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A Study on Antibacterial Activities of Curry Leaves (Murraya Koenigii)

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Abstract:

The primary aim of this study was to evaluate the antibacterial properties of aqueous and ethanolic extracts from Curry leaves *(Murraya koenigii)*. The leaves underwent successive solvent extraction using a Soxhlet apparatus with both water and ethanol. Prior to assessing antibacterial activity, the extracts were dissolved in dimethyl sulfoxide (DMSO). The antibacterial efficacy of the *M. koenigii* leaf extracts was tested against *Escherichia coli, Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* using the agar well diffusion method. The results indicated that the aqueous extracts produced inhibition zones of 21 mm for *E. coli* (highly sensitive) and 11 mm for *P. aeruginosa* (moderately sensitive), while the ethanolic extracts showed inhibition zones of 19 mm for *E. coli* (highly sensitive) and 9 mm for *P. aeruginosa* (moderately sensitive). In conclusion, the findings suggest that the aqueous extract of *M. koenigii* leaves demonstrates antibacterial activity comparable to the standard antibiotic amikacin, providing scientific evidence that this extract could serve as a natural adjunct in daily meals for the prevention and management of bacterial infections.

Keywords **-** *M. koenigii*, Curry leaves, Antibacterial activity, *E. coli, P. aeruginosa*.

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I. INTRODUCTION

Curry leaves, derived from *Murraya koenigii*, a small, aromatic deciduous shrub belonging to the Rutaceae family, are native to South Asia and widely distributed across countries like Sri Lanka, Bangladesh, China, and India. Within India, the plant's presence extends from the Himalayan foothills to regions like Maharashtra, Tamil Nadu, Kerala, and Assam (Bhusal and Thakur, 2021). Characterized by vibrant foliage, *Murraya koenigii* flourishes during the spring, summer, and monsoon seasons, only to shed its leaves during the winter months. Historical texts in Tamil and Kannada refer to *Murraya koenigii* as *'Kari'* signifying its traditional use as a flavouring agent in spiced sauces for vegetables. This widespread culinary application has established it as a highly popular spice and condiment throughout India. Furthermore, scientific literature suggests potential medicinal properties, with studies reporting that ethanol extracts of *M. koenigii* (IC_{50} 400 µg/ml) exhibit greater efficacy against *Mycobacterium smegmatis* compared to petroleum ether and water extracts. Notably, *M. koenigii* extract has demonstrated maximum activity against *M. bovis* BCG when used in conjunction with rifampicin, a first-line anti-tuberculosis drug. Given this established background regarding the botanical characteristics, traditional uses, and emerging antimicrobial potential of *Murraya koenigii*, the current research endeavours to further investigate and elucidate the antibacterial activities of extracts derived from its leaves.

II. MATERIAL AND METHODS

A. Test materail

The leaves of *M. koenigii* were collected from local provinces at Bhopal (M.P.).

B. Extract preparation

The methodology employed for extracting bioactive compounds from *Murraya koenigii* leaves followed a rigorous solvent extraction process. Initially, freshly harvested leaves were meticulously cleansed under running tap water to remove surface contaminants, followed by air-drying to eliminate residual moisture. The dried leaves were then mechanically reduced to a coarse powder to increase the surface area available for solvent interaction. Sequential solvent extraction was subsequently performed using a Soxhlet apparatus, a technique that facilitates continuous hot extraction, ensuring efficient recovery of target compounds. A gradient of solvents, arranged in ascending order of polarity (water and ethanol), was utilized to selectively extract compounds based on their solubility characteristics. After each extraction phase, the plant material was thoroughly air-dried to remove any remaining solvent before proceeding with the next solvent. The resulting extracts were then concentrated using a rotary flash evaporator, a method that employs reduced pressure to facilitate the evaporation of the solvent at lower temperatures, thereby minimizing the risk of thermal degradation of the extracted compounds. Finally, the concentrated extracts were carefully preserved in airtight containers and stored at a controlled temperature range of 4-5°C to maintain their integrity and prevent degradation prior to antibacterial activity testing. Before evaluation of antibacterial properties, the extracts were solubilized in dimethyl sulfoxide (DMSO), a common polar aprotic solvent known for its ability to dissolve a wide range of organic compounds (as corroborated by Raja and Devi, 2010).

C. Pathogenic microorganisms

The multiple antibiotic-resistant pathogenic microorganisms viz. Escherichia coli, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* were isolated from clinical samples of local hospital in and around Bhopal and confirmed by various microscopic evaluation like Gram's staining (Gram, 1884). Motility, capsule and spore formation as per the procedure prescribed by Collins and Lyne (Collins and Lyne, 2010). All the bacterial pathogens were further confirmed by suitable biochemical tests and used for antimicrobial activity studies (Barrow and Feltham, 1993).

D. Antibacterial activity

The antibacterial activity of leaf extracts of *M. koenigii* was determined by agar well diffusion technique. Muller Hintor agar plates were spread with an overnight culture of each bacterial strain. The well was made by sterile standard cork-borer and 100 mg/ml solution of extract added to each well. Then bacterial plates incubated at 37^0C for 24 hours after which diameter of zones of inhibition were measured (mm) by using Hi Antibiotic Zone Scale-C (Himedia). Each assay was performed in triplicate and means values are reported. Standard antibiotic strip of Streptomycin (100 µg/disc) for each bacterium along with DMSO were used as positive and negative controls respectively.

E. Statistical analysis

Each experiment was repeated in triplicate sets and the mean values for the inhibition zones from the triplicate results were employed to represent the antibacterial activity of the curry leaf extracts. Results were recorded as S (sensitive), I (intermediate sensitive) or R (resistant). The results of sensitivity tests were expressed as (0) = (R) for no sensitivity, + for (below 6 mm) = (R) for low sensitivity, ++ (7- 12mm) = (I) for moderate sensitivity and +++ (13mm & above) = (S) for high sensitivity (Rajendran et al., 2014).

III. RESULTS

The dried leaf powder of *M. koenigii* was subjected to successive solvent extraction in their increasing order of polarity such as hot water and ethanol. The extracts were concentrated and dissolved in DMSO for determination of the antibacterial activity (Raja and Devi, 2010). The zone of inhibition of pathogenic microorganisms viz. E. *coli, P. aeruginosa* and *K. pneumonia* was compared with standard antibiotic amikacin. The results of antibacterial activity of extracts of *M. koenigii* leaves were represented in Table 1. Results revealed that the zone of inhibition of aq. extracts of leaves of *M. koenigii* for *E. coli* and *P. aeruginosa* was found to 21 mm (highly sensitive) and 11 mm (moderate sensitive) respectively. Similarly, the zone of inhibition of ethanolic extracts of leaves of *M. koenigii* for *E. coli* and *P. aeruginosa* was found to be 19 mm (highly sensitive) and 9 mm (moderate sensitive) respectively. Results depicted that among the leaf extracts of *M. koenigii*, aq. extracts showed excellent antibacterial activity than ethanolic extracts. Furthermore, it was observed that aq. extract of *M. koenigii* leaves possess highly sensitive antibacterial activity (21 mm) against *E. coli* which was almost equal to that of standard antibiotic amikacin (22 mm).

Table 1: Antibacterial activity of extracts of *M. koenigii* leaves

S. No.	Bacterial strains	Leaf extracts of M. koenigii		
		Amikacin	Aq. Extract	Et. Extract
		Zone of Inhibition (mm)		
1	E. coli	22.00+++	21.00+++	19.00+++
2	P. aeruginosa	24.00+++	11.00++	9.00++
3	K. pneumonia	20.00+++	0.00	0.00

0 = No Sensitivity; + = Low Sensitivity; ++ = Moderate Sensitivity; +++ = Highly Sensitivity

IV. DISCUSSION

A. Throughout history, humans have depended on plants not only for sustenance but also for medicinal purposes, largely due to the presence of secondary metabolites such as alkaloids and phenolic compounds, which possess therapeutic properties. The use of medicinal plants for the treatment of various ailments can be traced back to ancient civilizations, with countries like India utilizing crude plant materials for medicinal purposes since the Vedic period. This long-standing tradition underscores the importance of exploring the antibacterial properties of these plants in contemporary research. In this context, our study focused on evaluating the antibacterial activities of both aqueous and ethanolic extracts derived from the leaves of *Murraya koenigii*, commonly known as curry leaves.

B. The results of our investigation revealed significant antibacterial activity in the solvent extracts of *M. koenigii* leaves, as evidenced by the formation of clear zones of inhibition against various test organisms, including *Escherichia coli* and *Pseudomonas aeruginosa*. This antibacterial efficacy is likely attributable to the diverse array of phytochemicals present in the leaves, which exist in varying concentrations. The findings align with previous research, which has established that plants are a rich source of potent antibacterial agents. For instance, a study conducted by Rajendran et al., (2014) demonstrated that *M. koenigii* exhibited substantial inhibition against several bacterial strains, including Proteus mirabilis and *Staphylococcus aureus*, with inhibition zones measuring up to 18 mm and 15 mm, respectively. Such results highlight the potential of *M. koenigii* as a valuable resource in the search for natural antibacterial compounds.

C. Further supporting our findings, research by Das and Biswas (2012) indicated that the ethyl acetate and dichloromethane fractions of methanolic leaf extracts of *M. koenigii* displayed mild antibacterial activity against *E. coli*, producing inhibition zones ranging from 9 mm to 11 mm. This suggests that while the antibacterial effects may vary in strength, *M. koenigii* consistently demonstrates the ability to inhibit both Gram-positive and Gram-negative bacteria. Notably, our study corroborates these earlier findings, particularly regarding the susceptibility of E. coli to the acetone extract, which yielded a notable inhibition zone of 16 mm. Collectively, these studies reinforce the significance of *M. koenigii* in the realm of medicinal plants, emphasizing its potential as a source of effective antibacterial agents.

D. Mathur et al., (2010) have provided compelling evidence that the methanolic extract of curry leaves exhibits significant inhibitory effects against a range of bacterial pathogens, including *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus uberis, Pseudomonas aeruginosa, Escherichia coli, Corynebacterium gravis*, and *Bacillus cereus*. This research reinforces existing scientific literature that highlights the potent antimicrobial properties of curry leaves. The findings suggest that these natural extracts could serve as a viable alternative to conventional antibiotics, particularly in light of the growing concerns surrounding antibiotic resistance and the adverse effects associated with synthetic antimicrobial agents. In our investigation, we observed that the antibacterial activity of curry leaves against *E. coli* was comparable to that of the standard antibiotic amikacin. Notably, the use of curry leaves as a treatment option presents a significant advantage, as it does not carry the potential negative side effects commonly associated with antibiotic therapies. This finding is particularly relevant in clinical settings where the side effects of antibiotics can lead to patient non-compliance or additional health complications. The ability of curry leaves to provide similar antibacterial efficacy without the drawbacks of traditional antibiotics positions them as a promising candidate for further research and application in medical treatments. The implications of these findings are profound; suggesting that curry leaves could serve as a powerful natural remedy for bacterial infections. Their therapeutic effects, coupled with the absence of adverse side effects, make them an attractive alternative for treating infections in humans. As the medical community continues to seek effective strategies to combat bacterial resistance and minimize the reliance on synthetic antibiotics, the incorporation of natural products like curry leaves into treatment protocols could represent a significant advancement in the field of antimicrobial therapy.

V. CONCLUSIONS

The results of the study demonstrated that the aqueous extract of curry leaves *(Murraya koenigii)* exhibited antibacterial properties comparable to those of the standard antibiotic amikacin. This finding is significant as it highlights the potential of *M. koenigii* as a natural alternative to conventional antibiotics, particularly in an era where antibiotic resistance is a growing concern. The extract's efficacy in combating bacterial pathogens suggests that it could serve as a valuable addition to dietary practices aimed at enhancing health and preventing infections. Furthermore, the current research provides a robust scientific basis for the incorporation of *M. koenigii* leaves into daily meals. By utilizing this natural remedy, individuals may not only enjoy the culinary benefits of curry leaves but also harness their medicinal properties. The study underscores the importance of exploring plant-based solutions in the fight against bacterial infections, which could lead to more sustainable and accessible healthcare options. In conclusion, the findings advocate for the broader use of *M. koenigii* as a preventive measure against bacterial infections. As the global health landscape continues to evolve, integrating such natural extracts into our diets could play a crucial role in maintaining public health. This research not only enriches the existing body of knowledge regarding the antibacterial effects of plant extracts but also encourages further investigation into their potential applications in both culinary and medicinal contexts.

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