



Antimicrobial potential of *Naringi crenulata* (Roxb.) nicolson: Comparative analysis of leaf and stem extracts

D Nageshwer Rao, B Rajani*

Department of Botany, Osmania University, Telangana, India

Abstract

The present study investigates the antimicrobial potential of *Naringi crenulata* (Roxb.) Nicolson stem and leaf extracts against bacterial and fungal pathogens. Solvent extracts (hexane, ethyl acetate, and methanol) were prepared using the Soxhlet extraction method and screened for antibacterial activity against *Staphylococcus aureus*, *Bacillus cereus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Enterobacter aerogenes*, and *Pseudomonas aeruginosa*, as well as antifungal activity against *Candida albicans* and *Aspergillus niger* using the agar well diffusion method.

Leaf extracts demonstrated greater antimicrobial efficacy compared to stems, with ethanol and hexane extracts exhibiting the highest activity. *S. aureus* was highly susceptible to ethanol leaf extract (4.4 cm zone of inhibition), whereas *B. cereus* showed significant inhibition with hexane (2.7 cm) and ethanol extracts (0.64 cm). *P. aeruginosa* exhibited slight inhibition with ethanol extract (1.015 cm), while *E. coli* and *E. aerogenes* were resistant to all treatments. Strong antifungal activity was observed, particularly against *C. albicans* and *A. niger*, with ethyl acetate and methanol leaf extracts producing the highest inhibition.

Phytochemical analysis revealed the presence of alkaloids, flavonoids, glycosides, tannins, and saponins, which may contribute to the observed bioactivity. The findings suggest that *N. crenulata* leaf extracts, especially ethanol and ethyl acetate fractions, possess promising antimicrobial properties. These results provide a scientific basis for further isolation of active compounds and their potential application in natural antimicrobial formulations.

Keywords: *Naringi crenulata*, antimicrobial activity, phytochemicals, medicinal plants, bacterial inhibition, antifungal properties

Introduction

Naringi crenulata (Roxb.) Nicolson, commonly referred to as bitter orange or wild lime, is a medicinal plant belonging to the Rutaceae family (Allayie *et al.*, 2016) [3]. Native to India and found across Sri Lanka and Southeast Asia, it flourishes in dry deciduous forests, scrublands, and riverbanks. Taxonomically classified under the order Sapindales, subfamily Aurantioideae, it shares close botanical relationships with citrus species (Ramya, 2014) [18]. Traditionally, it has been valued in indigenous medicine for managing fevers, digestive issues, skin ailments, and inflammatory conditions (Kuruvella *et al.*, 2024) [12]. Modern pharmacological research has validated its therapeutic relevance, highlighting its antimicrobial, antioxidant, anticancer, hepatoprotective, and neuroprotective properties (Neelam Singh, 2011) [15]. These medicinal attributes are linked to its rich phytochemical profile, comprising alkaloids, flavonoids, tannins, glycosides, and saponins—bioactive compounds recognized for their diverse pharmacological effects (Yesudanam *et al.*, 2023) [26].

Bacterial and fungal infections remain a global health challenge, contributing to high morbidity and mortality rates (Lakshmi *et al.*, 2024) [13]. The increasing prevalence of multidrug-resistant (MDR) bacteria has rendered conventional antibiotics less effective, necessitating the urgent search for alternative antimicrobial agents (Aagalya *et al.*, 2024; Bhatt *et al.*, 2021) [1, 5]. Opportunistic fungal pathogens, such as *Candida albicans* and *Aspergillus niger*, are also responsible for severe infections, particularly in immunocompromised individuals (Rawson *et al.*, 2023) [19]. The current treatment landscape relies heavily on synthetic

antibiotics and antifungal drugs, but emerging resistance and drug-related side effects have fueled interest in plant-derived antimicrobials as safer and more sustainable therapeutic options (Aagalya *et al.*, 2024) [1].

Herbal medicine has played a pivotal role in infection management, with various plant extracts demonstrating potent antibacterial and antifungal activity (Esmael *et al.*, 2024) [9]. Natural products, particularly secondary metabolites like tannins, flavonoids, alkaloids, and glycosides, have shown inhibitory effects on microbial growth by disrupting cell walls, membranes, or metabolic pathways (Gupta Himanshu Upadhyay, 2023) [10]. Several medicinal plants, such as *Azadirachta indica*, *Curcuma longa*, and *Embllica officinalis*, have exhibited significant antimicrobial activity, reinforcing the potential of botanical extracts as therapeutic agents (Izah *et al.*, 2003; Majhi *et al.*, 2024) [14]. While *Naringi crenulata* has been extensively studied for its pharmacological properties, its antibacterial and antifungal efficacy remains largely underexplored.

The plant has also shown nanobiotechnological applications, with its extracts being used in the green synthesis of silver nanoparticles (AgNPs), which have exhibited strong antibacterial activity against MDR pathogens (Chinnathambi *et al.*, 2023) [6]. Additionally, its bioactive compounds have demonstrated cytotoxic effects against HER2+ breast cancer cells, reinforcing its diverse pharmacological potential (Vallinayagam *et al.*, 2021) [25]. However, while its antimicrobial applications are gaining attention, there is a lack of comprehensive studies evaluating its comparative efficacy across different bacterial and fungal pathogens.

This study aims to systematically evaluate the antibacterial and antifungal activity of various solvent extracts (hexane, ethyl acetate, and methanol) of *Naringi crenulata* stems and leaves. The research investigates their efficacy against Gram-positive and Gram-negative bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*) and fungi (*Candida albicans*, *Aspergillus niger*). By identifying the most potent extracts and comparing their effectiveness, this study seeks to bridge the existing knowledge gap and explore *N. crenulata* as a promising natural source for antimicrobial drug development.

Methodology

1. Collection and Preparation of Plant Extracts

The plant *Naringi crenulata* was collected, and its stems and leaves were separated for extraction. The plant materials were thoroughly washed, shade-dried, and finely powdered (Sudheer *et al.*, 2014). The powdered samples were subjected to solvent extraction using hexane, ethyl acetate, and methanol. The extraction was performed using the Soxhlet apparatus, where 100 g of plant material was continuously extracted with 500 mL of each solvent for 24 hours (Veni Madhavi *et al.*, 2025) [20]. The extracts were concentrated using a rotary evaporator under reduced pressure to remove the solvents (Allakonda *et al.*, 2024) [2]. The dried extracts were stored at 4°C in sterile containers until further use (Pravalika *et al.*, 2021; Sujatha *et al.*, 2022) [17, 22].

2. Percentage yield was calculated as

$$\text{Yield (g/100 g)} = (W_1/W_2) \times 100$$

Where,

W_1 = weight of the crude extract residue obtained after solvent removal

W_2 = weight of plant powder packed in the extractor (Dokuparthi *et al.*, 2021) [8]

3. Preparation of Test Solutions

Each extract was dissolved in acetone at a concentration of 25 mg/mL and 12.5 mg/mL. The solutions were vortexed for uniform mixing and filtered using Whatman No. 1 filter paper to remove any plant residues. The filtered solutions were stored in sterile vials at 4°C until antimicrobial testing (Thiban *et al.*, 2012) [23].

4. Microbial Strains and Culture Conditions

The antibacterial activity of the extracts was tested against six bacterial strains: *Staphylococcus aureus*, *Bacillus cereus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Enterobacter aerogenes*, and *Pseudomonas aeruginosa*. The bacterial cultures were obtained from a microbiology laboratory and maintained on nutrient agar slants at 4°C. Before experimentation, each bacterial strain was revived by inoculation into nutrient broth and incubated at 37°C for 24 hours. The bacterial cultures were adjusted to a turbidity equivalent to 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) to ensure uniform bacterial inoculum density (Dash *et al.*, 2023) [7].

5. Antibacterial Assay

The antimicrobial activity of *Naringi crenulata* extracts was evaluated using the agar well diffusion method. Mueller-

Hinton agar plates were prepared by pouring 20 mL of sterile molten agar into sterile Petri dishes and allowing it to solidify. The bacterial cultures were uniformly spread on the agar surface using a sterile cotton swab. Wells of 6 mm diameter were punched into the agar using a sterile cork borer, and 40 μ L of each plant extract at concentrations of 25 mg/mL and 12.5 mg/mL were added into separate wells. The plates were left undisturbed for 30 minutes to allow diffusion of the extract into the agar medium (Aswany *et al.*, 2023) [4].

6. Incubation and Measurement of Zone of Inhibition

The inoculated plates were incubated at 37°C for 24 hours, and the antibacterial activity was assessed by measuring the zone of inhibition (ZOI) around each well using a digital Vernier caliper. The diameters of the inhibition zones were recorded in centimeters, and inhibition areas were calculated using the formula πr^2 , where r represents the ZOI radius. All experiments were performed in triplicates to ensure reproducibility (Poorniammal *et al.*, 2022) [16].

7. Data Analysis and Interpretation

The mean ZOI and standard deviation were calculated for each extract against the tested bacterial strains. The effectiveness of the extracts was interpreted based on the ZOI measurements, where larger inhibition zones indicated stronger antibacterial activity. The results were compared across different solvents to determine which extract exhibited the highest antimicrobial potential. The sensitivity of Gram-positive and Gram-negative bacteria to the extracts was analyzed to understand variations in antimicrobial susceptibility. The findings were used to infer the potential of *Naringi crenulata* as a source of antibacterial compounds, particularly from its leaves, which exhibited the highest activity (Tumpa *et al.*, 2024) [24].

Results and Discussion

1. Extraction Yield

The extraction yield of *Naringi crenulata* leaves and stems varied depending on the solvent used. Methanol exhibited the highest yield for both leaves (3.20%) and stems (2.80%), indicating its efficiency in extracting polar compounds. Ethyl acetate showed a moderate yield, with 2.60% for leaves and 2.10% for stems, suggesting its effectiveness in extracting semi-polar compounds. Hexane, a non-polar solvent, resulted in the lowest yield, with 2.10% for leaves and 1.20% for stems, implying limited solubility of non-polar compounds. The overall higher yield from leaves compared to stems suggests a greater abundance of extractable bioactive compounds in leaves, reinforcing their suitability for antimicrobial and phytochemical studies (Table 1).

Table 1: Percentage yield

Plant Part	Hexane Extract (%)	Ethyl Acetate Extract (%)	Methanol Extract (%)
<i>Naringi crenulata</i> Leaves	2.10%	2.60%	3.20%
<i>Naringi crenulata</i> Stems	1.20%	2.10%	2.80%

The phytochemical analysis of *Naringi crenulata* revealed variations in bioactive compounds between leaves and stems based on the solvent used. Carbohydrates were present in all

leaf extracts but absent in methanol and hexane stem extracts. Glycosides were consistently detected across all extracts, indicating their widespread presence. Alkaloids were present in hexane and ethyl acetate extracts but absent in methanol, suggesting their solubility in non-polar to semi-polar solvents. Saponins were detected only in methanol extracts, confirming their high polarity. Tannins were present in ethyl acetate and methanol extracts but absent in hexane, aligning with their semi-polar nature. Proteins, steroids, and flavonoids were absent in all extracts. These findings suggest that methanol is efficient for extracting polar compounds, while hexane and ethyl acetate favor alkaloids and glycosides (Table 2).

Table 2: Phytochemical Profile of *Naringi crenulata*

Name of the test	<i>Naringi crenulata</i> leaves			<i>Naringi crenulata</i> Stems		
	Hexane Extract	Ethyl acetate Extract	Methanol Extract	Hexane Extract	Ethyl acetate Extract	Methanol Extract
Test for carbohydrates	+ve	+ve	+ve	-ve	+ve	-ve
Test for protein	-ve	-ve	-ve	-ve	-ve	-ve
Test for steroids	-ve	-ve	-ve	-ve	-ve	-ve
Test for glycosides	+ve	+ve	+ve	+ve	+ve	+ve
Test for alkaloids	+ve	+ve	-ve	+ve	+ve	-ve
Test for saponins	-ve	-ve	+ve	-ve	-ve	+ve
Test for flavonoids	-ve	-ve	-ve	-ve	-ve	-ve
Test for tannins	-ve	+ve	+ve	-ve	+ve	+ve

The antimicrobial evaluation of different solvent extracts of *Naringi crenulata* revealed selective activity against certain microbial strains, while others remained resistant. The hexane extract exhibited weak antibacterial activity, with minimal inhibition against *Bacillus cereus* (0.167 cm) and no activity against other bacterial strains, indicating that non-polar compounds in *Naringi crenulata* have limited antibacterial properties. The methanol extract showed slight inhibition against *Bacillus cereus* (0.0942 cm) but was ineffective against all other bacteria, suggesting that polar phytochemicals in the extract do not significantly contribute to antibacterial activity. The ethyl acetate extract did not exhibit any measurable inhibition against any bacterial strains, indicating that semi-polar compounds were not effective against these microbes. Gram-negative bacteria such as *Escherichia coli*, *Enterobacter aerogenes*, and *Pseudomonas aeruginosa* were completely resistant to all extracts, highlighting their intrinsic resistance due to their outer membrane, which acts as a barrier against many antimicrobial compounds. Similarly, *Staphylococcus aureus* and *Staphylococcus epidermidis* exhibited no inhibition, suggesting that *Naringi crenulata* extracts lack potent antibacterial effects against these Gram-positive pathogens. However, the extracts showed notable antifungal activity. The hexane extract demonstrated strong inhibition against *Candida albicans* (1.96 cm), while the ethyl acetate extract exhibited moderate inhibition against *Aspergillus niger* (0.921 cm). These findings suggest that non-polar and semi-

polar compounds in *Naringi crenulata* might be more effective against fungal pathogens than bacterial ones. These results indicate that *Naringi crenulata* extracts have limited antibacterial activity but exhibit promising antifungal potential, particularly against *C. albicans* and *A. niger*. Further studies should focus on isolating and identifying specific bioactive compounds responsible for this antifungal activity (Figure 1-3).

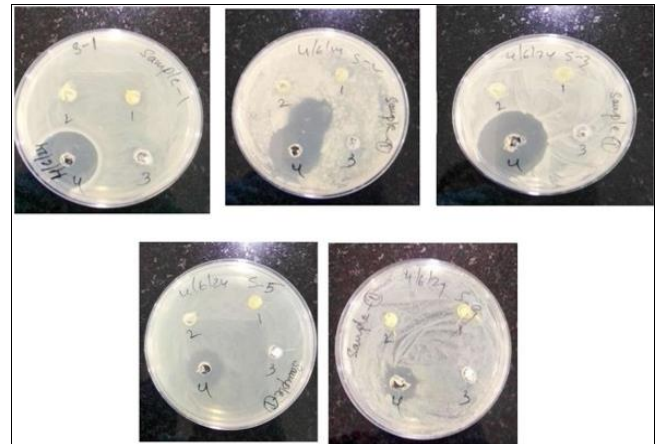


Fig 1: ZOI of n-hexane extracts of *N. crenulata* stem against *S. aureus*, *B. cereus*, *S. epidermidis*, *Enterobacter*, *P. aeruginosa*

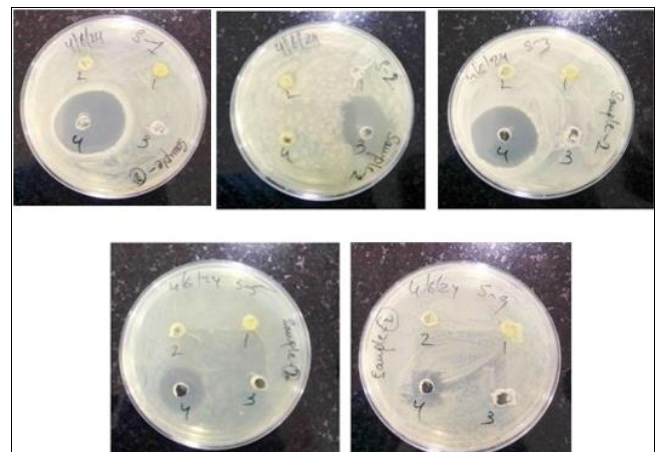


Fig 2: ZOI of Ethyl acetate extract of *N. crenulata* stem against *S. aureus*, *B. cereus*, *S. epidermidis*, *Enterobacter*, *P. aeruginosa*

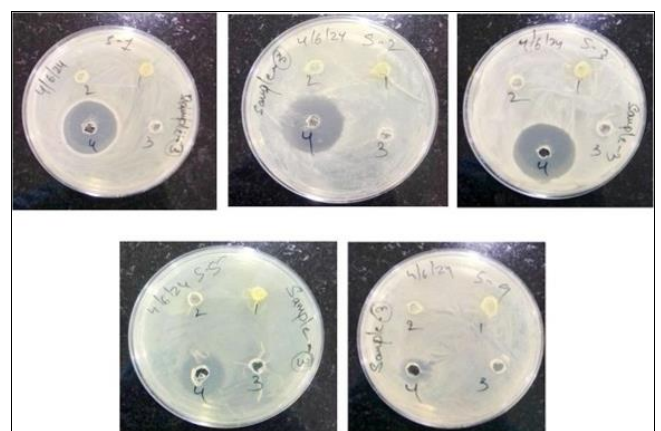


Fig 3: ZOI of Methanol extract of *N. crenulata* stem against *S. aureus*, *B. cereus*, *S. epidermidis*, *Enterobacter*, *P. aeruginosa*

The antimicrobial efficacy of *Naringi crenulata* leaf extracts exhibited varying degrees of inhibition across bacterial and fungal species. *Staphylococcus aureus* displayed the highest

sensitivity to the ethanol extract, forming a 4.4 cm inhibition zone, suggesting strong antibacterial potential. *Bacillus cereus* also responded to treatment, with hexane extract producing the largest inhibition zone (2.7004 cm), followed by ethanol extract (0.64 cm) and methanol extract (0.083 cm). However, *Staphylococcus epidermidis* exhibited complete resistance to all extracts, indicating its lower susceptibility.

Gram-negative bacteria showed minimal to no inhibition. *Escherichia coli* and *Enterobacter aerogenes* were unaffected by all tested extracts, reflecting their innate resistance. *Pseudomonas aeruginosa* displayed a slight response, with ethanol extract demonstrating the highest inhibitory effect (1.015 cm), while ethyl acetate (0.1884 cm) and hexane extract (0.0628 cm) exhibited weak activity.

In contrast, the plant extracts exhibited remarkable antifungal properties. *Candida albicans* showed the greatest inhibition with ethyl acetate extract (2.303 cm), followed by methanol extract (1.071 cm) and hexane extract (0.856 cm). Similarly, *Aspergillus niger* responded well, with hexane extract (1.717 cm) and ethyl acetate extract (1.66 cm) exhibiting nearly equivalent activity.

Overall, these results indicate that ethanol and ethyl acetate extract of *Naringi crenulata* leaves are highly effective against *S. aureus* and *B. cereus*, while demonstrating strong antifungal potential against *C. albicans* and *A. niger*. The lack of activity against Gram-negative bacteria suggests a potential limitation in their antibacterial spectrum, possibly due to structural differences in bacterial cell walls (Figure 4-6).

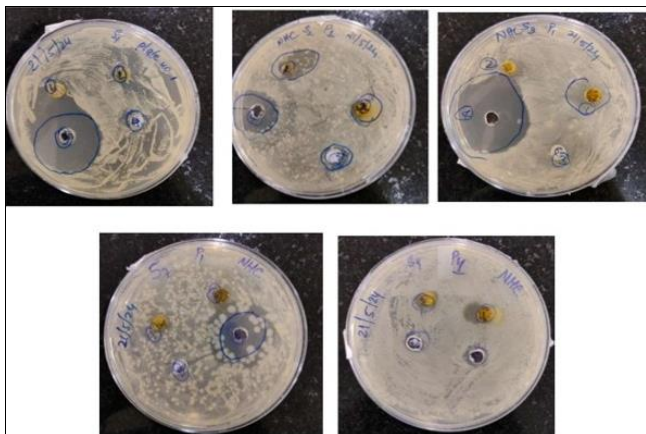


Figure 4: ZOI of n-hexane extract of *N. crenulata* leaf against *S. aureus*, *B. cereus*, *S. epidermidis*, *Enterobacter*, *P. aeruginosa*

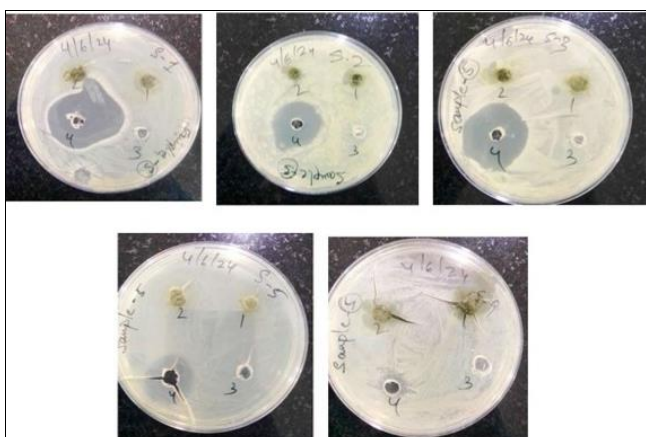


Figure 5: ZOI of Ethyl acetate extract of *N. crenulata* leaf against *S. aureus*, *B. cereus*, *S. epidermidis*, *Enterobacter*, *P. aeruginosa*

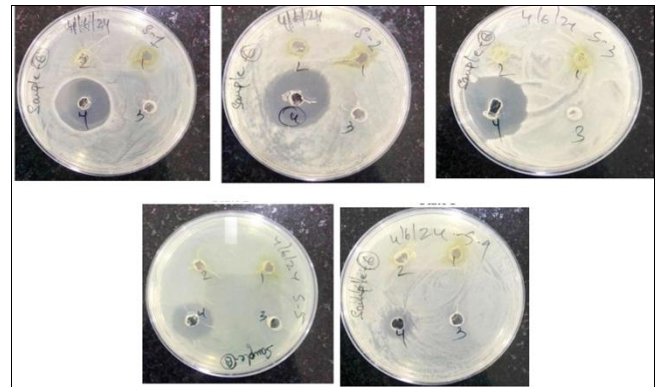


Fig 6: ZOI of Methanol extract of *N. crenulata* leaf against *S. aureus*, *B. cereus*, *S. epidermidis*, *Enterobacter*, *P. aeruginosa*

Summary and Conclusion

The antimicrobial activity of *Naringi crenulata* varied significantly between stems and leaves. Leaf extracts exhibited stronger antibacterial and antifungal effects than stem extracts, with notable inhibition against *Staphylococcus aureus*, *Bacillus cereus*, *Candida albicans*, and *Aspergillus niger*. Ethanol leaf extract showed the highest activity against *S. aureus* (4.4 cm), whereas stem extracts failed to inhibit the strain. Similarly, *B. cereus* showed greater susceptibility to leaf extracts, particularly the hexane extract (2.7004 cm), while stem extracts produced only weak inhibition. The antifungal potential was also more pronounced in leaf extracts, especially against *C. albicans* and *A. niger*, whereas stem extracts showed limited or no activity. Additionally, Gram-negative bacteria remained largely resistant to both stem and leaf extracts, with only *Pseudomonas aeruginosa* showing slight inhibition. These findings suggest that the leaves of *Naringi crenulata* contain a higher concentration of bioactive compounds responsible for antimicrobial effects, making them a more potent source for therapeutic applications.

This study highlights the significant antimicrobial and anthelmintic potential of *Naringi crenulata*, with leaf extracts demonstrating superior activity compared to stems. Ethanol and hexane leaf extracts exhibited strong inhibition against *Staphylococcus aureus*, *Bacillus cereus*, *Candida albicans*, and *Aspergillus niger*, while Gram-negative bacteria remained largely resistant. The anthelmintic evaluation revealed promising effects, with notable paralysis and death of helminths. The presence of bioactive phytochemicals such as alkaloids, flavonoids, tannins, and glycosides likely contribute to these effects. These findings suggest *N. crenulata* as a potential natural alternative for antimicrobial and anthelmintic therapies, warranting further phytochemical characterization and mechanistic studies.

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Conflict of Interest

The authors declare no conflicts of interest relevant to this article.

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