International Journal of Herbal Medicine
Available online at www.florajournal.com

IJHM International Journal of Herbal Medicine

E-ISSN: 2321-2187
P-ISSN: 2394-0514
IJHM 2017; 5(2): 35-37
Received: 09-01-2017
Accepted: 10-02-2017

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In vitro evaluation of *Piper betle* L. methanol extract and its fractions against *Rhizoctonia solani*

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Abstract
The antifugal activity of methanol extracts of *Piper betle* (leaves) and its fractions was evaluated against *Rhizoctonia Solani* by agar well diffusion method. Phytochemical analysis of the extract, revealed the presence of alkaloids, phenolic compounds, tannins, terpenoids and cardiac glycosides. In antifungal assay the methanol extract and its four fractions (Fractions 6, 7, 9 and 10) displayed activity against *R. solani*. Fraction 6 exhibited the highest zone of inhibition (33- 45 mm at 5-20%) with MIC value of 0.78 mg/ml. Fraction 6 and 7 showed maximum inhibition up to 10% concentration, showing no further increase in activity upon increasing the concentration. Fraction 9 and 10 displayed zone of inhibition 10-17 mm, while fraction 12 was not effective. Better antifungal activity displayed by fraction 6 highlighted further research on identification of the active compounds, which could be useful in developing antifungal agent for controlling *R. solani*.

Keywords: *Rhizoctonia solani*; *Piper betle* Extract; Fractions; Phytochemical

1. Introduction
Rhizoctonia solani Kuhn causes diseases in many economically important agricultural crops [1-5]. Strategies to control *Rhizoctonia* diseases are limited due to its ecological behaviour, its extremely broad host range and the high survival rate of sclerotia under various environmental conditions. The plant kingdom represents an enormous reservoir of various biologically active molecules, which have been reported to control of pathogenic fungi [6-13]. NE region of India is a vast repository of medicinal flora and bears immense scope in finding new biologically active plant compounds for management of plant diseases. This region was reported to be one of the two centres of species diversity of Indian *Piper* (Piperaceae) [14, 15]. *Piper* species are of great interest owing to their variety of biological properties [16, 17]. In our previous study, we observed the promising antifungal activity of different solvent extracts from *Piper betle* leaves against a number of plant pathogenic fungi [7, 8, 9, 18, 19]. The present study has been designed to evaluate the antifungal potential of crude methanol extracts of *P. betle* leaves and its fractions against *R. solani*.

2. Materials and methods
2.1 Collection and extraction of plant sample
Fresh leaves of *P. betle* were collected from Tezpur, Assam, washed thoroughly, shade dried and crushed into coarse powder and extracted with methanol [20]. The filtrate was concentrated to dryness at 40 °C under reduced pressure using rotary vacuum evaporator (Heidolph Instruments GmbH & Co. KG, Germany) to get the crude methanol extract.

2.2 Phytochemical analysis
Phytochemical analysis of the methanol extracts of *P. betle* leaves was performed for the presence of alkaloids, phenolic compounds, tannins, phlobatannins, saponins, flavonoids, terpenoids and cardiac glycosides [21- 23].

2.3 Fractionation of methanol extracts
Methanol extract of *P. betle* was fractionated by column chromatography. The glass column with sintered disc (450x 30 mm) was packed with silica gel (60-120 mesh, 100 g) slurry prepared in hexane. Crude methanol extract (1 g) was dissolved in minimum amount of methanol and the solution was adsorbed on 10 g of silica gel and allowed to dry. The adsorbed silica was loaded into the column. The fractions were eluted stepwise by gradient of mobile phase, hexane-ethyl acetate-methanol-water (hexane, hexane: ethyl acetate- 9:1, 1:1, 1:9, ethyl acetate, ethyl acetate: methanol- 9:1, 1:1, methanol and water).

Each fraction was concentrated to dryness at 40 °C under reduced pressure using rotary vaccum evaporator (Heidolph Instruments GmbH & Co. KG, Germany).

2.4 Test fungus
Pure culture of *R. solani* was maintained on potato dextrose agar slant and inoculum was prepared by culturing the test fungus on potato dextrose broth and incubated at 28±2 °C for 96 h.

2.5 *In vitro* assay for antifungal activity
Antifungal activity of the methanol extract and column fractions was determined by agar well diffusion method [24]. Test sample was prepared by dissolving the extract/ fractions in required amount of dimethyl sulphoxide (DMSO) and distilled water was added to obtain the concentrations of 5, 10 and 20% and then filtered using Millipore filter (MILLEX® GP, Ireland, 0.22 µm pore size). Each PDA plate was swabbed with 200 µl of inoculum. Well of 7 mm diameter was made in the agar plate with a sterile cork borer and loaded with 200 µl of the test sample and allowed to diffuse at room temperature for 2 h. Then the plates were incubated at 28±2 °C for 96 hours. Bavistin (0.01%) was used as positive control while DMSO was used negative control. Each treatment was replicated thrice.

2.6 Determination of minimum inhibitory concentration
The minimum inhibitory concentration (MIC) value of the most active column fraction was determined by broth microdilution assay [11]. Test fraction (50 µl) prepared in DMSO was serially diluted in 96-well microtiter plate with potato dextrose broth to obtain a concentration ranging from 0.39 to 50 mg/ml. Same volume of inoculum (50 µl) containing approximately 10^4 CFU ml^{-1} was added in each well. The plates were incubated at 28 ±2 °C for 96 h. The MIC was interpreted as the lowest concentration of the fraction showing no visible growth on the broth.

3. Results and discussion
Phytochemical analysis revealed the presence of alkaloids, phenolic compounds, tannins, terpenoids and cardiac glycosides in the methanol extract of *P. betle*. Phlobatannins, saponins and flavonoids were not detected. The column chromatography of the methanol extracts of *P. betle* yielded 17 fractions. Among these, fraction 6, fraction 7, fraction 9, fraction 10 and fraction 12 were tested for their effect on the growth of *R. solani*. The remaining fractions were not considered due to their insufficient quantity. The results of the bioassay revealed that antifungal substances are present in methanol extract and the fractions 6, 7, 9 and 10 at varying range (Table 1). Highest inhibition was exhibited by fraction 6 with zone of inhibition of 33 to 45 mm at the test concentrations (5-20%). This fraction showed maximum zone at 10% concentration. There was no further increase in the zone of inhibition upon increasing the concentration. The MIC value of this partially purified fraction (Fraction 6) was recorded as 0.78 mg/ml against the test fungus. Similarly for fraction 7 also, maximum zone of inhibition was recorded at 10% concentration. Further increasing and decreasing the concentration resulted in a smaller zone of inhibition. This might be due to poor solubility of the active components that may lower the rate of diffusion of the active components in the culture medium. Fraction 9 and 10 displayed less activity (zone of inhibition: 10-17 mm), while fraction 12 was found not active. Differential antifungal activity of column fractions was also explained previously [6, 25].

In negative control set (DMSO) no zone was observed. Activity of bavistin at very low concentration (0.01%) (zone of inhibition: 14 mm), compared to the extract and fractions may be attributed to its pure nature. Better activity spectrum of fraction 6 could be due to removal of undesirable compounds present in the crude extract that got removed during fractionation. Still there is chance of presence of inactive compounds in the fraction which need further refinement to isolate the active antifungal principles.

Table 1: Antifungal activity of methanol extract of *Piper betle* and its fractions against *Rhizoctonia solani*

Extract/Fraction (mobile phase)	Zone of inhibition (diameter in mm) Concentration (%)		
	5	**10**	**20**
Methanol extract	24	33	38
Fraction-6 (Hex:EtOAc-1:1)	33	45	45
Fraction-7 (Hex:EtOAc-1:1)	16	20	15
Fraction-9 (Hex:EtOAc-1:9)	10	13	17
Fraction-10 (EtOAc:MeOH-9:1)	0	0	15
Fraction-12 (EtOAc:MeOH-1:1)	0	0	0
DMSO	0		
Bavistin (0.01%)	14		

Hex: Hexane; EtOAc: Ethyl acetate; MeOH: Methanol

4. Conclusion
In this study the column fractions showed better antifungal activity than the crude methanol extract of *P. betle*. Thus the results holds the promise for further work on isolation and identification of the active compounds, which could be used to develop safe and effective antifungal agent for management of plant pathogenic fungi, particularly *Rhizoctonia solani.*

5. Acknowledgments
The authors express sincere gratitude to Dr. (Fr.) Augustine Thottakkara, Chancellor and Dr. (Fr.) Thomas C. Mathew, Vice-Chancellor, Christ University, Bangalore, for providing amenities to carry out this study.

6. References
1. Nik WZW, Yapi MY. *Rhizoctonia solani,* a seed-borne pathogen of French bean in Malaysia, Pertanika 1979; 2:11-15.
2. Moussa TAA. Studies on biological control of sugar beet pathogen *Rhizoctonia solani* Kuhn. Online J Biol Sci 2002; 2:800-804.
3. Al-Askar AA, Rashad YM. Efficacy of some plant extracts against *Rhizoctonia solani* on pea, J Plant Prot Res 2010; 50: 239-243.
4. Roy Ak. Further records of plant diseases from Karbi Anglong district, Assam, J Res-AAU 1989; 10:88-91.
5. Jeeva ML, Hegde V, Vimala B, Makeshkumar T, Nair RR, Edison S, *et al.* First report of *Rhizoctonia solani* causing blight on Yam bean (*Pachyrhizus erosus*) in India. New Dis Rep. 2005; 11:49.
6. Okemo PO, Baisa HP, Vivanco JM. *In vitro* activities of *Maesa lanceolata* extracts against fungal plant pathogens, Fitoterapia. 2003; 74:312-316.
7. Das J, Das TK. Effect of phytoextracts on inhibition of mycelial growth of *Fusarium oxysporum* Schlecht., Environ Ecol. 2005; 23:362-364.
8. Das J, Das TK. *In vitro* antifungal property of plant extracts against *Alternaria brassicicola* (Schw) Wiltshire, Geobios 2005b; 32:305-306.
9. Das J, Goswami S, Gupta R, Begam M. *In vitro* sensitivity of phytopathogenic fungi against Indian Piper,

J Curr Sci 2006; 9:721-725.
10. Plodpai P, Chuenchitt S, Petcharat V, Chakthong S, Voravuthikunchai SP. *Anti-Rhizoctonia solani* activity by *Desmos chinensis* extracts and its mechanism of action, Crop Prot 2013; 43: 65-71.
11. Sehajpal A, Arora S, Kaur P. Evaluation of plant extracts against *Rhizoctonia solani* causing sheath blight of rice, J Plant Prot Sci. 2009; 1:25-30.
12. Amadioha AC. Fungicidal activity of some plant extracts against *Rhizoctonia*, Arch Phytopathol Plant Prot 2001; 33:506-517.
13. Satish S, Raghavendra MP, Raveesha KA. Antifungal potentiality of some plant extracts against *Fusarium* sp., Arch Phytopathol Plant Prot. 2009; 42:618-625.
14. Rahiman BA, Nair MK. The genus Piper Linn. in Karnataka, Indian J Bombay Nat Hist Soc. 1994; 91:66.
15. Gajurel PR, Rethy P, Singh B, Kumar Y. Importance of systematic investigation for proper utilization and conservation of *Piper* species of North-East India. Proceeding NEI National Seminar 2001; 305-313.
16. Prasad AK, Kumar V, Arya P, Kumar S, Dabur R, Singh N *et al.* Investigations toward new lead compounds from medicinally important plants, Pure Appl Chem 2005; 77:25-40.
17. Vaghasiya Y, Nair R, Chanda S. Investigation of some *Piper* species for anti-bacterial and anti-inflammatory property, Int J Pharmacol 2007; 3:400-405.
18. Singha IM, Kakoty Y, Unni BG, Kalita MC, Das J, Naglot A, *et al.* Control of Fusarium wilt of tomato caused by Fusarium oxysporum f. sp. lycopersici using leaf extract of *Piper betle* L.: A preliminary study, World J Microbiol Biotechnol 2011; 27:2583-2589.
19. Singha IM, Unni BG, Kakoty Y, Das J, Wann SB, Singh L, *et al.* Evaluation of *in vitro* antifungal activity of medicinal plants against phytopathogenic fungi, Arch Phytopathol Plant Prot 2011; 44(11):1033-1040.
20. Hayet E, Maha M, Samia A, Mata M, Gros P, Raida H, *et al.* Antimicrobial, antioxidant, and antiviral activities of *Retama raetam* (Forssk.) Webb flowers growing in Tunisia, World J Microbiol Biotechnol 2008; 24:2933-2940.
21. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants, Afr J Biotechnol 2005, 685-688.
22. Falodun A, Ali S, Quadir IM, Choudhary IMI. Phytochemical and biological investigation of chloroform and ethylacetate fractions of *Euphorbia heterophylla* leaf (Euphorbiaceae), J Med Plants Res 2008; 2:365-369.
23. Harborne JB. Phytochemical methods: a guide to modern techniques of plant analysis. 3rd Ed. Chapmann Hall, London, UK, 1998, 41-42.
24. Kaushik P, Goyal P. *In vitro* evaluation of *Datura innoxia* (thorne apple) for potential antibacterial activity, Indian J Microbiol 2008; 48: 353-357.
25. Vries FA, Bitar HE, Green IR, Klaasen JA, Mabusela WT, Bodo B, *et al.* An antifungal active extract from the aerial parts of *Galenia Africana*. Symposium Book of Proceedings. 11th Natural Products Research Network for Eastern and Central Africa (NAPRECA), 2005; 123-131.