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Phytochemical Profiling and In Vitro Antibacterial Evaluation of Methanolic Bark Extract of *Murraya koenigii* (L.) Spreng

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ABSTRACT

The global rise in antimicrobial resistance (AMR) has intensified the search for plant-derived alternatives with therapeutic potential. *Murraya koenigii (*L.) Spreng., a plant valued in traditional medicine, is rich in bioactive compounds, though its bark has not been extensively studied. This research aimed to analyze the phytochemical constituents and evaluate the antibacterial properties of the methanolic extract of *M. koenigii* bark sourced from the Botanical Garden of VJ's College of Pharmacy, Rajahmundry. The bark was dried, ground, and extracted using methanol via maceration. Standard qualitative tests were used to identify secondary metabolites such as alkaloids, flavonoids, tannins, saponins, triterpenoids, and cardiac glycosides. The extract's antibacterial activity was tested against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* using the agar well diffusion method, with Amikacin as the positive control and methanol as the negative control. The extract demonstrated a rich phytochemical profile and showed dose-dependent antibacterial effects. Maximum inhibition was observed at 400 mg/mL with zones of 15.3 mm (*S. aureus*), 13.8 mm (*E. coli*), and 12.5 mm (*P. aeruginosa*). The study indicates that *M. koenigii* bark methanolic extract possesses significant antibacterial activity, suggesting its potential as a plant-based antimicrobial agent.

Keywords: *Murraya koenigii*, Methanolic extract, phytochemical screening, Antibacterial activity, Agar well diffusion.

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INTRODUCTION

The emergence of antimicrobial resistance (AMR) has become one of the foremost global health concerns. Misuse and overuse of antibiotic in clinical and agriculture setting have led to the development of resistant microbial strains, significantly reducing the effectiveness of many conventional drugs[1]. As a result, researchers are exploring alternative therapeutics sources, particularly from medicinal plants known for their diverse pharmacological properties[2].

Murraya koenigii (L.) spreng, commonly known as the curry leaf tree, is a plant native to the Indian subcontinent, widely cultivated across south and Southeast Asia. It belongs to the rutaceae family and holds great significance in the traditional medicine systems such as Ayurveda and unani, where it is employed to treat conditions ranging from gastrointestinal disorders to skin infection[3,4]. While the leaves are extensively used in both culinary and medicinal application, the bark has received relatively limited scientific attention, despite its historical use in treating ailments like inflammation, bites, and digestive issues[5].

The bark of murraya koenigii is a rich source of bioactive secondary metabolites, including alkaloids, flavonoids, tannins, saponins, and terpenoids [6]. Among these, carbazole alkaloids such as mahanimbine, girinimbine, and murrayanine have demonstrated significant pharmacology potential, including antimicrobial, antioxidant, and anticancer activities[7, 8]. These compounds are considered responsible for the therapeutic effects exhibits by various parts of the plant.

One of the key advantages of plant-based antimicrobial is their multi-targeted mechanism of action, which reduces the likelihood of resistance development comported to single-targeted synthesis drugs. Moreover, plant-derived substance is often biodegradable, less toxic, and more accessible in resource-limited regions[9, 10]. Given these benefits, medicinal plants such as M. Koenigii are valuable sources for novel antimicrobial agents.

Microorganism develop resistance through various mechanism, such as enzymatic drug degradation, alteration of drug-binding targets, reduced membrane permeability, efflux pump expression, and biofilm formation[11]. This defense mechanism compromises the effectiveness of antibiotic and contributes to the global AMR crisis. This situation underscores the importance of discovering alternative slur on that may act either as stand-alone treatment or as adjunct therapeutics to conventional antibiotics.

The methanol extract of M. Koenigii bark, prepared using maceration techniques, has been observed to retain a wide spectrum of phytochemical [6]. Methanol is a preferred solvent in phytochemical investigational due to its polarity and ability to extract both polar and semi-polar constituents. Several studies have documented that methanol extract of the plant exhibit

antibacterial activity against both gram-positive and gram-negative bacteria, including Escherichia coli, staphylococcus aureus, and pseudomonas aeruginosa [12, 13].

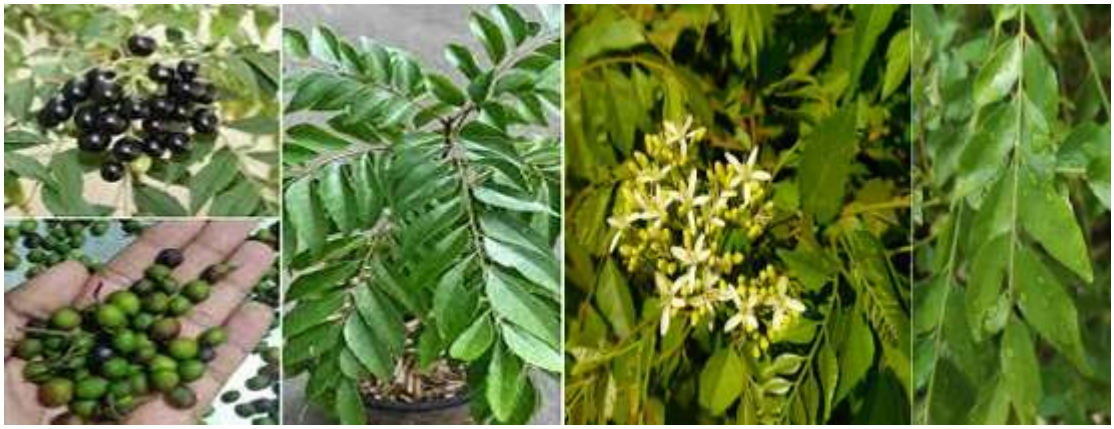

Figure 1: Curry leaves

In-vitro testing of antibacterial efficacy often employs the agar well diffusion method (also known as the cup-plate methods), which is recognized for its simplicity and cost-effectiveness. In this method, plant extracts are introduced into well on agar plate's inoculation with bacterial strains. The inhibition zone formed around the well reflects the antimicrobial potency of the extract[14]. In the present investigation, this method was used to evaluate the antibacterial activity of M. Koenigii bark extract compared to a standard antibiotic, Amikacin.

The phytochemical screening of the extract revealed the presence of alkaloids, flavonoids, tannins, triterpenoids, saponins, and cardiac glycosides – each known for contributing to antimicrobial membrane, inhibiting nucleic acid synthesis, or interfering with enzymatic system[6,15]. Such multi-model interaction makes plant-derived antimicrobial less prone to resistance development an potentially more effective over longer periods.

Beyond antimicrobial activity, many constituents found in M. Koenigii bark offer additional pharmacological benefits. For instance, Carbazole alkaloids have shown cytotoxic effects on cancer cell lines, while flavonoids and phenolic compounds are widely studied for their anti-inflammatory and antioxidant effects[8,16]. These complementary properties further enhance the therapeutic value of the plant.

In light of these attributes, this study focuses on assessing the phytochemical content and evaluating the antibacterial potential of M. Koenigii bark methanolic extract. By comparing the results with standard antibiotic performance and utilizing validated microbiological methods, this wok aims to contribute to the broader search for effective, natural antimicrobial agents.

MATERIALS AND METHOD

Plant Collection and Authentication

The bark of *Murraya koenigii* (L.) Spreng. was collected from the Botanical Garden of VJ's College of Pharmacy**,** Rajamundry, Andhra Pradesh, India.

Pharmacognostic Classification

- **Kingdom:** Plantae
- **Division:** Magnoliophyta (Angiosperms)
- **Class:** Magnoliopsida (Dicotyledons)
- **Order:** Sapindales
- **Family:** Rutaceae
- **Genus:** *Murraya*
- **Species:** *Murraya koenigii* (L.) Spreng.
- **Common Name:** Curry leaf tree

The bark is rich in secondary metabolites such as carbazole alkaloids (mahanimbine, girinimbine), flavonoids**,** tannins**,** saponins**,** and terpenoids**,** which contribute to its therapeutic potential [17, 18].

Preparation of Methanolic Extract:

The freshly collected bark was washes with the distilled water, shape-derived for seven days, and then ground into coarse powder using a mechanical grinder. About 100g of powdered bark was subjected to maceration using 500ml of methanol for 72 hours with occasional stirring. The extract was filtered using whatman filter paper and concentrated using a rotary evaporator under reduced pressure. The crude methanolic extract was stored in an airtight container at 4^{o}C until further use [19].

Preliminary phytochemical screening:

The methanolic extract was subjected to qualitative phytochemical screening for the presence of major bioactive groups, including:

- Alkaloids ((Mayer's and Dragendorff's test)
- Flavonoids (Shinoda test)
- Tannins (Ferric chloride test)
- Saponins (Foam test)
- Triterpenoids (Salkowski test)
- Cardiac glycosides (Keller-Killiani test)

These tests were conducted following standard protocols [20].

Figure 2: Murraya koenigii Bark (Under shade drying)

Figure 3: Maceration & Filtration

Microorganisms Used

Three bacterial strains were used for antimicrobial testing:

- *Escherichia coli* (Gram-negative)
- *Staphylococcus aureus* (Gram-positive)
- *Pseudomonas aeruginosa* (Gram-negative)

The strains were obtained from the Microbiology laboratory of VJ's College of Pharmacy. Bacteria were maintained on nutrients agar slants at 4^{o}C and sub cultured prior to use.

Antibacterial activity (Agar well diffusion method)

The agar well diffusion method was used to asses antibacterial activity. Muellers Hinton agar plates were inoculated with 100µL of each bacterial suspension (adjusted to 0.5 McFarland standards). Wells of 6mm diameter were made and filled with 100µL of methanolic extract at concentration of 100 mg/mL, 200 mg/mL, and 400 mg/mL.

- Positive control: Amikacin (30 µg/disc)
- Negative control: Methanol (vehicle)

The plates were incubated at 37 ^{o}C for 24hours, and the zone of inhibition (in mm) was measured using a ruler. All experiments were conducted in triplicate, and average values were recorded [21].

Statistical analysis:

All experimental data were expressed as mean ± standard deviation (SD). The results were statistically analyzed using one-way ANOVA, and $p < 0.05$ was considered statically significant.

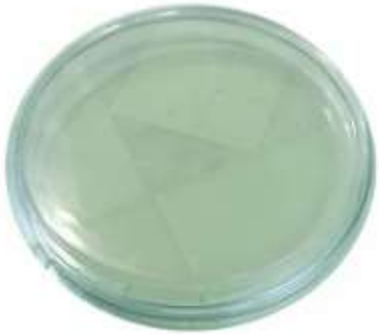

Figure 4: Nutrients Agar medium

Figure 5: Autoclave

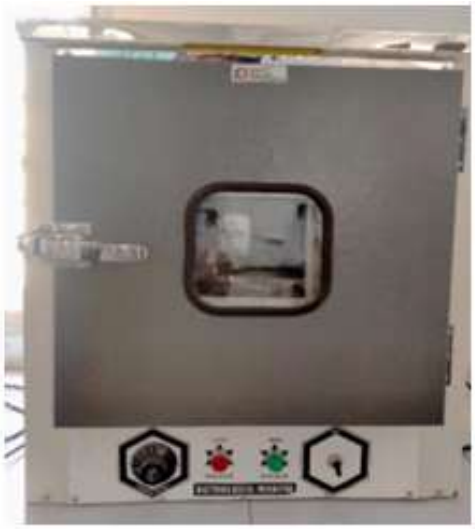

Figure 6: Bacteriological Incubator

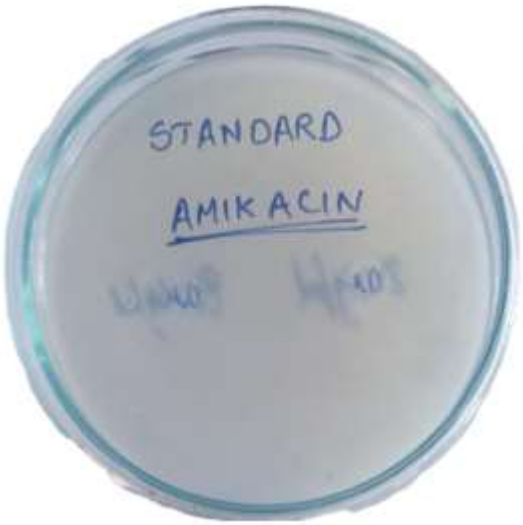

Figure 7: Standard Drug AMIKACIN 80 µg/ml & 100 µg/ml

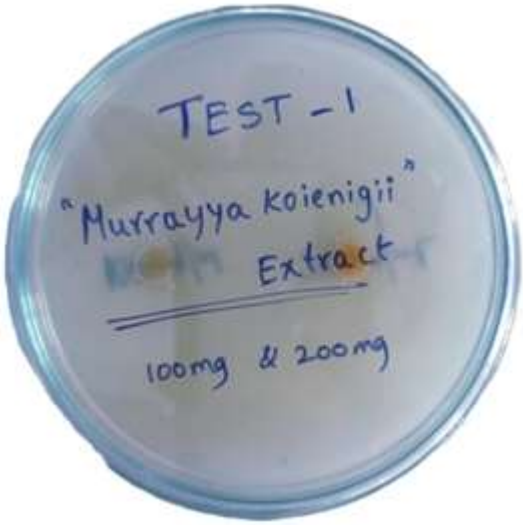

Figure 8: Test sample (Bark extract) 100 mg/ml & 200 mg/ml

RESULTS AND DISCUSSION

Preliminary phytochemical screening:

The methanolic extract of murraya koenigii bark revealed the presence of several secondary metabolites. The qualitative phytochemical analysis confirmed the presence of alkaloids, flavonoids, tannins, triterpenoids, cardiac glycosides, and saponins, while steroids and anthraquinones were absent (Table 1).

These phytoconstituents are known to possess antimicrobial, antioxidant, anti-inflammatory, and cytotoxic properties, supporting the traditional use of M.Koenigii in treating infections and inflammatory conditions. Carbazole alkaloids, such as mahanimbine and girinimbine, present in the bark, have been reported to exhibit strong antibacterial and anti-proliferative activities [22, 23].

Table 1 : Phytochemical Screening

Phytochemical test	Results
Alkaloid	+ve
Tannins	+ve
Saponin	+ve
Steroid	+ve
Anthraquinones	-ve
Triterpenoid	+ve
Cardiac glycoside	+ve

Antibacterial activity

The antibacterial activity of the methanolic extract was evaluated using the agar well diffusion method against three bacterial strains: staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. The extract exhibits dose-dependent inhibition against all test organisms (Table 2, Figure 1).

- At 100 mg/mL, mild inhibition zones were observed (mean zone diameters: S.aureus – 9.2 mm, E. coli – 8.6 mm, aeruginosa – 7.8 mm)
- At 200 mg/mL, the zone of inhibition increases moderately for all three strains.
- At the highest concentration of 400 mg/mL, the extract showed significant inhibition:

S. aureus – 15.3 mm

E. coli – 13.8 mm

P. aeuuginosa – 12.5 mm

The positive control, amikacin (30 µg/disc), showed higher inhibition zones (S. aureus – 22 mm, E. coli – 21 mm, P. aeruginosa – 20 mm), while the negative control (methanol) showed no activity, confirming the antimicrobial effect is due to the phytochemicals in the extract.

These results are in agreement with earlier studies that demonstrated significant antibacterial activity of murraya koenigii leaf and bark extract, attributed to the presence of bioactive compounds that disrupt microbial membrane, inhibit protein synthesis, or interfere with nucleic acid function [24, 25, 26].

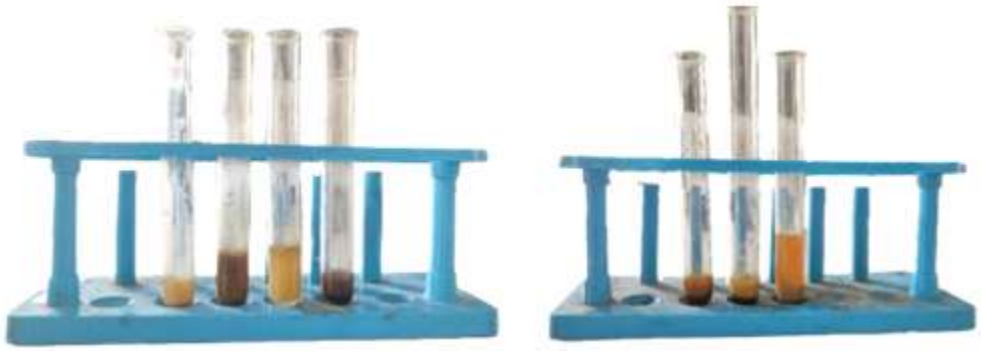

Figure 9: Results of Phytochemical analysis

Figure 10: Zone of inhibition of Amikacin

Figure 11: Zone of Inhibition of Bark extract

DISCUSSION:

The findings of the present study confirm that the ethanolic bark extract of murraya koenigii possesses board-spectrum antibacterial potential, especially against S. aureus, a common Gram-positive pathogen responsible for skin and wound infections. The effectiveness against Gram-negative strains (E. coli and P. aeruginosa) also suggests potential use against urinary and respiratory tract pathogens.

The mechanism of action may be attributes to:

- Alkaloids, which intercalate into microbial DNA or inhibit enzyme activity;
- Flavonoids and tannins, which disrupt cell membranes and inactivate microbial proteins;
- Saponins, known for their ability to form pores in microbial membrane, leading to cell lysis[27]

The dose-dependent activity of the extract reinforces the importance of phytoconstituent concentration in determining antimicrobial efficacy. Though not as potent as Amikacin, the natural origin, safety profile, and availability of plant based antimicrobial offer an alternative or adjunct approach in managing infections and combating antimicrobial resistance (AMR) [28].

CONCLUSION:

Murraya koenigii bark methanolic extract contains a wide range of bioactive phytochemicals with proven antibacterial properties. It showed significant, dose-dependent antibacterial activity against both Gram-positive and Gram-negative bacteria. The study supports the traditional medicinal use of the plant and its potential for development into plant-based antibacterial agents**.**

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