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In vitro investigation on the antibacterial activities of the leaves of *Naringi crenulata* (Roxb.) Nicols

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Abstract

Chloroform, ethyl acetate and methanol extracts of the leaves of *Naringi crenulata* were tested against four bacterial species such as *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Bacillus subtilis*. Among all the extracts, methanolic extract of the leaves (LME) was found to exhibit significant antibacterial effect at 1000 µg/ml with the zone of inhibition ranging from 18-22 mm. This antibacterial activity of LME of *Naringi crenulata* was determined using well diffusion assay. Minimum Inhibitory Concentration (MIC) value was determined by broth dilution assay. Among the four bacteria used, *Staphylococcus aureus* had significant MIC at 300 µg/ml. Furthermore, chromatographic analysis of the crude extract revealed the presence of nine distinct bioactive compounds. The results of the study suggest that the leaves of *Narinji crenulata* could be a potent source of natural drugs against these pathogenic bacteria and hence could lead to the development of effective drugs for the treatment of health effects caused by these test pathogens.

Keywords: *Naringi crenulata*, antibacterial activity, *LME, MIC*

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Introduction

The rising prevalence of microorganism showing resistance to antibiotics urges the mankind to develop new antimicrobial compound. Since antiquity, plants have been used to treat common infectious diseases the healing potential of many plants have been utilized by Indian traditional medicines like Siddha, Ayurvedha and Unani. Being non-toxic and easily affordable, there has been resurgence in the consumption and demand plants for medicinal plants (Jayashree and Maneemegalai, 2008). In 2001, researchers identified 122 compounds used in mainstream medicine which were derived from "ethnomedical" plant sources; 80% of these compounds were used in the same or related manner as the traditional ethnomedical use (Fabricant and Farnsworth, (2001). Many of the herbs and species used by humans to season food yield useful medicinal compounds (Lai and Roy, 2004). The screening of plant extracts and their products for antibacterial activity has shown that higher plants represent a potential source of novel antibiotic prototypes. Plant based antibacterials represent a vast untapped source of medicines even after their enormous therapeutic potential and effectiveness in the treatment of infectious disease hence, further exploration of plant antibacterials need to occur. The selection of crude plant extracts for screening programs is potentially more successful in initial steps than the pure compounds Even though hundreds of plant species have been tested for antibacterial properties; the vast majority of them have not yet been validated.

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-Malysia, in the southern Western Ghats, 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 oblonceolate wings, leaflets 5-9, opposite sessile, elliptic-obovate, apex emarginated 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 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 validation of antibacterial 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 same were verified and authenticated botanically by S. Aroumougame, University of Madras, 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 different solvents.

Plant extracts preparation

The finely ground leaves were extracted with different solvents such as, chloroform, ethyl acetate and methanol following the method (Eloff, 1998). Here, the extraction of the leaf powder was done with solvents in the ratio of 1:10 under shaking condition. The extracts were collected in different conical flasks and the same 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 re-dissolved in respective solvents to yield 10 mg/ml solutions for further analysis.

Antibacterial activity

The crude extracts were subjected to antibacterial screening against (1) *Escherichia coli* (MTCC 1687), (2) *Klebsiella pneumoniae* (MTCC 109), (3) *Staphylococcus aureus* (MTCC 96), and (4) *Bacillus subtilis* (ATCC 9372).

Well diffusion assay

Nutrient agar was prepared and poured in the Petridish. 24 hrs growing culture (*Klebsiella pneumoniae* and *Staphylococcus aureus*) 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, 2007).

Broth dilution assay

Dilution assay by standard method was used to compare the inhibition efficiency of the antibacterial agents. 5 ml of the Nutrient broth, 0.1 ml of the 24 hrs growing culture (*Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Bacillus subtilis*) and the different concentration (100 µg, 200 µg, 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.

$$\% \text{ viable cells} = \frac{\text{Control OD - Test OD}}{\textit{Control OD}} * 100$$

O.D – Optical Density

Thin Layer Chromatography

The methanolic leaf extract was loaded on pre-coated silica plates which were then developed using the solvents methanol, chloroform in the ratio of 0.5: 9.5. The spots were identified both in the UV light, far light and in the iodine chamber. Then R_f value was calculated as the ratio of distance travelled by the solute to the distance travelled by the solvent (Eloff, 1998).

Table 1. Qualitative analysis of the phytochemicals in the leaves of *Naringi crenulata*

Phytochemical Components	Methanol extract
Alkaloids	++
Carbohydrates	++
Glycoside	-
Saponins	-
Protein	++
Phenolic compound	++

+ = present; - = absent

Results and discussion

The *antibacterial* property of *Naringi Crenulata* investigated by well diffusion assay infers that Leaf Methanolic Extract (LME) exhibits higher activity

Fig. 1. The chromatogram of the methanolic leaf *Naringi crenulata*

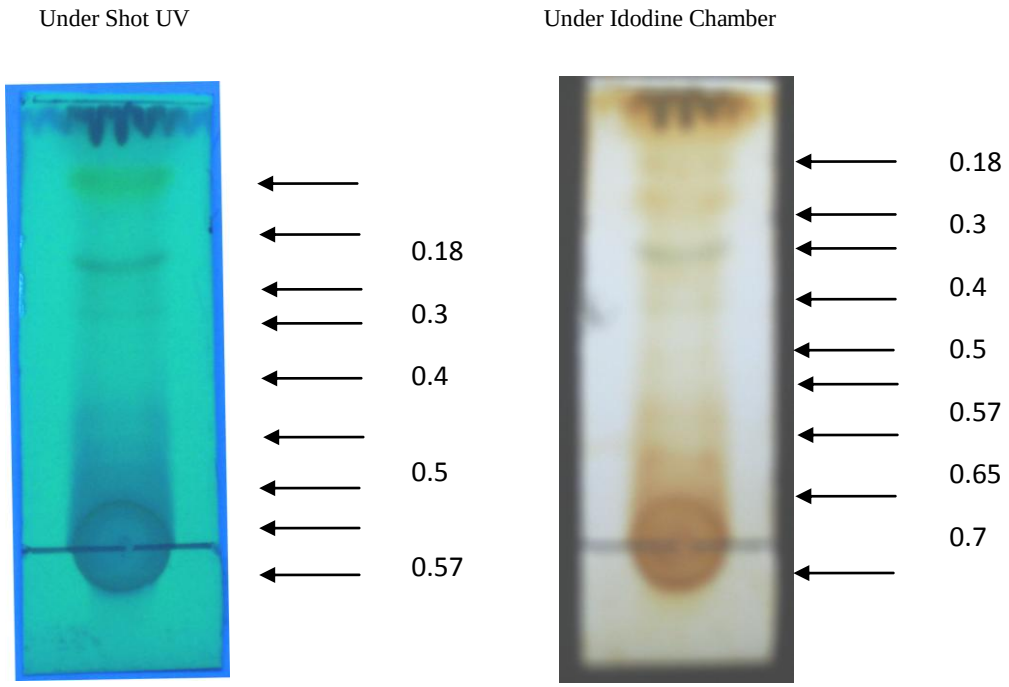

Table 2. *In vitro* antibacterial activity of different concentrations of chloroform, ethyl acetate and methanol extracts of the leave of *Naringi crenulata*

Organism	CE				EAE				ME			
	250	500	750	1000	250	500	750	1000	250	500	750	1000
Zone of inhibition (mm)												
E. coli	10	10	12	11	10	11	14	16	14	16	18	18
K.pneumoniae	11	12	17	18	11	14	18	17	16	18	21	22
S. aureus	10	10	11	13	10	10	10	10	16	19	20	21
B. subtilis	10	11	14	15	10	11	10	10	15	16	19	20

All the values are mean of three replicates. CE – Chloroform extract, EAE = Ethyl Acetate Extract, ME – Methanol Extract

Table 3. Minimum inhibitory concentration of methanol extract of leaves of *Naringi crenulata* against micro organisms (values are mean of three replicates)

Organisms	ME Leaf mg/ml									
	100	200	300	400	500	600	700	800	900	1000
E. coli	12	14	18	21	37	43	51	69	74	84
K. pneumoniae	26	33	46	53	56	61	67	73	78	79
S. aureus	34	42	50	60	66	74	76	79	83	57
B. subtilis	12	21	25	36	50	59	67	73	77	82

ME = Methanol extract

against the test pathogens when compared to other solvent extracts. The maximum zone of inhibition of LME (1000 µg/mL) was found to be 22 and 21 mm respectively against *Klebsiella pneumoniae* and *Staphylococcus aureus* (Table 2). Further, the IC_{50} of LME obtained from broth dilution assay was 400 µg/ml and 300 µg/ml, respectively against *Klebsiella pneumoniae, and Staphylococcus aureusi* (Table 3). The preliminary phytochemical screening of *Naringi crenulata* revealed the presence of phenolics and alkaloids in high amounts followed by absence of glycosides and saponins (Harborne, 1998; Sazada et al., 2009). Enormous amount of carbohydrates, proteins and amino acid were present (Table 1).

The chromatogram developed with methanol, chloroform in the ratio of 0.5: 9.5 revealed the presence of nine major compounds at R_f value of 0.92, 0.8, 0.7, 0.65, 0.57, 0.5, 0.4, 0.3 and 0.18 as visualized under iodine vapour and UV illumination (Fig. 1).

Conclusions

From the results obtained in this study, it is evident that the leaves of *Naringi crenulata* are effective against all the four pathogens. In addition, the results of phytochemical screening supports the fact that the phenolics and alkaloids present in higher amounts could be responsible for the inhibitory action of the leaf extracts. Also, the chromatogram developed, suggests that nine major compounds are present in the leaf extract of *Naringi crenulata* which could contribute to its antibacterial activity. These results reveal that the leaves of *Naringi crenulata* could be a potential source of traditional medicine for infections caused by (1) *Escherichia coli* (MTCC 1687), (2) *Klebsiella pneumoniae* (MTCC 109) (3) *Staphylococcus aureus* (MTCC 96) and (4) *Bacillus subtilis* (ATCC 9372.) necessary to elucidate the exact bioactive compound which is responsible for the destined antibacterial action.

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