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Evaluation of Antibacterial Activities of Curry Leaves *(Murraya koenigii)*

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Abstract
The main objective of the present study was to determine the antibacterial activities of aqueous (aq.) and ethanolic extracts of Curry leaves *(Murraya koenigii).* Leaves of *M. koenigii* was subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with water and ethanol. The extracts were dissolved in dimethyl sulfoxide (DMSO) before testing the antibacterial activity. The antibacterial activity of leaf extracts of *M. koenigii* for *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumonia* was determined by agar well diffusion technique. Results revealed that the zone of inhibition (mm) for pathogenic microorganisms *viz. E. coli, P. aeruginosa & K. pneumonia* was compared with standard antibiotic amikacin. 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. In conclusion, study findings confirmed that the aq. extract of *M. koenigii* leaves exhibited antibacterial activity at par with that of standard antibiotic amikacin. Therefore, current study supplies as a scientific evidence-based report that aq. extract of *M. koenigii* leaves could be effectively used as a natural aid in everyday meal for the prevention and control of bacterial infections.
Keywords: *M. koenigii*, Curry leaves, Antibacterial activity*, E. coli, P. aeruginosa*

Introduction

The World Health Organization (WHO) reports that drug-resistant microorganisms now account for an estimated 700,000 deaths a year and this number could increase to 10 million deaths each year if no action is taken.[1] In 2016 alone, 490,000 people developed multi-drug resistant tuberculosis globally.[2]

Indiscriminate use of antibiotics in today's world is one of the major causes for the rising emergence of multidrug resistant pathogenic strains that do not respond to the usual line of treatment. Therefore, the need to search for new antimicrobials remains unchallenged. Currently, in addition to antibiotics and chemically synthesized drugs, the trend to look out for alternative medicines such as natural or herbal medicines is increasing because they have no side effects or toxicity owing to their natural sources.[3]

Moreover, nowadays use of plant-based natural products in the treatment and prevention of diseases and health enhancement has led to the significant attention of the scientific community and the public. The availability of these medicinal plants provides a cost-effective source with no side effects to develop new drugs has drawn much attention among the researchers. Plant-based traditional medicine has a long history since ancient civilization and uses plant materials as a major ingredient in synthesizing drugs.[4]

Curry leaves (*Murraya koenigii*) are a popular leaf-spice used in very small quantities for their distinct aroma due to the presence of volatile oil and their ability to improve digestion. These leaves are widely used in Asian cuisines for flavoring foods. The leaves have a slightly pungent, bitter and feebly acidic taste, and they retain their flavor and other qualities even after drying. Curry leaf is also used in many traditional cultures namely Indian Ayurvedic and Unani prescriptions.[5]

M. koenigii leaf is an important leafy vegetable (Figure 1) that belongs to the Rutaceae family, and is native to India and the Southeast Asian Region. Curry leaves are natural flavoring agents with a number of important health benefits. They contain wide varieties of medicinal and pharmacological properties.[6,7] Literature reports evidenced that ethanol extracts of *M. koenigii* (IC_{50} 400 µg/ml) were found to be more effective against *Mycobacterium smegmatis* as compared to petroleum ether and water extracts. *M. koenigii* extract showed maximum activity against *M. bovis* BCG in combination with a first line anti-TB drug rifampicin.[8]

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Figure 1: Showing curry leaves (*Murraya koenigii*)

Furthermore, it is a widely accepted fact that the rapid development of deriving pharmacologically active drugs from medicinal herbs has a tremendous impact on current medicinal practices. With this background, the present study was designed with the main objective to determine the antibacterial activities of extracts of *M. koenigii* leaves.

Materials and Methods

Test material

The leaves of *M. koenigii* were collected from local provinces at Chikkaballapura District of Karnataka State.

Extract preparation

Leaves of *M. koenigii* were washed thoroughly under running tap water, dried on paper. Dried leaves were coarsely powdered and subjected to successive solvent extraction by continuous hot extraction (Soxhlet). The extraction was done with different solvents in their increasing order of polarity such as water and ethanol. Each time the material was air dried and later extracted with other solvents. All the extracts were concentrated by distilling the solvent in a rotary flash evaporator. The extracts were preserved in airtight containers and stored at 4-5^{o} C until further use. The extracts were dissolved in dimethyl sulfoxide (DMSO) before testing for the antibacterial activity.[9]

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 Chikkaballapura district headquarter and confirmed by various microscopic evaluation like Gram's staining.[10] Motility, capsule and spore formation as per the procedure prescribed by Collins and Lyne.[11] All the bacterial pathogens were further confirmed by suitable biochemical tests and used for antimicrobial activity studies.[12]

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^{0}C 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.

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.[13]

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.[9] The zone of inhibition of pathogenic microorganisms *viz. E. coli, P. aeruginosa* and *K. pneumonia* was compared with stander 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^{++}
	K. pneumonia	20.00^{+++}	0.00	0.00

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

Discussion

Historically, humans have relied heavily on plants for food and medicine since they contain secondary metabolites like alkaloids, phenolic compounds, and other compounds with therapeutic value. Due to their antimicrobial properties, medicinal plants have been used for treating human diseases for a very long time.[14] Countries like India have been using crude plants as medicine since Vedic period. Hence, in the current we aimed to determine the antibacterial activities of aq. and ethanolic extracts of *M. koenigii* leaves.

Results of the present study depicted that antibacterial activity of solvent extracts of leaves of *M. koenigii* was evident due to clear zone of inhibition against test organisms like *E. Coli, and P. aeruginosa.* The antibacterial activity of *M. koenigii* leaves could be due to different class of phytochemicals in different proportions.

Plants are sources of very potent and powerful drugs with antibacterial properties.[15,16] The study of Rajendran et al., revealed that the strongest inhibition zone against *Proteus mirabilis* (18 mm), *Staphylococcus aureus*, *Corynebacterium pseudotuberculosis* (15 mm), *K. pneumoniae* (15 mm) *P. aeruginosa* (14 mm), *Enterobacter aerogenes* (13 mm) and a moderate level zone of inhibition was observed with *Salmonella enterica* (11 mm), and *Streptococcus pyrogens* (10 mm) respectively. Also, in this study *M. koenigii* displayed antibacterial activity against the Gram-positive and Gram-negative bacteria, while *E. coli* showing maximum susceptibility to acetone extract with a zone of inhibition of 16 mm.[13] These findings were comparable with our study results.

Another study by Das and Biswas revealed that ethyl acetate and dichloromethane soluble partition of methanolic leaf extract of *M. koenigii* exhibited mild activity against *E. coli* forming zone of inhibition of 9 mm to 11 mm.[17] Furthermore, Mathur et al., have demonstrated that the methanolic extract of curry leaves inhibited *S. aureus*, *S. epidermidis*, *Streptococcus uberis, P. aeruginosa, E. coli, Corynebacterium gravis* and *Bacillus cereus*.[18]

This study has reiterated and supported the scientific literature evidence that curry leaves do indeed have powerful antimicrobial properties. In our study, it was discovered that curry leaves had antibacterial effects on *E. coli* that were comparable to the standard antibiotic amikacin without any of the potential negative side effects that people may experience from using antibiotics. Hence, the therapeutic antibiotics can be effectively replaced by curry leaves. It is clear from the foregoing that curry leaves are a powerful natural treatment for bacterial infections that has a therapeutic effect with no side effects when used to treat bacterial infections in humans.

Conclusions

The study findings confirmed that the aq. extract of curry leaves (*M. koenigii)* exhibited antibacterial activity at par with that of standard antibiotic amikacin. Therefore, current study supplies as a scientific evidence-based report that aq. extract of *M. koenigii* leaves could be effectively used as a natural remedy in everyday meal for the prevention and control of bacterial infections.

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