Journal of Medicinal Plants Research Vol. 4(13), pp. 1335-1338, 4 July, 2010
Available online at http://www.academicjournals.org/JMPR]
DOI: 10.5897/JMPR09.479
ISSN 1996-0875 ©2010 Academic Journals

Full Length Research Paper

In - vitro susceptibility testing of some *Phellinus* species against *Acinetobacter baumannii from* Maharashtra India

Mugdha H. Belsare[1*], Gauri S. Bapat[1], Kiran R. Ranadive[2], Jitendra G. Vaidya[1] and Subhash S. Deokule[1]

[1]Department of Botany, University of Pune, Maharashtra, India.
[2]Waghire College, Saswad, Tal. Purandhar, Maharashtra India.

Accepted 15 June, 2010

Antibacterial activity of methanol and ethyl acetate extracts of two *Phellinus* species were studied. The activity was evaluated by well assay method and microtiter plate dilution method using sixteen strains of *Acinetobacter baumannii* Bouvet and Grimont. The ethyl acetate extract of *Phellinus swieteniae* (Murr.) Herrera and Bondart showed activity against all strains of *Acinetobacter*. The minimum inhibitory concentration of ethyl acetate extract of *Phellinus merrillii* (Murr.) Ryv. was found to be in the range 0.71 - 1.42 mg/ml. The MIC value of methanol extract of both species was found to be much higher.

Key words: Antibacterial activity, *Phellinus*, *Acinetobacter*, medicinal mushrooms

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. *Phellinus* is a large and widely distributed genus of the family Hymenochetaceae Donk under the class, Basidiomycetes. In folk medicine, several species of *Phellinus* are known to improve health and has been used as remedy for various diseases. *Phellinus* has been used to treat abdominal pain, stomach problems, lymphatic tumor, and menses disorders. In Korea, this mushroom is used mainly to treat various cancers. In past decade, a few pharmacological actions of *Phellinus* have been reported. Kim et al. (2004) elucidated the anti-inflammatory and antinociceptive activities, in addition to its anti-angiogenic activity of the EtOH extract of *Phellinus linteus* (Berk and Curtis) Teng. Ethyl acetate extract of *P. rimosus* (Berk) Pilat exhibited significant *in vitro* antioxidant activity and also showed potent anti hepatotoxic activity against carbon tetrachloride - induced acute toxicity in rat liver (Ajith et al., 2002).

Two proteoglycans, PNW1 and PNM1, isolated from the mycelium of *P. nigricans* (Fr.) P. Karst. were shown to have antitumor and immunomodulating activities (Li et al., 2008). The hypoglycemic and antidibetic effect of the crude exopolysaccharides (EPS) produced from submerged mycelial culture of *Phellinus baumii* Pilat in streptozotocin (STZ) induced diabetic rats were investigated (Hwang et al., 2005).

Extracts of more than 75% of the surveyed polypore mushroom species showed antimicrobial activity and 45% of 204 mushroom species inhibited the growth of a wide variety of microorganism (Suay et al., 2000). Several different species of *Phellinus* are also believed to have antimicrobial activity. For example *Phellinus fastuosus* (Lév.) Ryv., has been demonstrated to exhibit antimicrobial activity (Sharifi et al., 2006). The methanol extract of *P. rimosus* (Berk) Pilat and *P. linteus* showed antimicrobial activity against a battery of pathogenic bacterial strains (Sheena et al., 2003; Hur et al., 2004).

*Corresponding author. bmugdha04@gmail.com. Tel: +91 901-167-8015.

Table 1. Inhibition Zone diameter of *Phellinus swieteniae* against bacterial strains.

Phellinus swieteniae		
Strains	**Inhibition zone diameter (mm)**	
	Ethyl acetate	**Methanol**
Aci 1	15.7 ± 0.52	12.9 ± 1.63
Aci 2	15.5 ± 4.32	12.0 ± 1.10
Aci 3	21.0 ± 3.10	15.5 ± 0.55
Aci 4	12.8 ± 1.60	13.8 ± 0.99
Aci 5	12.7 ± 0.82	11.7 ± 0.41
Aci 6	19.5 ± 0.84	NA
Aci 7	14.3 ± 2.42	12.7 ± 0.82
Aci 8	15.3 ± 2.66	11.7 ± 0.82
Aci 9	15.3 ± 0.82	13.3 ± 0.82
Aci 10	10.8 ± 0.41	11.7 ± 0.82
Aci 11	11.8 ± 1.33	11.2 ± 0.41
Aci 12	12.1 ± 0.66	10.5 ± 0.55
Aci 13	12.5 ± 0.55	15.2 ± 0.75
Aci 14	13.7 ± 0.52	12.0 ± 0.63
Aci 15	12.0 ± 0.63	14.3 ± 0.52
Aci 16	12.7 ± 0.52	12.2 ± 0.41

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

However, little information is available on antibacterial effects of *Phellinus merrillii* and *P. swieteniae* against multidrug resistant bacterial strains. Therefore, we examined the antibacterial effect of both of these *Phellinus* spp. Against *Acinetobacter baumannii* strains.

MATERIALS AND METHODS

Mushrooms

Phellinus samples used in the present study were identified in the Mycology Laboratory, Department of Botany, University of Pune; using the taxonomic keys (Larsen and Cobb-Poulle, 1990). Under the guidance of Prof. J.G. Vaidya. Samples were collected mainly from Western Maharashtra (mainly Konkan region).

Extraction

The extraction of mushroom fruit bodies was carried out using two solvents (Methanol and Ethyl acetate). The fruit bodies of each of the mushrooms were cut into small pieces and dried at 40ºC. The dried fruit bodies were crushed to powder form. Hundred grams of each of the powdered sample were extracted with 400 ml of solvent with initial warming for 24 h. Solution was filtered through Whatman filter paper no. 1 with Buchner funnel and filtrate was collected. This procedure was repeated two times. The filtrate obtained was concentrated in a Rotary Evaporater (Medica Instrument manufacturing Company) at appropriate temperature depending on solvent. The extract was collected and dissolved in same solvent (Harborne, 1984) used as test sample.

Test organisms

Two strains Aci 11, Aci 12 (NCIB 2886, NCIB 2890, NCL, Pune), Aci 13, Aci 14, Aci 15, Aci 16 (Joshi Hospital, Pune) and other strains (Department of Biochemistry, University of Pune) were procured. All strains were maintained on nutrient agar (NA) medium.

Antibacterial activity

This was determined using modified well diffusion method of 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 37ºC for 24 h.

Test samples (50 µl) were added to the wells (7 mm diameter). The plates were kept in the refrigerator for 30 min for pre-diffusion. Then the plates were incubated at 37ºC for 24 h. Negative controls were also prepared.

Minimum inhibitory concentration (MIC)

The MIC is the lowest concentration of the tested samples at which no growth is observed. The MIC was determined by using sterilized 96 wells microtitre plates, using the microdilution method. Fifty microliter of the extract and 200 µl of each inoculum were added to the well labeled as A. The inoculums and extract were mixed thoroughly. Only 100 µl of each inoculum's was added to the wells labeled B - H. The resultant mixture (100 µl) from well A was added to well B. The same procedure was repeated for inoculums mixtures from B-H., thus creating a serial dilution of the test sample (Ibrahim et al., 2009). Positive and negative controls were also prepared. The plates were incubated for 24 h at 37ºC. 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) (Annan and Houghton, 2008).

Table 2. Inhibition Zone of extracts of *Phellinus merrillii* against bacterial strains.

Phellinus merrillii		
Strains	**Inhibition zone diameter (mm)**	
	Ethyl acetate	**Methanol**
Aci 1	11.2 ± 0.75	12.8 ± 0.41
Aci 2	16.3 ± 0.52	14.2 ± 0.98
Aci 3	22.2 ± 0.75	NA
Aci 4	16.3 ± 1.03	12.0 ± 0.63
Aci 5	16.3 ± 1.03	16.5 ± 0.55
Aci 6	15.7 ± 7.23	16.7 ± 1.03
Aci 7	11.7 ± 0.82	15.0 ± 0.89
Aci 8	11.5 ± 0.55	14.7 ± 0.52
Aci 9	11.2 ± 0.41	16.7 ± 0.52
Aci 10	11.7 ± 1.03	16.7 ± 0.52
Aci 11	11.2 ± 1.17	10.7 ± 0.82
Aci 12	NA	10.6 ± 0.38
Aci 13	17.5 ± 0.84	20.2 ± 0.41
Aci 14	18.8 ± 0.41	11.5 ± 0.55
Aci 15	21.0 ± 0.63	11.2 ± 0.75
Aci 16	18.8 ± 0.75	12.7 ± 0.52

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

RESULTS AND DISCUSSION

The antimicrobial activities of different extracts tested were shown in Tables 1 and 2. All the four extracts showed moderate activity. These results were consistent with previous reports (Sheena et al., 2003; Hirasawa et al., 1999; Gbolagade and Fasidi, 2005). The results of these experiments indicated that the methanol extract of *P. merrillii* and *P. swieteniae* showed marked activity against almost all strains of Acinetobacter *baumannii.* The extracts of both mushroom showed measurable zone of Inhibition (ZOI). The diameter of ZOI of methanol extract of *P. merrillii* and *P. swieteniae* were 10.8 - 21.0 mm and 10.5 - 15.5 mm respectively (Table 1 and 2).

The ethyl acetate extracts of both *Phellinus* spp. showed moderate activity against all strains of *Acinetobacter*. The ZOI of ethyl acetate extract of *P. merrillii* and *P. swieteniae* were 10.6 - 16.7 and 11.2 - 22.2 mm, respectively (Table 1 and 2). The MIC value of methanol extract of both species was found to be much higher as compared to ethyl acetate extract which suggests that methanol extracts of *Phellinus* spp. may not be effective for antibacterial activity against *Acinetobacter baumannii.* The low MIC values (Ethyl acetate extract) [0.71 - 1.42 mg/ ml] obtained in *P. merrillii* against the bacterial strains indicates that this species shows significant activity. From the results obtained, it could be observed that ethyl acetate was the best solvent for extracting antimicrobial substances from these mushrooms.

Conclusion

A. baumannii has been consistently proven to be resistant to most of the antibiotics available; still it has shown positive support to the extracts studied. It can therefore be suggested that they are promising antimicrobial agents against *Acinetobacter*. The next report will be focused on identification of bioactive phytochemicals in *Phellinus* spp. The antimicrobial activity of *Phellinus* spp against different strains of multidrug resistant bacteria could also be tested in future studies.

ACKNOWLEDGMENT

Dr. Ghole Department of Biochemistry, University of Pune), Dr. Pradhan (Joshi Hospital, Pune) and Dr. D. V. Gokahle (NCL, Pune) are gratefully acknowledged for providing bacterial strains. The authors are grateful to Prof. M.R. Walher, Principal Waghire College, Saswad, Tal. Purandhar for his encouragement. The authors are also thankful to Mr. Belsare Hemant, Dr. Bhosle Shekhar,Garad Sandhya and Mr. Sonawane Amol for their support.

REFERENCES

Ajith TA, Janardhanan KK (2002). Antioxidant and antihepatotoxic activities of *Phellinus rimosus* (Berk) Pilat. J. Ethnopharmacol. 81: 387-391.

Annan K, Houghton PJ (2008). Antibacterial, antioxidant and fibroblast growth stimulation of aqueous extracts of Ficus asperifolia Miq.and Gossypium arboretum L., wound-healing plants of Ghana. J. Ethnopharmocol. 119: 141-144.

Gbolagade JS, Ishola OF (2005). Antimicrobial Activities of Some Selected Nigerian Mushrooms, Afr. J. Biomed. Res. 8: 83-.

Harborne JB (1984). Phytochemical Methods. Chapman and Hall, London, Ed – II: 288.

Hirasawa M, Naoto S, Tomotake N, Kazuo F, Kazuko T (1999). Three kinds of antibacterial substances from Lentinus edodes (Berk.) Sing. (Shiitake, an edible mushroom) Int. J. Antimicrob. Agents. 11: 151-157.

Hur JM, Chun HY, Seung HH, Sook HL, Yong OY, Jong CP, Kang JK (2004). Antibacterial effect of Phellinus linteus against methicillin - resistant *Staphylococcus aureus*, Fitoterapia 75: 603-605.

Hwang HJ, Sang WK, Jong ML, Ji HJ, Hyun OK, Hyun MK, Jong WY (2005). Hypoglycemic effect of crude exopolysaccharides produced by a medicinal mushroom Phellinus baumii in streptozotocin - induced diabetic rats. Life Sci. 76: 3069-3080.

Ibrahim H, Ahmad NA, Devi RS, Nor AMA, Mostura M, Rasadah MA, Khalijah A (2009). Essential oils of Alpinia conchigera Griff. And their antimicrobial activities. Food Chem. 113: 575-577

Kim SH, Song YS, Kim SK, Kim BC, Chang JL, Eun HP (2004). Anti-inflammatory and related pharmacological activities of the n-BuOH subfraction of mushroom Phellinus linteus. J. Ethnopharmacol. 93: 141-146.

Larsen MJ, Cobb-Poulle LA (1990). *Phellinus* (Hymenochaetaceae). A survey of the world texa. Oslo, Norway: Fungiflora.

Li X, Li LJ, Xu Z, Wen MT, Shan C, Li PZ (2008). Anti-tumor and immunomodulating activities of proteoglycans from mycelium of *Phellinus nigricans* and culture medium. Int. Immunopharmacol. 8: 909-915.

Sharifi A, Bhosle SR, Vaidya JG (2006). Evaluation of crude sesquiterpenoid extract of *Phellinus fastuosus* as a natural preservative Hind. Antibiotic, Bull. 47(48): 20-23.

Sheena N, Ajith TA, Thomas MA, Janardhanan KK (2003). Antibacterial Activity of Three Macrofungi, Ganoderma lucidum, Navesporus floccosa and *Phellinus rimosu*s Occuring in South India. Pharm. Biol. 41 (8): 564-567.

Suay I, Francisco A, Francisco JA, Angela B, Angeles CM, Teresa DeM, Juan BG, Antonio GdV, Juli´an G, Pilar H, Fernando P, Francisca VM (2000). Screening of basidiomycetes for antimicrobial activities, Antonie van Leeuwenhoek 78: 129-139.