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Inhibition Properties of Pleurotus Ostreatus against Clinical Pathogens by in-vitro Methods and its Phytochemical Screening

Dinesh M.D.[1*], Athira P.S.[1], Ajma N.[2], Abhisha N.C.[3] and Ashitha Carmel[4]
[1]Department of Microbiology, Pazhassiraja College, Pulpally, Wayanad, Kerala, India
[2]Department of Microbiology, E M E A College of Arts and Science, Kondotty, Malappuram, Kerala, India
[3]Department of Microbiology St. Xavier'S College for Womens, Aluva, Kerala, India
[4]Department of Microbiology, Mar Athanasious College, Kothamangalam, Ernakulam, India
md.dineshmd45@gmail.com

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Abstract

Inhibition properties of aqueous extracts of Pleurotus ostreatus were tested against bacterial pathogens such as Staphylococcus aureus, Pseudomonas and E.coli. The purpose of the antibacterial study by agar well cut method be evidence for good inhibition properties aligned with Staphylococcus aureus, Pseudomonas and E.coli. In Phytochemical tests presence of Proteins, reducing sugars and amino acids was investigated. The beyond clarification confirmed that bactericidal compounds possess in the Pleurotus ostreatus, which inhibit the growth of Staphylococcus aureus, Pseudomonas and E.coli.

Keywords: Clinical pathogens, Mushroom aqueous extracts, Phytochemical screening.

Introduction

A mushroom is the spore-bearing fruiting body of a fungus fleshy and spread out in nature commonly produced on the surface of soil or on its food source. Mushrooms hold long-chain polysaccharides, particularly alpha- and beta-glucans. These molecules have valuable effects on your immune functions. Last naturally-occurring compounds like fungal proteins, lectines, peptides, and laccases in mushrooms as well maintain your immune function. Mushrooms are a low-fat food eaten cooked, raw or as a decorate to a meal and an excellent supply of riboflavin, niacin and pantothenic acid. Mushrooms have effective antitumor, antibacterial, antiviral properties, act as haematological agents and involved in immune modulating treatments[1,2].

Pleurotus ostreatus is a variety of mushroom classes and it is edible mushroom. It's come underneath the family Pleurotaceae. It was first cultivate in Germany as a continuation assess during World War. Oyster mushrooms are scientifically known as *Pleurotus* and it is widely distributed all over India in the name of Dhin[3-5]. *P. eurofus* species as being convex, becoming plane or occasionally funnel shaped, cap 4-15cm in diameter or kidney shaped (If growing on the tops of logs), the gills running down the stem (if a stem is present), whitish or with a gray tinge, usually absent or rudimentary, when the mushroom is growing from the side of a log or tress[3]. *Pleurotus ostreatus* is an edible mushroom contains glucan, pleuran, guanide, mevinolin, superoxide dismutase, catalase and peroxidase these compound make it as invaluable medicinal applications[6]. Number of literaturatires is available on inhibitory properties *Pleurotus ostreatus* against bacterial pathogens and also fungal species. In this present study was to assess the antimicrobial activity, anti cariogenic activity, total protein estimation of selected mushroom and its phyochemical screening of aqueous extracts of *Pleurotus ostreatu.*

Materials and Methods

Bacterial strains: Clinical samples (*Staphylococcus aureus*, *Pseudomonas* and *E.coli)* were obtained from DDRC Laboratory, Wayanad, Kerala used for our study. The dental caries like *Streptococcus mutans* (MTCC no - 497) and *Streptococcus oralis* (MTCC no - 2696) were procured from M.T.C.C Chadigarh. The isolated microorganisms were identified on the investigation like morphological, biochemical and cultural characteristics on Manitol salt agar, Pseudomonas isolation agar and EMB agar. Dental caries strains were subcultured on Tood Hewith Agar plates. Strains were brought to pure culture on Nutrient agar plates and maintained at 4ºC.

Preparation of aqueous Extracts: *Pleurotus ostreatus* were procured from Regional Agricultural research station, Ambalavayal, Wayanad. The extracts were prepared by using distilled water as the solvent[7]. 20 g of sample of the Pleurotus *ostreatus* was extracted by soaking wet in 180 mL of distilled water in a beaker, stirred for about 6 min and left overnight. Thereafter, the solution was filtered using filter paper (Whatman No. 1) and the extracts were lyophilized.

Antimicrobial and anti- cariogenic activity of aqueous extracts of Pleurotus *ostreatus* against clinical pathogens by well

diffusion method. Loop full of bacterial suspensions was aseptically transferred in to sterile nutrient broth and kept for overnight incubation. From this 1ml of overnight culture mixed with sterile Muller Hinton agar plates. Muller Hinton agars were poured into sterile Petri dishes and it be left to set. The well was made with a sterile cork borer No. 4 in 10 mm in diameter and the agar discs were removed from the Petri plates. The well be packed with various concentrations of mushroom extract (20µl, 40 µl, 60µl and 80 µl respectively) and then held an incubator at 37ºC. After 24 hours, the inhibition zones were examined and it was calculated as millimeters in diameter[8]. The zones exist measured and values were tabularized.

Anti-cariogenic activity was performed using 1m.l of overnight culture of Streptococcal dental caries (*Streptococcus mutans* (MTCC no - 497) and *Streptococcus oralis* (MTCC no - 2696) suspension was aseptically transmit in to sterile Muller Hinton agar and mix well. Muller Hinton agars were poured into sterile Petri dishes and it be left to set. The well was made with a sterile cork borer No. 4 in 10 mm in diameter and the agar discs were removed from the Petri plates. The well be packed with various concentrations of mushroom extract (20µl, 40 µl, 60µl and 80 µl respectively) and then held an incubator at 37ºC. After 24 hours, the inhibition zones were examined and it was calculated as millimeters in diameter[8]. The zones exist measured and values were tabularized.

Phytochemical Studies of Plant Extract: Preliminary screening and identification of bioactive chemical element in the *Pleurotus ostreatus* were carried out in extracts as well as powder specimens by means of the standard trial[9-14].

Test for Saponins - About 0.5gm of each plant extract was shaken with water in a test tube. Frothing, which persist on warming was taken as preliminary evidence for the presence of saponins[9].

Test for Tannins - About 0.5gm of plant extract was stirred with 1ml of distilled water, filtered and a few drops of 1% ferric chloride was added to the filtrate. A blue-black, green or blue-green precipitate was taken as the evidence for the presence of tannins[10].

Test for Anthraquinone - About 0.5gm of extract was taken and 5ml of chloroform was added and shaken for 5min. The extract was filtered and filtrate was shaken with equal volume of 10% ammonia solution. A pink violet or red colour in ammoniacal layer indicates the presence of anthraquinone.

Test for Flavanoids - About 0.5gm of plant extracts were treated with 2ml of 2% sodium hydroxide solution. An intense yellow colour which turned to colourless on the drop wise addition of dilute acid indicates the presence of flavanoids[11].

Test for Phenol - To the plant extracts dissolved in water equal amount of ferric chloride was added. Deep bluish green colour indicates the presence of phenol.

Salkowsky test - 0.5gm of plant extract was dissolved in 2ml of chloroform. 2ml of conc. Sulphuric acid was carefully added to form a lower layer (chloroform layer). A reddish-brown colour at the interface indicates the presence of a steroidal ring[12].

Test for Proteins - Millon's test: To 2ml of plant extract, added 2ml of Millon's reagent and observed for two minutes for the formation of white precipitate. On gentle heating which may turned to red indicates the presence of proteins in it[13].

Test for Amino acids - Ninhydrin test: To 2ml of plant extract, added 2ml of Ninhydrin reagent. Violet colour indicates the presence of amino acid / proteins in it.

Test for Sugars - About 0.5 ml of the extract dissolved in water was taken. The volume was made upto 1 ml with distilled water. 4 ml of the Anthrone reagent was added. It was heated for 10 minutes in boiling water bath with lids closed. The tube was cooled rapidly. Blue black colour indicates the presence of sugars[14].

Test for Reducing Sugars - To the 5ml of Benedict's reagent, added 2ml of aqueous plant extract and boiled for 5min in boiling water bath. Red precipitate indicates the presence of reducing sugar.

Protein estimation of *Pleurotus ostreatus:* Determination of protein in Pleurotus *ostreatus* were performed by using Folin and Ciocalteau reagents[15]. 0.1% BSA was prepared and 1 ml was taken from this and mixed with 9 ml of distilled water (working standard solution). From this 0.2ml – 1ml was distributed into test tubes and was complete up to 1 ml with distilled water. 1ml distilled water separately served as blank. 0.1 ml of the test sample was thinned to 1 ml by distilled water. Alkaline Copper solution were added in all test tubes and left for 10 minutes at room temperature. Followed by 0.5 ml of Folin–Ciocalteau reagent was added and incubated at room temperature in dark for 30 minutes. Absorbance was read at 750 nm and values obtained were plotted on a graph and the standard graph was obtained. The total protein was extrapolated from the standard graph.

Results and Discussion

Clinical isolates were confirmed by gram staining, Biochemical characterisation and Cultural characteristics. (Table-1). Taking place gram staining isolates showed Gram positive cocci and gram negative rods correspondingly. Motility was checked by using hanging drop method and the isolate was found to be Non motile (Gram positive cocci) and Motile (Gram negative rods). The isolates showed gram negative rods appearance on Gram staining, subjected for biochemical and cultural categorization for further confirmation. Isolate 1 showed metallic sheen colonies on EMB agar and the isolate 2 indicated that blue – green colour with pigmented growth on pseudomonas isolation agar.

Table–1
Biochemical characterisation for Gram negative rods

Tests	Results	
	Isolate 1	**Isolate 2**
Indole	+	-
Methyl red	+	-
Voges Proskauer	-	-
Citrate utilization	-	+
Urease	-	-

Based on isolation and identification the collected isolates were confirmed as *Staphylococcus aureus*, *Pseudomonas* and *E.coli* . The Streptococcal dental caries obtained from MTCC were in addition used for assessing inhibitory properties of *Pleurotus ostreatus* extracts. Aqueous extracts of *Pleurotus ostreatus* were investigated by antibacterial activity by agar well diffusion method (Table-2).

The determination of antibacterial and anti- cariogenic activity activity by agar well diffusion method showed that *Pleurotus ostreatus* extracts tested exhibited antibacterial activity against *clinical isolates and Streptococcal dental caries* (Table-2 and Figure-1) About 80µl of *Pleurotus ostreatus* extracts produced zone of inhibition in the range of 9.4mm for *E.coli* and 8.5mm for *Staphylococcus aureus* When the concentrations of the extracts were decreased showed slight diminish in inhibition zones were observed. Phytochemical screening of selected herb showed the presence of Proteins, and amino acids (Table-3). The total protein content of *Pleurotus ostreatus* mushroom was calculated as 1.58mg/ml.

The above results indicated that observations some bactericidal compounds, which is present in the *Pleurotus ostreatus* that inhibit the growth of clinical pathogens and *Streptococcal* dental caries.

Table-2
Antibacterial activity of *Pleurotus ostreatus* against clinical isolates and Streptococcal species - Well cut Method

Organisms	Aqueous extracts of *Pleurotus ostreatus* Conc. µl/ml			
	80	**60**	**40**	**20**
Staphylococcus aureus [Zone of inhibition in (mm]	8.5	7.0	6.2	5.4
Pseudomonas [Zone of inhibition in (mm]	7.4	6.1	5.0	4.8
E.coli [Zone of inhibition in (mm]	9.4	8.2	7.1	6.2
Streptococcus mutans [Zone of inhibition in (mm]	6.2	5.1	4.2	2.8
Streptococcus oralis [Zone of inhibition in (mm]	5.4	4.6	3.2	2.1

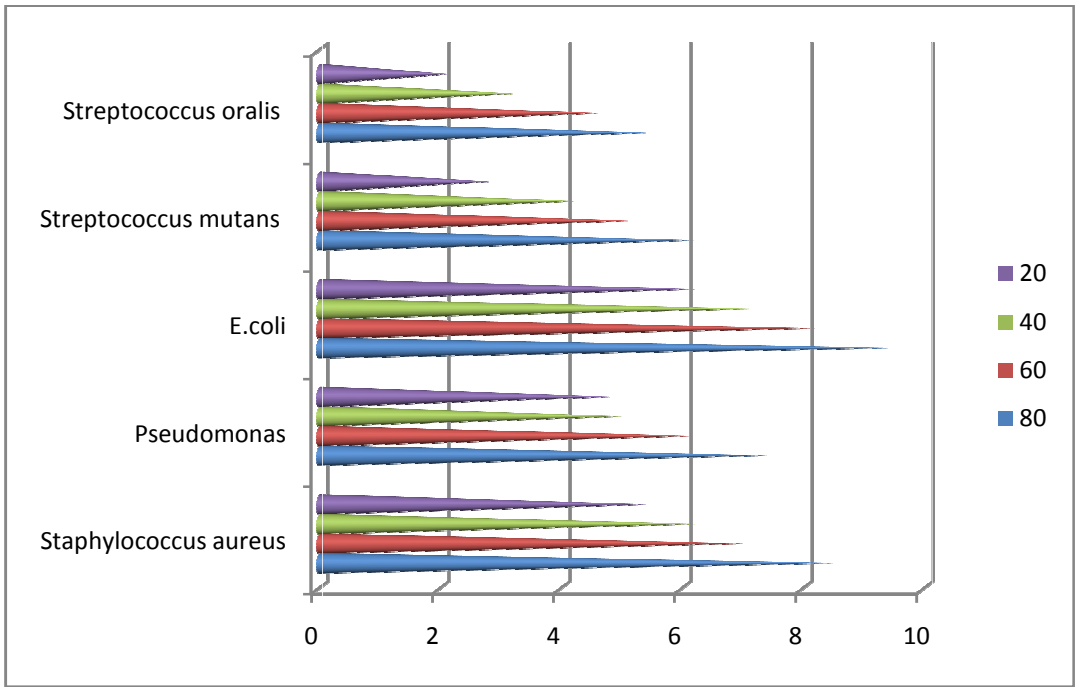

Figure-1
Comparison of inhibition properties of *Pleurotus ostreatus* against clinical pathogens and *Streptococcal* dental cries

Table-3
Phytochemical result

Tests	*Pleurotus ostreatus*
Saponin	**-**
Amino acids	**+**
proteins	**+**
Tannins	**-**
Anthraquinone	**-**
Flavanoids	**-**
Phenols	**-**
Salkowsky test	**-**
Benedict test	**-**
Anthrone test	**-**

Abbreviation: **- +** (Positive**) -** (Negative)

Conclusion

Pleurotus ostreatus is an edible mushroom broadly distributed in worldwide amid invaluable medicinal applications. In our do research we are investigated the inhibitory properties of *Pleurotus ostreatus* in opposition to clinical pathogens and Streptococcal dental caries. *Pleurotus ostreatus* show fine antimicrobial activity against all the selected pathogens. On phytochemical examination *Pleurotus ostreatus* showed the attendance of Amino acids, and Proteins. More research is required for the authentication of phytochemical compounds which point out antimicrobial activity.

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