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# Antibacterial and Antioxidant Potential of Leaf and Seed Extracts of *Murraya koenigii* (Linn.) Spreng

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Authors DKG and SJ have collected information from different sources, prepared, designed, analyzed and interpretated the data. Authors SD, US, MV have wrote the protocol. Author PD have been involved in formatting, editing and drafting the manuscript critically for important intellectual content and have given final approval of the version to be published*

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## ABSTRACT

India is known for its rich biodiversity and traditional healers have used flora to cure various human ailments. The same plants are still being used today for the benefit of mankind. Since the beginning of this century, there has been an increasing interest in the study of traditional uses of plants globally as natural products of plant origin is the most important resource for developing new drugs to treat various diseases. In the present study, *Murraya koenigii* has been evaluated for its antibacterial and antioxidative potential. The selection of the plant was based on the ethnobotanical data available on its traditional use. The extract of leaves and seeds in different solvents *viz.* acetone, chloroform, ethanol and methanol were examined for their antibacterial and antioxidant activities. The disc diffusion assay and MIC were used to determine antibacterial potential against four bacterial strains namely *Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus subtilis.* The different extracts were also assessed for their free radical scavenging activity using DPPH radical assay. Acetone leaf and seed extracts showed maximum activity against *S. aureus* and *K. pneumoniae,* respectively. However, methanolic leaf extract exhibited greatest free radical scavenging activity. As the leaf and the seed extracts of *Murraya koenigii* exhibited significant antibacterial and antioxidative properties, they could be exploited for their therapeutic potential.

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## 1. INTRODUCTION

Human beings have been utilizing plants for basic preventive and curative health care since time immemorial. As a matter of fact, the history of medicinal plants is as old as the history of civilization. In India, drugs of herbal origin have been used since ancient times in traditional systems of medicine such as Unani and Ayurveda. *Murraya koenigii* commonly known as curry leaf tree or Kari patta is a medicinally important herb of Indian origin and has been used for centuries in the Ayurvedic system of medicine [1]. Fourteen global species belongs to the genus *Murraya* and out of these only two *M. koenigii* and *M. paniculata* are available in India. *Murraya koenigii* is more popular than the latter due to its large spectrum of medicinal properties and also because of the use of its leaves for centuries as a natural flavouring agent in various curries and food items [2-6].

The plant has important phytochemicals which makes it a potential source of providing useful drugs for human use. However, systematic scientific studies are to be conducted for understanding the efficacy of whole plant or its parts for the treatment of various diseases. The therapeutic potential could be greatly affected by the solvent used due to extraction of the different phytocompounds which depends on the polarity of a particular solvent. So keeping this in view, the present study was planned to evaluate the antibacterial and antioxidant activities of leaf and seed extracts of the *Murraya koenigii* in different solvents.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material

Leaves and seeds of *Murraya koenigii* were collected and washed with tap water and then again washed with distilled water. The plant material was then air dried at room temperature for about 7 days. The dried material was further dried in oven at 30- 35℃ for about 2-3 days.

### 2.2 Extraction Procedure

The dried material of leaves and seeds was grinded separately with the help of a mixer grinder. The leaf and seed powder obtained was stored in air tight containers in refrigerator at 4℃. The powdered plant material was extracted using soxhlet apparatus in different solvents (acetone, chloroform, ethanol and methanol) (1:5 W/V) for 2-3 days. After extraction, the solvent was evaporated at 35℃ to 40℃ till thick slurry was obtained. The material was stored at 4℃ till further use.

### 2.3 Test Bacteria

The bacterial strains *Klebsiella pneumoniae (*MTCC NO 109)*, Staphylococcus aureus* (MTCC NO 96)*, Pseudomonas aeruginosa* (MTCC NO 2453) and *Bacillus subtilis* (MTCC NO 2057) were procured from IMTECH (Institute of microbial technology), Chandigarh. The bacterial culture was maintained on nutrient agar medium at 37℃.

### 2.4 Inoculum Preparation

Nutrient agar /broth (Himedia, India.) were used for the bacterial assays. Bacterial cultures were inoculated in 10 ml of sterile nutrient agar (NA) in culture tubes at 37℃ for 24 h. All the cultures were subcultured monthly and subsequently stored at 4℃.

### 2.5 Disc Diffusion Assay

Different concentration of the extracts (200 mg/ml, 100 mg/ml, 50 mg/ml and 25 mg/ml) were prepared by reconstituting the dried plant material with the respective solvents. The test bacterial strains were seeded into the respective medium in different petri plates by spread plate method 100 µl ($1\times10^6$ cells/ml) with the 24 h culture of bacterial growth in nutrient broth. After solidification of medium, whatman filter paper discs (6 mm in diameter) impregnated with the extracts of leaf and seed (100 µl each) were placed on test organism-seeded plates separately. Standard disc of ampicillin (20 µg/disc) and blank disc (impregnated with extraction solvents followed by evaporation) was used as positive and negative control, respectively. Petri Plates were incubated at 37℃ for 24 h for the growth of the microorganism to occur. The extracts having antimicrobial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized around the disc. The diameter of the inhibition zone obtained against the bacterial strains tested was measured after 24 h and noted down in

millimeter. The assay was performed in triplicate for each extract and the average zone of inhibition was recorded.

### 2.6 Determination of Minimal Inhibitory Concentration (MIC)

MIC was determined by micro broth dilution technique using serially diluted (2 fold) plant extract [7] with little modification. Equal volume of each extract and nutrient broth was mixed in separate wells of a micro titer plate 0.01 ml of standardized inoculum ($1\times 10^6$ cell/ml) and 0.01 ml of resazurin sodium salt indicator were added into each well. The plates were then incubated at 37℃ for 24 h in a B.O.D incubator. The positive control consisted of antibiotic (ampicillin), resazurin sodium salt indicator, broth media and bacterial inoculums on the other hand negative control used was broth media, resazurin sodium salt indicator and bacterial inoculum only. The lowest concentration (highest dilution) of the extract of leaves and seeds that showed no color change (purple to pink) as compared to the control was regarded as MIC for that particular bacterial strain.

### 2.7 Antioxidant Activity

The antioxidant activity of the extracts was investigated by DPPH (1, 1-diphenil-2 picrylhydrazyl) radical scavenging assay (Blois, 1958). The 2.5 ml of DPPH solution (0.5 mM) was added to 1 ml of different concentrations of plant extract (200 µg/ml, 400 µg/ml, 600 µg/ml, 800 µg/ml and 1000 µg/ml) and incubated in dark for 30 min. After incubation, the absorbance was measured at 517 nm. Ascorbic acid was used as standard. At least three repetitions were run for each assay. The scavenging activity of the extracts was calculated in % inhibition according to the formula given below:

$$\% \text{ Inhibition} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100$$

### 2.8 Statistical Analysis

In the present study, t-test was conducted to analyze the level of difference between leaf and seed extracts and One- way ANOVA was used to find out the level of difference among different leaf solvent extracts and seed solvent extracts (0.01 level of significance). Standard deviation was also determined for the zone of inhibition of different extracts against selected bacterial strains.

## 3. RESULTS

In the present investigation, leaf and seed extracts were prepared in acetone, chloroform, ethanol and methanol and evaluated for their antibacterial and antioxidative properties. The disc diffusion assay carried out with different concentrations of leaf extracts (Table 1) and seed extracts (Table 3) showed variable zone of inhibition against bacterial strains used in the study. The monitoring experiment for each extract was conducted thrice and the average of the three is presented in the table. It was also observed that the solvent had no inhibitory effect on any of the bacterial strains. Of the four leaf extracts, acetone and ethanolic extracts exhibited similar zone of inhibition (9 mm) against *Staphylococcus aureus.* Interestingly, the activity observed was even more than the positive control used i.e. ampicillin. These extracts did not exhibit any activity against any other bacterial strains used except for the acetone leaf extract which showed a zone of 6.3 mm against *Bacillus subtilis.* Methanolic and chloroform leaf extracts exhibited inhibitory effects only against *Pseudomonas aeruginosa* with a clear zone of 8.5 mm and 7.2 mm, respectively (Table 1). It is interesting to note that ampicillin was ineffective against these bacterial strains; thus the therapeutic potential of the plant is evident.

MIC was performed only with those extracts that exhibited significant antibacterial activity in disc diffusion assay. The MIC values of leaf extracts for different bacterial strains ranged between 0.62 mg/ml and 5.00 mg/ml (Table 2). The acetone leaf extract was found to be most effective against *Staphylococcus aureus* with MIC value of 0.62 mg/ml. However, MIC value of 5 mg/ml was obtained with *Bacillus subtilis.* The MIC value for *Staphylococcus aureus* was found to be more (1.25 mg/ml) with ethanolic leaf extract. The MIC values obtained with chloroform and methanolic leaf extract reflect that chloroform leaf extract was more effective to *Pseudomonas aeruginosa.*

When the seed extracts in different solvents were analyzed for their antibacterial potential, acetone seed extract exhibited activity against three bacterial strains - *Staphylococcus aureus, Klebsiella pneumoniae* and *Bacillus subtilis.* The maximum activity was shown against *Klebsiella pneumoniae* with 10 mm zone of inhibition (Table 3). Methanolic seed extract showed activity against *Bacillus subtilis* (9 mm zone of

inhibition) and *Staphylococcus aureus* (7.5 mm zone of inhibition). Ethanolic and chloroform seed extracts were effective against *Staphylococcus aureus* and *Bacillus subtilis,* respectively.

The micro broth dilution technique was used to find out the MIC values for different bacterial srains and it was observed that the values varied from 1.25 mg/ml to 5.00 mg/ml of the extracts in different solvents (Table 4). MIC value obtained for *Klebsiella pneumoniae* and *Staphylococcus aureus* was 1.25 mg/ml whereas the value was high (2.5 mg/ml) for *Bacillus subtilis.* However, MIC value obtained for *Staphylococcus aureus* with methanolic and ethanolic seed extract was similar (5.00 mg/ml).

The scavenging activity of the leaves and seeds extracts was investigated through the DPPH radicals. The results for scavenging activity of different extracts are represented graphically (Figs. 1 and 2) and $IC_{50}$ values obtained are represented in a tabular form (Table 5). When the activity of all the extracts was analysed, it was found that methanolic seed extract showed the highest radical scavenging activity with the lowest $IC_{50}$ value of 0.43 mg/ml followed by the methanolic leaf extract and ethanolic seed extract with an $IC_{50}$ value of 0.45 mg/ml. Leaf extract in acetone and ethanol and seed extract in chloroform also exhibited activity with the $IC_{50}$ values of 0.58, 0.62 and 0.79 mg/ml, respectively. Leaf extract in chloroform and seed extract in acetone showed less activity as compared to other extracts.

No significant difference was observed between leaf and seed extracts at 0.01 level of significance. Significant difference was observed among different solvent extracts of both leaf and seed at 0.01 level of significance.

**Table 1. Zone of inhibition of leaf extracts against selected bacterial strains**

| Leaf extracts | Microorganisms | Zone of inhibition (in mm) against different concentration (mg/ml) | | | | |
|---|---|---|---|---|---|---|
| | | 200 | 100 | 50 | 25 | Standard drug (ampicillin) |
| Acetone | *Klebsiella pneumoniae* | - | - | - | - | - |
| | *Staphylococcus aureus* | 9±0.66 | 8.6±1.3 | 7.3±0.42 | 6.9±0.54 | 12±0.86 |
| | *Pseudomonas auregiuosa* | - | - | - | - | - |
| | *Bacillus subtilis* | 6.3±0.42 | 6.1±0.54 | - | - | - |
| Chloroform | *Klebsiella pneumoniae* | - | - | - | - | - |
| | *Staphylococcus aureus* | - | - | - | - | 12±0.86 |
| | *Pseudomonas auregiuosa* | 7.2±0.24 | 7.0±0.67 | 6.8±0.42 | 6.3±0.42 | - |
| | *Bacillus subtilis* | - | - | - | - | - |
| Ethanol | *Klebsiella pneumoniae* | - | - | - | - | - |
| | *Staphylococcus aureus* | 8.9±0.43 | 8.1±0.24 | 7.3±0.17 | 6.9±0.11 | 12±0.86 |
| | *Pseudomonas auregiuosa* | - | - | - | - | - |
| | *Bacillus subtilis* | - | - | - | - | - |
| Methanol | *Klebsiella pneumoniae* | - | - | - | - | - |
| | *Staphylococcus aureus* | - | - | - | - | 12±0.86 |
| | *Pseudomonas auregiuosa* | 8.5±1.3 | 7.0±0.66 | 6.6±0.24 | 6.2±0.12 | - |
| | *Bacillus subtilis* | - | - | - | - | - |

**Table 2. MIC values for different bacterial strains with leaf extracts of *Murraya koenigii***

| Extract | Microorganism | MIC (mg/ml) |
|---|---|---|
| Acetone | *Staphylococcus aureus* | 0.62 |
| Acetone | *Bacillus subtilis* | 5.00 |
| Chloroform | *Pseudomonas auregiuosa* | 1.25 |
| Ethanol | *Staphylococcus aureus* | 1.25 |
| Methanol | *Pseudomonas auregiuosa* | 5.00 |

**Table 3. Zone of inhibition obtained with seed extracts against selected bacterial strains**

| Seed extracts | Microorganisms | Zone of inhibition (in mm) against different concentration (mg/ml) | | | | |
|---|---|---|---|---|---|---|
| | | 200 | 100 | 50 | 25 | Standard drug (ampicillin) |
| Acetone | *Klebsiella pneumoniae* | 10±0.32 | 7±0.24 | - | - | - |
| | *Staphylococcus aureus* | 8±0.66 | 7±0.10 | 6.5±0.10 | - | 12±0.86 |
| | *Pseudomonas auregiuosa* | - | - | - | - | - |
| | *Bacillus subtilis* | 8.5±1.4 | 7.5±0.67 | 6.5±0.11 | 6.2±0.03 | - |
| Chloroform | *Klebsiella pneumoniae* | - | - | - | - | - |
| | *Staphylococcus aureus* | - | - | - | - | 12±0.86 |
| | *Pseudomonas auregiuosa* | - | - | - | - | - |
| | *Bacillus subtilis* | 7.0±0.10 | - | - | - | - |
| Ethanol | *Klebsiella pneumoniae* | - | - | - | - | - |
| | *Staphylococcus aureus* | 7.0±0.96 | 7.0±0.10 | 6.5±0.11 | 6.2±0.02 | 12±0.86 |
| | *Pseudomonas auregiuosa* | - | - | - | - | - |
| | *Bacillus subtilis* | - | - | - | - | - |
| Methanol | *Klebsiella pneumoniae* | - | - | - | - | - |
| | *Staphylococcus aureus* | 7.0±0.03 | 6.5±0.32 | - | - | 12±0.86 |
| | *Pseudomonas auregiuosa* | - | - | - | - | - |
| | *Bacillus subtilis* | - | - | - | - | - |

**Table 4. MIC values for different bacterial strains with seed extracts of *Murraya koenigii***

| Seed extracts | Microorganism | MIC (mg/ml) |
|---|---|---|
| Acetone | *Klebsiella pneumoniae* | 1.25 |
| | *Bacillus subtilis* | 2.50 |
| | *Staphylococcus aureus* | 1.25 |
| Methanol | *Bacillus subtilis* | 1.25 |
| | *Staphylococcus aureus* | 5.0 |
| Ethanol | *Staphylococcus aureus* | 5.0 |
| Chloroform | *Bacillus subtilis* | 5.0 |

**Table 5. $IC_{50}$ values (mg/ml) of ascorbic acid and extracts of leaves and seeds in different solvents**

| Extract | IC50 value (mg/ml) |
|---|---|
| Acetone leaf extract | 0.58 |
| Chloroform leaf extract | 1.56 |
| Ethanolic leaf extract | 0.62 |
| Methanolic leaf extract | 0.45 |
| Acetone seed extract | 1.78 |
| Chloroform seed extract | 0.79 |
| Ethanolic seed extract | 0.45 |
| Methanolic seed extract | 0.43 |
| Ascorbic acid | 0.38 |

## 4. DISCUSSION

In the present investigation, acetone and methanol were found to be better solvent as compare to other two solvents. Acetone seed extract was found most effective against *K. pneumoniae* where as acetone leaf extract showed significant activity against *S. aureus*. *B. subtilis* was significantly inhibited by methanolic seed extract. Methanolic leaf extract was also found effective against *P. auregiuosa.*

The extraction of the different compounds depends upon the polarity of a particular solvent used. Some compounds are soluble in methanol whereas others are in ethanol, chloroform and acetone. Our result showed acetonic and methanolic extracts to be more effective probably due to the extraction of more bioactive compounds in them. This could also explain the variations observed in the antibacterial activity of the extracts prepared in different solvents.

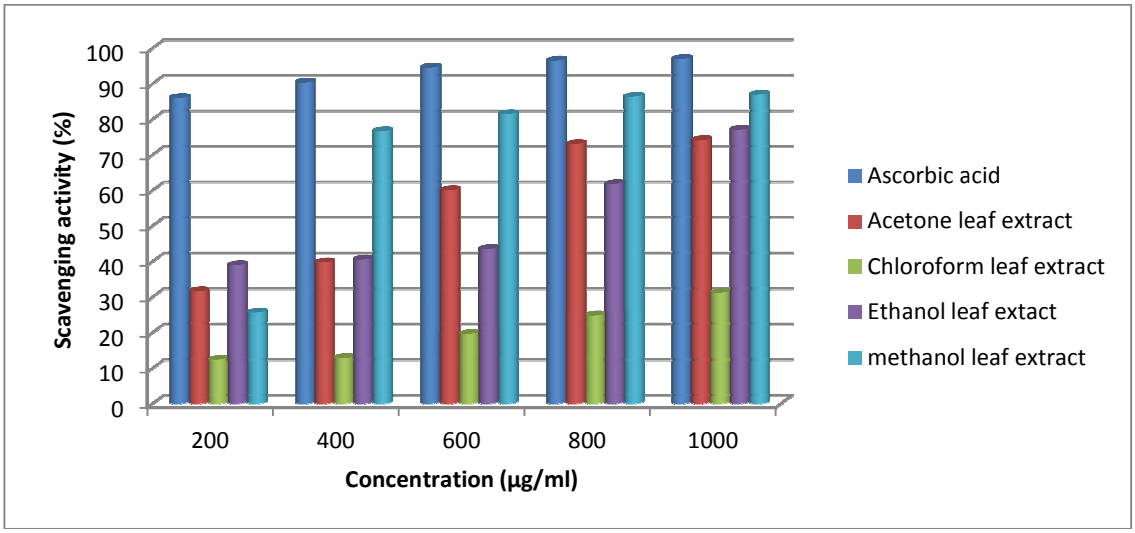


**Fig. 1. Scavenging activity of ascorbic acid and leaf extracts in different solvents**

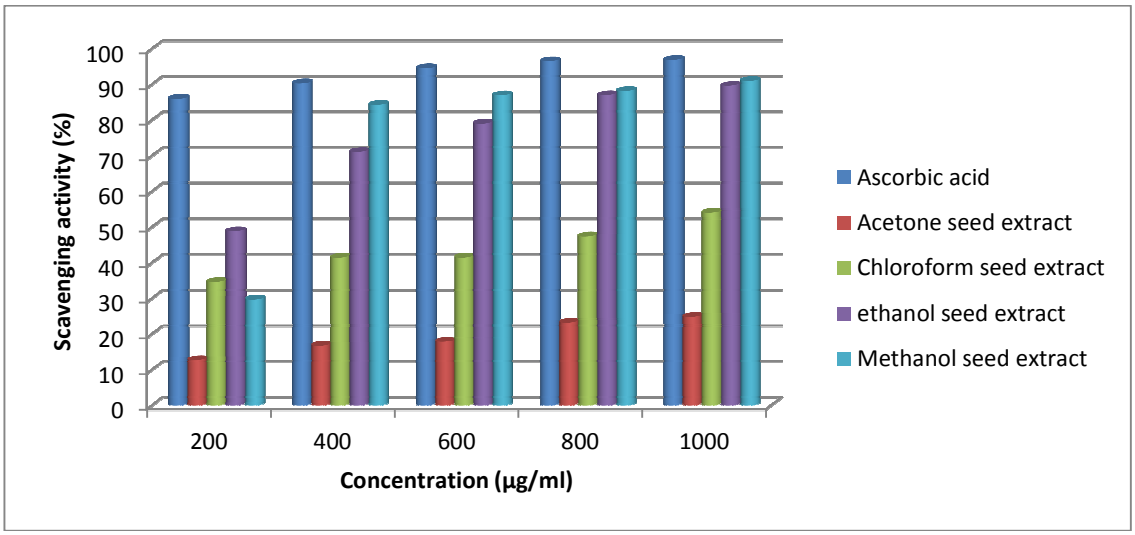


**Fig. 2. Scavenging activity of ascorbic acid and seed extracts in different solvents**

Studies carried out by other investigators have also shown the therapeutic potential of this plant. Akerel et al. [8] showed that crude extract and chloroform soluble fraction and petroleum ether soluble fraction of this plant showed a promising antibacterial activity. The antibacterial studies conducted by using methanolic extract also confirmed its effectiveness against *Salmonella typhi* and *Eschrichia coli* at 100 µg/ml and 200 µg/ml, respectively (Tanaka et al., 1998). Ajay et al. [9] showed antibacterial effect of essential oil from *M. koenigii* leaves against *B. subtilis, S. aureus, C. pyogenes, P. vulgaris, Pasteurella multicida* [9]. Upadhye et al. (2010) revealed that the extracts of *Murraya koenigii* roots are effective against *P. aeruginosa*, *E. coli* and *Salmonella abony* [10].

The ability of the extracts to inhibit the growth of several bacterial strains is an indication of the broad spectrum antimicrobial potential of various parts of *Murraya koenigii*, which makes the plant suitable for bioprospecting for novel antibiotic drugs. However, the quality and the quantity of the secondary metabolites of plants are function of geographical conditions and of the origin of the plant.

The leaves and seeds of *Murraya koenigii* also possess a very good antioxidant activity.

Methanolic extract of leaves and seeds showed significant antioxidant activity followed by ethanolic seed extract. However, Tomar [11] showed ethanolic leaf extract to possess better anti-oxidant activity in comparison with other extracts (chloroform and petroleum ether).

## 5. CONCLUSION

The present study on *Murraya koenigii* revealed that various extracts from leaf and seed exhibits antibacterial properties. Acetone leaf and seed extracts were found to be most effective against *S. aureus* and *K. pneumonia,* respectively. However, methanol leaf and seed extracts showed significant activity against *P. auregiuosa* and *B. subtilis,* respectively. Methanolic seed extract showed the highest radical scavenging activity followed by the methanolic leaf extract and ethanolic seed extract. The study carried out showed the potential of *M. koiengii* as a natural source of antibacterial and antioxidant bioactive compounds that can be used as additive in food and pharmaceutical industries. It can also be utilized for the development of new phytodrugs but only after investigation of its bioactivity, mechanism of action, pharmacotherapeutics and toxicity.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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