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ANTIBACTERIAL ACTIVITY OF *CARALLUMA FIMBRIYATA* AGAINST HUMAN PATHOGENIC BACTERIA

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ABSTRACT

The use of plants in treatment of burns, dermatophytes and infectious diseases is common in traditional medicine. Based on ethno pharmacological and taxonomic information, antibacterial activities of aqueous extracts of *Caralluma fimbriyata* were determined by *in vitro* by agar diffusion-method against some human pathogenic bacteria. The stem of *Caralluma fimbriyata*, belonging to the Asclopedaceae family and which have some ethnomedicinal applications were studied for antibacterial activity. 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*, isolated from stool and sputum sample. This study supports, the traditional medicines (herbal extracts) to cure many diseases like diarrhea, intestinal tract, throat, ear infections, fever and skin diseases.

KEYWORDS

Caralluma fimbriyata, Stool sample, Sputum sample and Disc diffusion method.

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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. The antimicrobials need to occur. Antimicrobials of plant ultimate goal are to offer appropriate and efficient origin have enormous therapeutic potential[1]. Human antimicrobial drugs to the patient. 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[2-4]. In recent of new antibacterial agents[5, 6].

Different extracts from traditional medicinal plants have been tested. Many reports 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[7]. The increasing interest on traditional ethno medicine may lead to discovery of novel therapeutic agents. Medicinal plants are finding their way into pharmaceuticals neutral ceuticals, cosmetics and food supplements. In this regard, plants have given western pharmacopoeia about 7000 different pharmaceutically important compounds and a number of top-selling drugs of modern time, e.g. quinine, artemisinin, taxol, camptothecin, etc.[8]. Until natural products have been approved as new antibacterial drugs, there is an urgent need to identify novel substances active towards highly resistant pathogens[9, 10]. Biomolecules of plant origin appear to be one of the alternatives for the control of these antibiotic resistant human pathogens[11]. 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[12-16]. Traditionally herbal extracts were known to be effective against microorganisms as a result; plants form the basis of modern medicine. Plants produce phytochemicals to protect themselves; but recent studies indicate that many phytochemicals can also protect humans against infectious diseases[17-20].

Caralluma geniculata 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[21]. The family Asclepiadaceae comprises about 200 genera and 2500 species[22] with a global distribution and represented in all types of habitats. A total of 16 species and 8 varieties of *Caralluma* occur in India out of which 5 species and 5 varieties are solely endemic to Peninsular India[23]. They grow in arid, rocky regions in the foot hills of Western Ghats and Eastern Ghats[24]. *Caralluma* species present in India are edible and also take part in traditional medicine of our country[25]. People in semi arid areas of Pakistan used the species of *Caralluma* for centuries as emergency foods[26]. Pallliyars 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[27] 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[28]. 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[29]. People of Puttaparthi Mandal belongs to Sri Sathya Sai taluk 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[30]. Farmers in Dindigul District, Tamilnadu, India believed that feeding leaves of *C. adscendens* R.Br. (Muyalkathu, Muyal Kurabu) in odd numbers i.e., 3,5,7 or 9 can relieve bloat and also the mixture of paste with ghee and leaves of *Carulluma* cure mastitis in animals. Roasted plants of *C. umbellata* Haw. (Chirukalli) is made in to paste and applied for indigestion by the malayali tribals in Kollihills of Tamilnadu, India[31]. Many recent studies revealed that *Caralluma* 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 geniculata* collected from Maruthvamalai, Kanyakumari M District, Tamilnadu, India and to determine their functional group using (FT-IR) spectral analysis.

MATERIALS AND METHODS

Collection of Plant materials

Stem of *Caralluma fimbriyata* collected from kovilpatti, in Manapparai Taluk, Trichy District and identified and a voucher specimen was deposited in the Rapinat Herbarium, St. Joseph's college, Tiruchirappalli, Tamilnadu, India.

Preparation of Extract

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

Sample collection

Samples for the study were collected from the patients of different Hospital at Trichy, Tamil Nadu, India. Clinical specimens such stool (10 samples), and sputum (5 samples) specimens of patient, were collected aseptically by using sterile cotton swabs. Stool and sputum specimens were 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 deviced 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 (Cappucino and Sherman, 1996).

Indole production test

The isolated cultures were inoculated in a sterile tryptone broth and incubated. 1ml of Kovac's reagent was added, after 15 minutes the results were observed. The development of bright red color ring at the interface of reagent and medium of indole and constitutes positive test, the absence of color change indicates the negative test.

Methyl Red test

The isolated cultures were inoculated in MR-VP broth and incubated for 24 hrs at 37°C. Five drops of methyl red reagent was added and the results were observed. The development of stable bright red color in the broth indicates sufficient acid production and constitutes a positive reaction. Yellow color indicates a negative reaction.

Vogesproskauer test

The isolates were inoculated in MR-VP incubated for 24 hrs at 37°C. After incubation added 0.5 ml alpha napthol followed by 0.2 ml of KOH and the well shaked the tubes gently to expose the medium to atmospheric oxygen and allow the medium to stands for 10-15 minutes and observed for colour change. Red or Brown colour indicates positive reaction; pale yellow colour change indicates negative reaction.

Citrate Utilization test

The isolates were inoculated in Simmons citrate agar slants and incubated at 37°C utilization of citrate contained in the medium from a blue color constituted the positive reaction. The absence of the blue color indicates negative reaction.

Triple Sugar Iron agar test

TSI prepared agar medium was prepared and made it as a slant with enough amount of butt. The isolates were inoculated by stabbing down the center of agar butt. The isolates were inoculated by stabbing down the center of agar butt carefully withdraw the inoculating needle carefully and then streaked the surface of the slant incubate the tube at 37°C for 18-24 hrs. The results after inoculation will be of three types

Acid butt, alkaline slant (Yellow butt red slant)
Acid butt, acid slant (Yellow butt yellow slant)
Alkaline butt, alkaline slant (red butt red slant).

Antimicrobial assay - Disc diffusion method

The modified agar Disc diffusion method was employed to determine the antibacterial activities 17. 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.

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 (NCCLS, 2002).

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 stool samples 3 isolates showed *E.coli* and S. *aureus*. The highest isolates were obtained in stool samples. 5clinical sputum samples 3 isolates showed, *S. aureus* and *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 fimbriyata* against *(*stool Sample A) *(*stool Sample B) and (sputum Sample A) (sputum Sample B) were investigated through agar disc diffusion method. The stool sample A (E.coli) showed zone of inhibition of 18mm and sample B (S. aureus) showed maximum zone of inhibition of 22 mm. (Table No.2) (Figure No.3). The sputum sample A (S. aureus) showed zone of inhibition of 17mm and sample B (Bacillus subtilis) showed maximum zone of inhibition of 21mm (Table No.1) (Figure No. 2).

Table No.1: Zone of inhibition of aqueous stem extracts of *Caralluma fimbriyata* against bacterial strains isolated from stool sample

S.No	Samples	Test organism (Stool sample)	Zone o f inhibition in mm		$X^2=\Sigma [0-E)^2]/E$ Observed crude
			Standard value	Observed value	
1	*Caralluma fimbriyata*	Sample A *(E.coli)*	20	18	0.2
2		Sample B *(S. aureus)*	20	22	0.2

Table No.2: Zone of inhibition of aqueous stem extracts of *Caralluma fimbriyata* against bacterial strains isolated from sputum sample

S.No	Samples	Test organism (Sputum sample)	Zone o f inhibition in mm		$X^2 = \Sigma [0 - E)^2]/E$
			Standard value	Observed value	Observed crude
1	*Caralluma fimbriyata*	Sample A (*S. aureus*)	20	17	0.45
2		Sample B (*Bacillus* sps)	20	21	0.45

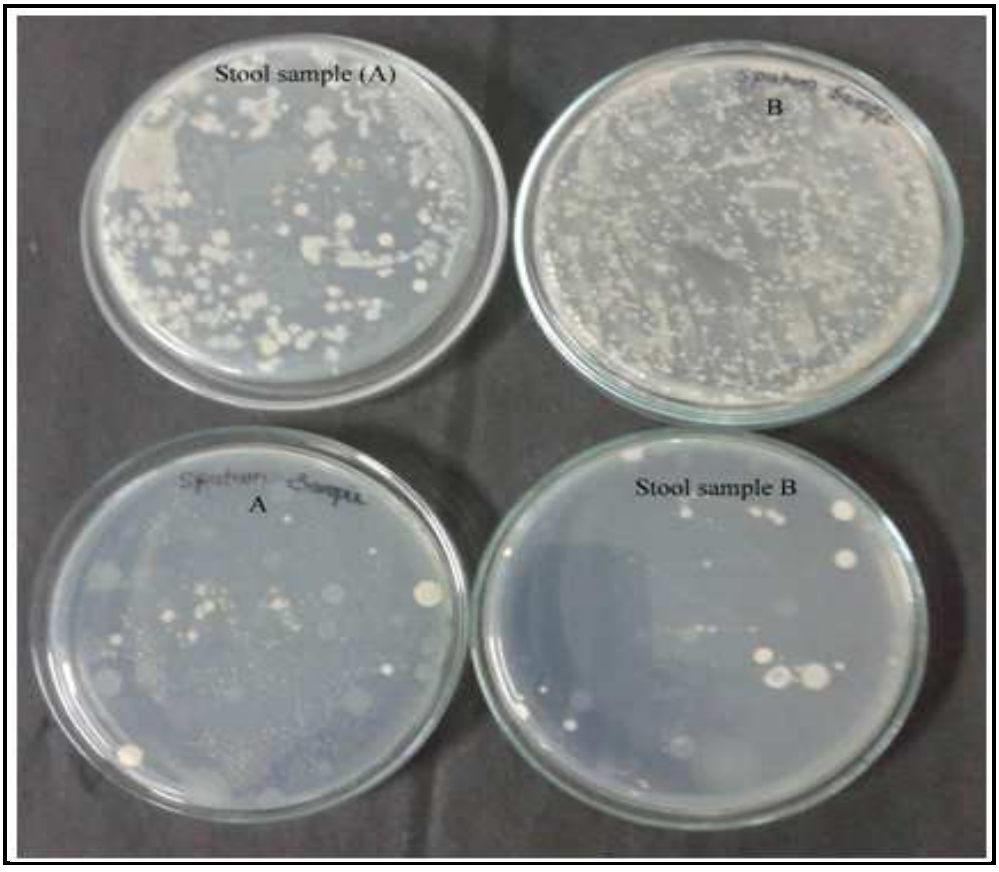

Figure No.1: Isolated colonies on Nutrient agar plates

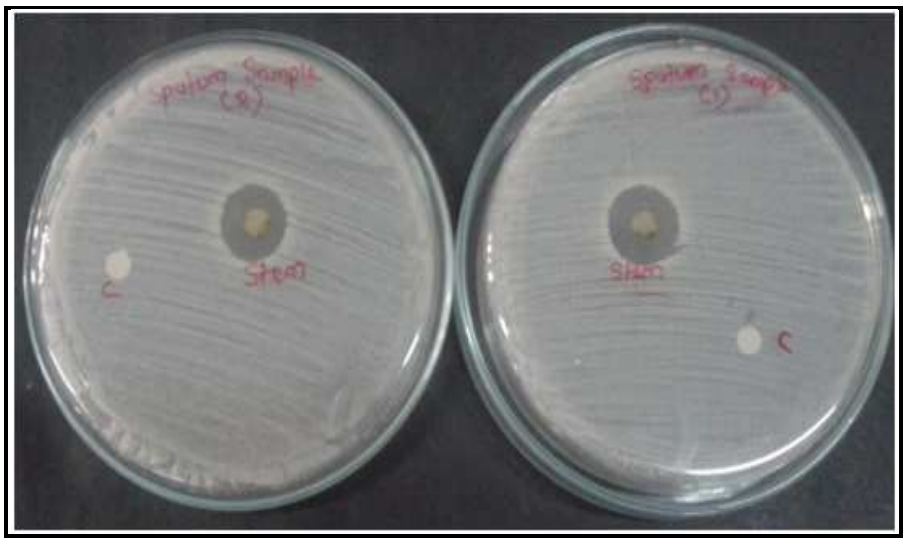

Figure No.2: Zone of inhibition of aqueous stem extracts of *Caralluma fimbriyata* against bacterial strains isolated from stool sample

Figure No.3: Zone of inhibition of aqueous stem extracts of *Caralluma fimbriyata* against bacterial strains isolated from sputum sample

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 fimbriyata* plant extract against the bacterial strain present in the stool sample *S. aureus* (22mm) than the *E.coli* 18mm.

In the earlier study reorted 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 fimbriyata* against the pathogenic bacteria present in the sputum sample. In this study reported the maximum zone of inhibition showed the Sample B *Bacillus subtilis* (21mm) than the sample A (*S.aureus* 17mm).

CONCLUSION

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.

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