

IN VITRO EVALUATION OF ANTIMICROBIAL ACTIVITIES OF CRUDE EXTRACTS FROM *MURRAYA KOENIGII* AGAINST PATHOGENIC BACTERIA

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ABSTRACT

Murraya koenigii, curry leaf is found almost throughout India up to an altitude of 1500 m. It is much cultivated for its aromaticity and is used in South India as a natural flavouring agent in various curries. The present study deals with the antimicrobial activities which confirmed that the methanol extracts of leaves are active against *K. pneumoniae* and *S. typhi* with maximum inhibition and moderate effect against other four bacteria. Which was then followed by Aqueous, chloroform and petroleum ether extracts showed better activity against *P. aeruginosa*, *E. coli* and *S. typhi*. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the water, ethanol, chloroform and pet ether extracts were in the range of 12.5 to 100.0 mg/ml and 50.0 to 100.0 mg/ml, respectively. Amongst the evaluated extracts, the ethanolic extract showed the strongest antibacterial effect. Hence, present study is useful towards authenticating the taken specimen to be a potent antimicrobial agent.

Keywords: Antibacterial, MBC, MIC, *Murraya koenigii*, Zone of inhibition

INTRODUCTION

The plants belonging to Rutaceae are herbs, shrubs and trees with glandular punctuate, commonly strongly smelling herbage comprising about 150 genera and 1,500 species that are further characterized by the common occurrence of spines and winged petioles. Shrubs or trees are up to 4 m tall and found in evergreen areas and in moist forest. Leaves of *Murraya koenigii* (L) Spreng (Mitha neem) are commonly used as flavoring agent in Indian curry preparation since ancient times. It is commonly found in the outer Himalayas, from the Ravi eastwards, ascending to 5,000 feet, in Assam, Chittagong, Upper and Lower Burma. It is also found in evergreen and deciduous forests of peninsular India, often as under wood¹. The leaves, bark and roots of *Murraya koenigii* can be used as a tonic and a stomachic^{2,3,4}. The Indian variety *M. koenigii* and Chinese variety *M. paniculata* are the two species available and both have some common medicinal properties. The macroscopic studies revealed the shape of leaves of *Murraya koenigii* (L.) Spreng as obliquely ovate or somewhat rhomboid with acuminate obtuse or acute apex, bipinnately compound with exstipulate in alternate arrangement. The microscopic studies showed stomata were found distributed on abaxial surface while the adaxial surface was without stomata. The type of stomata was noted as anomocytic one. The uniseriate multicellular trichomes were observed on both surfaces, more frequent on upper surface of midrib portion. The transverse section of leaf exposed a layer of epidermis composed of rectangular cells as outermost covering on both upper and lower layer. The upper epidermis was enveloped with deposition of cuticle⁵.

Phytochemically leaves are found to contain alkaloid⁶ and volatile oil. Traditionally, the plant is used as a stimulant, stomachic, febrifuge, analgesic and for the treatment of diarrhea, dysentery and insect bites⁷. The leaves are reported to have great medicinal value such as antibacterial, anti-inflammatory, anti-feedant etc.⁸. Chemical tests were employed in the preliminary phytochemical screening for various secondary metabolites such as carbohydrates, alkaloids, phytosterols, coumarins, flavonoids and alkaloids⁹. The aqueous extract of *M. koenigii* leaves showed hypoglycemic action in normal and alloxan diabetic dogs¹⁰. The essential oil from *M. koenigii* leaves showed antibacterial effect against *Bacillus subtilis*, *Streptococcus aureus*, *Corynebacterium pyogenes*, *Mycobacterium tuberculosis*^{11,12}. The chemical examination of strong odiferous oil occurring in the leaves and the seeds has been made, which exhibited a strong antibacterial and antifungal activity. An alkaloid, murrayacinine, is also found in this plant¹. It is an endangered medicinal plant valued for its digestive, carminative, anthelmintic and laxative property

since time immemorial. It is also used in diabetes, heart related problems, neural disorders, cancerous tumors and liver disorders. The seeds are also used for wound healing antioxidant, anti-inflammatory, analgesic and contraceptive activity¹³. Aqueous and methanol extracts of this plant treatment has caused significant increase in the insulin concentration in diabetic rats which confirms that extracts of *M. koenigii* leaves have a modulatory role in the treatment of diabetes mellitus¹⁴.

In the recent years, most of the fields of science and technology are performing experiments for the growth and welfare of mankind. In the field of medicine many different systems are practiced in India- Ayurveda, Unani, Siddha, Amchi and local health traditions. The most fast moving concept of medicine is nanomedicine. The efficiency of antibiotics in combating microbial infections was very promising shortly after their introduction. It was even thought that the microbial war was as good as over as was declared by the Surgeon General of the United States. However, resistance to these agents developed rapidly afterward and the problem of antibiotic resistance has remained a menace threatening the benefits of antibacterial agents. As a result, a solution to the issue of antimicrobial resistance is a matter of urgent importance. Natural products are viewed as a privileged group of structures which have evolved to interact with a wide variety of protein targets for specific purposes¹⁵.

In this study, different extracts of *M. koenigii* leaves were prepared and tests were performed to check the activity of leaves against different micro-organisms.

MATERIALS AND METHODS

Plant Materials

Fresh leaves of plant *Murraya koenigii* were collected in the winter season locally from in and around IISc campus, Bangalore, Karnataka (Southern India) in August 2011. The taxonomic identification of the plant was confirmed and processed for further investigations. Collected leaves were washed thoroughly under running water for 2-3 times. Washed leaves were dried under the shade for 45 days. The dried leaves were powdered and stored in a sterile bottle at room temperature.

Method of extraction

Dried leaves were subjected to extraction by weighing 20g of finely ground plant material and soaked in 200ml of ethanol, water, chloroform and petroleum ether to produce respective extracts. Soaking was performed in separate sterile bottles under dark

condition for 48-72 hours. Later they were filtered using muslin cloth and kept for drying¹⁶.

Preparation of test samples

For antimicrobial studies of the leaves, the concentrations of 50mg/ml of each extract were used for screening. Each solid extract was dissolved in its respective solvent to obtain a stock solution of 50mg/ml.

Sources and maintenance of organisms

Stock cultures of gram positive organisms (*Staphylococcus aureus*) and gram negative organisms (*Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumonia* and *Salmonella typhi*) were obtained from Department of Biotechnology, The Oxford College of Science, Bangalore. They were maintained on Nutrient Agar (Hi-media, Mumbai) slope at 4°C and sub-cultured into Nutrient broth by picking-off technique¹⁷. Twenty four hour old cultures were prepared freshly for every study.

Preparations of standard bacterial suspensions

The average number of viable *P. aeruginosa*, *E. coli*, *S. aureus*, *K. pneumoniae* and *S. typhi* organisms per ml of stock suspension of nutrient broth media was determined by means of surface viable counting technique. About 10^4 - 10^5 colony-forming units per ml were used. For every trial a fresh stock solution was prepared and the experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained¹⁸.

Antibacterial susceptibility test

To start the antibacterial activity of the prepared extracts 100µl of standardized bacterial stock suspension was thoroughly mixed with 100ml of sterile nutrient agar. 20ml of sterilized nutrient agar medium was plated in each of the petri plates and the plates were allowed to set. Using spread plate method, 1ml of standard bacterial solutions of 10^4 - 10^5 dilutions of the following microorganism's *P. aeruginosa*, *E. coli*, *S. aureus*, *K. pneumoniae* and *S. typhi* were inoculated using spreader. Each organism was inoculated in different petri plate. Using cork borer of 5mm diameter, wells were made in the solidified nutrient agar plates. Two of the wells were filled with the 100µl each of standard antibiotic ciprofloxacin and Dimethylsulfoxide (DMSO) which was considered as control respectively. The rest of the wells were filled with 100µl of test compounds such as ethanol, water, chloroform and petroleum ether extracts. The plates were incubated overnight at 37°C. Microbial growth was determined by measuring the diameter of inhibition zone. All well-agar diffusion techniques were performed in the triplicate for the strain and the antibacterial activity was expressed as average mean of the diameter (mm) of inhibition zone produced by various prepared extracts¹⁹.

Determination of Minimum Inhibitory Concentration (MIC)

To measure the MIC values, micro-broth dilution method was used²⁰. The reconstituted extracts was serially diluted 2-fold in nutrient broth medium to obtain various concentrations of the stock, 100, 50, 25, 12.5, 6.25, 3.125, 1.562, 0.781 mg/ml and were assayed against the test organisms. The minimum inhibitory concentration was defined as the lowest concentration able to inhibit any visible bacterial growth^{21,22}.

Determination of Minimum Bactericidal Concentration (MBC)

Equal volume of the various concentration of each extract and nutrient broth were mixed in micro-tubes to make up 0.5ml of

solution. 0.5ml of standard bacterial solutions of 10^4 - 10^5 dilutions was added to each tube²². The tubes were incubated aerobically at 37°C for 24 hours. Two control tubes were maintained for each test batch, containing extract without inoculum and the other containing growth medium and inoculum. The MBC was determined by sub-culturing the test dilution on nutrient agar and further incubated for 24 hours. The highest dilution that yielded no single bacterial colony was taken as the Minimum bactericidal concentration²³.

Statistical analysis

The results of the experiment are expressed as mean \pm SE of three replicates in each test. The data were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's multiple pairwise comparison tests to assess the statistical significance. $P \leq 0.05$ was considered as statistically significant, using software ezANOVA ver. 0.98.

RESULTS AND DISCUSSION

Antibacterial activities were performed against both Gram positive and Gram negative organisms. There was a significant variation in the antibacterial activities of the four extracts. Ethanol extract showed broad spectrum with standard ciprofloxacin as its activities were independent on Gram reaction. The zone of inhibition for *K. pneumoniae* was high, which was followed by *S. typhi*, *E. coli* and *P. aeruginosa*, where as *S. aureus* showed much less inhibition zone. Ethanol extract was found more effective than petroleum ether extract against all organisms except for *S. typhi*. Water extract showed low antibacterial activity with inhibition zones ranging between 4mm and 12mm for different bacteria tested. Ciprofloxacin, which was used as a positive experimental control against all the bacterial strains assayed, produced a zone of inhibition ranging from 12mm-20mm, while very negligible inhibitory effect could be observed for DMSO used as negative control. However, aqueous extracts showed moderate inhibitory effect against three bacterial strains viz., *P. aeruginosa*, *E. coli* and *S.aureus*. The results are presented in **table 1**.

The minimum inhibitory concentration (MIC) of the extracts for different organisms ranged between 12.5 to 100.0 mg/ml, while that of the ciprofloxacin control ranged between 3.125 and 6.25 mg/ml (**Table 2**). The ethanol extract has showed comparable results with standard. Both the extract and standard has showed better inhibition for *K.pneumoniae gram negative* strain. The minimum bactericidal activity (MBC) of the extract for different bacteria ranged between 50.0 to 100.0 mg/ml, while that of the ciprofloxacin 6.25 to 12.5 mg/ml (**Table 2**). Invariably ethanol extract maintained the same results as of MIC. A correlation was found between the antibacterial activity observed by agar diffusion assay and MIC, MBC determination which was the same case observed by Ramzi *et al.*²⁴.

Ethanol extracts have the higher solubility for more phytoconstituents, and hence consequently showed highest antibacterial activity. It indicates that leaves of *Murraya Koenigii* may possess compounds with antimicrobial properties which are effective against infectious diseases^{25,26}. The antifungal activity of leaves of *M. koenigii* is well documented²⁷. The antimicrobial activity of leaves of *M. koenigii* may be due to the presence of carbazole alkaloids. Carbazole alkaloids have been reported for their various pharmacological activities such as anti-tumor, anti-viral, anti-inflammatory, anti-convulsant, diuretic and anti-oxidant²⁸. The results from the present investigation indicate that this plant extract could possibly be used as an efficient antibiotic in its purified form.

Table 1: Antibacterial potential of leaf extracts of *Murraya Koenigii* against different bacteria.

S. No.	Organisms	Water (mg/ml)	Ethanol (mg/ml)	Chloroform (mg/ml)	Pet Ether (mg/ml)	Ciprofloxacin (mg/ml)	DMSO (mg/ml)
1	<i>P. aeruginosa</i>	4.43 \pm 0.18	8.33 \pm 0.15	7.23 \pm 0.18	5.17 \pm 0.15	15.27 \pm 0.09	2.07 \pm 0.03
2	<i>E. coli</i>	7.47 \pm 0.15	8.30 \pm 0.12	6.17 \pm 0.15	5.17 \pm 0.20	20.07 \pm 0.15	5.37 \pm 0.18
3	<i>S. aureus</i>	5.47 \pm 0.18	6.37 \pm 0.18	6.23 \pm 0.09	4.50 \pm 0.17	16.23 \pm 0.20	1.57 \pm 0.18
4	<i>K.pneumoniae</i>	10.33 \pm 0.20	12.43 \pm 0.23	11.27 \pm 0.18	8.23 \pm 0.15	20.20 \pm 0.15	5.33 \pm 0.20
5	<i>S. typhi</i>	12.23 \pm 0.15	9.23 \pm 0.15	10.23 \pm 0.15	12.13 \pm 0.19	12.07 \pm 0.18	3.00 \pm 0.06

The values are the mean of triplicates \pm S.E.

Table 2: The MIC and MBC profile of the extracts

Test bacteria	Water (mg/ml)		Ethanol (mg/ml)		Chloroform (mg/ml)		Pet ether (mg/ml)		Ciprofloxacin (mg/ml)	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
<i>P. aeruginosa</i>	25.0	50.0	25.0	50.0	25.0	50.0	50.0	100.0	6.25	12.5
<i>E. coli</i>	12.5	50.0	12.5	50.0	50.0	100.0	25.0	100.0	3.125	6.25
<i>S. aureus</i>	50.0	100.0	50.0	100.0	50.0	100.0	50.0	100.0	3.125	6.25
<i>K. pneumoniae</i>	12.5	25.0	12.5	25.0	12.5	25.0	25.0	50.0	6.25	12.5
<i>S. typhi</i>	12.5	25.0	12.5	25.0	12.5	25.0	50.0	100.0	3.125	6.25

CONCLUSION

The present study revealed that the various extracts of leaves of *M. koenigii* exhibited antimicrobial properties which explain the basis for its use in traditional medicines to treat skin infections. This property concludes the *M. koenigii* can be utilized for the preparation of effective drugs, as it has been reported for their various pharmacological activities. Results obtained will form the basis for selection of this plant for further investigation in the potential discovery of new natural bioactive compounds. Further studies which are aimed in that direction are in progress.

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