of the combination in capsule dosage form was found to be 100.42% of aspirin and 101.22% of clopidogrel bisulphate. The method was simple and had short run time of 10 min, which makes the method rapid. The results of the study indicate that the proposed HPLC method is simple, precise, accurate and less time consuming.

REFERENCES


Antifungal Activity of Millingtonia hortensis

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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 albicans. 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. In addition, resistance of fungal strains to existing antifungal drugs is becoming a major threat and therefore, there is a need for discovering
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.

**Millingtonia hortensis** Linn. (Syn Biognonia suberosa Roxb., Biognonin azedactha 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, Punjab 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 susceptibility 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 cerevisiae**. 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 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**.

### Table 1: Antifungal Activity of Millingtonia Hortensis

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

Antifungal activity of *Millingtonia hortensis* (n=3; means±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)
antifungal principal.

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Spectrophotometric Simultaneous Estimation of Ranitidine Hydrochloride and Ondansetron hydrochloride from Tablet Formulation

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Three simple, accurate, economical and reproducible UV spectrophotometric methods for simultaneous estimation of two component drug mixture of ranitidine hydrochloride and ondansetron hydrochloride from combined tablet dosage form have been developed. First developed method involves formation and solving of simultaneous equations at 267.2 nm and 314.4 nm. Second method was developed making use of first order derivative spectroscopy using 340.8 nm and 276.0 nm as zero crossing points for estimation of ranitidine hydrochloride and ondansetron hydrochloride respectively. Third method is based on two wavelength calculation, wavelengths selected for estimation of ranitidine hydrochloride were 266.1 nm and 301.8 nm and for ondansetron hydrochloride 305.7 nm and 319.2 nm. The results of analysis have been validated statistically and by recovery studies.

Ranitidine hydrochloride, chemically 1,1-ethenediamine-N-[2-[[5-[(dimethylamino)methyl]-2-furanyl]-methyl]thio]ethyl]-N'-methyl-2-nitro hydrochloride is an H₃-receptor antagonist indicated for the duodenal ulcer1. Literature survey reveals that for ranitidine hydrochloride HPLC3,4, spectrophotometric⁵ and capillary electrophoresis⁶,⁷ methods have been reported for its determination from human plasma and commercial formulation. Ondansetron hydrochloride, chemically 4H-carbazol-4-one-1,2,3,9-tetrahydro-9-methyl-3-[(2-methyl-1H-imidazole-1-yl) methyl] hydrochloride is a selective 5-HT₃ receptor antagonist indicated for the prevention of nausea and vomiting². Three HPLC⁸-¹⁰ and one LC¹¹ methods have been reported in literature for estimation of ondansetron hydrochloride from human plasma and commercial formulation. However no spectrophotometric method is yet reported for simultaneous analysis of two drugs from combined pharmaceutical dosage form.

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