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Antimicrobial activity and immune stimulating properties of Oyster mushrooms

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Antimicrobial property and the immune stimulating activity of three different species of Oyster mushrooms viz. *Pleurotus sajor-caju. P. ostreatus* and *P. florida* were studied. Petroleum ether extract and acetone extract of each of the three species of Oyster mushrooms were tested apainst *in vitro* growth of some common human pathogenic fungi like, *Aspergillus flavus. Aspergillus candidus, Candida albicans, Penicillum patullum* and *Rhizopus stolonifer* and bacteria like. *Bacillus subtilis. Staphylococcus aureus, Streptococcus* sp. *Escherichia coli* and *Vibrio chlerae.* It was recorded that *P. ostreatus* had broad spectrum antimicrobial activity as it exhibited highest potency to inhibit the growth of test fungal and bacterial strains. Petroleum ether extracts in almost all the cases were more pronounced to exert antimicrobial activity than the acetone extracts in almost all the cases were more pronounced to exert antimicrobial activity than the acetone extracts. For determination of immune stimulating property of oyster mushroom, estimation of haemoglobin, total count of leucocytes, differential count of leucocytes (granulocyte-agranulocyte ratio) and total count of peritoneal macrophages was done after oral administration of the aqueous suspension of mycelia mats of each of the three species of Oyster mushrooms to albino mice in different experimental set up. There was a marked increase in blood heamoglobin content and a steep rising in the total count of leucocytes and peritoneal macrophages and such enhanced trend in immune property was more pronounced in the mice treated with *P. sajor-caju* followed by *P. florida* treatment.

Key words : Antimicrobial property immune stimulating property Oyster mushrooms, *Pleurotus sajor-caju. Pleurotus ostreatus. Pleurotus florida*

INTRODUCTION

The *Pleurotus* spp. are generally called Oyster mushrooms and their pileus or cap is spatulate and stipe eccentric or lateral. More than 50 species of oyster mushrooms have been described throughout the world. As *Pleurotus* spp. are easy to grow and of broad adaptability to diverse climatic situations, they are cultivated world wide and their production has increased rapidly. It is the third most popular cultivated edible mushroom after *Agaricus bisporus* and *Lentinula edodes* (Chang, 1996). There can be year-round production of this mushroom using different species or varieties in different seasons. Therefore, *Pleurotus* will no doubt continue to make a global impact on the mushroom industry as well as in human welfare. It enjoys a high demand of premium price because of very good nutritional value as well as medicinal property. The oyster mushrooms are rich sources of non-starch carbohydrates, have a high content of dietary fibers, good quality proteins with most of the essential amino acids, vitamins and minerals (Bano and Rajarathanma, 1988; Opletal, 1993; Stamets, 1993). The protein content expressed as a percentage of dry weight ranges from 10 to 30% containing all the essential amino acids which comprises 40% of total amino acid content. The lipid content is 3-5%. Fresh mushrooms contain 3-28% carbohydrates and 3-32% dietary fibers on dry weight basis (Chang and Miles, 1993).

According to *Icons of Medicinal Mushrooms from China* (Yin *et al.*, 1989) *Pleurotus* species are used

in traditional Chinese medicines to prevent more than 30 diseases or disorders. *Pleurotus* species have hypoglycemic effect, antithrombotic and antimicrobial activity, inhibit tumor growth, and lower blood pressure and plasma lipid concentration (Gunde-Cimerman, 1999). In addition they have antioxidant activity (Manpreet *et al.,* 2004; Iwalokum *et al.,* 2007, Zheleva *et al.,* 2004 and Ulrike *et al.,* 2005) and high degree of immune stimulating property (Chang and Miles, 2004; Pradeep *et al.,* 2005; Jong *et al.,* 1991; and Chihara *et al.,* 1982). Many mushrooms have been reported as the source of new group of fungal immunomodulatory proteins which directly or indirectly stimulate the immune system by proliferating macrophages, leucocytes etc. The immune modulating action may exert antitumor activity through the stimulation of host's defense mechanism. Use of herbal medicines are increasing day by day not only by the developing countries but also by the developed countries in their primary health care system. Possibility of using bio-resources for extraction of drugs and medicines is the modern area of frontier research. In present study an attempt has been made to assay the antimicrobial property and immune stimulating activity of three common oyster mushrooms *viz. Pleurotus sajor-caju, P. ostreatus* and *P. florida.*

MATERIALS AND METHODS

Preparation of mushroom extracts

Fruit bodies of the three different species of oyster mushrooms were collected from authentic commercial growers of Burdwan district town area and were identified in the Department of Botany, Burdwan University, Burdwan. Voucher specimen of each of the three species of oyster mushrooms (PS, PO, and PF) were preserved in formalin acetic acid solution (4%) in Mycology and Plant Pathology laboratory, Department of Botany, Burdwan University, Burdwan. The fruit bodies were oven-dried in a hot-air oven at 40°C. The oven-dried fruit body samples were ground in mortar-pestle and made into powder. An aliquet of 3.5 g dry fruit body powder of each of the three test species of *Pleurotus* were extracted separately with 100 ml petroleum ether (20-80°C) and 80% acetone for 2 hr using soxhlet apparatus. The residual solvent was removed by evaporation at 40°C for 24 hr in a rotary evaporator. The resulting organic extracts were stored under refrigeration at 4°C for antimicrobial assay.

For assay of immune modulating activity of oyster mushrooms, mycelial mats of respective mushrooms were grown on potato dextrose broth and 10 days old mycelial mats were harvested, rinsed repeatedly with sterile distilled water. Moister was removed by absorption on filter paper, and then dried at room temperature in the laboratory. The air-dried mycelia were powdered and the powder was weighted and ground into a paste in aqueous suspension. The aqueous suspension of the mycelial mat was administrated orally to adult albino mice with the help of a canula or feeding tube at a dosage of 1.0 g mycelial paste per kg of body weight. Control group of mice were maintained which received no mycelial paste.

Assay of antimicrobial activity

Antifungal activity In vitro effect of extracts (petroleum ether and acetone extracts) of the test mushroom fruit bodies against some common pathogenic fungal and bacterial strains were studied. Antifungal activity of the mushroom extracts were determined following cup-plate assay (Royse and Ries, 1978) and food poisoning technique (Mondal *et al.,* 1995).

Cup plate assay : In cup plate method, 15 ml of sterilized PDA medium at pH 6.5 was plated in each of the Petridishes and allowed to solidify. A groove was made in the centre of the PDA plate with the help of a sterile cork borer aseptically in which 1 ml of each of the mushroom extracts were added. Five mm inoculum disc cut out by a sterile cork borer from the actively growing culture of each of the test fungal strains was placed on one side of the medium in the same plate and was incubated at 30 ± 1°C for 10 days (until full growth of the control plate was achieved). At the end of the incubation period, radial growth of the test fungal strain was measured separately and percentage of growth inhibition of the pathogen was recorded against the control set. Five replicaplates were made for each treatment. A standard antifungal antibiotic, 100 µl of fluconazole at a dose of 5 µg/ml was served as negative control to compare and assess the antifungal activity of the mushroom extracts.

Food poisoning assay : For designing the food poisoning technique, 15 ml of PDA medium was poured in each of the sterilized Petridishes and allowed to solidify. Just before solidification 1 ml of each of the mushroom extracts were added to each of the Petridishes separately and mixed thoroughly, inoculated with mycelial discs (5 mm) of each of the test fungi at the centre of the Petridishes and incubated at 30 ± 1°C for 10 days (until full growth of the control plate was achieved). At the end of incubation period, diametric growth of the test fungi were recorded and percentage growth inhibition was calculated against the control set. Five replicates were made for each treatment. A standard antifungal antibiotic, 100 µl of fluconazole at a dose of 5 µg/ml was served as negative control to compare and assess the antifungal activity of the mushroom extracts (Table 1)

Antibacterial activity : Antibacterial effects of the mushroom extracts were studied following agar-well diffusion technique (Akpata and Akinrimisi, 1977) with little modification. An overnight culture of each microbial isolates was emulsified with nutrient broth to a turbidity that was equivalent of 0.5 McFarland (10^8 cfu/ml). 100 µl of each standard inoculum was then poured on nutrient agar to attain a confluent growth (Bauer *et al.,* 1966). Well was made on the agar plates at the centre using a sterile cork borer and filled with 100 µl of the respective extracts of the test mushrooms. The plates were incubated. Bacterial control well contained 100 µl of ciprofloxacin at 5 µg per well. A well containing 100 µl of 1% (v/v) Tween-20 solution in phosphate buffer saline (pH 7.2) was served as positive control. Growth inhibition was measured as diameters of inhibitory zones and was compared to the antibiotic treated plate (Table 2).

Assay of Immune modulating/stimulating activity

Estimation of haemoglobin : Haemoglobin content was estimated following acid haematin method in which haemoglobin gets converted into acid haematin by the action of hydrochloric acid and was measured using haemoglobinometer. Content of haemoglobin was expressed as g/100 ml blood.

Total count of Leucocytes (TC) : It was done by haemocytometer method. For calculating total leucocyte content, blood was diluted with WBC diluting fluid, which removes the red cells by haemolysis and also accentuates the nuclei of the white cells. Counting was done under a microscope and by knowing the volume of the fluid examined and the dilution of the blood, the number of white cells per cu mm in undilute whole blood was calculated.

Differential count of leucocytes (DC or Granulocyte – Agranulocyte ratio) : It was done following the Leishman stain method. Blood sample smear was prepared and observed under microscope. Approximately 100 leucocytes were counted and from that observation the ratio of granulocyte and agranulocyte was calculated.

Count of peritoneal macrophages : Count of peritoneal macrophages was done following the method of Hudson and Hay (1980). Effect of *Pleurotus* spp. in basic immunological parameters is presented in Table 3.

RESULTS AND DISCUSSION

Present study had revealed antifungal and antibacterial activity of petroleum-ether extracts and acetone extracts of all the three test species of *Pleurotus.* However, *P. ostreatus* exhibited highest potency to deter or inhibit the growth of test fungal and bacterial strains. Both the solvent extracts were found to inhibit the growth of Gram-positive and Gram-negative bacteria as well as fungi tested *in vitro.* It is conclusive from the results that *P. ostreatus* has a broad spectrum antimicrobial activity (Table 1) and the observation has again been confirmed by compare and contrast with the activity of standard antibiotics *viz.* fluconazole and ciprofloxacin which signify the findings. In all the cases, petroleum-ether extracts were more pronounced to exert antimicrobial activity than the acetone extracts. The observed disparity in the susceptibility of the same fungal and/or bacterial strains with different solvent extracts like, petroleum-ether extract and acetone extract of the same *Pleurotus* strain might be due to the fact that the organic solvents used here have varying ability to extract bioactive substances from the same species of mushroom (Table 2). It is proved that the antimicrobial activity of *Pleurotus* is due to the presence of different phytochemical constituents like, phenolics, tannins, terpinoids etc. with

Table 1 Antimicrobial activity of three different species of oyster mushrooms against some human pathogenic fungi

Mushroom extract	Type of solvent	Test fungi	Colony diameter of the fungal pathogen (cm)	Growth inhibition of the fungal pathogen (%)*
Pleurotus ostreatus	Petroleum ether	*Aspergillus flavus*	2.0	77.78±0.04
		Aspergillus candidus	2.3	74.44±0.02
		Penicillium patulum	2.9	67.80±0.01
		Candida albicans	2.2	75.56±0.05
		Rhizopus stolonifer	2.5	72.22±0.09
	Acetone	*Aspergillus flavus*	2.1	76.67±0.07
		Aspergillus candidus	2.4	73.33±0.02
		Penicillium patulum	2.7	70.00±0.05
		Candida albicans	2.3	74.44±0.04
		Rhizopus stolonifer	2.4	73.33±0.08
Pleurotus florida	Peroleum ether	*Aspergillus flavus*	2.4	73.34±0.01
		Aspergillus candidus	2.7	70.00±0.03
		Penicillium patulum	2.8	68.88±0.08
		Candida albicans	2.5	77.22±0.05
		Rhizopus stolonifer	2.9	67.78±0.09
	Acetone	*Aspergillus flavus*	2.8	68.88±0.02
		Aspergillus candidus	2.9	67.80±0.06
		Penicillium patulum	2.9	67.80±0.01
		Candida albicans	2.7	70.00±0.04
		Rhizopus stolonifer	2.6	71.11±0.03
Pleurotus sajor-caju	Petroleum ether	*Aspergillus flavus*	2.6	71.11±0.10
		Aspergillus candidus	3.3	63.34±0.12
		Penicillium patulum	3.6	60.00±0.18
		Candida albicans	4.0	55.56±0.20
		Rhizopus stolonifer	3.6	60.00±0.14
	Acetone	*Aspergillus flavus*	3.0	66.67±0.13
		Aspergillus candidus	3.5	61.11±0.10
		Penicillium patulum	3.8	57.78±0.12
		Candida albicans	4.5	50.00±0.11
		Rhizopus stolonifer	3.5	61.11±0.17
Fluconazole antibiotic (100 µl at a dose of 5 µg/ml)	Aqueous	*Aspergillus flavus*	0	100±0.01
		Aspergillus candidus	0.8	91.11±0.05
		Penicillium patulum	1.5	83.33±0.09
		Candida albicans	1.0	88.89±0.03
		Rhizopus stolonifer	0	100±0.01
Control			9.0	—

* Data are the mean values of five replicates

CD at 5% → 2.11

Table 2 Antimicrobial activity of the mushroom extracts against some pathogenic bacteria

Mushroom	Solvent	Name of the bacteria	Growth status*
Pleurotus ostreatus	Petroleum ether	*Bacillus subtilis*	+ + + + + +
		Staphylococcus aureus	+ + + + +
		Streptococcus sp.	+ + + + +
		Escherichia coli	+ + + + + +
		Vibrio cholerae	+ + + +
	Acetone	*Bacillus subtilis*	+ + +
		Staphylococcus aureus	+ + + +
		Streptococcus sp.	+ + +
		Escherichia coli	+ + +
		Vibrio cholerae	+ +
Pleurotus florida	Petroleum ether	*Bacillus subtilis*	+ + + + +
		Staphylococcus aureus	+ + + +
		Streptococcus sp.	+ + + +
		Escherichia coli	+ + +
		Vibrio cholerae	+ +
	Acetone	*Bacillus subtilis*	+ + +
		Staphylococcus aureus	+
		Streptococcus sp.	+ +
		Escherichia coli	–
		Vibrio cholerae	–
Pleurotus sajor-caju	Petroleum ether	*Bacillus subtilis*	+ + +
		Staphylococcus aureus	+ + + +
		Streptococcus sp.	+ + +
		Escherichia coli	+ +
		Vibrio cholerae	–
	Acetone	*Bacillus subtilis*	+ +
		Staphylococcus aureus	–
		Streptococcus sp.	+
		Escherichia coli	–
		Vibrio cholerae	–
Ciprofloxacin antibiotic (100 µl at a dose of 5 µg/ml)	Aqueous	*Bacillus subtilis*	+ + + + + +
		Staphylococcus aureus	+ + + + + +
		Streptococcus sp.	+ + + + + +
		Escherichia coli	+ + + + + +
		Vibrio cholerae	+ + + + +
0.1%(v/v) Tween-20	Phosphate buffer saline (P^H 7.2)	*Bacillus subtilis*	G
		Staphylococcus aureus	G
		Streptococcus sp.	G
		Escherichia coli	G
		Vibrio cholerae	G

* Date are the mean values of five replicates; +=5-10 mm diameter of zone of inhibitions; ++=10-20 mm diameter of zone of inhibition; +++=20-30 mm diameter of zone of inhibition; ++++=30-50 mm diameter of zone of inhibition; +++++=50-70 mm diameter of zone of inhibition; ++++++=70-95 mm diameter of zone of inhibition; –= no zone of inhibition; G=bacterial growth around the Tweeen–20 control well.

mechanism of actions characterized by cell membrane lysis, inhibition of protein synthesis and activity of proteolytic enzymes and microbial adhensins (Cowan, 1999; Mothana *et al.,* 2000). However, the greater activity of the petroleum-ether extract of all of the *Pleurotus* species than the acetone extract provides an indication for a superiority of petroleum-ether over acetone in the extraction of antimicrobial substances from oyster mushrooms. (Tables 1 and 2).

Antibacterial activity of two edible mushrooms *viz., Agaricus bisporus* and *Pleurotus sajor-caju* against some human pathogenic strains like, *Escherichia coli, Enterobacter aerogenes, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* have been reported by Tambekar *et al.* (2006). Antimicrobial activity of different mushrooms has earlier been reported by several authors *viz., Pleurotus ostreatus* by Iwalokum (2007) and Hwang *et al.,* (2000), *P. florida* and *Lentitus edodes* by Rachel *et al.* (2009), Jin-Tong and Huang (2009) and *Pleurotus florida, Ganoderma lucidum, Fomes lignosus, Auricularia auricula* and *Trametes saepiara* by Fagade and Oyelade (2009).

Oral administration of all of the three species of oyster mushrooms to the mice showed a marked increase in blood haemoglobin content and a steep rising in the total count of leucocytes. However, such enhanced trend in haemoglobin content and total leucocytes count of blood was most pronounced in *P. sajor-caju* administrated mice followed by *P. florida* treatment (Table 3). It is also evident from the results that mice treated with different species of oyster mushrooms resulted into a tremendous increase in the total number of peritoneal macrophages and changes in granulocyte : agranulocyte ratio. Increase in number of macrophages was highest in *P. sajor-caju* treated mice followed by *P. florida* treatment. Thus it is apparent from the results that *P. sajor-caju* has highest immune-modulating or immune-stimulating potency among the three species of oyster mushrooms tested.

An increase in these blood components is very significant as they are essentially important parameters of body's defense system. Present study had revealed that oyster mushrooms under consideration had significant impact on stimulation of host's immune system. Increase in macrophage count, total count of leucocytes and granulocyte : agranulocyte ratio as observed in present investigation is very significant as they are fundamental parameters of immune system. It has been well established that macrophages play vital role for immune stimulation, tumor cell lysis and inhibition of tumor growth (Pradeep *et al.,* 2005; Cristina Lull *et al.,* 2005). An increase in granulocytes:agranulocytes ratio is a signal of immune stimulation. Immune system becomes operative by the coordinated interaction of granulocytes and agranulocytes. The granulocytes are responsible for phagocytosis and agranulocytes for formation of immunoglobulins or antibodies. Increase in total count of lymphocytes is an indication of enhancement of total number of agranulocytes and thereby alteration in the level of granulocytes:agranulocytes ratio. Any formulation which enhances the granulocyte: agranulocyte ratio may certainly be considered as a potent drug or medicine for immune stimulation. Such immune-stimulating properties of oyster mushroom is due to the presence of some protein hound polysaccharides belonging to 1, 3-β-D-

Table 3 : Immune stimulating property of oyster mushrooms

Name of the mushroom	Content of haemoglobin (g/100ml)	Total count of leucocyte (cells/ml × 10^{-6})	Granulocyte: Agranulocyte Ratio	No. of peritoneal macrophages (cells/mouse × 10^{-6})
Pleurotus sajor-caju	21.10±0.40	17.00±0.28	58.42±0.19	25±0.10
Pleurotus ostreatus	19.88±0.25	15.00±0.11	67.33±0.35	20±0.21
Pleurotus florida	20.31±0.18 *	15.50±0.57	56.44±0.15	21±0.16
Control	14.00±0.36	9.00±0.20	54.46±0.48	8.±0.11

Data are the mean value of five replicates; CD at 5% → 1.82

Glucans (Mizuno, 1995; Chihara, 1993; Sakagami and Takeda, 1993; Jong *et al.*, 1991;). It has been reported that some polysaccharides or polysaccharide-protein complexes of mushroom origin are able to stimulate the non-specific immune system and to exert antitumor activity through stimulation of host's defense mechanism (Reshetnikov *et al.*, 2001; Mizuno, 1999; Wasser and Weis, 1999). There are evidences that the β-D-glucans induce a biological response by binding to membrane complement receptor type-3 (CR3, alpha M β-2 integrrin or CD11b/CD18) on immune effector cells. The ligand-receptor complex can be internalized. The intercellular events that occur after glucan receptor binding have not been fully determined till now. In a recent experimental approach it could be shown that a β-D-glucan compound-'schizophyllan' produced by *Schizophyllum commune* is able to bind the mRNA poly(A) tail (Karinnga *et al.*, 2004). Molecular weight, degree of branching, number of subunits, as well as ultrastructural features significantly affect the biological activities of β-D-glucans (Adachi *et al.*, 2002). Zjawiony (2004) have reported that higher immune-stimulating property and antitumor activity seems to be correlated with higher molecular weight, lower level of branching and greater water solubility of β-D-glucan.

It is imperative from the present study that the test mushrooms have potential antimicrobial activity along with immune stimulating effects. Thus it is suggestive that the daily intake of these mushrooms can provide a natural line of coverage of many broad spectrum antibiotics to fight against the germs and pathogens as well as to potentate our body's immune defense.

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