Evaluation of antimicrobial potential of fruiting body extracts of *Pleurotus ostreatus* (oyster mushroom)

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Abstract

The present study was designed to evaluate the antimicrobial potential of the fruiting body of *Pleurotus ostreatus* against several pathogenic microorganisms. The fruiting body powder of *Pleurotus ostreatus* was extracted with different organic solvents viz. benzene, chloroform, acetone, ethanol, methanol, and in distilled water by maceration method in the order of increasing solvent polarity. Using the agar well diffusion technique, the extracts were tested for their antimicrobial activity against four Gram positive (*Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*) and seven Gram negative (*Aeromonas hydrophila*, *Alcaligenes faecalis*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*) bacterial strains. Four standard antibiotics viz., streptomycin, penicillin, tetracycline and ampicillin were also tested against all the bacterial strains for comparison of the results with the crude extracts; and it was found that a few extracts were more effective than the antibiotics. Zone of inhibition for the various extracts varied between 6.5 to 14 mm; ethanol extract exhibiting maximum antimicrobial activity against most of the tested pathogens. Among the microorganisms tested, *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, *Staphylococcus aureus* and *Salmonella typhimurium* were found to be more susceptible to the extracts as compared to the other microbes.

Keywords: Antimicrobial activity, *Pleurotus ostreatus*, fruiting body, organic solvent extracts, pathogenic microorganisms

1. Introduction

Infectious diseases account for a high proportion of health problems in most of the developing countries. Although several antimicrobial agents have been synthesized chemically, but an indiscriminate use of these commercial antimicrobial drugs has led to the development of resistance to the existing antibiotics by the microorganisms (Raghunath, 2008). The spread of such drug resistant pathogens is becoming one of the most serious threats to successful treatment of microbial diseases. Moreover, antibiotics are sometimes associated with adverse effects on hosts like hypersensitivity and these side effects sometimes become much worse than cure. This situation has generated the need, and has provided the necessary impetus for a continuous search for novel antimicrobial agents from different natural biological sources (Cordell, 2000). Natural antimicrobials can be derived from different plant parts, various animal tissues or from microorganisms (Cowan, 1999; Nair and Chanda, 2007). In recent times, there has been a renewed interest in traditional medicine and emphasis has been placed on the use of natural plant materials (Nascimento et al., 2000; Sibanda and Okoh, 2007) in the control and treatment of various infections and diseases, resulting into an increase in the demand for more drugs from plant sources. Different parts of medicinal plants have been used to cure specific ailments in India since ancient times. More recently, in addition to medicinal plants; research on drugs derived from fungal sources has also gained a momentum (Rosa et al., 2003). Many fungi, including mushrooms contain dozens of active constituents that together combine to give the mushrooms their therapeutic value (Stamets, 2002; Yamac and Bilgili, 2006).

Mushrooms belong to a special group of macroscopic fungi. It is estimated that about 140,000 different species of mushrooms exist, however, only about 10% are known. Half of them
present some nutritious properties, and some of them have been reported to present pharmacological properties (Lindequist et al., 2005). The medicinal use of mushrooms has a very long tradition in the Asian countries (Bendict and Brady, 1972; Wasser and Weiss, 1999), whereas their use in the western hemisphere has been slightly increasing only since the last decade. Mushrooms have been reported to contain dietary fibres, β-glucans, chitin, pectinous substances, natural antibiotics, phenolic compounds, flavonoids and several other secondary metabolites (Cohen et al., 2002; Carbonero et al., 2006). Both fruiting body and the mycelium of mushrooms contain compounds with wide ranging antimicrobial activity. Several studies report the effectiveness of different mushroom extracts against several microorganisms (Cohen et al., 2002; Gbolagade and Fasidi, 2005; Solak et al., 2006; Turkoglu et al., 2006; Barros et al., 2007; Demirhan et al., 2007). But, although a number of mushroom varieties with antimicrobial activities have been identified, a greater number still remain unidentified. In the present study, attempts have been made to examine Pleurotus ostreatus, an edible mushroom for its antimicrobial properties.

Pleurotus ostreatus, commonly known as the oyster mushroom is a common edible mushroom. It was first cultivated in Germany as a subsistence measure during World War I (Eger et al., 1976) and is now grown commercially around the world for food. However, the first documented cultivation was by Kaufert (Kaufert, 1936). Oyster mushroom is extremely delicious as well as confers various health giving properties. Traditionally, it has been used to strengthen veins and relax tendons. In China oyster mushroom is indicated for joint and muscle relaxation. In vivo research has shown that consumption of oyster mushrooms lowers cholesterol levels (Rop et al., 2009) because these mushrooms naturally contain lovastatin. Studies have shown they contain up to 2.8% lovastatin on a dry weight basis. Researches have also proven that alcoholic extracts, especially ethanol ones from Pleurotus strains have a strong inhibition effect of the oxidative stress at the level of the liver and brain. The latest researches show that the aqueous extracts of Pleurotus sajor-caju and Pleurotus pulmonarius also have a strong antiviral effect. Although widely cultivated for its culinary properties, the chemical compounds secreted by the oyster mushroom are only little known.

Thus, keeping in view the medicinal importance of mushrooms in our daily life and the limited reports on the detailed studies of Pleurotus ostreatus, the present investigation involves in vitro study of the antimicrobial activity of fruiting body extracts of Pleurotus ostreatus.

2. Materials and Methods

2.1 Collection of mushrooms

The mushroom, Pleurotus ostreatus used in this study was obtained from Haryana Agro Industrial Corporation, Agro R & D Centre for mushrooms, located at Murthal, Sonipat in Haryana, India. The fruiting bodies were washed thoroughly, initially with tap water and then with distilled water to remove any debris or dust particles; thereafter, they were allowed to dry in an oven at 40 °C. The dried mushrooms were grounded to a fine powder and stored in airtight containers until used for further studies.

2.2 Preparation of mushroom extracts

To 100 g of Pleurotus ostreatus (fruiting body powder), 500 ml of each solvent viz. benzene, chloroform, acetone, ethanol, methanol, and distilled water was added serially for preparing extracts in increasing order of solvent polarity (flow chart-1). Extraction with each solvent was done for 48 h at room temperature. After filtering through filter paper (Whatmann No.1), the supernatant in different solvents was recovered. This process was repeated twice and the respective solvent from the supernatant was evaporated in a rotary vacuum evaporator to obtain the crude extract. These extracts (increasing polarity) were stored at 4 °C until used for evaluating the antimicrobial activity.

2.3 Bacterial test organisms

A total of four Gram positive (Bacillus subtilis MTCC-1133, Micrococcus luteus MTCC-1809, Staphylococcus aureus MTCC-3160, Staphylococcus epidermidis MTCC-3086) and seven Gram negative (Aeromonas hydrophila MTCC-1739, Alcaligenes faecalis MTCC-126, Enterobacter aerogenes MTCC-2823, Escherichia coli MTCC-294, Klebsiella pneumoniae MTCC-3384, Pseudomonas aeruginosa MTCC-1035, Salmonella typhimurium MTCC-1253) pathogenic bacterial strains were used in this study for testing the antimicrobial activity of the crude mushroom extracts. The microorganisms were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India.

2.4 Antimicrobial assay

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Pleurotus ostreatus (Fruiting body powder)

Extraction with benzene (twice) 48 h at room temperature

Residue → Benzene extract

Extraction with Chloroform (twice) 48 h at room temperature

Residue → Chloroform extract

Extraction with Acetone (twice) 48 h at room temperature

Residue → Acetone extract

Extraction with Ethanol (twice) 48 h at room temperature

Residue → Ethanol extract

Extraction with Methanol (twice) 48 h at room temperature

Residue → Methanol extract

Extraction with Distilled Water (twice) 48 h at room temperature

Residue → Distilled Water Extract

Flow chart-1 Schematic representation of the extraction procedure of fruiting body of Pleurotus ostreatus prepared in increasing order of solvent polarity.
The antimicrobial activity of various crude extracts was evaluated using agar well diffusion assay (Perez et al., 1990). In this method, 100 µl of 24 h old culture of each test organism was inoculated on the agar plates and then spread on to the surface of the agar with the help of a sterilized glass spreader. After 30 minutes of inoculation of test microorganisms, wells (5 mm diameter) were prepared with the help of sterilized steel cork borer. Five wells were made in each plate, out of which four wells were loaded with 60 µl of different test mushroom extracts. One well loaded with the respective extraction solvent was used as control.

Sixty µl each of standard antibiotics viz. ampicillin, penicillin, streptomycin, and tetracycline were loaded in different wells in a separate plate to be used as a positive control and for comparison of results with the mushroom extracts. All the plates were then aerobically incubated at 30°C for 24 – 48 h. Antimicrobial activity of the extracts was determined by measuring the diameter of zone of inhibition and comparing it with the growth inhibition results obtained from the standard antibiotics. The diameter (in mm) of zone of inhibition was measured at cross angles and was taken as mean of three independent measurements. Antibacterial activity was recorded when the zone of inhibition was greater than 6 mm.

2.5 Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) is defined as the lowest concentration of the test sample that results in a complete inhibition of visible growth. The MIC for all the extracts was determined by using a modified agar- well diffusion method (Okeke et al., 2001; Ubalua and Oti, 2008). In this method, different dilutions of all the extracts (ranging from 100 µg/ml – 1000 µg/ml with a gap interval of 50 µg/ml) were prepared in their respective solvents. A 50 µl volume of each dilution was introduced in triplicate wells into nutrient agar (NA) plates already seeded with the standardized inoculums (10⁶ cfu/ml) of all the test bacterial strains. One well loaded with the respective extraction solvent was used as control. The test plates were incubated at 30°C for 48 h. The least concentration of each extract showing a clear zone of inhibition was taken as the MIC value.

3. Results

3.1 Antimicrobial assay

The results of the antimicrobial response shown by the different extracts are summarized in figures 1 to 4. All the extracts prepared exhibited variable degree of antimicrobial activity against the tested microorganisms. However, the data indicates that the extracts prepared in organic solvents consistently displayed better antimicrobial activity than that of the aqueous extract. The aqueous extract exhibited almost no antimicrobial activity against all the tested microorganisms. On the other hand, the organic solvent extracts showed a remarkable activity against some of the tested microorganisms. Among the five organic solvent extracts, benzene and chloroform fractions showed a higher zone of inhibition against the bacterial strains, followed by almost a comparable activity by the acetone and ethanol fractions. Least activity was exhibited by the methanol fractions.

Among the five organic solvent extracts prepared, highest antibacterial activity (14 mm zone of inhibition) was exhibited by the benzene extract against B. subtilis, and S. aureus, and by the chloroform extract against S. epidermidis. However, these extracts showed an inhibitory effect against lesser number of bacterial strains; maximum number (nine) of bacterial strains were found to be susceptible to ethanol and acetone extracts. Methanol extract showed lowest antimicrobial response as only four bacterial strains were found to be sensitive to this extract. Zone of inhibition for the various extracts was found to be in the range of 7-14 mm, 10-14 mm, 7-12 mm, 7-13 mm, and 7-12 mm, respectively, for the benzene, chloroform, acetone, ethanol and methanol.

Amongst the Gram positive bacterial strains tested, B. subtilis and S. epidermidis were found to be more resistant to the extracts as they formed a zone of inhibition against lesser number of extracts, as compared to the other two bacterial strains. S. aureus was the most sensitive Gram positive bacterial strain, exhibiting a zone of inhibition against four extracts. Amongst the Gram negative bacteria, E. aerogenes and K. pneumoniae were the most resistant bacteria (forming a smaller
Fig. 1: Antibacterial activity of various extracts of *P. ostreatus* prepared in the order of increasing solvent polarity against Gram positive bacteria. No activity was found in the aqueous extract.

Fig. 2: Antibacterial activity of standard antibiotics against Gram positive bacteria.
Fig. 3: Antibacterial activity of various extracts of *P. ostreatus* prepared in the order of increasing solvent polarity against Gram negative bacteria. No activity was found in the aqueous extract.

Fig. 4: Antibacterial activity of standard antibiotics against Gram negative bacteria.
zone of inhibition and against the least number of extracts. P. aeruginosa, A. hydrophila and S. typhimurium were the most sensitive strains (showing a greater zone of inhibition against larger number of extracts). A. faecalis and E. coli showed an intermediate antimicrobial response.

The effectiveness of the extracts against the various microorganisms was compared with the antibacterial response shown by the antibiotics viz., tetracycline, streptomycin, penicillin and ampicillin (Figures 2 & 4). All the bacterial strains were found to be more resistant to tetracycline and streptomycin as compared to penicillin and ampicillin. Although the standard antibiotics were found to form a bigger zone of inhibition, but some of the extracts showed a higher response than the response exhibited by the four antibiotics against a few microorganisms. All the extracts except the chloroform extract showed a higher response (10-13 mm zone of inhibition) than tetracycline (8.5 mm zone of inhibition) against A. hydrophila; benzene and ethanol also showed a response comparable to that of tetracycline against B. subtilis. Compared to streptomycin (12 mm and 10 mm), the chloroform extract (14 mm and 12 mm) exhibited a higher response against S. epidermidis and S. typhimurium. When the activity of the extracts was compared with that of penicillin; it was observed that among the Gram positive strains, benzene and ethanol extracts showed a higher response against B. subtilis and M. luteus. Amongst the Gram negative strains, chloroform extracts showed a higher activity against S. typhimurium and E. aerogenes, and ethanol was better than penicillin against A. faecalis. As compared to ampicillin, several extracts (benzene, ethanol and chloroform) showed a higher response against both Gram positive (B. subtilis and M. luteus) and Gram negative (A. hydrophila, A. faecalis and S. typhimurium) bacterial strains. Methanol extracts also exhibited a response similar to that of ampicillin against a few bacterial strains.

3.2 Minimum Inhibitory Concentration

All the active extracts were further subjected to determination of inhibitory concentration, the results being shown in table 1. Lower MIC values were exhibited by the ethanol and acetone extracts against most of the bacterial strains. Benzene and chloroform extracts showed comparatively higher MIC values than acetone and ethanol, indicating less effectiveness of these extracts. Methanol extracts exhibited the highest MIC values. Among the various bacterial strains tested, lowest MIC values were obtained for P. aeruginosa, followed by A. hydrophila, indicating that these bacteria were most sensitive to the P. ostreatus fruiting body extracts. The results of MIC assay confirmed the findings of antimicrobial assay, wherein it was reported that ethanol extracts showed a wider range of inhibition against the microorganisms tested; and that P. aeruginosa and A. hydrophila were among the most sensitive strains.

4. Discussion

Antibiotics provide the main basis for the therapy of bacterial infections. However, the high genetic variability of bacteria enables them to rapidly evade the action of antibiotics by developing antibiotic resistance. Thus, there has been a continuous search for new and more potent antibiotics. Hence, the last decade has witnessed a tremendous increase in the investigations on several alternate sources for effective human disease management. More recently, in addition to plants, mushrooms have attracted a lot of attention as being a potential source of several compounds having antimicrobial activity (Lindequist, 2005). Not many reports are available on the exploitation of mushrooms for the management of diseases. This is mainly due to a lack of information on the screening of a diverse population of mushrooms for their medicinal properties. Therefore, in the present investigation, Pleurotus ostreatus, an edible mushroom was evaluated for its antibacterial potential against a large number of pathogenic microorganisms.

Pleurotus represents one of the greatest untapped resources of nutritious food. Several species of Pleurotus are primarily consumed for their nutritive value and used industrially as a bioremediator (Solomko, and Eliseeva, 1988; Fountoulakis et al., 2002). The present study has further revealed the antimicrobial potential of P. ostreatus. Extracts of the fruiting body prepared in different solvents showed antibacterial activity (zone of inhibition in the range of 7-14 mm) against several pathogenic microorganisms. Similar results have been reported in earlier studies (Akyuz et al., 2010; Manjunathan and Kaviyarasan, 2010; Hacioglu et al., 2011). In the present study, it has been observed that most of the test organisms showed resistance to ethanol, chloroform and benzene extracts, the results being in accordance with the studies of Shahi et al. (2012). This resistance may be due to the presence of antibiotic resistance genes that may be located on plasmids of these organisms. Maximum antimicrobial response was observed with ethanol extracts. In previous studies also, ethanol extracts have been reported to
Table 1: Minimum inhibitory concentration (MIC) values of different leaf extracts of *P. dulce* against the tested bacterial strains.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Microorganism</th>
<th>Minimum Inhibitory Concentration (µg/ml)</th>
<th>Benzene</th>
<th>Chloroform</th>
<th>Acetone</th>
<th>Ethanol</th>
<th>Methanol</th>
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<tr>
<td></td>
<td></td>
<td>Fructing Body Extract (<em>P. ostreatus</em>)</td>
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<td></td>
<td><strong>Gram Positive Bacteria</strong></td>
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<td></td>
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</tr>
<tr>
<td>1.</td>
<td><em>Bacillus subtilis</em></td>
<td>250</td>
<td>-</td>
<td></td>
<td>250</td>
<td>-</td>
<td></td>
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<tr>
<td>2.</td>
<td><em>Micrococcus luteus</em></td>
<td>250</td>
<td>-</td>
<td></td>
<td>250</td>
<td>500</td>
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</tr>
<tr>
<td>3.</td>
<td><em>Staphylococcus aureus</em></td>
<td>500</td>
<td>1000</td>
<td>750</td>
<td>1000</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td><em>Staphylococcus epidermidis</em></td>
<td>-</td>
<td>250</td>
<td>1000</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td><strong>Gram Negative Bacteria</strong></td>
<td></td>
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<td>5.</td>
<td><em>Aeromonas hydrophila</em></td>
<td>250</td>
<td>-</td>
<td>250</td>
<td>250</td>
<td>500</td>
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<tr>
<td>6.</td>
<td><em>Alcaligens faecalis</em></td>
<td>1000</td>
<td>-</td>
<td>250</td>
<td>250</td>
<td>-</td>
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<tr>
<td>7.</td>
<td><em>Enterobacter aerogenes</em></td>
<td>-</td>
<td>500</td>
<td>1000</td>
<td>-</td>
<td>-</td>
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<tr>
<td>8.</td>
<td><em>Escherichia coli</em></td>
<td>-</td>
<td>-</td>
<td>1000</td>
<td>500</td>
<td>1000</td>
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<tr>
<td>9.</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>-</td>
<td>-</td>
<td>250</td>
<td>500</td>
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<td>10.</td>
<td><em>Pseudomonas aeruginosa</em></td>
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<td>250</td>
<td>250</td>
<td>250</td>
<td>-</td>
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<tr>
<td>11.</td>
<td><em>Salmonella typhimurium</em></td>
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<td>250</td>
<td>1000</td>
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</tr>
</tbody>
</table>

All the values are an average of three determinations.
- : No activity.

be effective against several microorganisms (Jonathan and Fasidi, 2003; Hacioglu et al., 2011). Cowan (1999) reported that the most active components are generally water insoluble, hence it is expected that low polarity organic solvents would yield more active extracts. In the present study also, the aqueous extract exhibited no antibacterial activity.

The antibacterial activity of the different extracts against the test organisms showed a high variability. This variability may be attributed to the genetic structure of mushroom species; physical and biochemical constituents; chemical differences among different extracts, solvents and test microorganisms. In similar studies, it has been reported that the extracts of various mushrooms inhibit the growth of microorganisms at different ratios (Iwalokun et al., 2007; Jagadish et al., 2008). The broad spectrum of antimicrobial activity obtained in the present study may be attributed to the presence of bioactive metabolites of various chemical types in mushroom compounds. The present study thus confirms the antimicrobial potential of fruiting body extracts of *Pleurotus ostreatus*. Further studies on the isolation and identification of the active compounds may provide a better source for developing new therapeutic agents.

5. Conclusion

The present study scientifically validates the antimicrobial potential of the traditionally important edible fungi, *Pleurotus ostreatus*. The results provide an important basis for the use of ethanol, benzene and chloroform extracts of the tested mushroom species for the treatment of infections associated with the pathogens used in this study, which could be useful for the development of new antimicrobial drugs. However, further studies related to the isolation and identification of the particular compounds responsible for the antimicrobial activity are underway. The antimicrobial mechanisms associated to each group of chemicals to which the isolated compounds may belong, will help in further explaining the inhibition potency of the tested samples.

References

activity of some edible mushrooms in the eastern and southeast Anatolia region of Turkey. Gazi University Journal of Science. 23(2): 125-130.


