**In vitro antifungal activity of Naringi crenulata (Roxb.) Nicols leaf extract**

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**Abstract**

Chloroform, ethyl acetate and methanol extracts of the leaves of *Naringi crenulata* were tested against three human pathogenic fungal species such as *Candida albicans*, *Candida tropicalis* and *Candida parapsilosis*. Among all the extracts, methanolic extract of the leaves (LME) was found to exhibit significant antifungal effect at 1000 µg/ml with the zone of inhibition ranging from 20-23 mm. This antifungal activity of LME of *Naringi crenulata* was determined using well diffusion assay. Minimum Inhibitory Concentration (MIC) value was determined by the broth dilution assay. Among the three fungi used, *Candida parapsilosis* had shown significant MIC at 300 µg/ml. The results of the study suggest that the leaves of *Naringi crenulata* could be a potent source of natural drugs against these human pathogenic fungi and hence could lead to the development of effective drugs for the treatment of antifungal effects caused by these test pathogens.

**Keywords**: Naringi crenulata, antifungal activity, LME, MIC, human pathogens

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**Introduction**

Plants play an important role in providing potentially valuable drugs to cure enormous diseases without causing side effects. Thus, there is a need to investigate the biological properties of medicinal plants in order to develop new drugs. Many of the herbs utilised by humans as seasonal product yield useful medicinal compounds (The Wealth of India, 2005). Enormous work had been carried out on herbal treatment of various diseases but there is need to work more (Alade and Irobi, 1993; Esimone et al., 2003) The main objective of this study was to analyse the antifungal activity of *Naringi crenulata*, a dicot plant, which was found to possess antibacterial activity (Samundeeswiari et al., 2012). The plant, *Naringi crenulata* (Roxb.) Nicols, belonging to the family of Rutaceae is commonly called as Mahavilvam in Tamil. It is distributed throughout India, Indo-Malaysia in the southern Western Ghats and South and Central Sahyadris.

*Naringi Crenulata* (Roxb.) Nicols is a tree 8-12 m tall; bark appeared dull brown yellow, smooth; spines are sharp; leaves compound, imparipinnate to 15 cm long, alternate, rachis with oblong series wings, leaflets 5-9, opposite sessile, elliptic-ovate, apex margined or obtuse, base acute, margin crenulated or irregularly serrulate, glandular, glabrous; flowers in axillary recemose, white, fragrant flowers; fruit globose berries, 2 seeded (Gamble, 1935; Sold and Nicols, 1979; Saldanha, 1996).

Various parts of this plant have been employed in indigenous medicine and are used as anti-epileptic, purgative, sudorific, colic trouble and cardialgia. Leaves are used for offering pookas for Lord Siva and used as a remedy for epilepsy (Subramaniam, 2011). The current
study focuses on the validation of antifungal activity of *Naringi Crenulata*.

**Materials and Methods**

**Plant material**

The fresh plants were collected from the fields located near Khalaahasthi temple, Andhra Pradesh (India). The plant was identified and verified using the voucher specimens with the P.G. Research Department of Botany, Govt. Arts College, Nandanam, Chennai-35. The leaves were then separated from the stem, carefully washed with tap water, rinsed with distilled water, and air-dried for 1 hr. Then the leaves were shade dried at room temperature for one week. Then they were ground into powder and subjected to extraction with different solvents.

**Plant extracts preparation**

The finely ground leaves were extracted with different solvents such as, chloroform, ethyl acetate and methanol following the method of Lai and Roy (2004). The extraction of the leaf powder was done with solvents in the ratio of 1:10 under shaker. The process was repeated thrice to attain maximum extraction. Then the solvents were evaporated and condensed to concentrate the extracts obtained. The concentrated residues were weighed and redissolved in respective solvents to yield 10 mg/ml solutions for further analysis.

**Antifungal activity**

The crude extracts were subjected to antifungal screening against (1) *Candida albicans* (MTCC 183), (2) *Candida tropicalis* (MTCC 184) and (3) *Candida parapsilosis* (MTCC 2509).

**Well diffusion assay**

Potassium dextrose agar medium was prepared and poured in the Petri dish. The 24 hrs growing culture of *Candida albicans* (MTCC 183), *Candida tropicalis* (MTCC 184), and *Candida parapsilosis* (MTCC 2509) were swabbed on it. The wells (10 mm diameter) were made by using cork borer and the different concentrations of the crude extract were loaded in the wells. The plates were then incubated at 37ºC for 24 hrs. The inhibition diameter was then measured (Fazeli et al., 2007).

**Broth dilution assay**

Dilution assay by standard method was used to compare the inhibition efficiency of the antifungal agents. 5 ml of the Potassium dextrose broth nutrient medium, 0.1 ml of the 24 hrs growing culture (*Candida albicans, Candida tropicalis* and *Candida parapsilosis*) and the different concentration (100, 200 and 1000 µg) of the drug dissolved in Dimethyl sulphoxide were added in the test tubes. The tubes were incubated at 37ºC for 24 hrs. The optical densities were measured spectrometrically at 600 nm. The percentage of viable cells was calculated using the following formula (Pyun and Shin, 2005; Cos et al., 2006).

\[
\text{OD-Optical Density:} \quad \text{Control OD} - \text{Test OD} \\
\% \text{ viable cells} = \frac{\text{Control OD}}{\text{Control OD} × 100}
\]

**Results and Discussion**

The antifungal property of *Naringi Crenulata* investigated by well diffusion assay infers that leaf methanolic extract (LME) exhibits higher activity against the test pathogens when compared to other solvent extracts. The maximum zone of inhibition of LME (1000 µg/mL) was found to be 23 and 20 mm respectively (Table 1) against *C. albicans* and *C. tropicalis*. Further, the IC₅₀ of LME obtained from broth dilution assay was 300, 400 and 500 µg/ml respectively against *C. albicans, C. tropicalis* and *C. parapsilosis* (Table 2).
Table 1. *In vitro* antifungal activity of different concentrations of chloroform, ethyl acetate and methanol extracts of the leaves of *Naringi crenulata* (CE - Chloroform extract, EAE - Ethyl Acetate Extract, ME - Methanol Extract).

<table>
<thead>
<tr>
<th>Organism</th>
<th>CE 250</th>
<th>500</th>
<th>750</th>
<th>1000</th>
<th>EAE 250</th>
<th>500</th>
<th>750</th>
<th>1000</th>
<th>ME 250</th>
<th>500</th>
<th>750</th>
<th>1000</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida albicans</em></td>
<td>12</td>
<td>14</td>
<td>14</td>
<td>18</td>
<td>12</td>
<td>14</td>
<td>16</td>
<td>17</td>
<td>13</td>
<td>15</td>
<td>19</td>
<td>23</td>
</tr>
<tr>
<td><em>Candida tropicalis</em></td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>16</td>
<td>11</td>
<td>12</td>
<td>11</td>
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<td>11</td>
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<td>20</td>
</tr>
<tr>
<td><em>Candida parapsilosis</em></td>
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<td>11</td>
<td>14</td>
<td>13</td>
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<td>16</td>
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<td>11</td>
<td>12</td>
<td>12</td>
<td>16</td>
<td>16</td>
</tr>
</tbody>
</table>

All the values are mean of three replicates

Table 2. Minimum inhibitory concentration of methanol extract of leaves of *Naringi crenulata* against microorganisms

<table>
<thead>
<tr>
<th>Organisms</th>
<th>ME Leaf mg/ml</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>100</td>
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<td><em>Candida albicans</em></td>
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</tr>
<tr>
<td><em>Candida tropicalis</em></td>
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</tr>
<tr>
<td><em>Candida parapsilosis</em></td>
<td>11</td>
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</tbody>
</table>

ME - Methanol extract

Conclusions

From the results obtained in this study, it is evident that the leaves of *Naringi crenulata* are effective against all the three fungal pathogens. Further studies are required to confirm its effectiveness as a topical antifungal agent. The leaves of *Naringi crenulata* could be a potential source of traditional medicine for infections caused by *Candida albicans, Candida tropicalis* and *Candida parapsilosis* necessary to elucidate the exact bioactive compound which is responsible for the destined antifungal action.

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References


