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Antibacterial Activity of a Mushroom - *Stereum ostrea*

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Abstract

The antimicrobial activity of crude culture filtrate and methanol extract of *Stereum ostrea* was evaluated by disc diffusion method against two Gram-negative bacteria- *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and three Gram-positive bacteria such as *Bacillus subtilis*, *Staphylococcus aureus* and *Micrococcus sps*. To determine the minimal inhibitory concentrations (MICs) we studied concentrations of 10-50 µl of crude and methanol extract of *Stereum ostrea* against selected bacteria. Crude culture filtrate was highly inhibitory when compared to methanol extract, which were evident through the increased zones of inhibition against Gram-negative and Gram-positive bacteria. Crude culture filtrate of *Stereum ostrea* showed highest and lowest percent of inhibition zone against *Bacillus subtilis* (15.9 mm) and *Klebsiella pneumonia* (9.1mm). These results indicate that crude culture filtrate of *Stereum ostrea* possesses potential broad spectrum antimicrobial activity.

Keywords: *Stereum ostrea*, Crude culture filtrate, Methanol extract, Antimicrobial activity, MIC

1. Introduction

Microbial diversity favors probability of identifying microorganisms that produce bioactive compounds, which can be developed into new molecules that fight against many pathogens. Natural products of microorganisms are resources for novel secondary metabolites and among them filamentous fungi remain the most promising source. Majority of the organisms containing new metabolites are disappearing rapidly and screening of the natural products has become costly and laborious process. According to Keller (2005) production of metabolites varies in function and specificity of a particular fungus. Dreyfuss and Chapela (1994) made an assumption that certain physical and biological change in natural environment favors the production of a diverse range of secondary metabolites. According to Bandow *et al.*, (2003) the mis-usage of antibiotics since the "Golden Age of Antibiotics" in 1950s had caused the threats of antibiotic resistant "superbugs" was reported to be one of the leading cause of deaths world-wide. Many pathogenic bacteria are becoming resistant to synthetic drugs and hence an alternative strategy is very much needed. Fungi represent an enormous source for natural products with diverse chemical structures and activities and apparently, majority of fungi inhabiting the world have not yet been described. Hence, today, many academic institutions are investing necessary resources to search for new natural products with significant pharmacological activity.

Stereum ostrea (*S. ostrea*) is a colorful mushroom belongs to Stereaceae, Basidiomycota. It is inedible due to its tough, leathery texture and is often called as 'False turkey tail'. It is saprophytic on the dead hardwoods, growing in dense overlapping clusters and widely distributed in the world (Kuo, 2005). This fungus has long been used for folk remedies even without any knowledge of which compounds are

responsible. The ethnobotanical uses of this mushroom to heal both plant and human diseases have been accumulated but scientific evidences are not yet well known. The main focus of present study lies in the investigation of a white-rot fungus- *S. ostrea* isolated from wood logs and their inhibitory activity against selected Gram-positive and Gram-negative bacteria.

1.1 **Materials and Methods**

The white-rot fungus *S. ostrea* (Blume & T. Nees:Fr.) Fr. was kindly supplied by Prof. M.A. Singaracharya, Department of Microbiology, Kakatiya University, Andhra Pradesh, India and was isolated from wood logs. Two Gram-negative bacteria such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and three Gram-positive bacteria such as *Bacillus* subtilis, *Staphylococcus aureus* and *Micrococcus sps.* were used in this study were obtained from Department of Botany, Sri Krishnadevaraya University, Anantapur, Andhra Pradesh, India. The strains of bacteria were maintained on nutrient agar medium at 37^0C for 24 h.

1.1.1 Antimicrobial Activity

The fungus *Stereum ostrea* was cultured in Koroljova broth (Koroljova *et al.* 1984) and was incubated at 30^0C on rotary shaker at 150 rpm for 6 days. After incubation the liquid culture was filtered through two layers of Whatman No. 1 filter paper. Methanol extract was prepared by taking 1g of *S. ostrea* biomass, which was mixed with 50 ml of absolute methanol for 2 days. Later the solution was collected and filtered through several layers of Whatman No. 1 filter paper and collected the filtrate for its antibacterial activity.

1.1.2 Assay for antibacterial studies

Antibacterial activities of crude as well as methanol extract were evaluated against selected bacteria. Fresh overnight cultures of inoculum (0.1 ml) of each culture containing 10^8 cells, was spread on agar plate. Three sterile paper discs (5mm diameter) were placed in each agar plate and on two discs crude, methanol extract of each 50µl was placed. On the third, absolute methanol was placed as a control. Ampicillin at 50ppm was placed as a positive control and distilled water was used as a negative control in all plates inoculated with selected bacteria. The bacterial cultures were incubated at 37°C for 24 h. The microbes were plated in triplicates and average zone diameter was noted in mm.

1.1.3 Determination of minimal inhibitory concentration

The minimal inhibitory concentration was aimed to find out the lowest concentration of the sample that inhibits the growth of tested microorganisms. The samples were used against 2 Gram-negative and 3 Gram-positive bacteria. The test was carried out using filter paper disc method by using different concentrations (10-50 µl) of crude and methanol extract. Three sterile Whatman paper discs (5 mm diameter) were soaked each with 10, 20, 30, 40 and 50 µl of the aliquots of crude and methanol extract and placed on bacteria (10^6 CFU/ml) seeded plate. Bacterial cultures were incubated at 37^0C for 24 h. After incubation of all cultures, zone of inhibitions of bacterial growth were observed. Each experiment was done in 3 replicates and average zone diameter was measured in mm. Controls were maintained with devoid of

samples.

2. Results and Discussions

Results obtained from the paper disc method in the present study relieved that crude culture filtrate and methanol extract of *S. ostrea* showed inhibitory effect against 2 Gram-negative bacteria - *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and 3 Gram-positive bacteria such as *Bacillus subtilis*, *Staphylococcus aureus* and *Micrococcus sps*. Crude culture filtrate was more effective against 5 bacteria and *Bacillus subtilis* was most inhibited 15.9 mm followed by *Micrococcus sps.* 13.5 mm, *Staphylococcus aureus* 13.0 mm, *Pseudomonas aeruginosa* 11.1 mm and least *Klebsiella pneumonia* 9.1 mm, indicating that the active principle against this organism was present in crude (Table 1). In methanol extract the highest and lowest inhibitory zones were 14.7 and 8.7 mm (*Micrococcus sps* and *Klebsiella pneumonia*), respectively (Table 1). Ampicillin at a concentration of 50ppm does not show any effect on *Staphylococcus aureus* and *Klebsiella pneumonia* where crude and methanol extracts of *S. ostrea* displayed inhibitory effect on those bacteria. The MIC is the lowest concentration of substance at which it plays to inhibit the growth of target microorganisms. We studied 10-50 µl concentration of crude and methanol extract against selected bacteria (Table 2). The MIC of bacterial inhibition in both crude and methanol extract was found 20 µl (*Bacillus subtilis*), 30 µl (*Micrococcus sps*., *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). The lowest MIC values of crude and methanol extract were against *Bacillus subtilis* suggesting that *S. ostrea* was most effective against *Bacillus subtilis* (Table 2).

A vast number of fungi have been utilized for isolation of potential compounds and biotransformation process. In particular fungi are well known to show antibacterial, antifungal, larvicidal, molluscicidal, antioxidant and free radical scavenging activities (Demain, 1999). Findings of the present investigation revealed the inhibitory action of *S. ostrea* in both crude and methanol extract were tested against selected strains of bacteria. Bacterial strain *Bacillus subtilis* was highly susceptible to *S. ostrea* for both crude as well as methanol indicating the potential of *S. ostrea*. In accordance to findings of Al-Fatimi *et al*., (2006) stated that the fungus *Podaxis pistillaris* was found to exhibit a strong antibacterial activity against several Gram-positive and Gram-negative bacteria such as *S. aureus*, *Micrococcus flavus*, *B. subtilis*, *Proteus mirabilis*, *Serratia marcescens* and *E. coli* using paper disc Method. Jonathan and Fasisdi (2003) reported that two edible Nigerian macrofungi *Lycoperdon pusilum* and *L. giganteum* were selectively active on a few clinical pathogenic organisms. Antibacterial activities in the present investigation indicate great variation among tested bacteria. Crude as well as methanol extract of *S. ostrea* were influential towards selected bacterial strains. In conclusion, newly isolated white-rot basidiomycetes fungi can be exploited for its antibacterial activity against clinically important bacteria.

3. Conclusion

The results of present study clearly indicated that crude and methanol extract of *S. ostrea* have been used *in vitro* to evaluate the antibacterial activity against clinical pathogens. Hence the present study suggests that *S. ostrea* contain a potential metabolite can be further utilized for biotechnological applications in agriculture and medicine.

References

Al-Fatimi, M.A.A., Julich, W.D., Jansen, R., Lindequist, U. (2006), "Bioactive components of the traditionally used mushroom Podaxis pistillaris", eCAM **3**: 87-92.

Bandow, J.E., Brotz, H., Leichert, L.I.O., Labischinski, H., Hecker, M. (2003), Proteomic approach to understanding antibiotic action", Antimicrobial Agents and Chemotherapy **47**: 948-955.

Demain, A.L. (1999), "Pharmaceutically active secondary metabolites of microorganisms", Applied Microbiology and Biotechnology **52**: 455-63.

Dreyfuss, M.M., Chapela, I.H. (1994), "The discovery of natural products with therapeutic potential" (ed.Gullo, V.P.); Butterworth-Heinemann, Stoneham: 49-80.

Jonathan, S.G., Fasidi, I.O. (2003), "Antimicrobial activities of two Nigerian edible macro-fungi *Lycoperdon pusilum* (Bat. Ex) and *Lycoperdon giganteum* (Pers.)", African Journal of Biomedical Research **6**: 85-90.

Keller, N.P., Turner, G., Bennet, J.W. (2005), "Fungal Secondary Metabolism -From biochemistry to Genomics", National Reviews in Microbiology **3** (1): 934-947.

Koroljova-Skorobogat'Ko, O.V., Stepanova, E.V., Gavrilova, V.P., Morozovaa, O.V., Lubimova, N.V., Dzchafarova, A.N., Jaropolov, A.I., Makower, A. (1998), "Purification and characterization of the constitutive form of laccase from the basidiomycetes *Coriolus hirsutus* and effect of inducers on laccase synthesis", Biotechnology Applied Biochemistry **28**: 47-54.

Kuo, M. (2005), "*Stereum ostrea*, retrieved from the MushroomExpert", Com, web site: http://www.mushroomexpert.com/stereumostrea.html

Table 1. Antibacterial activity of *S. ostrea* against tested bacteria and Zone of inhibition (mm)

Organism	Crude	Methanol extract	Ampicillin
Klebsiella pneumonia	9.1±0.56	8.7±0.98	5.3±1.12
Pseudomonas aeruginosa	11.1±1.25	10.8±0.16	6.9±0.05
Bacillus subtilis	15.9±0.33	15.0±0.57	10.3±1.15
Staphylococcus aureus	13.0±0.50	12.2±0.56	10.0±0.33
Micrococcus sps	13.5±0.56	11.6±1.20	8.7±0.57

Values are mean inhibition zone (mm) ± S.D of three replicates

Table 2. Minimal inhibitory concentration of *S. ostrea* against tested bacteria

Organism	MIC (µl)				
	10	20	30	40	50
Klebsiella pneumonia	+	+	–	–	–
Pseudomonas aeruginosa	+	+	–	–	–
Bacillus subtilis	+	–	–	–	–
Staphylococcus aureus	+	+	–	–	–
Micrococcus sps	+	+	–	–	–