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Cultivation of oyster mushroom (*Pleurotus* sp) using different substrates and evaluate their potentials of antibacterial and phytochemicals

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ABSTRACT

Mushroom is a variety of gilled fungi belongs to the class Basidiomycetes. It grows on tropical and northern temperate regions mainly in a moist condition. Mushroom act as a reservoir of nutrients. Edible mushroom plays a vital role in maintaining human health. The most commonly used edible mushroom is *Pleurotus* (oyster). It is rich in Protein, fibre, minerals and vitamins. It has a variety of fascinating properties like anti-inflammatory, anticancer, antiaging, analgesic and immune modulator property. The present study investigates the various phytochemicals and antibacterial potentials from the methanol, ethanol and aqueous extracts of fruit body mushroom. The cultivation of mushroom using different substrates such as paddy straw and corn straw. The mushroom extracts were subjected to qualitative phytochemical screening using standard methods. The crude extracts contain Alkaloids, Steroids, Flavonoids, Saponins, Phenols, Tannins, Proteins, Amino acids, Carbohydrates, Reducing sugar. The results indicated that the ethanolic extract had shown significant antibacterial activity against the test organisms. Enterococcal infection is a common infection prevailing nowadays. This study suggests that mushroom possess antibacterial potential against enterococcal species. So, intake of mushroom acts as a medicine for enterococcal infections.

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INTRODUCTION

Mushroom is a unique horticulture crop and edible fungi of commercial importance and their cultivation worldwide. (Shivhare et al., 2004). Mushrooms are increasingly being utilised as important food products for their significant role in human health, nutrition, and disease control (Chang & Miles, 1989). Mushrooms with a high flavour, texture and nutritional value have been identified as an excellent food source of malnutrition in developing countries (Eswaran and Ramabadran, 2000). Mushroom is a rich source of protein, Vitamins and minerals are used as both food and medicine. The vitamins of mushrooms are not eliminated by cooking, drying and freezing (Bhavani Devi and Nair, 1986). Mushroom requires Carbon, Nitrogen and inorganic compounds as their nutritional sources. It mainly depends on lignocelluloses for their carbon source.

Oyster mushroom is one of the most predominant edible mushrooms. They are good sources of dietary fiber and other valuable nutrients. It grows under natural conditions, i.e. living tree or dead woody branches of trees and primary decomposer. *Pleurotus* species is commonly known as a giant mushroom. Mushrooms are all around the world in its natural habit like forest environments (Bononi *et al*., 1999). Edible ligninolytic mushrooms with

high medicinal properties using the biotechnological and environmental field. The third most important cultivated mushroom was *Pleurotus* species for eatable purposes. It is a rich source of protein, minerals and vitamin C and B complex. They are frequently regarded as a therapeutic food having anticarcinogenic, anti cholesterolaemic and anti-microbial properties, and also prophylactic properties.

MATERIALS & METHODS

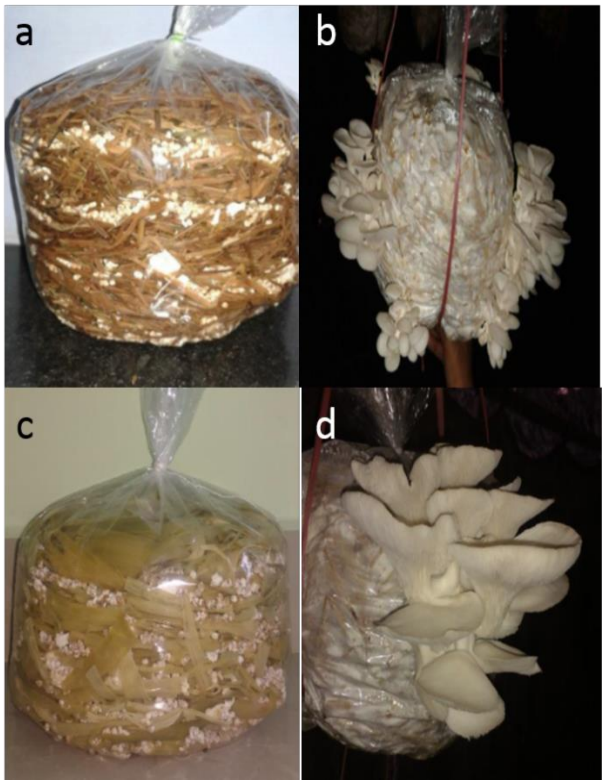

Figure 1: Cultivation of (Pleurotus) oyster mushroom using different substrates

Substrates for Mushroom Cultivation: Paddy straw (Anthra ponni) and Corn straw (Normal sugary (SU)).

These substrates were collected from different localities of Tiruchirappalli district. Collected substrates were dried and taken to the laboratory for mushroom cultivation.

Selection of mushroom variety: Oyster mushroom (*Pleurotus*) was selected, and mother culture (spawn) of this variety was procured from Local cultivators in Thennur, Trichy.

Preparation of substrates for mushroom cultivation: Substrates (Paddy straw and Corn straw) were chopped into 2-3 inches in length and dipped in water for 9 to 12 hours (Rajarathnam *et al*., 1979). Then the substrates were sterilized by autoclaving at 121^0C for 1 hour. About 60% of moisture content was allowed for the substrate to spawning. The mushroom bed was prepared in polythene bags size in 60 cm × 40 cm and 100 gauges of thickness with a layer method of spawning (Pani and Das 1998). The spawned bags were incubated in dark room for mycelia growth. After that, the bags were transferred into the cropping room for fruit body formation at 27 - 32˚C T and maintained 80 to 90% of relative humidity. In the cropping room, sufficient light and ventilation were maintained. 20% Potassium permanganate was prepared and sprayed over the gunny bags thrice a week in a mushroom cultivation room.

Mushroom bed substrates were fully covered with white mycelium indicates that spawn runned successfully. Then the holes were made on the surface of bags for pinheads formation to the outside of the bag. Humidity range was maintained by daily watering and spraying of the mushroom bed bags which avoids the drying of growing mushrooms and substrate also.

The appearance of pinheads and fruit bodies: The mycelium grows through the substrate and pinheads developed from the holes. And developed pinheads were allowed to grow into a mature fruiting body. The average incubation period for fruiting body formation was about 21 to 30 days. The thickenings of the mycelia in the bags, colonization of mycelia in the bags were an indication for the bags to be opened for fruiting. The appearance of those mushrooms occurs, and about 3 fleshes occur in a week continuously for a period of four weeks.

Harvesting of mature fruit bodies and yield: The fully grown mature fruit bodies were harvested from the bag by handpicked or clean scalpel was used to detach the fruit bodies at the base of the stipe from the bags. Measure the weight of the fruit bodies. The first harvesting onwards 15 - 21 days. After that primary harvest sprayed water to maintaining moisture content at 60-65% in beds, the final harvest can be taken from bed within 35 – 45 days. An average yield of harvested mushroom was around at 500-700g / 1kg of agro waste.

Collection and drying of mushroom: Matured fruiting bodies were harvested and transported to the laboratory. Collected mushrooms were washed with running tap water to remove dust and other particles. Then the mushrooms were cut into small pieces and shade dried in room temperature. After drying the mushroom was made into powders. This powder can be used for further studies.

Preparation of *Pleurotus* extracts: Dried samples of fruiting body powder were separately extracted with 100ml of various solvents like Methanol, Ethanol and Aqueous.

Table 1: Amount of cultivated oyster mushroom (Pleurotus) from different substrates

Substrates	Dry weight of substrates	Cultivated Mushroom Weight			Total weight
Paddy Straw	2000 g	685 g	585 g	560 g	1830 g
Corn Straw	2000 g	587 g	498 g	378 g	1463 g

Table 2: Preliminary phytochemical screening of Pleurotus harvested from paddy straw

Phytochemicals	Methanol	Ethanol	Aqueous
Alkaloids	+	+	+
Steroids	+	+	+
Terpenoids	+	_	+
Flavonoids	+	+	+
Saponins	+	+	+
Phenols	+	+	+
Tannins	+	+	+
Glycosides	+	+	+
Proteins	+	+	+
Amino acids	+	+	+
Carbohydrates	+	_	+
Reducing sugar	+	+	+

Table 3: Preliminary phytochemical screening of Pleurotus harvested from Corn straw

Phytochemicals	Methanol	Ethanol	Aqueous
Alkaloids	+	+	+
Steroids	+	+	+
Terpenoids	_	_	+
Flavonoids	+	+	+
Saponins	+	+	+
Phenols	+	+	+
Tannins	+	+	+
Glycosides	_	_	+
Proteins	+	+	+
Amino acids	+	+	+
Carbohydrates	+	+	+
Reducing sugar	+	+	+

+ indicates presence; - indicates the absence

Phytochemical analysis of *Pleurotus:* The phytochemical analysis of the mushroom extracts will be carried out by using standard methods.

Antibacterial activity: The extracts (Methanol and Ethanol) of *Pleurotus* was tested against three grams positive (*Streptococcus* sp, *Bacillus* sp, *Staphylococcus aureus*) and three-gram negative bacteria (*Escherichia coli, Proteus* sp, *Klebsiella* sp) by the disc diffusion method.

Disc diffusion method (Collins, 1987)

Antibacterial activity of two extracts (Methanol and Ethanol) was screened by the disc diffusion method. The 24 hours' culture was used to 10^{-8} and 10^{-6} CFU/µL, respectively. Then, 100µl of bacterial suspension was swabbed over the surfaces of sterilized Muller Hinton agar (MHA) in petriplates. The sterile disc (6 mm in size) were loaded with

different concentrations like 25µl, 50µl, 75µl, 100µl. All the discs were inoculated into MHA plates and incubated at 37˚C for 24 hrs. After the incubation period, the clear zone was measured. Streptomycin and Ampicillin were used as a standard disc.

RESULTS AND DISCUSSION

Selection of substrates

Two substrates were used for the cultivation of mushroom. Growth characteristic of mushroom was monitored on Paddy straw and Corn straw.

Cultivation of mushroom

The mushroom bed was prepared by using paddy straw and corn straw. The mycelial growth scattered throughout the mushroom bag. Pinhead has appeared on the mushroom bags on the 5th day. The pinheads were growing and developed into fruit bodies. After maturation of fruit bodies were harvested on the 18th day. The harvested Mushroom weight from 2 kg of agro wastes was tabulated (Table-1).

Table 4: Antibacterial activity of Pleurotus sp

Substrate	Extract	Test organism	Zone of inhibition (d ameter) (mm)			
			25µl	50µl	75µl	100µl
Paddy straw	Methanol	*E.coli*	5 ± 0.35	7 ± 0.49	9 ± 0.63	11 ± 0.77
		Klebsiella sp	3 ± 0.21	5 ± 0.35	5 ± 0.35	7 ± 0.49
		Bacillus sp	9 ± 0.63	8 ± 0.56	10 ± 0.7	15 ± 1.05
		Proteus sp	7 ± 0.49	9 ± 0.63	10 ± 0.7	7 ± 0.49
		Staphlylococcus aureus	6 ± 0.42	8 ± 0.56	11 ± 0.77	14 ± 0.98
		Streptococcus sp	8 ± 0.56	8 ± 0.56	10 ± 0.7	12 ± 0.84
	Ethanol	*E.coli*	5 ± 0.35	8 ± 0.56	6 ± 0.42	9 ± 0.63
		Klebsiella sp	6 ± 0.42	9 ± 0.63	10 ± 0.7	11 ± 0.77
		Bacillus sp	4 ± 0.28	10 ± 0.7	12 ± 0.84	16 ± 1.12
		Proteus sp	4 ± 0.28	8 ± 0.56	13 ± 0.91	17 ± 1.19
		Staphlylococcus aureus	7 ± 0.49	9 ± 0.63	11± 0.77	15 ± 1.05
		Streptococcus sp	6 ± 0.42	6 ± 0.42	9 ± 0.63	12 ± 0.84
Corn straw	Methanol	*E.coli*	6 ± 0.42	8 ± 0.56	10 ± 0.7	11 ± 0.77
		Klebsiella sp	8 ± 0.56	9 ± 0.63	13 ± 0.91	13 ± 0.91
		Bacillus sp	5 ± 0.35	6 ± 0.42	15 ± 1.05	12 ± 0.84
		Proteus sp	4 ± 0.28	9 ± 0.63	12 ± 0.84	17 ± 1.19
		Staphlylococcus aureus	7 ± 0.49	5 ± 0.35	11 ± 0.77	20 ± 1.4
		Streptococcus sp	8 ± 0.56	9 ± 0.63	9 ± 0.63	11 ± 0.77
	Ethanol	*E.coli*	8 ± 0.56	10 ± 0.7	13 ± 0.91	17 ± 1.19
		Klebsiella sp	7 ± 0.49	8 ± 0.56	12 ± 0.84	15 ± 1.05
		Bacillus sp	5 ± 0.35	7 ± 0.49	9 ± 0.63	11 ± 0.77
		Proteus sp	3 ± 0.21	6 ± 0.42	7 ± 0.49	9 ± 0.63
		Staphlylococcus aureus	6 ± 0.42	5 ± 0.35	8 ± 0.56	15 ± 1.05
		Streptococcus sp	4 ± 0.28	7 ± 0.49	9 ± 0.63	12 ± 0.84

Preparation of *Pleurotus* powder and their extract

Fruiting bodies were harvested and transported to the laboratory. The collected mushroom was washed with water to remove dust particles. Then washed mushroom was cut into small pieces and shade dry in room temperature. After drying the mushroom was made into powders. Then powdered mushrooms were subjected to extract with different solvents (Methanol, ethanol and aqueous).

Phytochemical analysis of *Pleurotus*

The phytochemicals found to be present in the extract of *Pleurotus.* Three different solvents were used namely Methanol, Ethanol and Aqueous. *Pleurotus* was subjected to the preliminary presentation of various phytochemical components (Table-2 & 3).

Antibacterial activity

Mushrooms have been extensive use in medicine for curing variety of ailments (Stamets, 1993).

More recently, in addition to mushrooms have attracted a lot of attention as being a potential source of several compounds having antimicrobial activity (Lindequist, 2005).

Antibacterial activity of *Pleurotus* was tested against six pathogens. Best result was observed in Methanol. Further studies were carried out by using this extract. The extracts (Methanol and Ethanol) of *Pleurotus* was tested against three grams positive (*Streptococcus* sp, *Bacillus* sp, *Staphylococcus aureus*) and three-gram negative bacteria (*Escherichia coli*, *Proteus* sp, *Klebsiella* sp). Out of six pathogens, *Bacillus* sp, *Staphylococcus aureus* was more sensitive to the *Pleurotus* extracts (Table 4).

CONCLUSION

The present study can be concluded that mushroom contains various phytochemical constituents like Alkaloids, Steroids, Terpenoids, Flavanoids, Saponins, Phenols, Tannins, Glycosides, Proteins, Amino acids, Carbohydrates, Reducing sugar. Among the three solvents, aqueous extract showed the best results followed by methanol and ethanol extract in both corn straw and paddy straw substrates.

The present study reveals that mushrooms contain effective phytocompounds and nutrients they exhibited significant antimicrobial activity so that they may be used for effective preparation of drugs and medicine. Enterococcal infection is a common infection prevailing nowadays. This study suggests that mushroom possess significant antibacterial against enterococcal species. So, intake of mushroom acts as a medicine for enterococcal infections.

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