**In vitro** evaluation of antioxidant and free radical scavenging activities of *Naringi crenulata*

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**Abstract**

This study aimed to evaluate the antioxidant activity of *Naringi crenulata* leaf extracts. Antioxidant potentials of the leaf extract in methanol were studied in *in vitro*. The methanolic extract of the leaves (LME) were subjected to assess their antioxidant potential activities using systems such as Ferric thiocyanate (FTC) and Thiobarbituric acid (TBA) test. Ferric thiocyanate (FTC) exhibit high resistance of lipid or lipid emulsions to oxidation in the presence of antioxidant being tested, were determined in the absorbance of red colour were measured at 500 nm. Thiobarbituric acid (TBA) has been used to screen the relative radical scavenging abilities of flavonoids and phenols present in the leaves of *Naringi crenulata* were measured at 532 nm showing, the peroxides are decomposed at lower molecular weight. Results of the study suggest that the leaves of *Naringi crenulata* possess significant antioxidant activity. Owing to these properties, this plant has the potential as natural source of antioxidants, capable of protecting against free radical mediated damage and may have applications in preventing and curing various diseases.

**Keywords**: LME, antioxidant activity, Ferric Thio Cyanate (FTC), Thio Butric acid (TBC)

Introduction

Plants produce a wide range of redox-active secondary metabolites with antioxidant activity, such as ascorbic acid, carotenoids, polyphenols, and enzymes which protect the cells from oxidative damage. Bioactive compounds commonly found in edible plant parts such as fruits, vegetables, flowers, and leaves have been shown to confer health benefits. Interestingly, many of them are known to contain large amounts of phenolic antioxidants (Yen et al., 2002). Some of the phenolic compounds present in natural products have higher antioxidant activities than those of synthetic antioxidants. Antioxidants provide protection to living organisms from damage caused by uncontrolled production of reactive oxygen species (ROS) and the concomitant lipid peroxidation, protein damage, and DNA strand breaking (Ghosal et al., 1996). Phenolic compounds in plant-derived foods and beverages have been shown to have important physiological properties and may be responsible for both detrimental and beneficial effects on human health (Chung et al., 1998; Singh et al., 2003). Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stoke, diabetes, Alzheimer’s diseases and cancer (Devesagayam et al., 2004).

The main objective of this study was to analyse antioxidant activity of *Naringi crenulata*, a dicot plant...
which was found to possess antibacterial activity and antifungal activity (Samundeeswari et al., 2012). *Naringi Crenulata* (Roxb) is a tree 8-12 m tall; bark appeared dull brown yellow, smooth. 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, south and Central Sahyadris.

*Naringi crenulata* (Roxb) Nicols is a tree 8-12 cm tall; bark appeared dull brown yellow, smooth; Spines are sharp; leaves compound, impair pinnae to 15 cm long, alternate, rachis with oblanceolate wings, leaflets 5-9, opposite sessile, elliptic-obovate, apex emarginated or obtuse, base acute, margin crenulated or irregularly serrulate, glandular, glabrous; Flowers in axillary racemose, white, fragrant flowers; Fruit globose berries, 2 seeded (Gamble 1935; Sold and Nicols, 1979). Various parts of this plant have been employed in indigenous medicine and it is used as antiepileptic, purgative, sudorific, colic trouble and cardialgia. Leaves are used for offering pojas for Lord Siva and used as a remedy for epilepsy (Subramanian, 2011). The current study, focuses on the antioxidant property of *Naringi crenulata* (Roxb) Nicols.

**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 with the P.G. Research Department of Botany, Government Arts College, Nandanam, Chennai. 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 in room temperature for one week. Then they were ground into powder and subjected to extraction with Methanol solvent.

**Preparation of Naringi crenulata plant powder**

The finely ground leaves were extracted with methanol solvent following the method (Lai and Roy, 2004). Here, the extraction of the leaf powder was done with methanol solvent in the ratio of 1:10 under shaking condition. The extract was collected in different conical flasks and the same was repeated thrice to attain maximum extraction. Then the concentrated solvents were evaporated and condensed to concentrate the extracts obtained. The concentrated residues were weighed and re-dissolved in methanol solvent to yield 10mg/ml solutions for further analysis.

**Results and Discussion**

**Ferric thio cyanate method: (FTC) Test (1)**

The plant sample of 4 mg in 99.5% ethanol were mixed with 2.51% linoleic acid in 99.5% ethanol (4.1 mL), 0.05 M phosphate buffer, pH 7 (8 mL) and distilled water (3.9 mL) and kept in screw cap containers under dark conditions at 40°C. To 0.1 mL of this solution, 9.7 mL of 75% ethanol and 0.1 mL of 30% ammonium thiocyanate were added. After 3 min, 0.1 mL of 2 M ferrous chloride in 3.5% HCl was added to the reaction mixture and the absorbance of the red color was measured at 500 nm (Fig. 1) each 24 h until one day after absorbance of the control reached maximum. The control and the standard were subjected to the same procedure as the sample except for the control, where there was no addition of sample, and for the standard 4 mg of sample were replaced with 4 mg of α-tocopherol or Butylated hydroxytoluene. The FTC method was used to measure the peroxide levels during the initial stage of lipid oxidation. Low absorbance values have indicated high levels of antioxidative activity. Table 1
details the absorbance values of different methanol extracts of *Naringi crenulata*. It is interesting to note that the methanolic extract exhibited higher antioxidant activity.

**Fig. 1.** Absorbance of the red color (at 500 nm)

Table 1. Ferric thiocyanate (FTC) method

<table>
<thead>
<tr>
<th>No of Days</th>
<th>Standard</th>
<th>Control</th>
<th>Test</th>
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<tbody>
<tr>
<td>1</td>
<td>2.001</td>
<td>0.898</td>
<td>0.248</td>
</tr>
<tr>
<td>2</td>
<td>1.839</td>
<td>0.229</td>
<td>0.242</td>
</tr>
<tr>
<td>3</td>
<td>1.829</td>
<td>0.230</td>
<td>0.220</td>
</tr>
<tr>
<td>4</td>
<td>1.829</td>
<td>0.198</td>
<td>0.198</td>
</tr>
<tr>
<td>5</td>
<td>1.687</td>
<td>0.232</td>
<td>0.146</td>
</tr>
<tr>
<td>6</td>
<td>1.637</td>
<td>0.141</td>
<td>0.141</td>
</tr>
<tr>
<td>7</td>
<td>1.590</td>
<td>0.127</td>
<td>0.127</td>
</tr>
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</table>

**Ferric thiocyanate (FTC) and Thiobarbituric acid (TBA) tests (2)**

The same samples as prepared for the FTC method were used in TBA test. To 1 mL of sample solution, 2 mL each of 20% aqueous trichloroacetic acid were added. This mixture was then incubated in a boiling water bath for 10 min. After cooling, it was centrifuged at 3000 rpm for 20 min and the absorbance of supernatant was measured at 532 nm (Fig. 2). Antioxidative activity was recorded based on absorbance on the eighth day. During the oxidation process, peroxides are gradually decomposed to lower molecular weight compounds, like malonaldehyde, which can be measured by TBA method on the final day of the incubation period. The anti-oxidative activity of *Naringi crenulata* methanol was high on 7th day of incubation (Table 2).

**Fig. 2.** Thiobarbituric acid (TBA) test

Table 2. Thiobarbituric acid (TBA) test

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<th>Control</th>
<th>Test</th>
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<tbody>
<tr>
<td></td>
<td>0.098</td>
<td>0.880</td>
<td>0.898</td>
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</table>

**Conclusion**

From the results obtained in this study, it is evident that the leaves of *Naringi crenulata* which have flavanoids are a class of polyphenolics that can be synthesized from the amino acid phenylalanine. Phenolic compounds are a large group of antioxidant compounds found in many food systems (Sun et al., 2002). According to Rice-Evans et al. (1997), number of hydroxyl groups and the amount and types of conjugation are two important factors in antioxidant potential of phenolic compounds. The better antioxidants are generally more conjugated and have numerous hydroxyl groups present (n=2 to 5), which enables the antioxidant to scavenge several radicals (table 1). Norhaiza et al. (2009) reported that *Naringi crenulata* showed significantly higher phenol and flavanoid content.

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Natural antioxidants constitute a broad range of compounds including phenolics, nitrogen compounds (Velioglu et al., 1998). Among bioactive compounds naturally occurring phenolic flavanoids have gained a particular interest because of their broad pharmacological activity. The leaf extract is the main source of antioxidants, which have shown radical scavenging activity and reducing potential. In the overall, the synthetic antioxidants such as butylated hydroxyytoluene (BHT) and ascorbic acid have been widely used for many years to retard lipid oxidation. However, the safety of using these synthetic antioxidants in food industry has become a concern among scientists and leading to current interest in uncovering natural antioxidants (Karimi et al., 2010).

References


