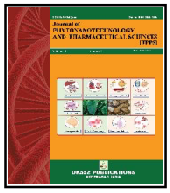
Journal of Phytonanotechnology and Pharmaceutical Sciences 5(1): 47-51, 2025

Online ISSN:2583-0376

http://jpps.ukaazpublications.com

DOI: http://dx.doi.org/10.54085/jpps.2025.5.1.7

Journal of Phytonanotechnology and Pharmaceutical Sciences



Original Article : Open Access

Anthelmintic potential of *Naringi crenulata* (Roxb.) Nicolson: A comparative study of leaf and stem extracts against parasitic worms

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Article Info

Article history

Received 10 January 2025

Revised 20 February 2025

Accepted 21 February 2025

Published Online 30 March 2025

Keywords

Naringi crenulata (Roxb.) Nicolson

Anthelmintic activity

Phytochemicals

Plant-based therapy

Helminthic infections

Abstract

Helminthic infections remain a significant global health burden, affecting over 1.5 billion people, particularly in regions with inadequate sanitation. The development of resistance to synthetic anthelmintics necessitates alternative treatments. This study evaluates the anthelmintic potential of *Naringi crenulata* (Roxb.) Nicolson leaf and stem extracts in different solvents (hexane, ethyl acetate, and methanol) against *Tubifex tubifex* and *Pheretima posthuma*, with albendazole as a reference. Extraction yield and phytochemical analysis revealed bioactive compounds, including glycosides, alkaloids, tannins, and saponins, contributing to anthelmintic activity. Among the extracts, ethyl acetate and methanol leaf extracts exhibited the highest potency, inducing paralysis within 33.94 and 44.25 min, respectively, and death within 42.68 and 55.67 min. The efficacy of these extracts was comparable to albendazole (paralysis: 25.3 min; death: 32.55 min), highlighting their potential as plant-based anthelmintics. The presence of key phytochemicals suggests mechanisms such as neuromuscular dysfunction and metabolic inhibition as contributors to worm mortality. Sustainable harvesting of leaves could optimize bioactive compound yields for pharmaceutical applications. Further studies should focus on isolating active constituents, *in vivo* efficacy validation, and potential synergistic interactions with conventional anthelmintics to mitigate resistance development. This research supports the integration of *N. crenulata* into anthelmintic therapy, offering a natural, effective, and sustainable alternative to synthetic drugs.

1. Introduction

Naringi crenulata (Roxb.) Nicolson, commonly known as the bitter orange or wild lime, is a medicinal plant belonging to the Rutaceae family (Kuruvella and Reddy Yellu, 2024). Native to India and widely distributed across Sri Lanka and Southeast Asia, it thrives in dry deciduous forests, scrublands, and riverbanks (Yesudanam and Jasmin, 2023). Taxonomically, it is classified under the order Sapindales, subfamily Aurantioideae, closely related to other citrus plants (Allayie *et al*., 2016; Ramya, 2014). Traditionally, *N. crenulata* has been used in indigenous medicine for treating fever, digestive disorders, skin infections, and inflammatory conditions (Chinnathambi *et al*., 2023; Ramachandiran *et al*., 2025). Recent scientific studies have validated its pharmacological potential, demonstrating neuroprotective, anticancer, antimicrobial, antioxidant, and hepatoprotective properties (Pratheeba *et al*., 2019; Vallinayagam *et al*., 2021). The plant is known to contain diverse bioactive compounds, including alkaloids, flavonoids, tannins, glycosides, and saponins, which contribute to its therapeutic effects (Neelam Singh, 2011).

Helminthic infections, caused by parasitic worms such as nematodes, trematodes, and cestodes, remain a significant global health burden, particularly in tropical and subtropical regions (Wu *et al*., 2022). These infections affect over 1.5 billion people worldwide, leading to malnutrition, anemia, cognitive impairment, and increased susceptibility to other infections (Arrais *et al*., 2022). The impact is most severe in economically disadvantaged communities with inadequate sanitation and hygiene. Current treatment options primarily rely on synthetic anthelmintic drugs such as albendazole, mebendazole, praziquantel, and ivermectin, which are widely used in helminth control programs (Chetty *et al*., 2020; Sanchez-Vegas and Villavicencio, 2022). However, these drugs face serious challenges, including drug resistance, reduced efficacy against certain helminths, and potential side effects with prolonged use. This necessitates the exploration of alternative, plant-based anthelmintic agents that are cost-effective, safe, and capable of overcoming drug resistance (Ramakrishnan *et al*., 2024; Banu *et al.,* 2024). Herbal medicines have historically been used for treating helminthic infections, with various plants exhibiting significant anthelmintic properties (Bhattacharjee *et al*., 2024; Kethireddy *et al.,* 2011). Medicinal plants contain secondary metabolites such as alkaloids, flavonoids, tannins, and glycosides, which interfere with helminth neuromuscular function or metabolism, leading to paralysis and death (Saikia, 2024). Several plants, including *Azadirachta indica, Punica granatum, Embelia ribes*, and *Ananas comosus*, have demonstrated strong anthelmintic activity. While *N. crenulata* has been extensively studied for its pharmacological effects, its anthelmintic potential remains largely unexplored. Given its diverse phytochemical composition and traditional use in treating infections, it is hypothesized that its extracts may exhibit potent anthelmintic activity.

This study aims to evaluate the anthelmintic activity of different solvent extracts of *N. crenulata* (hexane, ethyl acetate, and methanol) obtained from stems and leaves against *Tubifex tubifex* and *Pheretima*

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posthuma. The study aims to evaluate the anthelmintic potential of *N. crenulata* by assessing worm paralysis and death times after treatment with different extracts. It compares the efficacy of stem and leaf extracts, identifies the most effective solvent extract, and analyzes their potency against Albendazole to determine their potential as alternative anthelmintic agents. By investigating the anthelmintic potential of *N. crenulata*, this study aims to fill the gap in existing research and contribute to the development of plant-based anthelmintic therapies that are safer, more sustainable, and effective in combating helminthic infections.

2. Materials and Methods

2.1 Collection and preparation of plant extracts

Naringi crenulata (Roxb.) Nicolson leaves and stems are collected from Tandur, Vikarabad District, Telangana, and authenticated from Osmania University, Hyderabad. A voucher specimen (OUAS-204) was deposited in the herbarium for reference. The stems and leaves were separated from plant for extraction. The plant materials were thoroughly washed, shade-dried, and finely powdered. The powdered samples were subjected to solvent extraction using hexane, ethyl acetate, and methanol (Dokuparthi and Reddy, 2021). 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 h (Pravalika and Sujatha, 2021; Sujatha *et al*., 2022; Naaz *et al.,* 2024; Banu *et al.,* 2025). The extracts were concentrated using a rotary evaporator under reduced pressure to remove the solvents. The dried extracts were stored at 4°C in sterile containers until further use (Allakonda *et al*., 2024; Sudheer Kumar Dokuparthi *et al*., 2014).

2.2 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 (Veni Madhavi *et al*., 2025)

2.3 Selection and maintenance of test organisms

The anthelmintic activity of *N. crenulata* was evaluated using two model helminths, *Tubifex tubifex* and *Pheretima posthuma*. These organisms were selected due to their physiological similarities to parasitic helminths and their established use in anthelmintic screening. Live specimens of *T. tubifex* and *P. posthuma* were collected from natural freshwater sources and acclimatized in the laboratory. The worms were maintained in dechlorinated water at room temperature and were used within 24 h of collection to ensure optimal viability during experimentation (Banu *et al.,* 2024; Siddiqui and Patni, 2018).

2.4 Preparation of plant extracts

For the anthelmintic study, stem and leaf extracts of *N. crenulata* were reconstituted in distilled water at a concentration of 25 mg/ml.

2.5 Experimental design and treatment groups

The study included treatment groups with *N. crenulata* stem and leaf extracts in hexane, ethyl acetate, and methanol (25 mg/ml), along with a standard drug, albendazole (25 mg/ml), and negative control (distilled water).

2.6 Anthelmintic assay procedure

Freshly collected and healthy worms (*T. tubifex* and *P. posthuma*) were selected for the study. In each experimental group, six worms of each species were placed in petri dishes containing 10 ml of the respective test solution. The standard drug (albendazole) was included as a positive control, while worms in the negative control group were placed in distilled water. The plates were maintained at room temperature under static conditions for continuous observation (Ghosh *et al*., 2011).

2.7 Assessment of anthelmintic activity

The anthelmintic efficacy of the extracts was determined by recording two key parameters: paralysis time and death time of the worms. Paralysis time was noted when the worms became completely immobile even upon stimulation with a needle. Death time was recorded when the worms failed to respond to external stimuli and showed no movement for a prolonged period. Observations were made at regular intervals until all worms in the test solution were either paralyzed or dead (Bhatt *et al*., 2021; Bhutada *et al*., 2024).

The mean paralysis time and mean death time for each treatment group were recorded. The results were compared with the standard reference drug, albendazole, to determine the relative efficacy of each extract. The difference in response between *T. tubifex* and *P. posthuma* was also analyzed to evaluate species-specific sensitivity to the extracts.

3. Results

3.1 Extraction yield

The extraction yield of *N. 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: Extraction yield

Plant part	Hexane extract (%)	Ethyl acetate extract (%)	Methanol extract (%)
N. crenulata leaves	2.10%	2.60%	3.20%
N. crenulata stem	1.20%	2.10%	2.80%

3.2 Phytochemical profile

The phytochemical analysis of *N. 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 favour alkaloids and glycosides (Table 2).

Table 2: Phytochemical profile

Name of the test	*N. crenulata* leaves			*N. crenulata* stem		
	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

3.3 Anthelmintic activity

The anthelmintic activity of *N. crenulata* extracts was assessed by measuring the paralyzing time and death time of the worms after treatment with various stem and leaf extracts at a concentration of 25 mg/ml. A negative control (no extract) and albendazole, a standard anthelmintic drug, were used as references for comparison. The negative control exhibited no noticeable effect on the worms, with both the paralyzing time and death time extending beyond 480 min, indicating that in the absence of treatment, the worms remained unaffected. Similarly, the stem n-hexane extract of *N. crenulata* (NCSH) also showed no significant anthelmintic activity, as both the paralyzing time and death time exceeded 480 min, making it ineffective in comparison to other treatments. In contrast, the stem ethyl acetate extract (NCSEA) displayed moderate anthelmintic activity, with a paralyzing time of 196.34 min and a death time of 226.54 min. This shows a marked improvement over the negative control and the stem n-hexane extract, indicating that ethyl acetate extraction from the stem contains compounds with some anthelmintic properties. The stem methanol extract (NCSM) showed stronger activity, with a paralyzing time of 117.9 min, though its death time was slightly longer at 256.04 min, suggesting that while it paralyzed the worms more quickly, it took longer to kill them. The leaf extracts demonstrated significantly stronger anthelmintic activity compared to the stem extracts. The leaf n-hexane extract (NCLH) reduced the paralyzing time to 73.32 min and the death time to 121.57 min, showing enhanced effectiveness over the stem n-hexane extract. The leaf ethyl acetate extract (NCLEA) was particularly potent, with a paralyzing time of 33.94 min and a death time of 42.68 min, closely approaching the efficacy of albendazole, the standard drug. This suggests that the leaf ethyl acetate extract contains highly effective bioactive compounds against worms. The leaf methanol extract (NCLM) also demonstrated strong activity, with a paralyzing time of 44.25 min and a death time of 55.67 min, further highlighting the potency of the leaf extracts. Albendazole used as a standard reference, had the most rapid effect, with the shortest paralyzing time of 25.3 min and a death time of 32.55 min, confirming its superior anthelmintic efficacy (Table 2 and Figure 1). However, the leaf ethyl acetate and methanol extracts of *N. crenulata* approached its effectiveness, indicating that these extracts possess significant anthelmintic potential.

Table 3: Effect of *N. crenulata* on helminths

Treatment	Paralyzing time (min)	Death time (min)
Negative control	>480	>480
N. crenulata stem n-hexane extract NCSH (25 mg/ml)	>480	>480
N. crenulata stem ethyl acetate extract NCSEA (25 mg/ml)	196.34 ± 2	226.54 ± 1
N. crenulata stem methanol extract NCSM (25 mg/ml)	117.9 ± 1.53	256.04 ± 2.08
N. crenulata leaf n-hexane extract NCLH (25 mg/ml)	73.32 ± 2	121.57 ± 1.53
N. crenulata leaf ethyl acetate extract NCLEA (25 mg/ml)	33.94 ± 1.53	42.68 ± 2
N. crenulata leaf methanol extract NCLM (25 mg/ml)	44.25 ± 2.08	55.67 ± 1.53
Albendazole (25 mg/ml)	25.3 ± 1.53	32.55 ± 1.53

Data is represented as mean ± SD

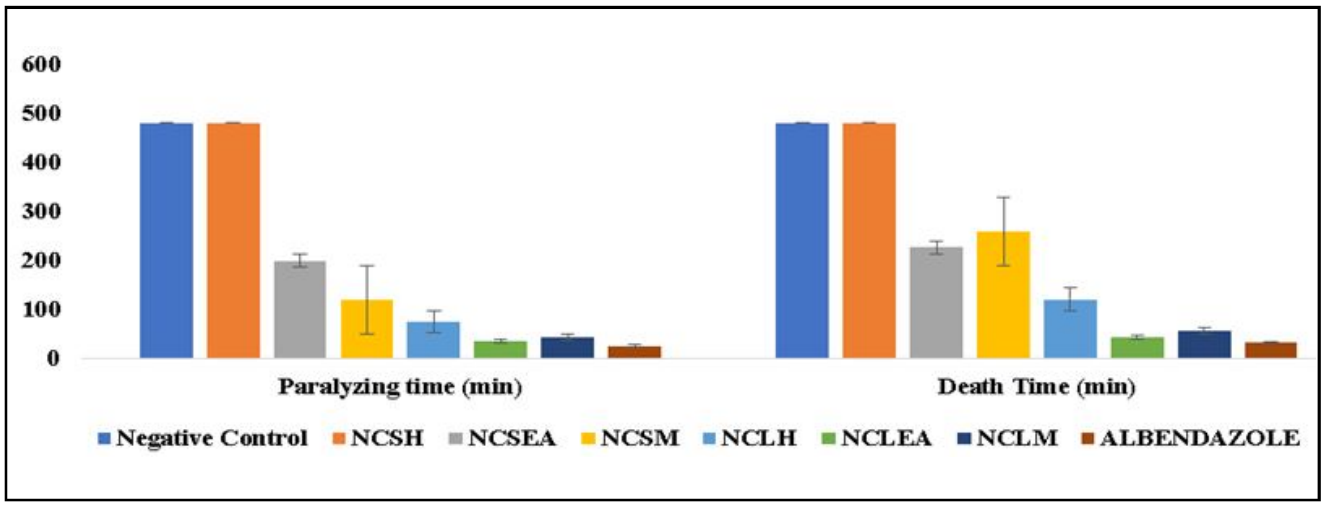

Figure 1:Antiheminthic activity of *N. crenulata*.

4. Discussion

The study assessed the anthelmintic efficacy of different solvent extracts of *N. crenulata* leaves and stems against *T. tubifex* and *P. posthuma*, using albendazole as a standard reference. The extraction yield varied among solvents, with methanol yielding the highest bioactive compounds, followed by ethyl acetate and hexane. Phytochemical analysis indicated the presence of glycosides, alkaloids, tannins, and saponins in varying concentrations, which contribute to the plant's anthelmintic properties. The leaf extracts exhibited stronger activity than the stem extracts, with the ethyl acetate and methanol extracts demonstrating significantly lower paralysis and death times, closely approaching the efficacy of albendazole. The hexane extracts showed weaker activity, reinforcing the hypothesis that polar and semi-polar solvents are more effective in extracting anthelmintic compounds. The variations in response between *T. tubifex* and *P. posthuma* further highlighted species-specific sensitivities, suggesting that different helminths may respond variably to plant-derived treatments. The findings emphasize the potential of *N. crenulata* as an alternative anthelmintic agent, particularly its leaf extracts in ethyl acetate and methanol. These extracts significantly reduced worm viability, indicating their potent bioactivity. Compared to synthetic drugs, plant-derived compounds offer the advantage of reduced side effects and a lower likelihood of developing resistance. However, the study suggests that further purification and isolation of the active constituents are necessary to enhance efficacy and optimize dosages. Additionally, *in vivo* studies are required to validate these findings and determine the safety and pharmacokinetics of the extracts in higher organisms. By demonstrating the strong anthelmintic potential of *N. crenulata*, this research contributes to the ongoing search for sustainable, plant-based therapies for parasitic infections.

5. Conclusion

This study identifies *N. crenulata* as a promising candidate for plant-based anthelmintic therapies, with ethyl acetate leaf extracts demonstrating potent activity. The extract induced paralysis in 33.94 min and death in 42.68 min, approaching albendazole's efficacy. Phytochemical analysis suggests glycosides, alkaloids, and tannins contribute to its anthelmintic effects by disrupting neuromuscular function and metabolism. Sustainable harvesting of leaves could optimize therapeutic yields. Future research should focus on isolating active compounds, conducting *in vivo* studies, and exploring synergistic effects with conventional drugs to develop effective, resistance-mitigating treatments for helminthic infections affecting over 1.5 billion people worldwide.

Acknowledgments

The authors express their gratitude to the Department of Botany, Osmania University, and Biogenic Products Pvt. Ltd. for their technical support.

Conflict of interest

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

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Citation **D. Nageshwer Rao and B. Rajani (2025). Anthelmintic potential of *Naringi crenulata* (Roxb.) Nicolson: A comparative study of leaf and stem extracts against parasitic worms. J. Phytonanotech. Pharmaceut. Sci., 5(1):47-51. http://dx.doi.org/10.54085/jpps.2025.5.1.7**