Antifungal Activity of *Millingtonia hortensis*

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Antifungal activities of different extracts of *Millingtonia hortensis* were investigated against various fungal pathogens. Methanol extract was found to have stronger activity than fluconazole against yeast like fungi: 4 fold against *Candida krusei* with 4 µg/ml minimal inhibitory concentration and 2 fold (MIC- 2 µg/ml) against *Sacharomyces cerevisiae*, though it showed the same activity as fluconazole against *Candida glabrata*. Aqueous extract also exhibited 4 fold stronger activity against *Candida krusei* (MIC- 4 µg/ml) and 4 fold (MIC; 2 µg/ml) against *Sacharomyces cerevisiae*. Chloroform and ethyl acetate extract showed lower activities against all fungal pathogens except for *Candida krusei*, compared with the standard. Against the filamentous fungus, *Trichosporon cutaneum*, all extracts showed less activity than the standard.

Key words: Antifungal, *Candida*, fluconazole

Fungal infection in humans can be classified as suprafacial and systemic mycosis, depending on the area of the body primarily affected. In patients with impaired immune system, there is high mortality due to systemic fungal infections. Although, azole derivatives, such as fluconazole and itraconazole are widely used in clinical settings, there are major drawbacks in their spectra, safety and pharmacokinetic properties[1-4]. In addition, resistance of fungal strains to existing antifungal drugs is becoming a major threat[5-6] and therefore, there is a need for discovering

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TABLE 1: ANTIFUNGAL ACTIVITY OF MILLINGTONIA HORTENSIS

Organism	A	B	C	D	Fluconazole
Candida albicans 1122	8.2±0.10	64.2±0.05	32.1±0.01	16.2±0.01	0.13±0.05
C. albicans 1162	64.1±0.05	64.1±0.01	32.3±0.05	32.4±0.01	32.1±0.01
C. glabrata 90030	8.1±0.01	16.3±0.10	32.1±0.01	16.3±0.10	8.4±0.01
C. krusei 7661	4.3±0.05	4.3±0.01	16.4±0.01	16.1±0.10	16.2±0.05
C. tropicalis 780	8.1±0.05	32.2±0.01	32.2±0.10	16.4±0.01	2.4±0.10
C. parapsilosis 826	16.3±0.01	32.4±0.05	32.2±0.05	16.1±0.05	2.1±0.01
Saccharomyces cerevisiae 250	4.2±0.10	2.3±0.01	16.1±0.01	8.4±0.05	8.3±0.10
Cryptococcus neoformans 513	8.3±0.05	8.1±0.05	32.3±0.01	16.3±0.01	4.1±0.01
Trichosporon cutaneum 517	8.1±0.01	8.3±0.10	32.4±0.05	16.2±0.05	4.3±0.05
Aspergillus funigatus 526	64.4±0.01	64.1±0.01	64.1±0.10	32.1±0.01	64.1±0.05

Antifungal activity of *Millingtonia hortensis* (n=3; mean±SD) against fungal pathogens. A, B, C and D refers to methanol extract, aqueous extract, chloroform extract and ethyl acetate extract, respectively. Minimum inhibitory concentration (MIC, µg/ml)

and develop more effective antifungal agents. These agents are required in the treatment of candidosis of mucous membrane, mouth, vagina and alimentary tract in normal patients, as well as in HIV positive and terminally ill patients[7].

Millingtonia hortensis Linn. (Syn *Biognonia suberosa* Roxb., *Biognonia azedachta* Koen.) is an important medicinal plant in Southern Asia, ranging from India, Burma, Thailand and Southern China. In India, it is popularly known as Indian cork tree[8-10]. The leaves of *Millingtonia hortensis* are used as antipyretic, sinusitis, cholagogue and tonic in folklore medicine[11]. The present study was carried out to screen the various extracts of this plant against the fungal pathogens, so that a lead can be obtained to carry out further activity guided phytochemical studies. The leaves extract constituting mainly flavanoids, tannins and alkaloids showed significant activity against microbes causing candidosis of mouth, vagina and alimentary tract. The leaves were collected from Hamirpur (HP India), in the month of March, 2001. A voucher (Voucher ID: MH 096-01) was deposited in Herbarium, Department of Botany, Punjabi University, Patiala, India. The leaves were shade dried and powdered. The extraction was carried out at room temperature in water, methanol, chloroform and ethyl acetate. The extracts were dried under reduced pressure. Fluconazole (0.2% Diflucan inj., Pfizer Co., Ltd) was used as a standard agent in this study. Concentrations were adjusted at 6.4 mg/ml of each extract with 100% dimethyl sulfoxide and diluted with RPMI 1640 broth (Sigma Chemicals, St. Louis, MO, U.S.A.) for 50 fold final strength. For antifungal susceptive test, a two fold dilution series of each extract was prepared at 100 fold final strength. The MIC assay was preformed on various fungal strains (Table 1) and all strains were maintained at -80° in RPMI 1640 (pH 7.1). Except for *Aspergillus fumigatus*, minimum inhibitory concentration (MIC) values were determined by serial diluting methods in liquid media using microtiter plate, according to the method of the National Committee for Clinical Laboratory Standards (NCCLS)[12]. MICs were evaluated after incubation for 48 h at 35° (except for *Cryptococcus neoformans* which was for 72 h at 35°) and adjusted to 2×10^3 cells/ml. For *Aspergillus fumigatus*, MIC values were determined by serial diluting methods in liquid media using microtiter plate following the method of NCCLS[13]. Other steps were same as above the concentration was adjusted to 2×10^4 cells/ml. Antifungal activity of various extracts of *Millingtonia hortensis* against fungal pathogens was investigated by measuring their MIC using NCCLS macrobroth dilution method. Fluconazole was used as a standard agent for evaluation of their activities. As mentioned in Table 1, each extract exhibited wide range of antifungal activity. Methanol extract was found to have 4 fold fungicidal activity (MIC of 4 µg/ml) than fluconazole against yeast like fungi, *Candida krusei* and 2 fold lower (MIC- 4 µg/ml) against *Saccharomyces cerevisia.* However, the methanolic extract showed same activity against *Candida glabrata* as compared to the standard. Aqueous extract also exhibited 4 fold higher activity against *Candida krusei* (MIC- 4 µg/ml) and 4 fold (MIC- 2 µg/ml) higher against *Saccharomyces cerevisiae*. Chloroform and ethyl acetate extracts showed lower activity against all fungal pathogens except for *Candida krusei*. Against the filamentous fungus, *Trichosporon cutaneum*, all extracts showed less activity as compared with the standard. Aqueous, as well as ethyl acetate extracts showed activity against azole resistant *Candida albicans* 1162 compared to that of fluconazole. These results suggest that methanol and aqueous extracts should be subjected to phytochemical investigations, for isolation of the

antifungal principal.

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REFERENCES

1. Lee, S.H., Lee J.R., Lunde, C.S. and Kubo, S., **Planta Med**., 1999, 65, 204.
2. Dixon, D.M., McNeil, M.M. and Cohen, M.L., **Public Health Rep**., 1996, 111, 226.
3. Georgopapadakou, N.H. and Walsh, T.J., **Science**, 1994, 262, 371.
4. Rex, J.H., and Rinaldi, M.G., **Antimicrob. Agents Chemother.**, 1995, 39, 1.
5. Margaret, M. and Hudson, T., **J. Antimicrob. Chemother.**, 2001, 48, 345.
6. Iwata, K., **Eur. J. Epidermiol.**, 1992, 8, 407.
7. Ternberg, S., **Science**, 1994, 266, 1632.
8. Blatter, E. and Millard W.S., In; Some Beautiful Indian Trees, Vol. 6, The Bombay Natural History Society, Mumbai, 1954, 106.
9. Gamble, J.S., In; Flora of the Presidency of Madras, Vol. 09, Botanical Survey of India, Calcutta, 1957, 699.
10. Hooker and Dalton, J., In; The Flora of British India, Vol. 04, L. Reeva and Co, England, 1954, 376.
11. Anulakanapakorn, K. and Bunyapraphatsara, N., **J. Sci. Soc. Thailand**, 1998, 13, 71.
12. National committee for clinical laboratory standards guidelines, National committee for clinical laboratory standards, Wayne Pa., 1997, M27-A.
13. National committee for clinical laboratory standards guidelines, National committee for clinical laboratory standards, Wayne Pa., 1998, M38-P.

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