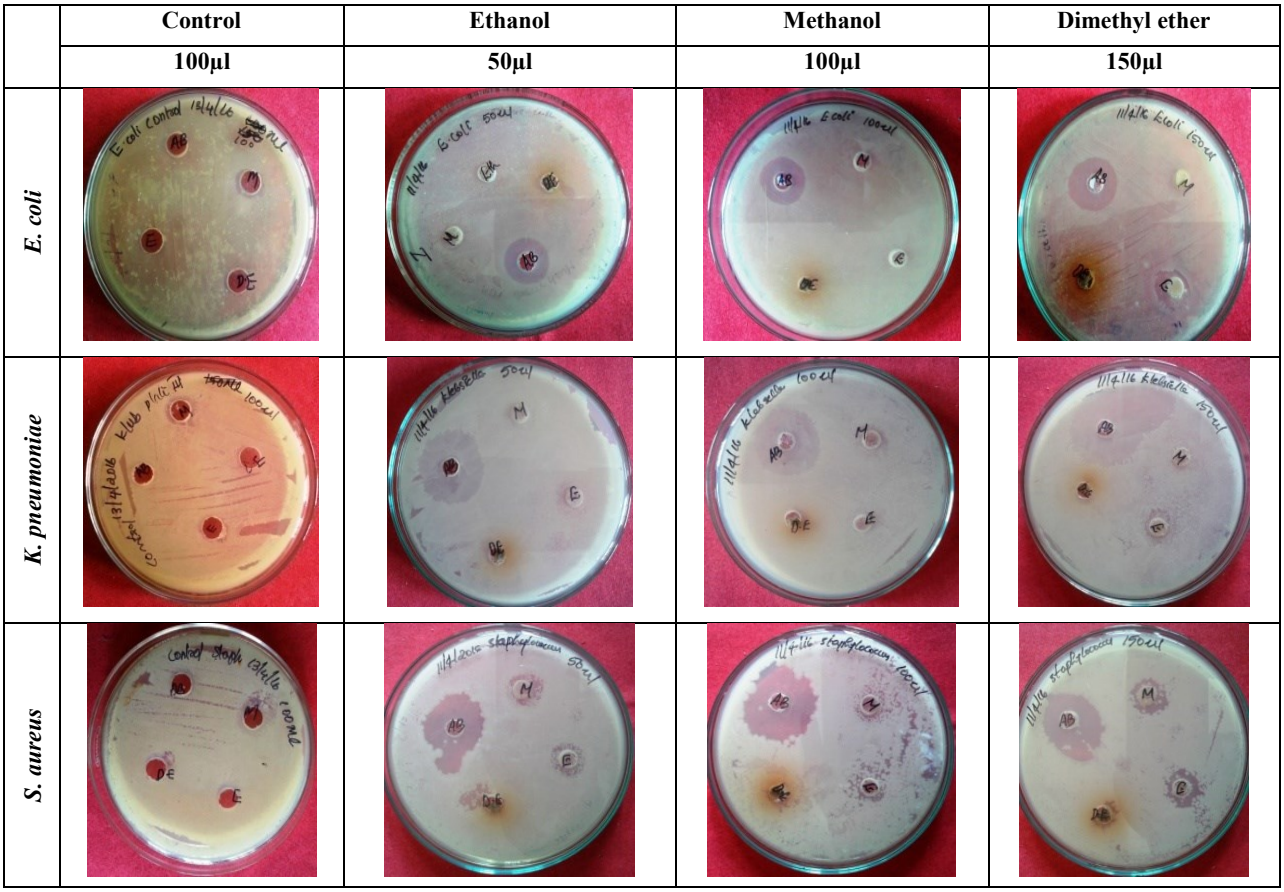
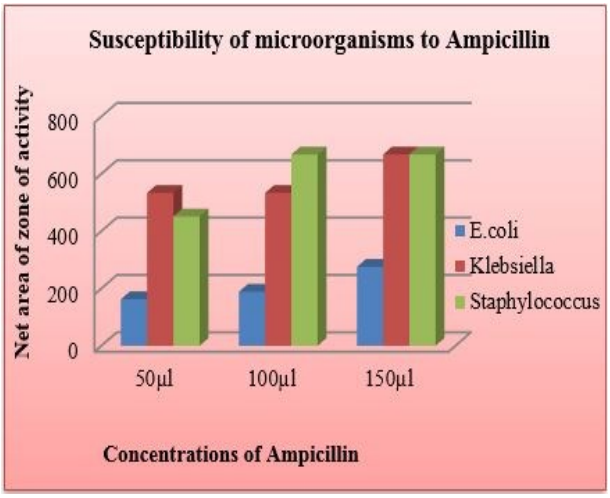
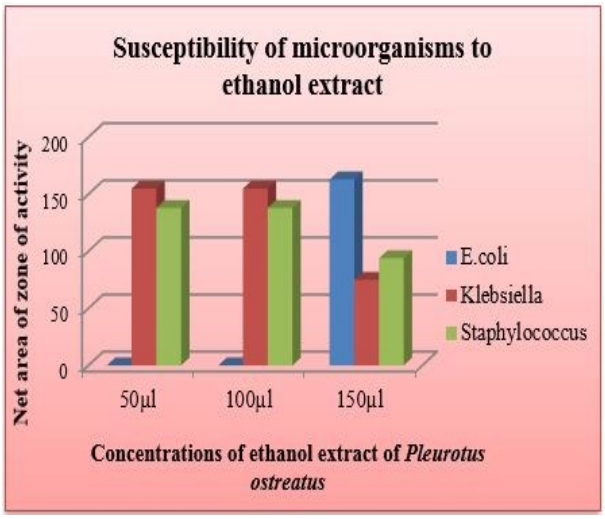


Fig. 1. Study of antimicrobial activity of *P. ostreatus* by agar well-diffusion method.

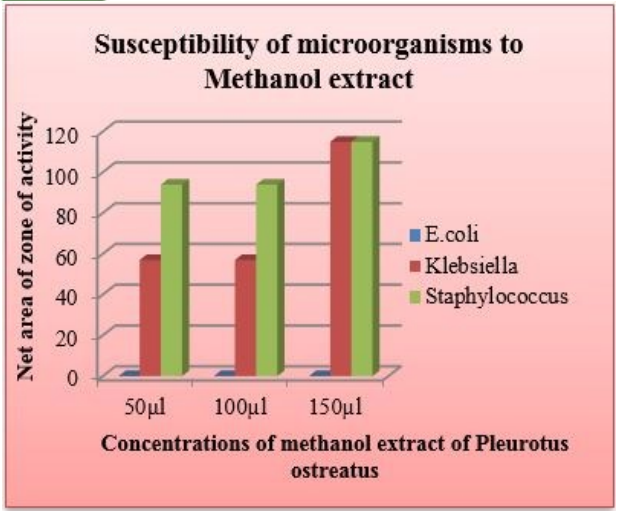
The susceptibility of the three micro-organisms viz., *E. coli*, *K. pneumonia* and *S. aureus* to different solvent-based extracts (ethanol, methanol, dimethyl ether) of *P. ostreatus* and an antibiotic control (ampicillin) can be graphically represented as follows :



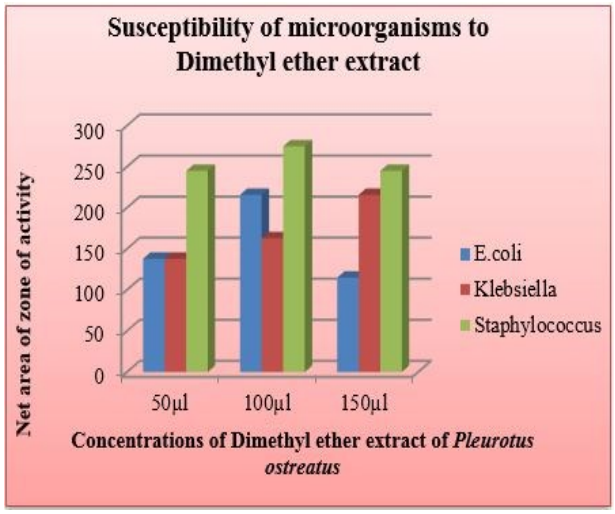
Graph 3: Susceptibility of microorganisms to Ampicillin (control).



Graph 4: Susceptibility of microorganisms to ethanol extract



Graph 5: Susceptibility of microorganisms to methanol extract.



Graph 6: Susceptibility of microorganisms to dimetylether extract.

The dimethyl ether-based extract of *P. ostreatus* showed the best results in all the plates. The highest susceptibility was shown by *S. aureus* to dimetyl ether extract of *P. ostreatus* at an amount of 100µl added; followed by *K. pneumonia*. The least susceptibility was shown by *E. coli*.

IV. Discussion

In this work, the anti-oxidant and anti-microbial activities of edible mushroom, *P. ostreatus* were studied. In the present study, the anti-oxidant activity of *P. ostreatus* was studied by two different assays, viz, using ascorbic acid as standard and red Radish extract as standard. The ascorbic acid content of *P. ostreatus* was evaluated. It showed a moderate level of ascorbic acid with a moderate scavenging effect. Thus, the anti-oxidant activity of *P. ostreatus* can be exploited commercially in preparations of drugs and cosmetics to reduce senescence and ageing. In the present study, the anti-microbial activity of *P. ostreatus* was tested against three pathogenic bacteria viz., *E. coli, K. pneumonia* and *S. aureus* at various volumes of three different solvent-based extracts viz., ethanol, methanol and dimethyl ether. It was observed from the study that dimethyl ether- based extract of *P. ostreatus* showed the highest anti-microbial activity against *S. aureus,* followed by *K. pneumonia* and the least seen in *E. coli.* The diseases caused by *S. aureus* ,like skin diseases, infections, rashes, etc., can be cured by treatment with medicinal preparations like ointments and skin creams having extract of *P. ostreatus* as an ingredient, resulting a synergistic effect against the diseases. Thus, these properties of *P. ostreatus* could be exploited commercially.

V. Conclusion

It can be stated that, tested mushroom extracts have a moderate anti-oxidant and anti-microbial activity. Based on these results, it can be concluded that mushrooms are good, safe and natural source of anti-oxidants. The main function of anti-oxidant is to reduce the effect of ageing caused due to senescence of cells. Mushroom extracts could be used in preparation of cosmetics like face creams, body creams,. etc. As mushroom *P. ostreatus* is edible, it is also suitable for consumption. *S. aureus* is a bacterium which causes number of diseases like skin rashes and also results in the infection of various tissues of the body. *S. aureus* was seen to be susceptible to the extract of mushroom, *P. ostreatus* in various concentrations. High dosage of mushroom extract can be used in medicinal preparations like ointments for skin rashes and other skin diseases etc. The cultivation of *P. ostreatus* on a large-scale being a very profitable business to cultivators shall pave way to the enhancement of the economic status of any nation.

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