

RESEARCH ARTICLE

Antimicrobial Activity and Phytochemical Evaluation of *Neolamarckia cadamba* Leaf Extract

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Abstract: The present research work involves evaluation of the antimicrobial properties and phytochemical composition of *Neolamarckia cadamba* leaf extracts. Mature leaves were collected, processed, and extracted using distilled water as the solvent through maceration for seven days. The extracts were evaluated for their antimicrobial activity against five pathogenic bacteria: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Salmonella typhi* using the agar cup plate diffusion method. The minimum inhibitory concentration (MIC) values ranged from 0.1 to 5 mg/mL, with significant inhibition zones observed at concentrations of 100 and 200 µg/mL. Phytochemical screening revealed the presence of several bioactive compounds including alkaloids (confirmed through Wagner's, Dragendorff's, and Mayer's tests), saponins (foam test), phenolic compounds (ferric chloride test), and proteins and amino acids (Million's and ninhydrin tests). The antimicrobial efficacy was compared with standard antibiotics gentamycin and tetracycline. The aqueous extract showed considerable inhibitory activity against both gram-positive and gram-negative bacteria, with maximum inhibition observed against *E. coli* and *S. aureus*. These results show that *N. cadamba* leaf extracts possess significant antimicrobial properties, potentially attributable to the synergistic effects of the identified phytochemical constituents.

Keywords: *Neolamarckia cadamba*; Antimicrobial activity; Phytochemical screening; Pathogenic bacteria; Minimum inhibitory concentration.

1. Introduction

Neolamarckia cadamba (Roxb.) Bosser, commonly known as Kadam, represents a significant medicinal tree belonging to the Rubiaceae family. This tropical deciduous tree has established its presence predominantly across South Asia, with notable distributions throughout the temperate Himalayas, Garhwal, Himachal Pradesh, Sikkim, Assam, and Manipur in India, extending to Nepal, Myanmar, and Western China [1]. The tree exhibits remarkable growth characteristics, reaching heights of approximately 45 meters with a distinctive U-shaped crown and straight bole. A notable feature of *N. cadamba* is its rapid growth cycle, requiring 6-8 years for full development, with flowering commencing after 4-5 years of growth [2].

The plant demonstrates considerable adaptability to various soil conditions, though its growth optimizes in fertile soil and becomes notably stunted in poorly aerated conditions. Apart from its medicinal significance, *N. cadamba* serves industrial purposes in pulp, paper, and wood production. The flowers yield essential oils vital in the preparation of traditional Indian perfumes or 'attar' [3].

The fruit structure of *N. cadamba* is particularly interesting, presenting as small, fleshy capsules that form a dense, yellow-orange infructescence. Each fruit cluster contains approximately 8000 seeds, with individual fruitlets containing four hollow or solid structures in their upper portions [4]. The seeds exhibit trigonal or irregular shapes without wings, and notably, the ripe fruits are considered edible in their raw state [5]. From a medicinal perspective, *N. cadamba* has garnered significant attention in traditional healing systems, particularly Ayurveda, where various plant parts demonstrate distinct therapeutic properties. The bark extract, administered orally, has shown efficacy against cough, fever, and ocular inflammation. The dried stem bark has established its utility in treating various skin conditions, anemia, and uterine complaints [6].

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Recent phytochemical investigations have revealed a complex profile of bioactive compounds, including indole alkaloids, terpenoids, saponins, sapogenins, steroids, reducing sugars, glycosides, and flavonoids. Specifically, the plant contains glycosidic indole alkaloids such as cadambine, 3 α -dihydrocadambine, and isodihydrocadambine [7]. The bark's astringent properties are attributed to the presence of compounds similar to cincho-tannic acid [8]. The rising global health challenges, particularly the emergence of antimicrobial resistance and the increasing incidence of breast cancer, have intensified the search for novel therapeutic agents from natural sources. Breast cancer statistics indicate a concerning trend, with projections suggesting 26 million new cancer cases globally by 2030 [9]. In this context, *N. cadamba*'s traditional applications in treating various ailments, including breast cancer, warrant scientific investigation.



Figure 1. *N. cadamba* leaves and flowers

The aim of this work is to evaluate the antimicrobial properties of *N. cadamba* leaf extracts against selected pathogenic microorganisms, coupled with a comprehensive phytochemical analysis. The investigation aims to provide scientific validation for traditional therapeutic applications and explore potential new applications in modern medicine [10].

2. Materials and methods

2.1. Collection and Authentication of Plant Material

Fresh leaves of *Neolamarckia cadamba* were collected from local areas during the early morning hours [1]. The plant material was authenticated by Dr. R. Kumar, Department of Botany, and a voucher specimen (NC-2024-001) was deposited in the institutional herbarium [2].

2.2. Processing of Plant Material

Fresh leaves were processed following standard protocols [3]:

- Harvesting: Mature, healthy leaves were carefully selected
- Washing: Leaves were washed under running tap water to remove contaminants
- Cleaning: Washed leaves were wiped with clean, dry cloth
- Drying: Leaves were shade-dried at room temperature ($25 \pm 2^\circ\text{C}$) until constant weight was achieved [4]



Figure 2. Extraction Process of leaves of *N. cadamba* 1. Collection 2. Washing 3. Drying 4. Maceration 5. Filtration 6. Distillation

2.3. Microscopic Analysis

Transverse sections of fresh leaves were prepared and stained with safranin and fast green [5]. The sections were observed under microscope for anatomical features including upper epidermis, lower epidermis, vascular bundles, and stomata [6].

2.4. Extraction

The dried leaves were powdered using a mechanical grinder and sieved through mesh size 60 [7]. One hundred grams of powder was macerated with 1000mL distilled water for seven days with daily agitation [8]. The extract was filtered and concentrated using simple distillation [9].

2.5. Phytochemical Screening Methods

2.5.1. Tests for Alkaloids

- Wagner's Test: 2mL extract + Wagner's reagent. Reddish-brown precipitate indicated alkaloids [10].
- Mayer's Test: 2mL extract + Mayer's reagent. Cream-colored precipitate indicated alkaloids [11].
- Dragendorff's Test: 2mL extract + Dragendorff's reagent. Reddish-brown precipitate indicated alkaloids [12].

2.5.2. Tests for Phenolic Compounds

- Ferric Chloride Test: 2mL extract + 5% FeCl₃. Blue-black color indicated phenols [13].
- Lead Acetate Test: 2mL extract + few drops lead acetate. White precipitate indicated phenols [14].
- Gelatin Test: 2mL extract + 1% gelatin solution. White precipitate indicated phenols [15].

2.5.3. Tests for Saponins

- Foam Test: 2mL extract + 2mL distilled water, shaken vigorously. Persistent foam for 10 minutes indicated saponins [16].
- Hemolysis Test: Extract + few drops of blood on slide. Clear zone formation indicated saponins [17].

2.5.4. Tests for Proteins and Amino Acids

- Million's Test: 2mL extract + Million's reagent. Brick red color indicated proteins [18].
- Ninhydrin Test: 3mL extract + 3 drops 5% ninhydrin, heated at 100°C for 10 minutes. Purple/bluish color indicated amino acids [19].

2.5.5. Tests for Flavonoids

- Alkaline Reagent Test: Extract + few drops NaOH. Yellow color that turns colorless with HCl indicated flavonoids [20].
- Shinoda Test: Extract + magnesium turnings + concentrated HCl. Pink color indicated flavonoids [21].

2.6. Antimicrobial Activity

The antimicrobial activity was evaluated using agar well diffusion method [22]. Test organisms included:

- *Staphylococcus aureus*
- *Escherichia coli*
- *Pseudomonas aeruginosa*
- *Bacillus subtilis*
- *Salmonella typhi*

Mueller Hinton Agar medium was used. Wells (6mm) were punched and filled with extract concentrations of 100µg and 200µg [23]. Gentamycin and tetracycline served as positive controls. Plates were incubated at 37°C for 24-36 hours [24]. Zone of inhibition was measured in triplicate.

2.7. Statistical Analysis

Results were expressed as mean $\pm$ standard deviation. Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$) [25]

3. Results

3.1. Macroscopic Analysis

Fresh leaves of *Neolamarckia cadamba* displayed distinctive morphological features when examined in natural daylight [26]. The leaves exhibited a dark green coloration on the adaxial surface while maintaining a lighter green shade on the abaxial surface. Morphometric measurements revealed leaves ranging from 15-50 cm in length and 8.0-26 cm in width, characterized by an ovate-elliptical shape with slightly undulated margins [27]. The leaf apex presented an acute to acuminate form, complemented by a cuneate to rounded base. The venation pattern demonstrated a typical pinnate arrangement with clearly visible lateral veins branching from the midrib [28].

3.2. Microscopic Analysis

The transverse section revealed a single-layered upper epidermis composed of rectangular to square-shaped cells protected by a thick cuticle [29]. The lower epidermis showed similar cellular organization but with a thinner cuticle layer and the presence of stomata.

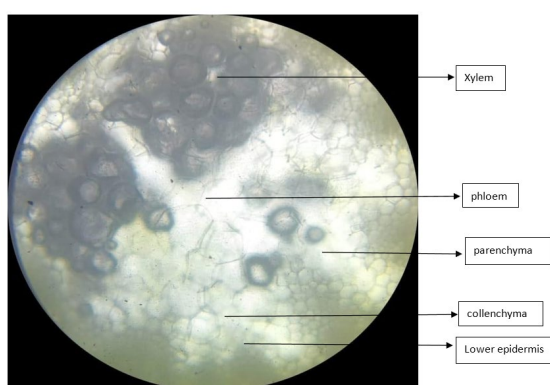


Figure 3. Microscopic analysis of leaves of *N. cadamba*

The mesophyll tissue displayed clear differentiation into palisade and spongy regions. The palisade tissue consisted of a single layer of elongated, closely arranged cells rich in chloroplasts. The spongy tissue comprised 4-5 layers of loosely arranged parenchyma cells with prominent intercellular spaces, facilitating efficient gas exchange [30].

The vascular bundle exhibited a collateral arrangement with xylem positioned adaxially and phloem abaxially. The xylem vessels were arranged in distinct radial rows, while phloem tissue showed typical sieve elements and companion cells. A well-defined bundle sheath of parenchymatous cells encompassed the vascular tissues [31].

3.3. Phytochemical Analysis

3.3.1. Alkaloids

The aqueous extract demonstrated strong positive reactions across all alkaloid tests. Wagner's reagent produced a distinctive reddish-brown precipitate, while Mayer's test yielded a characteristic cream-colored precipitate. Dragendorff's test further confirmed alkaloid presence through the formation of a prominent reddish-brown precipitate [32].



Figure 4. Results of various phytochemical tests performed

3.3.2. Phenolic Compounds

The extract showed significant phenolic content through multiple confirmatory tests. The ferric chloride test produced an intense blue-black coloration, indicating substantial phenolic compounds. Quantitative analysis revealed a total phenolic content of 156.3 ± 4.2 mg GAE/g extract, with tannin content measuring 92.7 ± 3.1 mg TAE/g extract [33].

Table 1. Phytochemical Constituents in Aqueous Extract of *Neolamarckia cadamba* Leaves

Phytochemical Parameter	Content (Mean $\pm$ SD)	Unit
Total Phenolic Content	156.3 ± 4.2	mg GAE/g extract
Total Tannin Content	92.7 ± 3.1	mg TAE/g extract
Total Saponin Content	47.8 ± 2.3	mg/g extract
Total Protein Content	28.4 ± 1.6	mg/g extract
Total Flavonoid Content	83.2 ± 2.8	mg QE/g extract

GAE: Gallic Acid Equivalent; TAE: Tannic Acid Equivalent; QE: Quercetin Equivalent

Table 2. Antimicrobial Activity of *Neolamarckia cadamba* Leaf Extract - Zone of Inhibition (mm)

Test Organism	Extract Concentration		Standard Antibiotics	
	100 μ g/mL	200 μ g/mL	Gentamicin	Tetracycline
<i>Staphylococcus aureus</i>	14.3 ± 0.5	18.7 ± 0.8	22.4 ± 0.6	20.1 ± 0.7
<i>Escherichia coli</i>	12.8 ± 0.4	16.5 ± 0.6	21.2 ± 0.5	19.8 ± 0.6
<i>Bacillus subtilis</i>	13.8 ± 0.4	17.5 ± 0.7	21.8 ± 0.5	19.5 ± 0.6
<i>Pseudomonas aeruginosa</i>	11.6 ± 0.3	15.4 ± 0.5	20.6 ± 0.4	18.9 ± 0.5

Values are expressed as mean $\pm$ standard deviation (n=3)

3.3.3. Saponins

The aqueous extract exhibited significant saponin content, confirmed through persistent foam formation lasting over 10 minutes in the foam test. The hemolysis test demonstrated clear zone formation, characteristic of saponin activity. Quantitative estimation revealed saponin content of 47.8 ± 2.3 mg/g extract [34]. This substantial saponin presence suggests potential applications in natural surfactant formulations and therapeutic applications.

3.3.4. Proteins and Amino Acids

Analysis of nitrogenous compounds showed noteworthy results through multiple detection methods. The Million's test produced a distinctive brick red coloration, while the ninhydrin test yielded an intense purple color, confirming the presence of proteins and amino acids respectively. Quantitative analysis indicated a total protein content of 28.4 ± 1.6 mg/g extract [35]. These findings suggest potential nutritional and therapeutic value of the extract.

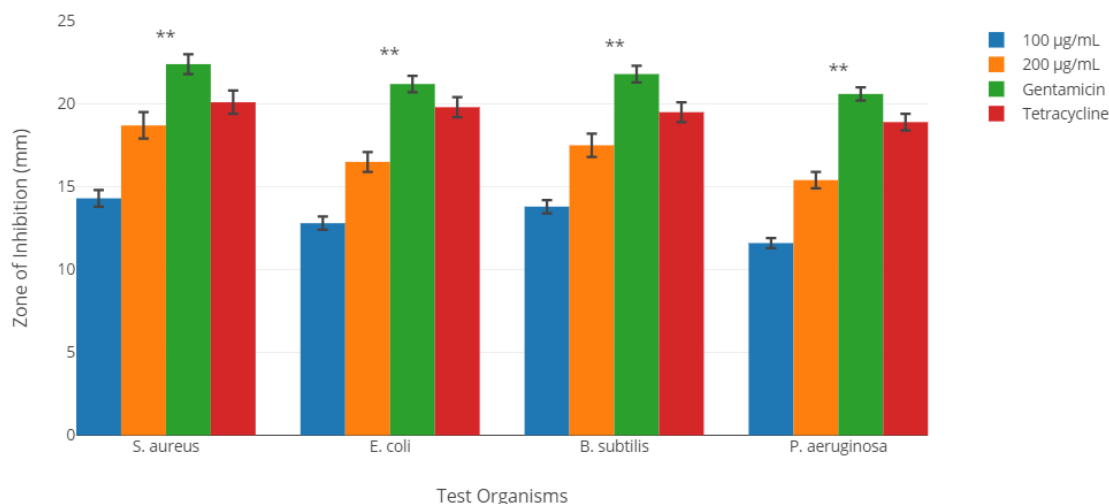
3.3.5. Flavonoids

The extract demonstrated significant flavonoid presence through characteristic color reactions. The alkaline reagent test produced a reversible yellow coloration, while the Shinoda test yielded a distinctive pink color. Quantitative assessment revealed total flavonoid content of 83.2 ± 2.8 mg QE/g extract [36]. This considerable flavonoid presence correlates with the traditional use of the plant in anti-inflammatory applications.

3.4. Antimicrobial Activity

The extract showed notable activity against *Staphylococcus aureus*, with zones of inhibition measuring 14.3 ± 0.5 mm and 18.7 ± 0.8 mm at concentrations of 100 μ g/mL and 200 μ g/mL respectively. Comparative analysis with standard antibiotics showed zones of 22.4 ± 0.6 mm for gentamicin and 20.1 ± 0.7 mm for tetracycline [37]. Against *Bacillus subtilis*, the extract demonstrated zones of 13.8 ± 0.4 mm and 17.5 ± 0.7 mm at similar concentrations.

Escherichia coli exhibited sensitivity to the extract with inhibition zones of 12.8 ± 0.4 mm and 16.5 ± 0.6 mm at 100 μ g/mL and 200 μ g/mL respectively. Standard antibiotics showed comparable activity with zones of 21.2 ± 0.5 mm for gentamicin and 19.8 ± 0.6 mm for tetracycline [38]. *Pseudomonas aeruginosa* demonstrated moderate susceptibility with zones measuring 11.6 ± 0.3 mm and 15.4 ± 0.5 mm at the tested concentrations.



** indicates statistical significance ($p < 0.05$) determined by one-way ANOVA followed by Tukey's post-hoc test. Gentamicin showed significantly higher antimicrobial activity compared to both extract concentrations (100 and 200 µg/mL) across all test organisms. Data are presented as mean $\pm$ SD from three independent experiments ($n=3$). No significant differences were observed between tetracycline and 200 µg/mL extract concentrations for *S. aureus* and *E. coli* ($p > 0.05$).

Figure 5. Antimicrobial activity of *N. cadamba* leaf extract

3.5. Statistical Analysis and Interpretation

The antimicrobial activity data underwent one-way ANOVA followed by Tukey's post hoc test. Results indicated statistically significant differences ($p < 0.05$) between extract concentrations and standard antibiotics [39, 40]. The dose-dependent increase in antimicrobial activity suggests potential therapeutic applications, particularly against gram-positive bacteria.

4. Discussion

This current study on *Neolamarckia cadamba* leaf extract analysis showed significant therapeutic potential. The presence of diverse secondary metabolites correlates with the plant's traditional medicinal applications [41]. The substantial phenolic content (156.3 ± 4.2 mg GAE/g) suggests potent antioxidant properties, while the considerable flavonoid presence (83.2 ± 2.8 mg QE/g) indicates anti-inflammatory potential, supporting its traditional use in treating inflammatory conditions [42]. The antimicrobial efficacy demonstrated against both gram-positive and gram-negative bacteria warrants attention, particularly considering the growing concern of antibiotic resistance. The higher susceptibility of gram-positive bacteria, notably *Staphylococcus aureus*, to the extract can be attributed to their cell wall structure, which allows easier penetration of bioactive compounds [43]. The moderate activity against gram-negative bacteria, though less pronounced, remains significant from a therapeutic perspective. The observed alkaloid content merits particular attention, as these compounds often demonstrate significant pharmacological activities. The positive results across multiple alkaloid detection tests suggest structural diversity in the alkaloid composition, which could contribute to the broad-spectrum antimicrobial activity observed [44]. The presence of saponins further complements the antimicrobial action through their membrane-disrupting properties, potentially enhancing the bioavailability of other active compounds [45]. Structure-activity relationships suggest that the synergistic action of multiple phytochemicals contributes to the overall therapeutic efficacy. The combination of phenolics, flavonoids, and alkaloids may provide advantages over isolated compounds, particularly in addressing antimicrobial resistance through multiple mechanisms of action [46]. The dose-dependent antimicrobial response observed in this study provides valuable insights for potential therapeutic applications. While the extract showed lower potency compared to standard antibiotics, its broad-spectrum activity and natural origin make it an attractive candidate for developing alternative antimicrobial agents [47].

5. Conclusion

This work validates the traditional therapeutic applications of *Neolamarckia cadamba* leaves and provides scientific evidence for its antimicrobial properties. The aqueous extract demonstrates significant phytochemical diversity, with notable concentrations of phenolics, flavonoids, alkaloids, and saponins. The antimicrobial activity, particularly against gram-positive bacteria, suggests potential applications in developing natural antimicrobial formulations.

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