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## Evaluation of the Antimicrobial Potential of *Caralluma fimbriata* Stem Extracts

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**Abstract:**

Medicinal plants have long been recognized as valuable sources of bioactive compounds with significant therapeutic potential. The present study aimed to evaluate the antimicrobial activity of stem extracts of Caralluma fimbriata against selected fungal and bacterial pathogens. Shade-dried stems of C. fimbriata were extracted successively using different solvents, including hexane, ethyl acetate, petroleum ether, and methanol. The antimicrobial activity of the crude extracts was assessed using the disc diffusion method. Antifungal activity was evaluated against Aspergillus niger, Microsporum canis, and Fusarium oxysporum, while antibacterial activity was assessed against Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae, and Escherichia coli. Among the tested extracts, the methanolic extract exhibited the highest antimicrobial activity. The methanol extract showed maximum antifungal activity against Aspergillus niger with a zone of inhibition of 3.8 ± 0.115 cm, followed by Microsporum canis (2.9 ± 0.176 cm) and Fusarium oxysporum (1.7 ± 0.088 cm). Similarly, the methanol extract demonstrated strong antibacterial activity against Pseudomonas aeruginosa with a zone of inhibition of 5.1 ± 0.132 cm, followed by Proteus mirabilis (4.7 ± 0.099 cm), Klebsiella pneumoniae (3.9 ± 0.043 cm), and Escherichia coli (3.2 ± 0.085 cm). The results indicate that Caralluma fimbriata possesses potent antimicrobial properties and may serve as a promising natural source for the development of antimicrobial agents.

**Keywords:** Caralluma fimbriata, Antimicrobial Activity, Antifungal Activity, Antibacterial Activity, Methanolic Extract

## Introduction:

The emergence of antimicrobial resistance among pathogenic microorganisms has become a major global health concern. The increasing resistance of bacteria and fungi to conventional antimicrobial agents has necessitated the search for novel therapeutic alternatives from natural sources. Medicinal plants have been extensively investigated as reservoirs of bioactive compounds possessing antimicrobial, antioxidant, anti-inflammatory, and anticancer properties.

Caralluma fimbriata is a succulent medicinal plant belonging to the family Apocynaceae (formerly Asclepiadaceae). It has been

traditionally used in various indigenous systems of medicine for the treatment of diabetes, obesity, inflammation, and gastrointestinal disorders. The plant contains several bioactive phytoconstituents, including pregnane glycosides, flavonoids, saponins, and phenolic compounds, which contribute to its therapeutic potential.

Although C. fimbriata has been widely studied for its anti-obesity and antidiabetic activities, limited information is available regarding its antimicrobial properties. Therefore, the present study was undertaken to investigate the antifungal and antibacterial activities of stem extracts of Caralluma fimbriata prepared using different solvent systems. The antimicrobial efficacy of the extracts was evaluated against selected fungal and bacterial pathogens using the disc diffusion method.

**Collection of plant material and preparation**

The plant samples were collected from Rajasthan. Fresh samples of Caralluma fimbriata were chopped into small pieces and dried in shade on laboratory benches. The dried samples were then ground into a powder using a commercial heavy-duty blender. The powdered samples were stored in sealed plastic bags, which were further kept inside paper sample bags and stored in cupboards at room temperature until use.

**Extraction**

To evaluate the medicinal importance of the plant, the shade-dried stems of Caralluma fimbriata were powdered to a fine consistency (40-mesh size) using a pulverizer. Subsequently, 100 g of the powdered material was packed in filter paper and successively extracted with ethyl acetate followed by methanol using a Soxhlet extractor for 8–10 hours each. The crude extracts obtained were concentrated using a rotary evaporator, and the resultant extracts were used for further studies.

**Antimicrobial studies**

i. **Antifungal assay**

The antifungal activity of the stem extract residues was tested against fungal strains, namely Microsporum canis, Fusarium oxysporum, and Aspergillus niger. All test organisms were obtained as gift cultures from Dr. Agarwal's Eye Hospital, Chennai. Aspergillus niger was selected as the test organism for determining antifungal activity.

The disc diffusion method was employed for the antifungal assay. Sterile paper discs (HiMedia, SD067; 6 mm diameter) were impregnated with the extract fraction residues dissolved in ethanol to obtain a concentration of 700 µg/disc.

The test fungi were maintained on Potato Dextrose Agar (PDA) plates. The composition of PDA medium is given below.

**Potato Dextrose Agar (PDA) Medium**

| Component | Quantity |
|---|---|
| Potato infusion (from 200 g potatoes) | As required |
| Dextrose | 20.0 g |
| Agar | 18.0 g |
| Sterile distilled water | 1000 mL |

The prepared medium was sterilized by autoclaving at 121°C and 1.1 kg/cm² pressure and subsequently used. PDA plates were seeded with actively growing fungal cultures using a sterile cotton swab.

ii. **Antibacterial assay**

Clinical isolates of Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Proteus mirabilis were obtained from Dr. Agarwal's Eye Hospital, Chennai, and used as test organisms. For the assay of fraction residues, Pseudomonas aeruginosa was selected as the test organism.

The bacterial cultures were subcultured and routinely maintained on both Nutrient Agar and Mueller–Hinton Agar media. However, Mueller–Hinton Agar was used for inoculum preparation and antibacterial activity studies.

**Nutrient Agar**

| Component | Quantity |
|---|---|
| Beef Extract | 3.0 g |
| Peptone | 5.0 g |
| Sodium Chloride | 5.0 g |
| Agar | 15.0 g |
| Final pH | 7.2 |

The above ingredients were dissolved in 1.0 litre of distilled water and sterilized at 121°C (1.1 kg/cm² pressure) for 15 minutes.

**Mueller–Hinton Agar**

The medium contained the following ingredients per 1.0 litre of water:

| Component | Quantity |
|---|---|
| Casein Hydrolysate (Enzymic) | 17.5 g |
| Beef Infusion | 30.0 g |
| Soluble Starch | 1.5 g |
| Agar | 20.0 g |

The final pH of the medium was adjusted to 7.4 ± 0.2 (at 25°C). The medium was sterilized at 121°C under 1.1 kg/cm² pressure for 15 minutes before use.

## Results and discussion

### Antimicrobial properties of crude solvent extract of caralluma fimbriata

### Antifungal activity

Four different solvent systems were used in the crude extract preparation of C. fimbriata. Extract residues of the sample obtained with these solvent systems were dried and redissolved in ethanol and used at a concentration of 700 µg/disc.

In the preliminary experiments, Aspergillus niger alone was used as the test pathogen. Methanol extract of C. fimbriata exhibited maximum activity with an inhibition zone (diameter) of 3.8 cm (Table 4). The petroleum ether extract of C. fimbriata exhibited only 65% or less than 65% activity against the pathogen as compared to the maximum activity.

The ethanol extracts of C. fimbriata exhibited only 40% or less than 40% activity against the test organism. Antifungal activity of the hexane extract of C. fimbriata was very poor.

Based on these observations, further studies were restricted to the methanol extract of C. fimbriata. The methanol extract of Caralluma fimbriata was active against Aspergillus niger as compared to other test

organisms (Table 5 & Plate I). Antifungal activity of the stem extract was 76.3% against Microsporum canis and 44.7% against Fusarium oxysporum. There is no effect in the control.

**Table 4: Antifungal Activity of the Crude Solvent Extracts of C. fimbriata**

| S. No. | Solvent Extract | Antifungal Activity of Caralluma fimbriata (% Maximum Activity) |
|---|---|---|
| 1 | Hexane | 5 |
| 2 | Ethyl Acetate | 40 |
| 3 | Petroleum Ether | 65 |
| 4 | Methanol | 100 |

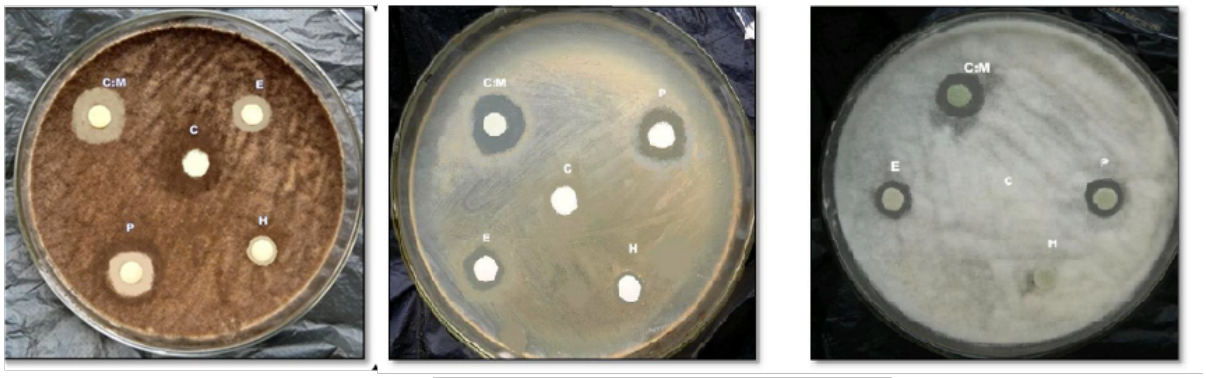
Extract residue dissolved in 1.0 mL ethanol, loaded on paper discs (700 µg residue/disc), and tested on plates seeded with Aspergillus niger. Maximum activity observed was 3.8 cm (for Caralluma fimbriata extract).

**Table 5. Antifungal Activity of the Crude Methanol Extract of C. fimbriata**

| S. No. | Test Fungus | Zone of Inhibition (cm) ± S.E. |
|---|---|---|
| 1 | Aspergillus niger | 3.8 ± 0.115 (100) |
| 2 | Microsporum canis | 2.9 ± 0.176 (76.3) |
| 3 | Fusarium oxysporum | 1.7 ± 0.088 (44.7) |
| 4 | Methanol | 100 |

Extract residues of the stem extract were used at a concentration of 700 µg residue/disc and tested on plates seeded with the respective test fungus. Percentage maximum activities (%) are given in parentheses. Results are expressed as the mean of ten replicates.

No inhibitory effect was observed in the control group.




*ASPERGILLUS NIGER* *MICROSPORUM CANIS* *FUSARIAM OXYSPORUM*

H-Hexane, M-Methanol, E-Ethyl acetate, P-Petroleum Ether, C-Control

Plate I. Antifungal Activity of the Crude Solvent Extract Residue of the Stem Extract

**Antibacterial activity**

The antibacterial activity of the crude solvent extracts of the stem sample was evaluated using the same four solvent

systems employed for the antifungal assay, namely hexane, ethyl acetate, methanol, and petroleum ether. Pseudomonas aeruginosa was used as the test organism (Table 6).

In this preliminary investigation, the stem extract prepared using methanol proved to be more effective than the other solvent systems in inhibiting the growth of Pseudomonas aeruginosa on Mueller–Hinton agar plates.

The petroleum ether extract exhibited only 76% of the maximum activity against the test organism (Table 6), whereas the methanol extract showed the maximum antibacterial activity. The ethyl acetate extract exhibited approximately 45% of the maximum activity against the test organism (Table 6).

The hexane extract appeared to be least effective in inhibiting the growth of Pseudomonas aeruginosa.

Based on these observations, further experiments on the antibacterial activity of the stem extract were carried out using the methanolic extract.

**Table 6. Antibacterial Activity of the Crude Solvent Extracts of C. fimbriata.**

| S. No. | Test Bacteria | Zone of Inhibition (cm) ± S.E. |
|---|---|---|
| 1 | Pseudomonas aeruginosa | 5.1 ± 0.132 (100) |
| 2 | Proteus mirabilis | 4.7 ± 0.099 (92) |
| 3 | Klebsiella pneumoniae | 3.9 ± 0.043 (76) |
| 4 | Escherichia coli | 3.2 ± 0.085 (62) |

Extract residue dissolved in 100 mL ethanol, loaded on paper discs (700 µg residue/disc), and tested on seeded plates.

Maximum activity (zone of inhibition) against Pseudomonas aeruginosa was 5.1 cm for the Caralluma fimbriata extract.

The methanol extract of C. fimbriata was prepared as described earlier and tested at a concentration of 700 µg/disc by the disc diffusion method against four pathogenic bacteria, namely Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Escherichia coli. The results are presented in Table 7 and Plate II. The extract residues of the stem sample recorded maximum activity against Pseudomonas aeruginosa with an inhibition zone of 5.1 cm for Caralluma fimbriata. Proteus mirabilis, Klebsiella pneumoniae, and Escherichia coli were also effectively inhibited by the extract residues of the stem sample (Plate II). Against these organisms, the extract residue exhibited 62% to 92% of the maximum activity (Table 7).

There was no effect in the control group.

**Table 7. Antibacterial Activity of the Methanol Extract Residue of the Sample.**

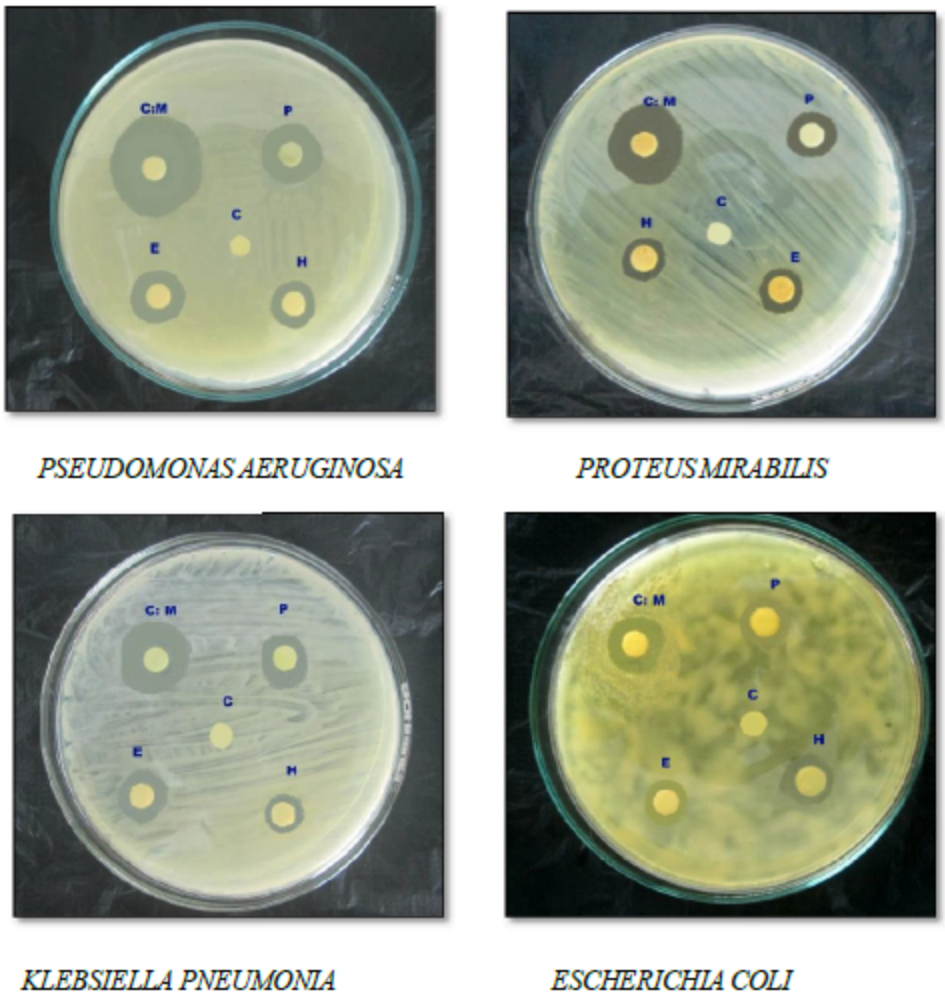
| S. No. | Solvent Used for Extraction | Antibacterial Activity (% Maximum Activity) |
|---|---|---|
| 1 | Hexane | 15 |
| 2 | Ethanol | 45 |
| 3 | Petroleum Ether | 76 |
| 4 | Chloroform : Methanol (2:1) | 100 |

700 µg extract residue/disc was used in the assay.

Values given in parentheses indicate the percentage of maximum activity (% maximum activity).

Results are expressed as the mean of ten replicates.

No inhibitory effect was observed in the control group.




*PSEUDOMONAS AERUGINOSA* *PROTEUS MIRABILIS*

*KLEBSIELLA PNEUMONIA* *ESCHERICHIA COLI*

H-Hexane, M-Methanol, E-Ethyl acetate, P-Petroleum Ether, C-Control

Plate II. Antibacterial Activity of the Crude Solvent Extract Residue of the Stem Sample

**Conclusion**

The present investigation demonstrated that the stem extracts of Caralluma fimbriata possess significant antimicrobial activity against a range of fungal and bacterial pathogens. Among the various solvent extracts evaluated, the methanolic extract exhibited the highest antifungal and antibacterial activities, indicating that methanol was the most effective solvent for extracting antimicrobial constituents from the plant material.

The methanol extract showed maximum inhibition against Aspergillus niger among the fungal species tested and against

Pseudomonas aeruginosa among the bacterial species tested. The observed antimicrobial activity may be attributed to the presence of bioactive phytochemicals such as flavonoids, phenolics, glycosides, and other secondary metabolites present in the plant.

The findings suggest that Caralluma fimbriata represents a promising natural source of antimicrobial compounds and may be useful for the development of alternative therapeutic agents against microbial infections. Further studies involving phytochemical characterization, isolation of active constituents, and in vivo evaluation are warranted to establish its therapeutic potential.

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