Research Article

PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL PROPERTIES OF PLEUROTUS FLORIDA AND PLEUROTUS EOUS AGAINST SOME HUMAN PATHOGENS:
A COMPARATIVE STUDY

Muthukumaran P*, N. Saraswathy, R. Kogilavani, S. Udhaya Bhaskar and S. Sindhu
Department of Biotechnology, Kumaraguru College of Technology, Coimbatore, Tamil Nadu, India

*Corresponding Author Email: m.kumaran005@gmail.com

Article Received on: 20/12/13 Revised on: 11/01/14 Approved for publication: 21/01/14

DOI: 10.7897/2230-8407.050218

ABSTRACT

In this study, Two Oyster mushrooms, Pleurotus florida and Pleurotus eous which are edible varieties that commonly available in South India were taken to investigate the phytochemical screening and antimiicrobial activity. Preliminary phytochemical screening was done for aqueous methanolic extract of P. florida and P. eous. The screening tests were performed for various phyto constituents like phenols, flavonoids, terpenoids, saponins, steroids, tannins, glycosides, anthraquinones and Phlobatamins. For antimicrobial activity, three bacterial pathogens such as Klebsiella pneumonia - MTCC 109, Pseudomonas aeruginosa - MTCC 424, Staphylococcus aureus - MTCC 737 and three fungal pathogens such as Aspergillus fumigatus - MTCC 343, Aspergillus brasiliensis - MTCC1344, Candida albicans - MTCC 227 were used for antimicrobial activity against the aqueous - methanolic extracts of Pleurotus florida and Pleurotus eous by using agar well diffusion assay. The inhibitory effect of Pleurotus eous showed the greater activity than the Pleurotus florida. The zone of inhibition was maximum for the Pleurotus eous extract of 7 mm and 6 mm against Klebsiella pneumonia - MTCC 109 and Aspergillus fumigatus - MTCC 343 respectively.

Keywords: Pleurotus florida, Pleurotus eous, phytochemical, antimicrobial activity.

INTRODUCTION

Mushroom accumulates a variety of secondary metabolites, including phenolic compounds, saponins, tannins, terpenoids and flavonoids. Apart from these compounds, mushroom are rich in Protein, carbohydrates, vitamins, minerals and other therapeutically important compounds. Also, a mushroom phenolic compound has been found to be an excellent antioxidant and synergist that is not mutagenic. The antimicrobial property of several plants has been studied for several decades. More recently, in addition to plants, mushrooms have attracted a lot of attention as being a potential source of several compounds having antimicrobial property1. Both fruiting bodies and the mycelium of mushrooms contain compounds with wide ranging antimicrobial activity. Several studies report the effect of different mushroom against several microorganisms2. Studies have revealed that most of the test organisms showed resistance to organic solvent extracts of mushrooms. This resistance may be due to the presence of antibiotic resistance genes that may be located on plasmids of these organisms. From these reports, it is focused that mushrooms are a vital sources of medicinal compounds that may use to cure different disorders and prevent pathogenic micro-organisms. They are several medicinal mushrooms were used as medicine for curing various types of diseases such as Antibacterial, antiviral, anticancer, anti-inflammatory, and some medicinal mushroom extracts are used as immunomodulator and some extend, it can used to treat cardiovascular diseases3.10. Oyster species of Mushrooms such as Pleurotus florida and Pleurotus eous are edible and highly nutritious mushroom which is common in tropical part of India, west Africa, Europe, North America and southern part of Asia. Pleurotus florida is also commonly called as White oyster mushroom and Pleurotus eous is general denoted as pink oyster mushroom. Both species are rich in vitamin C and vitamin B complex and the protein content varies between 1.6 to 2.5 %. It has most of the mineral salts required by the human body. With this medical and nutritional importance of mushrooms, antimicrobial potential of two commonly available oyster mushrooms were taken into this studies.

Aims and Objectives

The main objective of this work is to study the antimicrobial activity of two species of oyster mushrooms such as Pleurotus florida and Pleurotus eous against some human pathogens. In the view of above background a study was undertaken with following aims and objectives:

- Preliminary phytochemical screening from Pleurotus florida and Pleurotus eous
- Antimicrobial properties of aqueous methanolic extract of Pleurotus florida and Pleurotus eous against some human pathogens
- Preliminary phytochemical screening from Pleurotus florida and Pleurotus eous
- Collection of Mushroom for phytochemical extraction.
- Preparation of Pleurotus florida and Pleurotus eous aqueous-methanolic extract.
- Phytochemical analysis of Pleurotus florida and Pleurotus eous.

Antimicrobial properties of aqueous methanolic extract of Pleurotus florida and Pleurotus eous against some human pathogens

- Preparation of the inoculums.
- Antibacterial activity of aqueous-methanolic extract of Pleurotus florida and Pleurotus eous by agar-well diffusion method.
- Antifungal activity of aqueous-methanolic extract of Pleurotus florida and Pleurotus eous by agar-well diffusion method.
MATERIALS AND METHODS
Sample collection
The two species of oyster mushrooms such as Pleurotus florida and Pleurotus eous were used. The fruiting bodies of P. florida were purchased at the local market in Coimbatore, Tamil Nadu, India. The spawns of P. eous were collected from TNAU, (Bill number: 530924) Coimbatore, Tamil Nadu, India and the fruiting bodies were cultivated under suitable conditions. The fruiting bodies of two mushrooms were dried and then powdered and stored in air tight plastic bags for solvent extraction. The dried mushroom powders were stored at room temperature (28 ± 2°C) in order to be used conveniently in the study. For antimicrobial studies, three bacterial pathogens such as Klebsiella pneumonia - MTCC 109, Pseudomonas aeruginosa - MTCC 424, Staphylococcus aureus - MTCC 737 and three fungal pathogens such as Aspergillus fumigatus -MTCC 343, Aspergillus nidulans - MTCC1344, Candida albicans - MTCC 227 are procured from the Microbial Type Culture Collection (MTCC), IMTECH (Institute of Microbial Technology), Chandigarh, India and stored at -20°C for further studies.

Preliminary phytochemical screening from Pleurotus florida and Pleurotus eous
Preparation of Pleurotus florida and Pleurotus eous extracts
The dried powder was subjected to aqueous methanolic extraction (1:1). About 15 g of mushroom powder were extracted by using Soxhlet’s extractor for 24 h, at 65°C using distilled water: methanol (1:1). The organic solvent in the extracts were removed with a rotary evaporator. Then, the obtained concentrated samples were dried and stored in sterile capped bottles under refrigeration condition (4°C) prior to use for subsequent assays.

Phytochemical analysis of Pleurotus florida and Pleurotus eous
The phytoconstituents present in the aqueous methanolic extracts of Pleurotus florida and Pleurotus were determined qualitatively[11,13].

Antimicrobial properties of aqueous methanolic extract of Pleurotus florida and Pleurotus eous against some human pathogens
Preparation of the Inoculum
An overnight culture of each microbial pathogen were inoculated in nutrient broth (Three Bacterial pathogens - Klebsiella pneumonia - MTCC 109, Pseudomonas aeruginosa - MTCC 424, Staphylococcus aureus - MTCC 737), Czapek yeast extract agar for fungi (Two fungus - Aspergillus fumigatus - MTCC 343, Aspergillus brassiellis - MTCC1344) and Malt yeast agar for yeast (Candida albicans - MTCC 227) and incubated favourable temperature and time.

Antimicrobial activity of aqueous methanolic extract of Pleurotus florida and Pleurotus eous by agar well diffusion assay
The determination of the inhibitory effect of the extracts of P. eous on test bacteria was carried out by agar well diffusion method. Bacterial cultures were grown at 37°C for 24 h in the nutrient broth. The culture suspensions were adjusted by comparing against McFarland. Petri dishes with 10 ml of nutrient agar were prepared, previously inoculated with 100 μl of the culture suspensions. The wells (7.0 mm) were made and the extract which is dissolved in DMSO was added to the wells (100 μl) and the same volume (100 μl) of DMSO was used as a control. The inoculated plates were incubated for 24 h. After incubation, the diameter of the inhibition zone was measured with calipers. The measurements were basically from the edge of the zone to the edge of the well[14].

RESULTS
Preliminary phytochemical screening from Pleurotus florida and Pleurotus eous
In addition to the in vitro antioxidant activity assays, phytochemical analysis was also carried out, where the two different edible mushrooms extract was used. Tests for phenols, tannins, anthroquinones, flavonoids, steroids, terpenoids, glycosides, saponins were carried out after extraction with water and methanol. The results of phytochemical analysis are shown in Table 1.

Antimicrobial activity of aqueous-methanolic extract of Pleurotus florida and Pleurotus eous by agar well diffusion assay
The antimicrobial activities of the aqueous-methanolic extracts of Pleurotus florida and Pleurotus eous were tested three bacterial pathogens such as Klebsiella pneumonia - MTCC 109, Pseudomonas aeruginosa - MTCC 424, Staphylococcus aureus - MTCC 737 and three fungal pathogens such as Aspergillus fumigatus - MTCC 343, Aspergillus nidulans - MTCC1344, Candida albicans - MTCC 227. The inhibitory effect of Pleurotus eous showed the greater activity than the Pleurotus florida used against pathogenic bacteria and fungi. The results of antimicrobial activity are shown in Figure 1, 2 and 3.

DISCUSSIONS
As per the Table 1, the Preliminary phytochemical screening tests were done for both oyster mushrooms. The tests revealed the presence of phenols, saponins, tannins, terpenoids, flavonoids, and anthroquinones. Tannins and phlobatannins were absent in both mushroom extracts of P. florida and Pleurotus eous. The preliminary screening tests may be useful in the detection of the bioactive compounds and subsequently may lead to drug discovery and development. Antimicrobial activity of aqueous methanolic extract of Pleurotus florida and Pleurotus eous by agar well diffusion produced zone of growth of inhibition which are shown in Figure 1 and 2. Aqueous methanolic extract of Pleurotus eous showed stronger inhibitory activity against test organisms when compared with aqueous methanolic extract of Pleurotus florida. Figure 3 showed that antibacterial and antifungal activity of Pleurotus eous against Klebsiella pneumonia - MTCC 109 and Aspergillus fumigatus - MTCC 343. The inhibitory effect of Pleurotus eous showed the greater activity than the Pleurotus florida. The zone of inhibition was maximum for the Pleurotus eous extract of 7 mm for Klebsiella pneumonia - MTCC 109 Figure 3(a) and 6 mm for Aspergillus fumigatus- MTCC 343 Figure 3 (b). Similarly it shows maximum inhibitory activity against Staphylococcus aureus - MTCC 737 (4 mm) and Candida albicans - MTCC 227 (3 mm). But in case of Pleurotus florida shows lesser inhibitory activity against both the test organisms such as Staphylococcus aureus - MTCC 737 (3 mm) and Candida albicans - MTCC 227 (2 mm) respectively (Figure 1 and 2).
Table 1: Phytochemical screening of Aqueous - methanolic extract of *Pleurotus florida* and *Pleurotus eous*

<table>
<thead>
<tr>
<th>Phytochemical constituent</th>
<th>Aqueous - methanolic extract of <em>Pleurotus florida</em></th>
<th>Aqueous - methanolic extract of <em>Pleurotus eous</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenols</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anthroquinones</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ Indicates the presence of the phytochemical constituents, - Indicates the absence of the phytochemical constituents

Figure 1: Antibacterial activity of aqueous- methanolic extracts of *Pleurotus florida* and *Pleurotus eous*

Figure 2: Antifungal activity of aqueous- methanolic extracts of *Pleurotus florida* and *Pleurotus eous*

Figure 3(a): *Klebsiella pneumonia*  
Figure 3(b): *Aspergillus fumigatus*

Figure 3: Antimicrobial activity of aqueous methanolic extract of *Pleurotus florida* and *Pleurotus eous* by agar well diffusion assay
CONCLUSION
The phytochemical analysis done on the mushrooms extracts and it showed that *P. florida* and *P. eous* contained various phytochemicals such as phenols, saponin, flavonoids, steroids, terpenoids. This study showed that the aqueous - methanolic extracts of two different oyster mushroom species: *P. florida* and *P. eous* shows the inhibitory effects on various human pathogens such as *Klebsiella pneumonia* - MTCC 109, *Pseudomonas aeruginosa* - MTCC 424, *Staphylococcus aureus* - MTCC 737, *Aspergillus fumigatus* - MTCC 343, *Aspergillus nidulans* – MTCC 1344 and *Candida albicans* - MTCC 227. The aqueous methanolic extracts of *P. eous* showed greater activity than that of the *P. florida* aqueous-methanolic extracts against all six test organisms. In this the study, the constituents of *Pleurotus eous* having high antimicrobial activity and it can used for further studies to unravel novel treatment strategies for diseases associated with human pathogens; further investigations; that would identify the bioactive compound from the both species of *Pleurotus*.

ACKNOWLEDGEMENT
Authors are thankful to the Dr. A. Manickam, Professor and Head, Department of Biotechnology, Kumaraguru College of Technology, Coimbatore, Tamil Nadu, India for providing valuable guidance and support. And also thankful to Dr. J. Prakasham, Professor and Head, Department of Plant Pathology, Tamil Nadu, India Agricultural University (TNAU), Coimbatore, Tamil Nadu, India for providing mushroom spawn and guidance.

REFERENCES


Source of support: Nil, Conflict of interest: None Declared