

## Antimicrobial efficacy of *Murraya koenigii* (Linn.) Spreng. root extracts

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*In vitro* antimicrobial efficacy of root extracts of *Murraya koenigii* (Linn.) Spreng. was assessed by disc diffusion method against four bacterial strains (*Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi*) and three fungal strains (*Aspergillus niger*, *Candida albicans* and *Trichophyton rubrum*). Minimum inhibitory concentration was also determined. The most susceptible bacterial and fungal strains were *S. aureus* and *T. rubrum*, respectively. The root extracts in organic solvents (hexane, methanol and chloroform) showed good antimicrobial activity. However, aqueous extracts could not exhibit any activity. Results of the present investigation indicate that root of *M. koenigii* possess antimicrobial properties and hence can be exploited for future natural plant based antimicrobial agents.

**Keywords:** Antibacterial, Antifungal, Antimicrobial, Curry leaf tree, *Murraya koenigii*, Rutaceae, *Staphylococcus aureus*, *Trichophyton rubrum*.

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### Introduction

Medicinal plants are naturally gifted with invaluable bioactive compounds which form the backbone of traditional medicines. The use of plants as medicines antedates history. Approximately 80% of the 4,000 million inhabitants of the earth rely on herbal medicines for their primary health care<sup>1</sup>. There has been an increasing interest worldwide on therapeutic values of natural products from plants due to disenchantment with modern synthetic drugs<sup>2,3</sup>. Moreover, the clinical efficacy of many existing antibiotics is also being threatened by the emergence of multidrug-resistant pathogens. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Therefore, there has been a tremendous upsurge in the demand for the drugs from natural sources.

*Murraya koenigii* (Linn.) Spreng., a member of the family Rutaceae, is a deciduous to semi-evergreen aromatic tree found throughout India. Traditionally, it is used as an analgesic, febrifuge, stomachic, carminative and for the treatment of dysentery and skin eruptions<sup>4,5</sup>. Curry leaf tree is commonly used as spice due to the aromatic nature of leaves. Carbazole alkaloids<sup>6</sup>, the major constituents of the plant are known to possess cytotoxic, antioxidative, antimutagenic and anti-inflammatory activities<sup>7-9</sup>. The

leaves are rich in mono-terpenoids and sesquiterpenoids which exhibited antifungal activities<sup>10</sup>. Minor furano-coumarins are also reported from seeds<sup>11</sup>. In the present investigation, an attempt has been made to investigate antimicrobial screening of root extracts of *M. koenigii* against pathogenic microorganisms.

### Materials and Methods

#### Plant material

The plants of *M. koenigii* were collected from the campus of University of Rajasthan, Jaipur during the month of July 2007. The plant was identified and voucher specimen was deposited to the Herbarium, Department of Botany, University of Rajasthan, Jaipur (RUBL NO.-20397). Roots were separated, washed thoroughly with distilled water, shade dried, powdered using blender and stored in air tight container until for further use.

#### Preparation of extracts

The powdered roots (500 g) were extracted with hexane, methanol and chloroform using Soxhlet's apparatus for 12-14 h. The organic extracts were separately filtered with Whatman No. 1 paper and evaporated to dryness on water bath to obtain semi-solid mass. However, aqueous extraction of roots is performed by using hot water maceration. The dried extracts were stored at 5°C in the refrigerator until further study.

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### Antimicrobial screening

#### Test microorganisms

*In vitro* antimicrobial activity was evaluated against seven pathogenic microorganisms, Gram positive *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923), Gram negative *Escherichia coli* (ATCC 25922), *Salmonella typhi* (ATCC 3492) and fungal strains, *Aspergillus niger* (ATCC 322), *Candida albicans* (ATCC 4718) and *Trichophyton rubrum* (ATCC 2327). All these test microorganisms were obtained from Batra Hospital and Medical Research Centre (BHMRC), New Delhi. The bacterial cultures were grown and maintained on Nutrient Broth medium at 37°C for 24 h while the fungal cultures were maintained on Potato Dextrose Agar slants and incubated at 27°C for 48 hours.

#### Antimicrobial assay

Antimicrobial assay of crude extracts was performed against seven test pathogenic strains by disc diffusion method<sup>12</sup>. The nutrient agar and potato dextrose agar plates were seeded with suspension (10<sup>6</sup>cfu/ml) of the bacterial and fungal strains, respectively. The sterilized Whatman no.1 filter paper disc (6 mm) were impregnated with (1000 µg/ml) of extract, dried and placed aseptically on seeded plates with the help of a sterile forceps. Later on these plates were kept at room temperature for 30min (Pre diffusion time). The standard discs (6 mm) impregnated with antibiotics Streptomycin (2 µg/ml) and Fluconazole (2 µg/ml) were used as control. The plates were incubated at 37°C for 24 h and 25°C for 48 h for bacteria and fungi, respectively. The diameter of the inhibition zone (mm) was measured and in each case the activity index was calculated. The

experiment was repeated three times and the mean values calculated for conclusion.

Minimum inhibitory concentration of extract against tested microorganisms was determined by broth dilution method<sup>13</sup>. For broth dilution, 1 ml of standardized suspension of a strain (10<sup>6</sup>cfu/ml) was added to each tube containing extracts at various concentrations in nutrient broth medium. The tubes were incubated at 37°C for 24 h (for bacterial strains) and 25°C for 48 h (for fungal strains) and observed for visible growth after vortexing the tubes gently. The experiment was repeated two times. The minimum inhibitory concentration (MIC) is taken as the lowest concentration of the extracts at which there is turbidity after incubation.

### Results and Discussion

The antimicrobial efficacy of the extracts of *M. koenigii* roots was quantitatively assessed on the basis of inhibition zone, activity index (Table 1) and minimum inhibitory concentration (Table 2). In the present investigation, all the extracts (hexane, methanol and chloroform) were found to be effective against tested pathogenic strains except aqueous extract. Methanol extract showed more pronounced antimicrobial activity than other extracts. Among the tested bacterial strain, the most susceptible bacterium to the extract (hexane, methanol and chloroform) was *S. aureus*, which is known to play significant role in skin diseases<sup>14</sup>. It indicates that roots of *M. koenigii* may possess compounds with antimicrobial properties which are effective against infectious diseases. Earlier, the extracts of *M. koenigii* were shown to possess bioactive compounds having anti-oxidative, anti-mutagenic and hypoglycemic activities<sup>8,15,16</sup>.

Table 1 — Antimicrobial activity of various organic extracts of roots of *Murraya koenigii*

Test organisms	Organic extracts								Control	
	Hexane		Methanol		Chloroform		Aqueous		Streptomycin (2µg/ml)	Fluconazole (2µg/ml)
	IZ	AI	IZ	AI	IZ	AI	IZ	AI		
<i>Bacillus subtilis</i>	7.00	0.31	10.00	0.45	7.00	0.31	-	-	22.00	-
<i>Escherichia coli</i>	7.00	0.29	10.00	0.41	9.00	0.31	-	-	24.00	-
<i>Salmonella typhi</i>	6.00	0.33	9.00	0.50	6.00	0.33	-	-	18.00	-
<i>Staphylococcus aureus</i>	9.00	0.45	15.00	0.75	10.00	0.50	-	-	20.00	-
<i>Aspergillus niger</i>	6.00	0.35	9.00	0.52	8.00	0.47	-	-	-	17.00
<i>Candida albicans</i>	6.00	0.33	10.00	0.55	8.00	0.44	-	-	-	18.00
<i>Trichophyton rubrum</i>	6.00	0.30	14.00	0.70	8.00	0.40	-	-	-	20.00

IZ = Inhibition zone (mm); AI= Activity Index; - = No activity; Values are means of three replicates.

Table 2 — Determination of MIC of various organic extracts of roots of *Murraya koenigii*

Test organisms	Organic extracts			
	Hexane	Methanol	Chloroform	Aqueous
<i>Bacillus subtilis</i>	0.625	0.312	0.625	-
<i>Escherichia coli</i>	0.625	0.312	0.312	-
<i>Salmonella typhi</i>	0.625	0.625	0.625	-
<i>Staphylococcus aureus</i>	0.312	0.078	0.312	-
<i>Aspergillus niger</i>	0.625	0.312	0.625	-
<i>Candida albicans</i>	0.625	0.312	0.625	-
<i>Trichophyton ruburam</i>	0.625	0.156	0.312	-

MIC: Minimum inhibitory concentration (mg/ml) of the extract; Values are means of two replicates.

In antibacterial screening, the methanol extract showed maximum inhibitory effect against *S. aureus* (IZ=15.00 mm; AI=0.75) with MIC value of 0.078 mg/ml while hexane extract showed minimum inhibitory effect against *E. coli* (IZ=7.00 mm; AI=0.29) with MIC value of 0.625 mg/ml. However, aqueous extract showed no inhibitory effect against tested four bacterial strains (*Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi*).

In antifungal screening, the methanol extract showed maximum inhibitory effect against *T. ruburam* (IZ =14.00 mm; AI=0.70) with MIC value of 0.156 mg/ml while hexane extract showed minimum inhibitory effect against *T. ruburam* (IZ=6.00 mm; AI=0.30) with MIC value of 0.625 mg/ml. Aqueous extract also showed no inhibitory effect against tested three fungal strains (*Aspergillus niger*, *Candida albicans*, and *Trichophyton rubrum*). The antifungal activity of leaves of *M. koenigii* is well documented<sup>17,18</sup>.

The results from the present study indicate that this plant extract could possibly use as antibiotics. The antimicrobial activity of roots of *M. koenigii* is due to presence of carbazole alkaloids<sup>6</sup>. The present investigation supports the antimicrobial traditional use of this plant. However, antimicrobial activities of its essential oils were previously demonstrated<sup>10</sup>. *In vitro* antimicrobial activity of extracts against pathogens justifies the folk medicinal use of curry leaf tree for the treatment of diarrhoea, dysentery and skin eruptions.

## Conclusion

The present investigation revealed that the various extracts from the roots of *M. koenigii* exhibited antimicrobial properties which explain the basis for its

use in traditional medicines to treat skin infections. The methanol extracts exhibited significant inhibitory activity against pathogenic microorganisms. It showed maximum inhibitory effect against *S. aureus* and *T. rubrum*, bacterial and fungal strains respectively.

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