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Research Article

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Phytochemical and Antimicrobial Screening of the Polar and Non-Polar Solvent Stem Extract of *Caralluma Fimbriyata*

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ABSTRACT

The present study deals with the polar and non polar stem extracts of Caralluma fimbriyata. In this study polar (Methanol, Ethanol, Water) and non-polar (Chloroform, Petroleum Ether) solvent extracts of Caralluma fimbriyata were investigated for their phytochemical and antimicrobial activity. Phytochemical analysis revealed the presence of the Tannins, phenols, alkaloids, flavonoids, terpenoids, glycosides and steroids. Polar extracts showed more phytochemicals than the non polar extracts. The glycosides are found to be present in non polar extracts that are absent in the polar solvent. The microorganisms employed were E.coli, Streptococcus aureus, Streptococcus epidermidis, Klebsiella, Bacillus subtilis and Proteus. Among the three aqueous, ethanol and methanolic extracts were found to more active towards the organisms tested than the non polar extracts. The analysis revealed maximum activity of polar solvent against bacteria in order of the Proteus, staphylococcus aureus, Bacillus Sp., E.coli, Streptococcus epidermidis, Klebsiella Sp., Whereas non polar solvent extracts showed their maximum activity on bacteria in order to Staphylococcus aureus, Bacillus, E.coli, Proteus, Klebsiella, Streptococcus epidermidis. Due to the presence of various active phytochemicals present in Caralluma fimbriyata may be attribute to the broad spectrum inhibition zone against microorganisms, which may be their individual or combined action

Key words*:Caralluma fimbriyata, Polar, non polar, Stem extracts, Antibacterial.*

INTRODUCTION

Plants have the capability an extensive diversity of chemical compounds that are used to carryout vital natural functions, and to protect against attack from predators. On long-term many of these phytochemical have valuable effects when consumed by humans, and their usage is effective in the treatment of various diseases. So far at slightest 12,000 such compounds have been isolated; a number predictable to be less than 10% of the total[1-2]. *Caralluma* belongs to the family Asclepiadaceae, has about hundred species, dispersed in various countries which includes Spain, Saudi Arabia, Africa, Middle East, India, and Pakistan. In Pakistan, two species of *Caralluma* is found, *C. edulis* and *C. tuberculata*[3]. *Caralluma* has dominant medicinal importance found in the dry regions of the world and possess anti-inflammatory and anti-tumor activity[4-6]. Due to the presence of the pregnane glycosides in *Caralluma* it possesses anti-tumor and anti-cancer properties[7,8]. Traditionally in Pakistan both urban and rural population, used *caralluma* as an anti-diabetic therapeutic agent[11]. In semi arid areas of Pakistan *Caralluma* species have been used for centuries as emergency foods[12-13] and other *Caralluma* species for their anti-hyperglycemic activity[14] and joints pain[15].

The genus *Caralluma* (Asclipiadaceae), which are comprises about 200 genera and 2500 species. The member of the genus is small plant, erect, fleshy. They have four grooved stems, round shape devoid of leaves and small flowers in several varieties of dark colors. The species of Caralluma found in India are edible and form part of the traditional medicine system of the country[16].

The genus *Caralluma fimbriyata* is a very variable herbs, up to 1 m. in height, with fleshy, almost leafless stems, deep purple-brown flowers, and 10-12 cm slender follicles, distributed in peninsular India from Andhra Pradesh and Maharashtra to Kerala up to 600 m. The herb cotains hydrocarbon, n-pentatriacontane and a glycoside. In addition to *Caralluma* species commonly used in treatment of rheumatism, diabetes, leprosy, antipyretic and anthelmintic, for tumor, fungal diseases, snake, scorpion bite and antinociceptive activity[17-19].

MATERIALS AND METHODS

(i) Collection of Plant materials

Stem of *Caralluma fimbriyata* 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.

(ii) Phytochemical Studies

The extract was subjected to preliminary phytochemical tests to determine the group of secondary metabolites present in the plant material. Condensed extracts were used for preliminary screening of phytochemicals such as alkaloids, steroids, and phenols, glycosides, terpenoids and saponins, tannins, flavonoids .

(iii) Preparation of different solvent extracts

The fresh plants of *Caralluma fimbriyata* were carefully washed with tap water, rinsed with distilled water, and air dried for one hour. Then it was cut into small pieces, dried in room temperature for two weeks, grounded into powder with the help of hand mill and stored in room temperature. The *Caralluma fimbriyata* whole plant powder was macerated in different solvents including methanol 95% (v/v), ethanol, water, chloroform, petroleum ether at room temperature, undergoing mechanical shaking for 4 hours followed by filtration. The extracts obtained were concentrated in a rotary evaporator at 40°C and the residue was extracted twice again analogously, there by obtaining the crude solvent extracts.

(iv) Test Microorganisms

The microorganisms used in this study includes *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumonia*, *Proteus Sp.*, *Escherichia coli*, and *Bacillus Sp*., obtained from the Department of Microbiology, Jamal mohamed college ,Tiruchirappalli. The bacterial strains were cultured on respective selective media and stored at 20°±2°C.

(v) Preparation of inoculums

Exactly 18 hour broth culture of the test bacteria isolates was suspended into sterile nutrient broth .The broth culture is maintained as a inoculum.

(vi) Antimicrobial assay – Disc diffusion method

The modified agar Disc diffusion method was employed to determine the antibacterial activities17. 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, its 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

The phytochemicals that are present in the root screened by different screening tests. The stem extracts revealed the presence of the Tannins, phenols, alkaloids, flavonoids, anthocyanins, terpenoids, cyogenic glycosides and steroids.(Table 1) showed the presence of tannins, phenols, alkaloids flavonoids, anthocyanins, terpenoids, glycosides and steroids both in polar and non polar solvents but during the

phytochemical analysis polar solvents when reacted with the phytochemical tests more rapidly than the non polar solvents. (Figure.1and 2).

The antibacterial activities of *Caralluma fimbriyata* stem extracts was assayed and revealed the data on effect of plant extracts on the growth of series of bacterial strains *E.coli, Staphylococcus aureus, Klebsiella , Bacillus subtilis, Streptococcus epidermidis,Proteus.* Among the two polar and nonpolar solvent extracts tested methanol extracts of stem showed broad inhibition zone on the bacteria *Proteus, staphylococcus aureus, Bacillus, E.coli, Streptococcus epidermidis, Klebsiella.*. But when compared with the zones of inhibition of ethanol and water extracts, Petroleum ether extracts are also showed their maximum activity on bacteria in order *Pseudomonas Sp., E.coli, Klebsiella pneumonia, Staphylococcus aureus* and *Bacillus subtilis.(Table 2)(Figure 3 and 4).* Further studies are needed to isolate and characterize the bioactive principle compounds to develop new antibacterial drug.

Table1. Phytochemical screening test of stem extracts of *Caralluma fimbriyata*

S. No	Phytochemicals	Polar solvents			Nonpolar solvents	
		methanol	ethanol	water	chloroform	Petroleum ether
1	Tannins	+	+	+	+	+
2	Phenols	+	+	+	+	+
3	Saponin	+	+	+	+	+
4	Alkaloids	+	+	+	+	+
5	Flavonoids	+	+	+	+	+
6	Anthocyanins	+	+	+	+	+
7	Amino acids	+	+	+	+	+
8	Carbohydrates	-	+	+	+	+
9	Terpenoids	+	+	+	+	+
10	glycosides	+	+	+	+	+
11	Steroids	+	+	+	+	+

(+) = Present, (-) = Absent

Fig.1: Phytochemical screening test of polar and non polar stem extracts *of Caralluma fimbriyata*

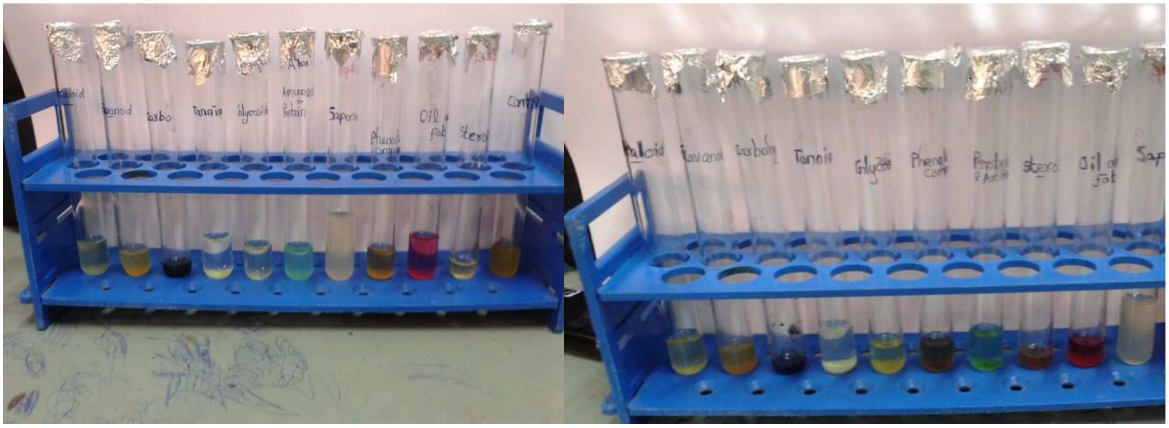

Table 2. Antibacterial activity of polar extracts of *Caralluma fimbriyata*

S. No	Sample	Test organisms	Standard value	Conc. in µg	Zone of inhibition in mm		
					Methanol	Ethanol	Water
1		*E.coli*	20	128	14	24	11
2		*Bacillus Sp.,*	20	128	23	19	13
3	*Caralluma*	*S.aureus*	20	128	23	20	15
4	*fimbriyata*	*S.epidermidis*	20	128	22	23	16
5		*Proteus Sp.,*	20	128	24	25	17
6		*Klebsiella Sp.,*	20	128	22	13	10

Table 3. Antibacterial activity of Non polar extracts of *Caralluma fimbriyata*

S. No	Sample	Test organism	Standard value	Conc.in µg	Zone of inhibition in mm	
					Petroleum ether	Chloroform
1	*Caralluma fimbriyta*	*E.coli*	20	128	26	27
2		*Bacillus*	20	128	27	29
3		*S.aureus*	20	128	56	55
4		*S.epidermidis*	20	128	24	18
5		*Proteus*	20	128	24	26
6		*Klebsiella*	20	128	23	27

Fig.2: Antibacterial activity of polar extracts of *Caralluma fimbriyata*

Fig.3: Antibacterial activity of Non polar extracts of *Caralluma fimbriyata*

DISCUSSION

The therapeutic value of medicinal plants lies in the various chemical present in it. The bioactivity of plant extracts is attributed to phytochemical constituents. For instant plant rich in phytochemicals are reported to have major group of phenolic compounds for their antiviral properties,antimicrobial and tannins which inhibit the bacterial growth by damaging the cell membrane. The data on antimicrobial activity are given in the Table 2 and 3. Its clearly showed that all the extracts have antimicrobial activity almost equivalent to that of control. Methanol, ethanol, chloroform, petroleum ether and aqueous extracts shown better activity, against *E.coli, Bacillus SP., Proteus Sp., S. aureus, S. epidermidis, Klebsiella Sp.* Aqueous extracts were more effective against *Proteus Sp.,* and S. *aureus.* Methanol extract was more effective against *Proteus Sp., S.aureus and Bacillus Sp. C*hloroform extracts were shown more activity against *S.aureus.* The antibacterial potential of plant was compared according to their zone of inhibition against the several pathogenic bacteria. The plant powder from various extracts possesses showed their activity against the bacteria. Methanol extracts of *C. nilagiriana* showed high antibacterial activity against *P.aeroginosa* with about (30± 1.84 mm) inhibition zone. *P.aeroginosa* and *Bacillus subtilis,* especially those with multi drug resistance, are among the most difficult to treat with conventional antibiotics 27. In the present study the growth of *Bacillus* was remarkably inhibited by the methanol extracts of *C. fimbriyata* (23mm) (Table-3). However the methanol extract showed low activity against another bacterium *E.coli* (14mm) in comparison to that of aqueous extract and methanol extract. The aqueous extracts showed more activity in *Proteus* and *S. aureus* (17mm) and (15mm) that the methanol extract and chloroform extract showed low activities. The chloroform extract showed high activity on the pathogen *S.aureus* (56 mm) inhibition zone. Among the six selected pathogens, against *C. fimbriyata,* five different extracts showed better antibacterial activity . The inhibitory activity of plant extracts generally depends on the concentration, type of parts used and microbes tested. The accumulation and concentration of secondary metabolites which are responsible for inhibitory activity varies according to the plant parts. It may be the reason for the variation in the inhibitory activity of extracts of *Caralluma fimbriyata* due to the presences of alkaloids, tannins, flavonoids, terpenoids, glycosides, amino acids and carbohydrates. In this plant, further studies are needed to isolate and characterize the bioactive principle compounds to develop new antibacterial drug.

CONCLUSION

Preliminary phytochemical studies shows the presence of alkaloids, glycosides, tannins, saponin, flavonoids, steroid, and phenol were observed. As per our knowledge different solvent extracts of *Caralluma fimbriyata* stem showed the antibacterial action in dose dependent on different pathogenic strains. Further studies are needed for confirmation of antibacterial action by isolating pure chemical constituents and also identify which compound is responsible for antibacterial action of *Caralluma fimbriyata*.

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