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Screening of mushroom *Phellinus switeniae* (Murr.) S. Herrera and Bondart against clinical isolates of *Acinetobacter baumannii* Bouvet & Grimont

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ABSTRACT

Antibacterial activity of chloroform and methanol extracts of *Phellinus switeniae* (Murr.) S. Herrera and Bondart were studied. The activity was evaluated by well assay method and microtiter plate dilution method using sixteen strains of *Acinetobacter baumanii* Bouvet & Grimont. The methanol extract of *Phellinus switeniae* showed activity against all strains of *Acinetobacter* but no activity has been found in case of chloroform extract of *Phellinus switeniae* against all strains of bacteria. Methanol extract of *Phellinus switeniae* was found to be efficient against human pathogenic *Acinetobacter*, as low MIC values seen.

Introduction

Mushrooms have been proved to be one of the most productive sources producing a large and diverse variety of secondary metabolites with significant bioactivities. Extracts of medicinal mushrooms are also extensively used in Chinese traditional medicine to treat viral and other microbial infections, cardiovascular diseases, diabetes and also for hepatoprotection. Researchers have reported antimicrobial activity of several mushrooms (Gao et al., 2005; Kim & Fung, 2004). Methanolic extracts from three macro polypore *Ganoderma lucidum, Navesporus floccose* and *Phellinus rimosus* showed antibacterial activity against a battery of pathogenic bacterial strains (Sheena et al. 2003). The methanol extract and its fractions of the fruit body of *Phellinus linteus* showed antibacterial effect against methicillin resistant *Staphylococcus aureus* (Hur et al. 2004).

The chloroform and ethyl acetate extracts of the dried mushroom have antibacterial activity against *Streptococcus mutans* and *Prevotella intermedia* (Hirasawa, Shouji, Neta,

Fukushima, & Takada, 1999).Both fruiting body and the mycelium contain compounds with wide-ranging antimicrobial activity.

In recent years, multiple drug resistance in human pathogenic microorganisms has developed, due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This situation has forced scientists to search for new antimicrobial substances from various plants which are good sources of novel antimicrobial chemotherapeutic agents (Karaman et al., 2003). The reason for this study is that antimicrobial activities of *Phellinus switeniae* against *Acinetobacter* have not yet been reported in the literature. Therefore, the aim of the present work is to screen antimicrobial potentials of *Phellinus switeniae* (Chloroform and methanol extracts) on MDR *Acinetobacter baumannii.*

Materials and methods

Mushrooms

The samples used in present investigation were identified *Phellinus* species. These samples were identified in mycology lab, Department of Botany, University of Pune with traditional taxonomic methods. Samples were collected mainly from Western Maharashtra (mainly Konkan region).

Extraction

The fruit bodies of mushrooms were cut into bits and dried at 40º C. The dried samples were crushed to powder form with blender and 50.0gm each of the powdered samples were soaked in 200ml of solvent in flask. The flasks were covered with aluminum foil and allowed to stand overnight for extraction with initial warming. It was filtered through Whatman filter paper no.1 and the filtrate obtained was concentrated in a rotary evaporator at 50º C. Extract was collected and dissolved in same solvent.

Microorganisms

Strains of the *Acinetobacter baumannii* were procured from National Chemical Laboratory (NCL), Joshi Hospital and Department of Biochemistry, University of Pune, Pune. All strains were maintained on Nutrient agar (NA) medium.

Antibacterial activity

It was determined using modified well diffusion method by M. Hirasawa *et al.* (1999). Antibacterial activity was assayed by measuring the diameter of zone of inhibition against *Acinetobacter* seeded in Nutrient agar (NA) plates. The plates were prepared by inoculating 20 ml of nutrient agar with 100 µl of *Acinetobacter* grown in Nutrient broth (NB) at 37º C for 24 hours.

Test sample extracts (50 µl) were added to the wells (7 mm diameter). The plates were kept in the refrigerator for 30 minutes for pre-diffusion. Then the plates were incubated at 37º C for 24 hours. Negative controls were also prepared.

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Minimum Inhibitory Concentration (MIC)

The lowest concentration of antimicrobial agent that resulted in complete inhibition of microorganisms represents MIC. The minimum inhibitory concentration (MIC) was determined by using sterilized 96 wells microtitre plates, using the microdilution method. Serial dilution was done for the test extracts (H. Ibrahim et.al 2009). Positive and negative controls were also prepared. The plates were incubated for 24 hours at 37° C. Turbidity or bacterial growth was determined after addition of 50 µl of 2-(4-Iodo phenyl) - 3-(4-nitro phenyl) 5-phenyltetrazolium chloride (I.N.T) (Himedia) (K. Annan, P. J. Houghton 2008).

Results and discussion

The results of these experiments indicated that the methanol extract of *P. switeniae* showed marked activity against almost all strains of *Acinetobacter baumannii.* The extracts of mushroom showed measurable zone of inhibition (ZOI). The diameter of ZOI of methanol extract and chloroform extract of *P. switeniae* were10.5 - 15.5 mm and 10.3 - 21.8 mm respectively. But no activity has been found in case of chloroform extract of *Phellinus switeniae* against all strains of bacteria.

MIC values of methanol extract of *Phellinus switeniae* were in the range of 2.33- 4.66 mg/ml which suggests that methanol extracts of *Phellinus switeniae* may be effective for antibacterial activity against *Acinetobacter baumanii.*

Table 1. Zone diameter of extracts of *Phellinus switeniae* against bacterial strains

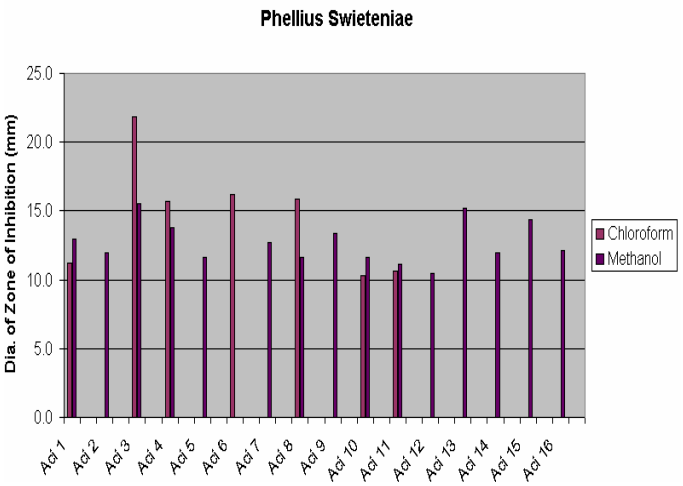
Inhibition Zone Diameter (mm) of extracts of *Phellinus swieteniae*		
Strains	**Chloroform**	**Methanol**
Aci 1	11.2 ± 0.4	12.9 ± 1.63
Aci 2	NA	12.0 ± 1.10
Aci 3	21.8 ± 0.4	15.5 ± 0.55
Aci 4	15.7 ± 0.6	13.8 ± 0.99
Aci 5	NA	11.7 ± 0.41
Aci 6	16.2 ± 0.4	NA
Aci 7	NA	12.7 ± 0.82
Aci 8	15.8 ± 0.2	11.7 ± 0.82
Aci 9	NA	13.3 ± 0.82
Aci 10	10.3 ± 0.5	11.7 ± 0.82
Aci 11	10.7 ± 0.7	11.2 ± 0.41
Aci 12	NA	10.5 ± 0.55
Aci 13	NA	15.2 ± 0.75
Aci 14	NA	12.0 ± 0.63
Aci 15	NA	14.3 ± 0.52
Aci 16	NA	12.2 ± 0.41

Values are mean ± SD,
n = 6,
NA - no activity

Conclusion

The present study indicated that there was no antimicrobial activity detected against any of the MDR *Acinetobacter baummannii* Bouvet & Grimont strains by the chloroform extract of *Phellinus switeniae.* Methanol extract of *Phellinus switeniae* showed significant anti- Acinetobacterial activity as the low MIC value. Studies are underway to isolate and identify the bioactive component from the effective extracts.

Figure 2. Activity of Extracts *Phellinus switeniae* against *Acinetobacter baumanii*



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