

## **In-Vitro Evaluation of Antibacterial activity of plant Caralluma fimbriata extract**

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### **ABSTRACT**

The use of plants in treatment of burns, dermatophytes and infectious diseases is common in traditional medicine<sup>1</sup>. Based on pharmacological and taxonomic information, antibacterial activities of aqueous extracts of *Caralluma fimbriata* were determined by in vitro using agar (disc) diffusion method against some human pathogenic bacteria<sup>2</sup>. The stem of *Caralluma fimbriata*, belonging to the Asclepiadaceae family and which have some ethnomedicinal applications were studied for antibacterial activity. Sun dried powdered stem materials of selected plant were extracted with aqueous. The aqueous extracts were evaporated to dryness using rotary flash evaporator. The antibacterial screening of aqueous extract carried out in vitro on the following bacteria viz., *E. coli*, *Bacillus subtilis*, *Staphylococcus aureus*. This study gives idea about, the traditional medicines (herbal extracts) to cure many diseases like diarrhea, intestinal tract, throat, ear infections, fever and skin diseases.

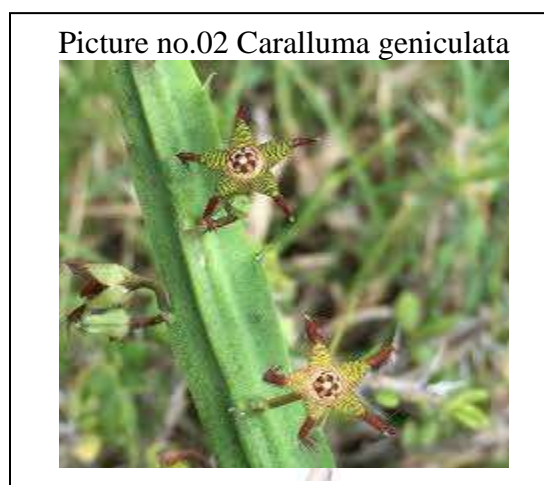
**Key Words:** *Caralluma fimbriata*, herbal extracts, Antibacterial activity

### **INTRODUCTION**

Plant based antimicrobials represent a vast untapped genetic mechanisms of resistance and to continue studies source for medicines and further exploration of plant to develop new drugs, either synthetic or natural<sup>3</sup>. Antimicrobials activities of plant ultimate goal are to offer appropriate and efficient origin have enormous therapeutic potential<sup>4</sup>. Infections particularly those involving microorganisms i.e. Over the past twenty years there has been a lot of bacteria, fungi, viruses, they cause serious infections in interest in the investigation of natural materials as

sources tropical and subtropical countries of the world<sup>5-7</sup>. In recent of new antibacterial agents, different extracts from traditional medicinal plants have been tested. Many research have show the effectiveness of traditional herbs against microorganisms, as a result, plants are one of the bedrocks for modern medicine to attain new principles<sup>8</sup>. The increasing interest on traditional ethno medicine may lead to discovery of novel therapeutic agents. Medicinal plants are finding their way into pharmaceuticals nutraceuticals, cosmetics and food supplements<sup>9</sup>. In this regard, plants have given western pharmacopoeia about 7500 different pharmaceutically important compounds and a number of top selling pharmaceutical drugs of current time, e.g. quinine, artemisinin, taxol, camptothecin, etc<sup>10</sup>. Until natural products have been approved as new antibacterial drugs, there is an urgent need to identify novel substances must be active towards highly resistant pathogens.<sup>11, 12</sup> Biomolecules of plant origin appear to be one of the alternatives for the control of these antibiotic resistant human pathogens<sup>13</sup>. Hence, more studies pertaining to the use of plants as therapeutic agents should be emphasized, especially those related to the control of antibiotic resistant microbes. The objective of this research was to evaluate the potentiality of plant extracts on standard microorganism strains as well as on the multi-drug resistant bacteria. Plants produce bioactive molecules in a diverse range making them a rich source of different types of medicines<sup>14</sup>. In old medicine therapy herbal extracts were known to be effective against microorganisms as a result plants form the basis of modern medicine. The plants produce phytochemicals to protect themselves but recent studies shows that many phytochemicals can also protect humans against infectious diseases<sup>15</sup>. *Caralluma geniculate* (Picture no.02 ) is an attractive, succulent medicinal plant of the family Asclepiadaceae. It is an endemic plant distributed in Maruthuvamalai, Aramboli and Valliyur hills of Kanyakumari District, Tamilnadu, India<sup>16</sup>. The family Asclepiadaceae comprises about 200 genera and 2500-3000 species with a global distribution and represented in all types of habitats. A total of 16 species and 8-10 varieties of *Caralluma* occur in India out of which 5 species and 5 varieties are solely endemic to Peninsular India<sup>17</sup>. They grow in arid, rocky regions in the foot hills of Western Ghats and Eastern Ghats<sup>18</sup>. *Caralluma* species present in India are edible and also take part in traditional medicine of our country<sup>19</sup>. People in semi-urban areas of Pakistan used the species of *Caralluma* for centuries as emergency foods. Palliyars of Western Ghats, Tamilnadu used the stems of *C. adscendens* R.Br. var. *attenuata* (Wight) Grav. and Mayuranathan (Periyasirumankeerai) and *C. lasiantha* (Wight) N.E.Br (Sirumankeerai) as edible plant whereas, Karuppusamy documented that Paliyan tribes of Sirumalai Hills, Southern India utilized burned stems of *C. umbellata* (Roxb.) Haw. (Kallimulayan local name) in direct fire and eaten for five days regularly in empty stomach to cure ulcer and sliced stem of *C. adscendens* (Roxb.) Haw. with salt was taken orally for diuretic condition<sup>20</sup>. Similarly 10 grams of fresh rootless plant of *C. lasiantha* Wight and N.E.Br (Sirumankeerai) was taken as such twice a day for a period of three days to reduce body heat<sup>21</sup>. People of Puttaparthi Mandal belongs to Sri Sathya Saitaluk of Anathapur District, Andhra Pradesh used succulent stems of *C. adscendens* Grav. and Mayur (Telugu Name-Kundelu Kommulu) and *C. umbellata* Haw. (Telugu Name Kundeti Kommulu) to treat inflammation and stomach disorders<sup>22</sup>. Farmers in Dindigul District, Tamilnadu, India

believed that feeding leaves of *C. adscendens* R.Br. (Muyalkathu, MuyalKurabu) in odd numbers i.e., 3,5,7 or 9 can relieve bloat and also the mixture of paste with ghee and leaves of *Caralluma* cure mastitis in animals. Roasted plants of *C. umbellata* Haw. (Chirukalli) is made in to paste and applied for indigestion by the malayaltribals in Kollihills of Tamilnadu, India<sup>23</sup>. Many recent studies revealed that *Carallumafimbriata* is an important medicinal plant. Keeping the values of *Caralluma* in mind the present investigation was carried out to screen the biomolecules present in aqueous, petroleum ether, chloroform, ethanol and acetone extracts of the aerial part of *Caralluma fimbriata* (Picture no.01) collected from village-Bhambarde, Taluka-Shirur, District-Pune Maharashtra, India and to determine their functional group using (FT-IR) spectral analysis.

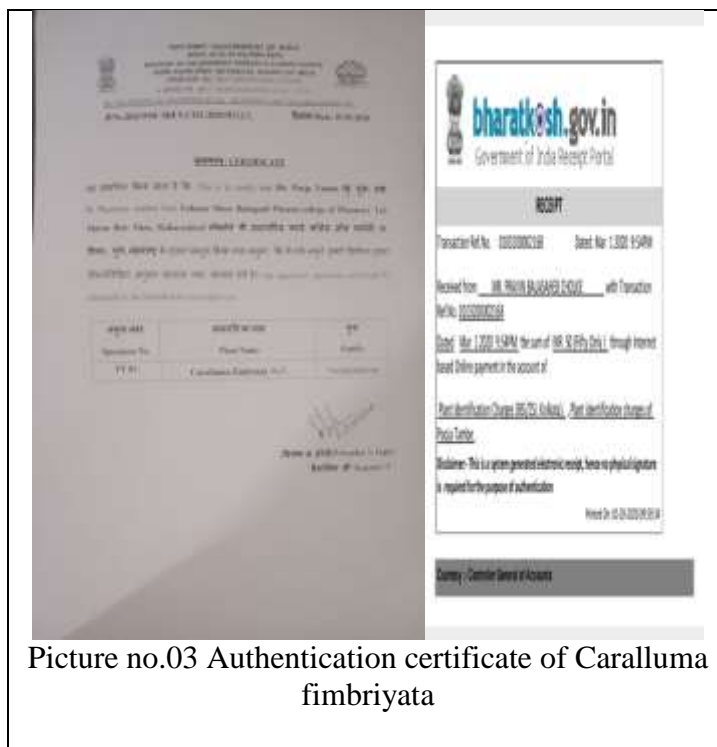


## EXPERIMENTAL

### MATERIALS & METHOD PREPARATION OF EXTRACT:

#### Collection of Plant materials:

Stem of *Caralluma fimbriata* collected from village-Bhambarde, Taluka-Shirur, District-Pune Maharashtra, India, and identified a voucher specimen no, PT 01,BSI/WRC/IDEN.CER./2020/H3/83 was deposited in the Government of India, Ministry Of Environment, Forests & Climate Change, Botanical Survey of India, Western Regional Center, 7.Koregoan Road, Pune-41100.



Picture no.03 Authentication certificate of *Caralluma fimbriata*

### Preparation of Extract:

The whole plant was collected and dried under shade, powdered and sieved through sieve no.14 (Mesh size- 1410 $\mu$ ) and stored in air tight containers.

### Sample collection:

Samples for the study were collected from Savitribai Phule Pune University Department of Microbiology, Bacteriaculture was collected by using sterile container. The bacterial strains were cultivated at 37°C and maintained on nutrient agar and they are maintained by sub culturing.

### Identification of Bacteria:

The procedure devised most of the bacteriological assessment of rods, cocci and spiral into two large additional groupings. After adding Gram stain cells that look identical become separable as purple Gram positive organisms and pink Gram negative organisms.

### Microscopic Observation:

The bacterial isolates were gram stained and observed under a high power magnifying lens in light microscope.

**Biochemical characterization of organisms:**

The following Biochemical tests were performed to identify the isolated bacteria<sup>24-26</sup> (Cappucino and Sherman, 1996).

1. Indole production test
2. Methyl Red test
3. Vogesproskauer test
4. Citrate Utilization test
5. Triple Sugar Iron agar test

**Antimicrobial Assay: (Disc Diffusion Method)**

The modified agar Disc diffusion method was employed to determine the antibacterial activities<sup>27</sup>. Agar disc diffusion method allows better diffusion of the extracts' into the medium thus enhancing contact with the organisms. Paper discs may act as a barrier between the extract and the organisms thus; preventing total diffusion of active components absorbed by the discs into the medium and may be responsible for the observed differences.

The standardized 24 hour old broth culture of the test organisms swabbed onto sterile Muller Hinton Agar plates. Then the sterile discs are placed on the muller hinton agar plates. The plates were then incubated at 37°C for 24 hours. At the end of the incubation period, inhibition zones formed on the agar plates were observed, measured and tabulated for various bacterial strains used<sup>28</sup>.

**Chi-Square Test:**

In this study chi-square test was applied. The purpose of chi-square test was to decide whether the set of observed data agrees with the standard antimicrobial disc susceptibility test<sup>29,30</sup>.

**RESULTS****Microscopic Observation:**

The morphological characteristics of the isolated strains were shown. Gram stain was made for respective strain and observed under light microscope. Among the 10 clinical samples A&B isolates showed E.coli and S. aureus. The highest isolates were obtained in samples C clinical Bacillus subtilis.

**Biochemical Characteristics:** The biochemical characteristics of the isolated strains were identified.

### Antimicrobial Screening:

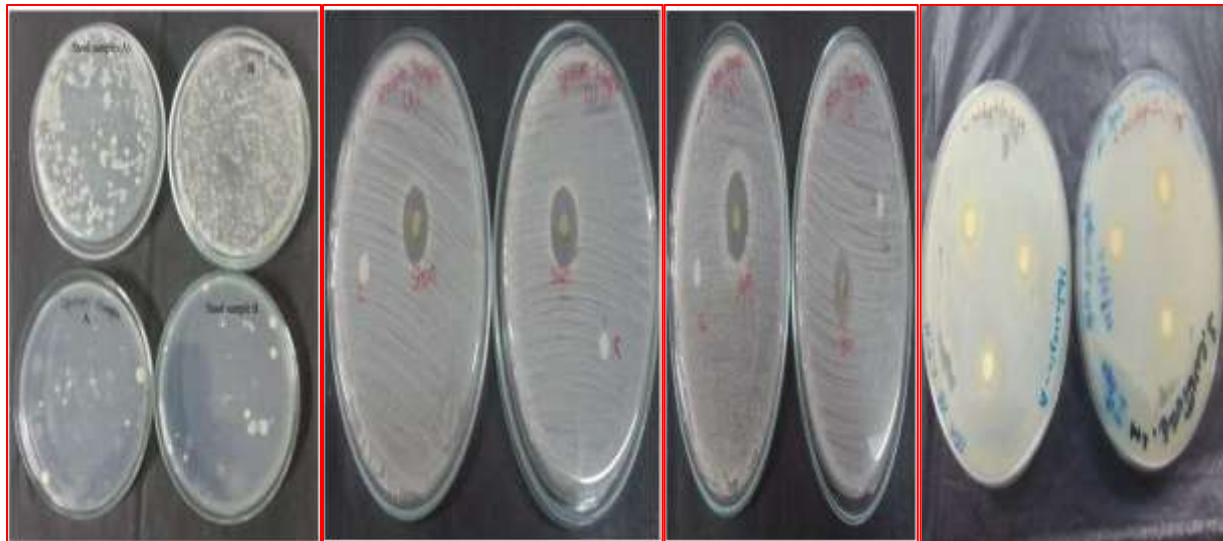
The antibacterial activity of aqueous extracts of medicinal plant *Caralluma fimbriata* against (Sample A) (Sample B) and (sputum Sample C) were investigated through agar disc diffusion method. The sample A (*E.coli*) showed zone of inhibition of 18mm and sample B (*S. aureus*) showed maximum zone of inhibition of 19 mm. (Table No.01) (Picture No.04) & sample C (*Bacillus subtilis*) showed maximum zone of inhibition of 20mm.

### DISCUSSION

Plants are important source, potentially useful structure for the development of new chemotherapeutic agents. The first step towards this goal was invitro antibacterial activity assay (Tona et al., 1998). The antimicrobial activity of antibiotics can be administered through various ways to treat both human and veterinary diseases. (White and Hancock, 2007; Nelson et al., 2007; William and Cromie, 2000). In the earlier study R.Gopinath and M.Prakash showed the prevalence of *Enterococcus faecalis* from 100 various clinical samples. *Enterococcus faecalis* was found to be most predominant in stool samples. Plant extract gave a zone of inhibition of around 18-21mm for all the strains. In the present study reported that the maximum zone of inhibition of *Caralluma fimbriata* plant extract against the bacterial strain present in the stool sample *S. aureus* (19mm) than the *E.coli* 18mm. In the earlier study reported this traditional usage stems from the fact that Tridax is associated with antibacterial activity (Mundada and Shivhare, 2010). We studied the efficacy of aqueous and ethanolic extracts of Tridax as antibacterial agents against human pathogens including nosocomial strains. In the present study showed the antimicrobial activity of *Caralluma fimbriata* against the pathogenic bacteria present in the sputum sample. In this study reported the maximum zone of inhibition showed the Sample C *Bacillus subtilis* (20mm) than the sample A (*S.aureus* 19mm). The present study has revealed the importance of natural products to control antibiotic resistant bacteria which are being a threat to human health. This scientific study can serve as an important platform for the development of inexpensive, safe and effective medicines.

Table no.01: Zone of inhibition of extracts of *Caralluma fimbriata* against bacterial strains.

Sr. No.	Samples	Test organism (sample)	Zone of inhibition in mm		$\chi^2 = \sum (O - E)^2 / E$ Observed crude
			Standard value	Observed value	
1	Caralluma fimbriata	Sample A ( <i>E.coli</i> )	20	18	0.2
2		Sample B ( <i>S. aureus</i> )	20	19	0.2
		Sample B ( <i>Bacillus</i> sps)	20	20	0.45



Picture no 04:  
Disc diffusion method (zone of inhibition)

#### ACKNOWLEDGEMENT:

Authors are sincerely thankful to LokneteShriDadapatilPharate College of Pharmacy, MandavganPharata, Shirur, Pune.(M.S.) for providing necessary facilities for work and authors also thankful to Savitribai Phule Pune University, Department of Microbiology, Pune for providing facilities.

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