



ISSN: 2277- 7695

TPI 2014; 3(6): 65-69

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www.thepharmajournal.com

Received: 11-06-2014

Accepted: 31-07-2014

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## Screening of antibacterial and phytochemical analysis of *Caralluma fimbriata*

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### ABSTRACT

Medicinal plants have played a vital role in drug production. Using plant extracts directly has proved to be risk-free. In the current studies, Crude extracts of *Caralluma fimbriata* stem were used to investigate its antibacterial activity along with their phytochemical screening. Antibacterial assay was carried out by agar disc diffusion technique. Zones of inhibition around the bacterial colonies showed the antibacterial properties of plant extract. Extracts of *Caralluma fimbriata* stem were effective against all test organisms *Escherichia coli*, *Proteus spp*, *Pseudomonas spp*, *Bacillus* and *Staphylococcus aureus*. The inhibitory effect was compared to standard antibiotic gentamycin. The preliminary phytochemical screening showed that the whole plant was rich in alkaloid, flavanoid, tannin, sterols, saponin, carbohydrate, tannin, oil and fat, aminoacid, protein, gum and mucilage.

**Keywords:** *Caralluma fimbriata*, antibacterial, disc diffusion method, phytochemical screening

### 1. Introduction

*Caralluma fimbriata* an edible succulent cactus is a perennial herb growing in dry parts of Tamil Nadu, India. It belongs to the family Apocynaceae is also a well-known as famine food, appetite suppressant and thirst quencher among tribal population. Genus *Caralluma* comprises about 200 genera and 2500 species [1]. *Caralluma* have found medicinal uses in the treatment of Rheumatism, Diabetes, Leprosy, Antiseptics and Disinfectants [2]. An investigation was carried out to find out the effect of *Caralluma fimbriata* extract on appetite, food intake, and anthropometry in adult Indian men and women [3]. The extract of *Caralluma fimbriata* in the form of capsules has been released under the trade name GENASLIM for body weight control. Microorganisms have developed resistance to many antibiotics. There is need to develop alternative antibiotics drugs from plant. one approach is to screen local medicinal plants which represent rich source of novel antimicrobial agents. The present study was carried out to investigate the antibacterial properties of *Caralluma fimbriata* extract.

Phytochemicals in fruits, vegetables, spices and traditional herbal medicinal plants have been found to play protective role against many human chronic diseases including cancer and cardiovascular disease (CVD).

The medicinal importance of a plant is due to the presence of some special substances like alkaloids, flavanoids, tannin, sterols, saponin, oil and fat, protein and amino acid, gum and mucilage glycosides, phenolic compounds and tannins, etc. The active principles usually remain concentrated in the storage organs of the plants [4]. Considering all these facts, the present study was designed to investigate the presence of various phytochemicals in the plant extracts of *Caralluma fimbriata*, a plant which evokes various therapeutic effects.

### 2. Materials and Methods

#### 2.1 Collection of Plant Material

The plant *Caralluma fimbriata* was collected from the region of kovilpatti, Tiruchirappalli district, Tamilnadu India. The collected plant was dried under shade. After drying, it was powdered and used for our studies.

#### 2.2 Chromatography

Column chromatography is used to purify liquids by separating an organic solvents from a mixture of solvent.

### 2.2.1 Preparation of Leaf Extract

The leaf extract was prepared by grinding the mixture in mortar and pestle containing 22 ml of acetone, 3 ml of petroleum ether, and calcium carbonate. The pigments were filtered and mixed with 20 ml of petroleum ether and 20 ml of 10% aqueous sodium chloride solution. The separating funnel was shaken carefully and the lower was allowed to drain in to the beaker.

### 2.2.2 Preparation of Column

A plug of cotton is placed to the bottom of the column so that silica and soil won't fall out. Slurry of silica was prepared and poured into the column carefully. It is allowed to settle and sand is added.

### 2.2.3 Loading of Sample

The sample was added using pasture's pipette carefully above the sand. The eluent is added on top of the sand. The mobile phase slowly flows down through the silica gel column by gravity leaving behind zone of colour and a component was eluted from the column.

## 2.3 Antibacterial Screening

### 2.3.1 Microbial Strains Used

Different microbial strains were used to evaluate the antimicrobial effect of which two were gram positive bacterial strains (i.e.) *Staphylococcus aureus*, *Bacillus and Staphylococcus epidermidis* and three were gram negative bacterial strains (i.e.) *E. coli*, *Klebsiella*, *Proteus*. The strains were obtained from MTCC, Chandigarh, India and maintained on agar slants.

### 2.3.2 Disc Diffusion Method

Disc diffusion method was carried out for antibacterial susceptibility testing according to the standard method to assess the presence of antibacterial activities of the plant extract [5, 6, 7]. Muller Hinton agar plates were prepared. Overnight nutrient broth culture of test organisms were seeded over the MHA plates using sterile cotton swab so as to make lawn culture. The discs which has been impregnated with aqueous extracts of leaf were placed on the MHA plates with the control disc and subjected to antibacterial screening. The plates were then incubated at 37 °C for 18 to 24 hrs depending on the species of bacteria used in this test. After the incubation, the plates were examined for inhibition zone.

### 2.4 Chi-Square Test ( $\chi^2$ )

In this study chi-square test ( $\chi^2$ ) was applied. The purpose of chi-square test ( $\chi^2$ ) was to decide whether the set of observed data (Antibiogram of microorganisms) agrees with the standard antimicrobial disc susceptibility test (NCCLS, 2002)

### 2.5 IR Spectrum Analysis

FTIR relies on the fact that the most molecules absorb light in the infra-red region of the electromagnetic spectrum. This absorption corresponds specifically to the bonds present in the molecule. The frequency ranges are measured as wave numbers typically over the range 4000-600  $\text{cm}^{-2}$ -1.

### Procedure

FTIR spectrum of the compound obtain from column chromatography was done using Shimadzu IR Affinity 1 instrument.

## 2.6 Phytochemical Analysis

The freshly prepared crude extract was qualitatively tested for the presence of biochemical constituents [8,9].

### 2.6.1 Test for Alkaloid

#### Mayer's test:

5 ml aqueous extract was added with 2 ml of 1% HCl. Mayer and Wagner's reagent was then added to mixture. Turbidity of the resulting precipitate was taken as an evidence for the presence of alkaloid

### 2.6.2 Test for Flavonoids

#### Ferric chloride test

To 1 ml of aqueous extract, 1 ml of 10% lead acetate solution was added. The formation of a yellow precipitate was taken as a positive test for flavonoids.

### 2.6.3 Test for Tannin

#### Lead acetate test

About 2 ml of the aqueous extract was stirred with 2 ml of distilled water and few drops of  $\text{FeCl}_3$  Solution were added. Formation of green precipitate was indication of presence of tannins.

### 2.6.4 Test for Sterols

#### Liebermann Burchard Reaction

A small amount of extract of sample and a few crystal of sodium nitrate were taken in a dry test tube and heated gently for a minute. It was cooled and 0.5 ml of concentrated sulphuric acid was added.

### 2.6.5 Test for Saponins

#### Foam Test

5 ml of aqueous extract was shaken vigorously with 5 ml of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication of the presence of saponins.

### 2.6.6 Tests for Glycosides

#### Legal test

A portion of extract was hydrolysed with HCl for few hours on a water bath and the hydrolysate was subjected to legal's test to detect the presence of different glycosides. To the hydrolysate 1 ml of sodium nitroprusside was added and then it was made with NaOH solution. If the extract produced pink to red colour, it indicates the presence of glycosides.

### 2.6.7 Test for Proteins

#### Biuret Test

Test solution was treated with 10% sodium hydroxide solution and two drops of 0.1% copper sulphate solution and observed for the formation of violet/pink color.

### 2.6.8 Test for Free Amino Acids

#### Ninhydrin Test

Test solution when boiled with 0.2% solution of Ninhydrin, would result in the formation of purple color suggesting the presence of free amino acids.

### 2.6.9 Test for Carbohydrate

#### Molisch's test

Test solution was mixed with few drops of Molisch's reagent. This was followed by addition of 2 ml of concentrated

sulphuric acid down the side of the test tube. The mixture was then allowed to stand for 2-3 minutes. Formation of red or dull violet colour at the interphase of the two layer to show a positive result for the presence of carbohydrate.

**2.6.10 Test for Fixed Oils and Fatty Acids**

Few drops of 0.5N alcoholic potassium hydroxide were added to small quantity of various extract along with a drop of Phenolphthalein. The mixture was heated on a water bath for 1-2 hrs. Formation of soaps or particle neutralization of alkali indicates the presence of fixed oils and fats.

**2.6.11 Test for Phenolic Compound**

Small amount of various extracts were taken separately in water and tested for the presence of phenolic compounds with dilute ferric chloride solution. Violet colour indicates the presence of phenolic compound.

**2.6.12 Test for Gums and Mucilage's**

About 10ml of the extract was added to 25 ml of absolute alcohol with stirring and filtered. The precipitate was dried in air and examined for its swelling properties and for the presence of gum and mucilages.

**3. Result and Discussion**

In earlier study reported that the antimicrobial activity of the aqueous extract of *Caralluma adscendens* was more effective against *E. coli*, *S. aureus*, and it showed less antimicrobial activity against *Shigella sonnei* and *B. pumilus* [10].

In present study the antibacterial activity of the aqueous extract of *Caralluma fimbriata* was assayed in vitro by disc

diffusion method against 5 bacterial strains. The gram positive bacterial strains revealed that the zone of inhibition of *S. aureus* (17 mm), *Bacillus subtilis* (16 mm) and gram negative bacterial strains showed *Proteus*(16 mm) , *Pseudomonas aeruginosa* (15 mm), *E. coli* (13 mm) compared with compound the gram positive bacterial strains revealed the zone of inhibition that *S. aureus* (24 mm), *Bacillus subtilis* (21 mm) and gram negative bacterial strains *Proteus Sp.*, (24 mm), *Pseudomonas aeruginosa* (20 mm), *E. coli* (13 mm). The Maximum Zone of inhibition were observed in compound. (Table 1 & 2) (Fig.1).

The chi-square values obtained respectively which has less than the calculated table value.  $\chi^2 (0.05) = 3.841$  at 5% level of significance. Above results lead to the conclusion that the data was consistent with the hypothesis, the diameter of inhibition zone obtained from the observed data showed the similarities with experimental data.

In earlier study reported that the phytochemical analysis of the aqueous stem extracts of *Caralluma fimbriata* showed the presence of Alkaloid, protein and amino acid, glycosides, steroids, sterols, flavanoids and the absence of tannin and phenolic compound.

In present study the aqueous stem extracts of *Caralluma fimbriata* were subjected to suitable chemical tests to confirm the presence of phytochemicals such as Alkaloid, protein and amino acid, glycosides, steroids, sterols, saponins, flavanoids tannin and phenolic compound oil and fatty acid, gum and mucilages and carbohydrates.(Table 3)

The FT – IR spectrum is used to obtain the graph and band stretching was interpreted. (Table 4) & (Fig 2).

**Table 1:** Zone of inhibition formed by aqueous extracts of *Caralluma fimbriata* stem against bacterial strains

S. No	Sample	Bacterial strains	Zone of inhibition in Diameter (mm)		$\chi^2 = \sum [O - E]^2 / E$
			Standard value	Observed value (Aqueous)	
1.	Caralluma fimbriata	<i>S. aureus</i>	20	17	0.45
2.		<i>B. subtilis</i>	20	16	0.8
3.		<i>Proteus Sp.</i> ,	20	16	0.8
4.		<i>pseudomonas</i>	20	15	1.25
5.		<i>E. coli</i>	20	13	2.45

Table value  $\chi^2(0.05) = 3.841$ , Chi-square value significance at 5% level.

**Table 2:** Zone of inhibition formed by coloumn compound of *Caralluma fimbriata* stem against bacterial strains

S. No	Sample	Bacterial strains	Zone of inhibition in Diameter (mm)		$\chi^2 = \sum [O - E]^2 / E$
			Standard value	Observed value(Aqueous)	
1.	Caralluma fimbriata	<i>S. aureus</i>	20	24	0.8
2.		<i>B. subtilis</i>	20	21	0.05
3.		<i>Proteus Sp.</i> ,	20	24	0.8
4.		<i>Pseudomonas Sp.</i> ,	20	20	0
5.		<i>E. coli</i>	20	14	1.8

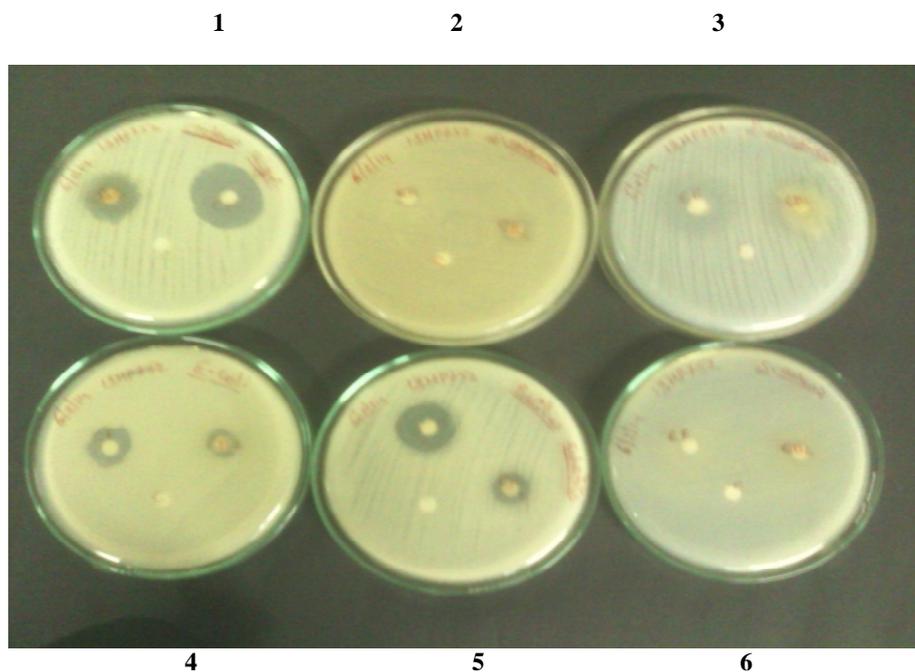
Table value  $\chi^2(0.05) = 3.841$ , Chi-square value significance at 5% level.

**Table 3:** Phytochemical analysis

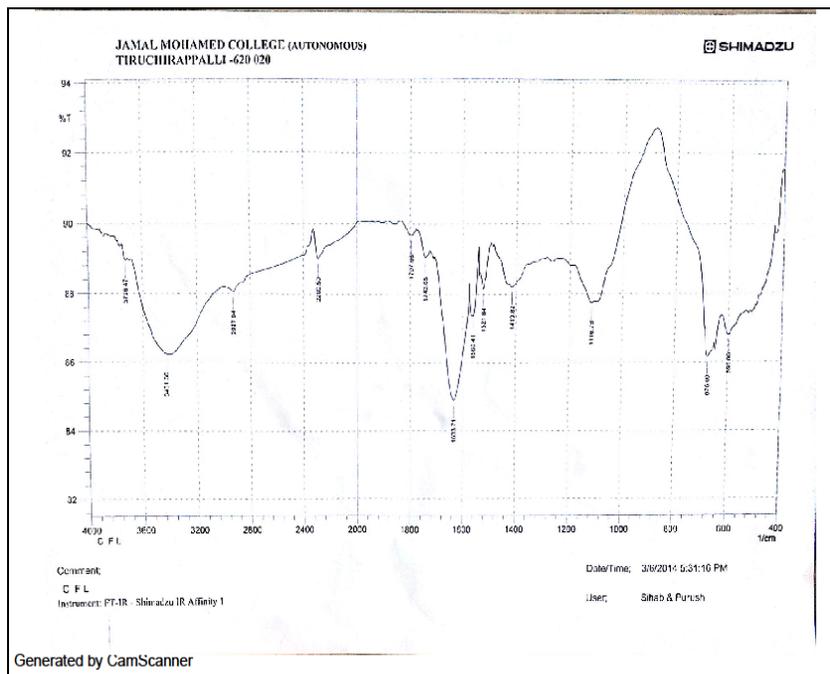
S. NO	Phytochemical constituents	Aqueous Extract
1	Alkaloids	Positive
2	Flavanoids	Positive
3	Carbohydrates	Positive
4	Glycosides	Positive
5	Sterols	Positive
6	Saponins	Positive
7	Oils and fats	Positive
8	Tannins	Positive
9	Phenolic compounds	Positive
10	Proteins and amino acids	Positive
11	Gums and mucilage	Positive

**Table 4:** IR spectrum analysis of crude sample of *Caralluma fimbriata* stem extract

S. No	Peak	Stretching	Interpretation
1	596.00	C-Br stretching	Halogen
2	675.09	-C-Cl stretching	Halogen
3	1116.78	-C-F stretching	Halogen
4	1413.82	-C-C stretching	Aromatics
5	1521.84	N=O stretching	Nitro compounds
6	1560.41	N=O stretching	Nitro compounds
7	1743.00	C=O stretching	Acid anhydrides
8	1797.66	C=O stretching	Acid anhydrides
9	2289.50	N-H stretching	Amino acids
10	2927.94	C-H stretching	Alkanes
11	3431.36	O-H stretching	Alcohol
12	3726.47	N-H stretching	Amides



**Fig 1:** Zone of inhibition formed by stem extracts of *Caralluma fimbriata* stem against bacterial strains



**Fig 2:** IR spectrum analysis of crude sample of *Caralluma fimbriata* stem extract

#### 4. Conclusion

The present study showed that the antibacterial activity of the stem extracts of *Caralluma fimbriata* against pathogenic organisms. These results may help in standardization, identification and in carrying out for further research in *Caralluma fimbriata*. Hence this plant is highly valuable in medicinal usage for the treatment of various human ailments.

#### 5. Acknowledgements

The authors are thankful to Principal and Management of Jamal Mohamed College (Autonomous), Trichirappalli for offering facilities to carry out this study.

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