Antimicrobial Properties Of Crude Solvent Extract Of *Caralluma Fimbriata*

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ABSTRACT

The phytochemistry of the genus *Caralluma* is characterized by many pregnane glycosides. Other chemical constituent s include flavones, glycosides, megastigmane glycosides, saponins and several flavonoids. The appetite suppressing pr operties of *C. fimbriata* could be attributed to the pregnane glycosides, which are present in the plant species belongin g to the Apocynaceae family (Kunert *et al.,* 2008). Eleven pregnane glycosides have been isolated from the plant extra ct from which, four (compounds 10–13) contain a novel genin.

Among the great variety of secondary compounds found in plants, phenolics and terpenoids represent the main antimic robial agents currently known. Other compounds such as alkaloids, flavonoids, tannins and coumarin have been identif ied as antimicrobial agents.

Antifungal activity of the stem sample extract residues were tested against fungi namely, *Microsporum canis, Fusariu m oxysporum* and *Aspergillus niger.* Disc diffusion method was adopted for antifungal assay and bioassay and Muller/Hinton agar plates used for antibacterial assay.

KEYWORDS: Caralluma fimbriata, Caralluma Antifungal, Bioassay, Antibacterial Assay, Edible Cactus

INTRODUCTION

Caralluma fimbriata is such a promising and potential plant on the aspect of ethnobotany, phyto chemistry and pharma cology (Priya *et al.*, 2012).

Caralluma, a cactus plant belongs to family Asclepiadaceae is a succulent, perennial herb, grow to a height of 1 to 10 f t and grow in different regions of India. The members of genus *Caralluma* are erect and fleshy. They have quadrangul ar stem, devoid of leaves and small flowers in several varieties of dark colour. The species of *Caralluma* found in Indi a are edible and form a part of traditional medical system of country (Al,Yaha , 2000). There are six different species o f *Caralluma* they are, *Boucerosea lasiantha (BL), Caralluma adscendens var.annuata (CAA), Caralluma stalagmifera (CS), Caralluma longipetale (CL), Caralluma adscendens varfimbriata (CAF), Boucerosea umbellate (UMB L)* distributed in peninsular India. This succulent Cactus contain glycosides, hydrocarbons, saponins as major phytoco nstituents and reported for various biological activities such as rheumatism, diabetes, leprosy, antinociceptive, antipyre tic, antihelminthic, antiobese activities. (Rao *et al.*,1998; Jadge *et al.*,2009; Zakaria *et al.,* 2001; Venkatesh *et al*.,2003; Wadood *et al.,1999*; Lawrence *et al.*,2004; Abdel,Sattar *et al.*, 2002). To attract the Pharmaceutical companies, resear chers elucidate the bioefficacies of phytomedicines of the potencial medicinal herbs.

ANTIMICROBIAL STUDIES ANTIFUNGAL ASSAY

Antifungal activity of the stem sample extract residues were tested against fungi namely, *Microsporum canis, Fusariu m oxysporum* and *Aspergillus niger.* Disc diffusion method was adopted for antifungal assay.

The test fungi were maintained on Potato Dextrose Agar (PDA) plates.

ANTIBACTERIAL ASSAY

Clinical isolates of *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Proteus mirabilis* were gift ed by Dr Agarwal's eye Hospital, Chennai, used as the test organisms. For the assay of fraction residues, *Pseudomona s aeruginosa* was used as the test organism.

BIOASSAY

Disc diffusion method (Bauer *et al.,* 1996) was adopted for the determination of antibacterial activity of the extract resi dues. From the stock cultures of various test organisms, inoculumwas prepared by subculturing each of the organisms on Muller/Hinton agar at 37ºC. Seeding of Muller/Hinton agar plates was done using the 24 hr culture with a cotton s wab under aseptic conditions. The discs loaded with extract residues were aseptically placed on top of the seeded medi um and gently pressed to ensure contact. The plates were then incubated at 37ºC. After overnight incubation, the plates were observed for zones of inhibition. Commercial antibiotics *viz.* Tetracycline and Amoxycillin were also tested in a similar manner.

EXPERIMENTAL RESULTS

1. 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 □g/disc. In the preliminary experiments, *Aspergillus niger* alone was used as test pathogen. Methanol extract of *C. fimbriata* ex hibited maximum activity with an inhibition zone (diameter) of 3.8 cm (Table 1). The petroleum ether extract of *C. fi mbriata* exhibited only 65% or less than 65% activity against the pathogen as compared to maximum activity. The etha nol extracts of *C. fimbriata* exhibited exhibit 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 observation further studies were restricted to methanol extract of *C. fimbriata*. The Methanol extract of *Caralluma fimbriata* was active against *Aspergillus niger* as compared to other test organism (Table 2 & Plate I). Antifungal activity of the stem extract was 76.3% against *Micr osporum canis* and 44.7% in *Fusarium oxysporum.* There is no effect in control.

Table 1: Antifungal activity of the crude solvent extracts of *C .fimbriata*

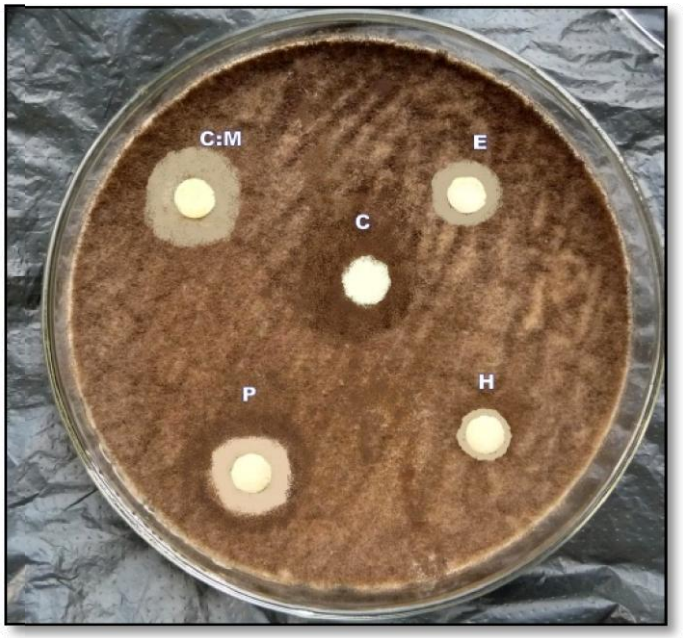
S. No	**Solvent extract**	**Antifungal activity *Caralluma fimbriata* (% Maximum activity)**
1.	Hexane	5
2.	Ethyl acetate	40
3.	Petroleum Ether	65
4.	Methanol	100

- Extract residue dissolves in 1.0 ml ethanol, loaded on paper disc (700 □g residue/ disc) and tested on plates seeded with *Aspergillus niger*.
- Maximum activities were 3.8 cm (for *Caralluma fimbriata* extract).

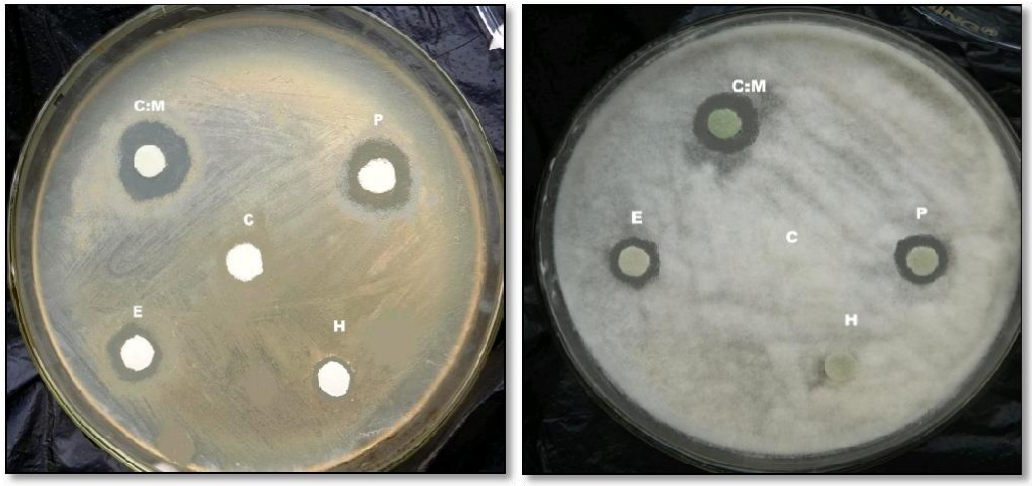
Table 2: Antifungal activity of the crude Methanol extracts of *C. fimbriata*

	Test fungus	**Zone of inhibition of *Caralluma fimbriata* (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)

- Extract residues of the stem extract were used at a concentration of 700 □g residue/ disc and tested on plates seeded with test fungus.
- % Maximum activities are given in parentheses.
- Results are average of ten replicates.
- No effect in control.



ASPERGILLUS NIGER



MICROSPORUM CANIS ***FUSARIAM OXYSPORUM***

H-Hexane **M- Methanol**

E- Ethyl acetate **P-Petroleum Ether**

C-Control

[Plate I:AntifungalActivity of The Crude Solvent Extract Residue of The stem extract]

2. ANTIBACTERIAL ACTIVITY

The antibacterial activity of the crude solvent extracts of the stem sample, the same four solvent systems used for anti fungal assay namely, hexane, ethyl acetate, methanol and petroleum ether. The bacteria *Pseudomonas aeruginosa* were used as test organism (Table.3). In this preliminary investigation, the leaves extract prepared with a mixture of methan ol proved to be more effective than the other solvent system used in inhibiting the growth of *Pseudomonas aeruginosa* on Muller/Hinton agar plates. Petroleum ether exhibit only 76% maximum activity against the test organism (Table.3). While in methanol of the leaves extract were able to exhibit only 45% maximum activity against the test organism (T able.3). The hexane extract appeared to be poor growth of *Pseudomonas aeruginosa*. Based on these observations, furt her experiments on the antibacterial activities of the stem extract were carried out to methanol extracts.

Table 3: Antibacterial activity of the crude solvent extracts of *C. fimbriata*

S.No	Test bacteria	Zone of inhibition of *Caralluma fimbriata* (cm) □ S.E.
1.	*Pseudomonas aeruginosa*	5.1 □ 0.132 (100)
2.	*Proteus mirabilis*	4.7 □ 0.099 (92)
3.	*Klebsiella pneumonia*	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 □g residue/ disc) and tested on seeded plates.
- Maximum activities (zone of inhibition) for *Pseudomonas aeruginosa* were 5.1 cm (for *Caralluma fimbriata* extract).

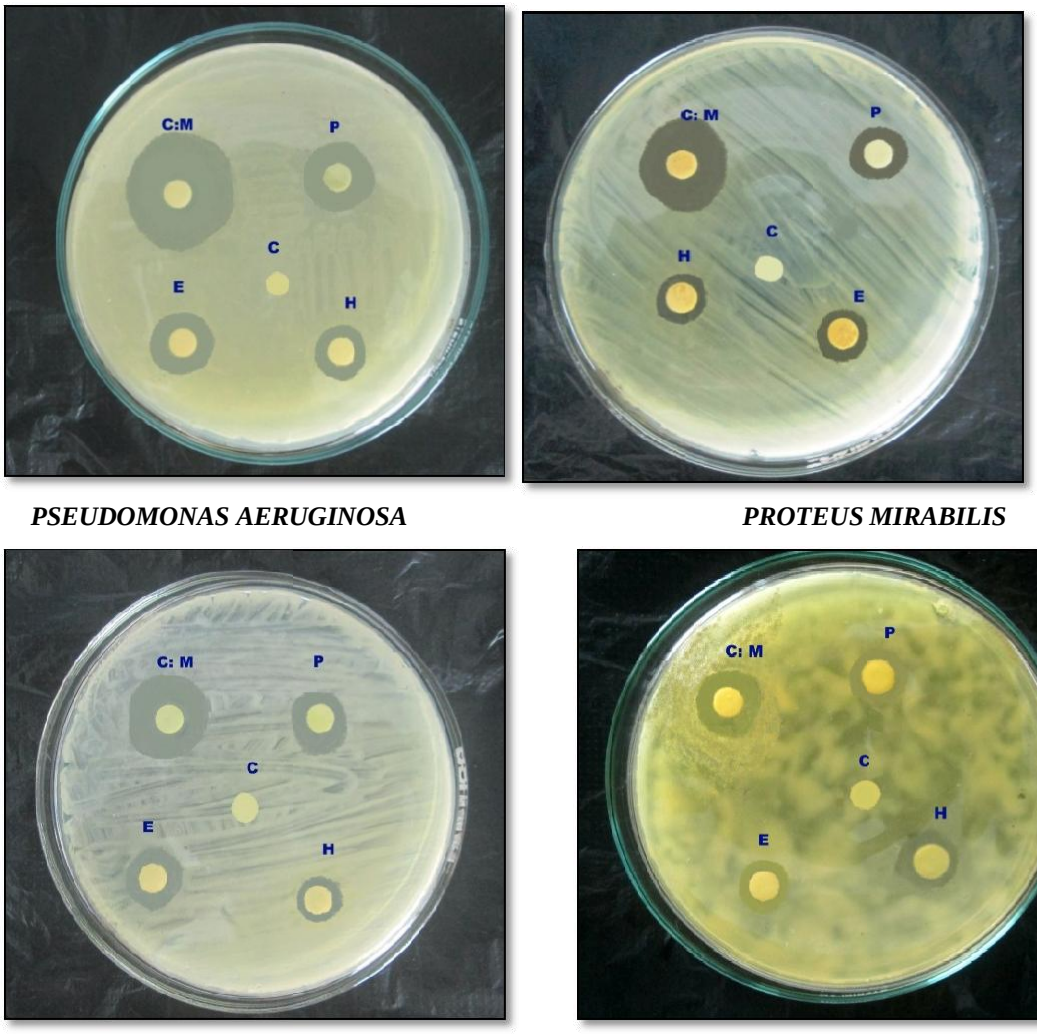
The methanol extract of *C. fimbriata* was prepared as described earlier and testes at a concentration of 700 □g/disc by disc diffusion method against four pathogenic bacteria namely, *Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumonia and Escherichia coli*. The results are presented in Table 7 & Plates II. The extract residues of stem sample recorded maximum activity against *Pseudomonas aeruginosa* with an inhibition zone of 5.1 cm for *Caralluma fimbri ata*. *Proteus mirabilis, Klebsiella pneumonia* and *Escherichia coli* were also effectively inhibited by the extract residue s of the leaves sample plate II) against this organism the extract residue of sample exhibited 62% to 92% of maximum activity (Table 4). There is no effect in control.

Table 4: 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 □g extract residue/ disc was used in the assay.
- Values given in parentheses indicate % maximum activity.
- Results are average of ten replicates

- No Effect in control.



PSEUDOMONAS AERUGINOSA ***PROTEUS MIRABILIS***

KLEBSIELLA PNEUMONIA ***ESCHERICHIA COLI***

H- Hexane **M- Methanol**

E- Ethyl acetate **P-Petroleum EtherC-Control**

Plate II: Antibacterial activity of the crude solvent extract residue of the stem sample

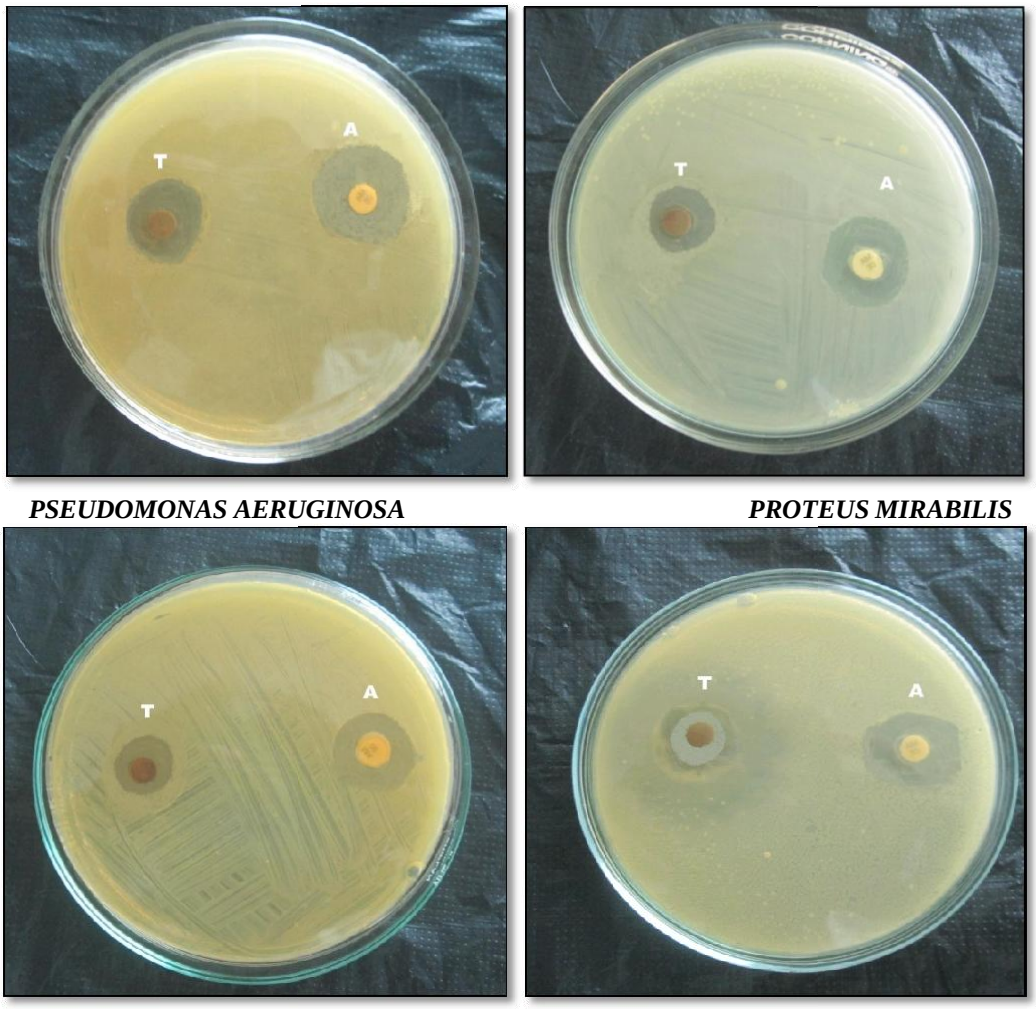
2.1. BIOASSAY

The antibacterial activities of the methanol extract of the *C. fimbriata* sample against the four pathogenic bacteria were compared with the standard antibiotics (Tables 5 & Plates III). Effect of the extracts in *Pseudomonas aeruginosa, Kle bsiella pneumonia, Proteus mirabilis* and *Escherichia coli* used more than 139% to 188% of the maximum activity obt ained with some of the standard antibiotics. Thus methanol extract of *C. fimbriata* appeared to be effective in antibacte rial principles(s) as indicated by the inhibition of the growth of the test bacteria pathogens in the agar diffusion method

Table 5: A comparison of the antibacterial activity of the crude methanol) extract residue of *Caralluma fimbriat a* with standard antibiotics

S. No	Test bacteria	Width of inhibition zone in cm		
		Caralluma fimbriata	Tetracycline	Amoxycillin
1.	*Pseudomonas aeruginosa*	5.1 □0.132 (144)	2.5 □ 0.064 (69.4)	3.6 □ 0.033(100)
2.	*Proteus mirabilis*	4.7 □ 0.099 (188)	2.1 □ 0.031(84)	2.5□ 0.057(100)
3.	*Klebsiella pneumonia*	3.9 □ 0.043 (185)	1.8 □ 0.072 (85.7)	2.1□ 0.047(100)
4.	*Escherichia coli*	3.2 □ 0.085 (139)	2.0 □ 0.034 (86)	2.3□ 0.058 (100)

Solvent extract residues of the experimental sample were used at a concentration of 700 □g/disc. **Standard antibiotics were used at a concentration of 700 □g/disc. Values given in parentheses indicate % maximum activity.**



PSEUDOMONAS AERUGINOSA ***PROTEUS MIRABILIS***

KLEBSIELLA PNEUMONIA ***ESCHERICHIA COLI***

T – Tetracycline A - Amoxycillin

Plate III: Antibacterial activity of known antibiotics against the test bacterial pathogen

CONCLUSION

Four different solvent systems were used in the crude extract preparation of *C. fimbriata* extract residues of the sample obtained with Hexane, Ethyl acetate, Petroleum ether and methanol. In the preliminary experiments, *Aspergillus niger* alone was used as test pathogen. Based on these observation further studies were restricted to methanol extract of *C.fi mbriata*. The Methanol extract of *Caralluma fimbriata* was active against *Aspergillus niger* as compared to other test o rganism.

The methanol extract of *Caralluma fimbriata* was active against *Aspergillus niger* as compared to *Microsporum canis* and *Fusarium oxysporum.*

Antibacterial activity was performed using the same solvent system of antifungal assay, Methanol proved to be more e ffective than the other solvent system. The methanol extract of *C. fimbriata* was tested against four pathogenic bacteria namely, *Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumonia and Escherichia coli*. The extract residues of stem sample recorded maximum activity against *Pseudomonas aeruginosa*. *Proteus mirabilis, Klebsiella pneumoni a.*and *Escherichia coli* were also effectively inhibited by the extract residues of the extract against this organism.

The antibacterial activities of the methanol extract of the *C. fimbriata* sample against the four pathogenic bacteria were compared with the standard antibiotics. Methanol extract of *C. fimbriata* appeared to be effective.

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