



**ANTIBACTERIAL ACTIVITY OF THE WHOLE PLANT OF
CARALLUMA NILAGIRIANA KUMARI ET SUBBA RAO –
AN ENDEMIC MEDICINAL PLANT SPECIES.**

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ABSTRACT

The methanolic, aqueous and chloroform extracts of the endemic medicinal plant species of *Caralluma nilagiriana* were studied for antibacterial activities against five microorganisms. The zone of inhibition of various extracts was compared with standard tetracycline(30 μ g/ml). The antibacterial activity justifies its use in traditional medicine. In *Salmonella typhi*, *Escherichia coli*, and *Staphylococcus aureus* all the three extracts(30 μ g/ml) was found to have significant antimicrobial activity, but less than that of standard tetracycline. In *Klebsiella pneumonia* all the three extracts were highly active when compared to standard. But in *Pseudomonas aeruginosa* the methanolic extracts strongly inhibited the colonial growth against the standard. The other two extracts found to have antimicrobial potency but less than that of standard.

KEYWORDS: *Caralluma nilagiriana*, tetracycline, antimicrobial activity.



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INTRODUCTION

Plants have been used as healers and health rejuvenators since time immemorial. Even now, WHO recognizes that medicinal plants play an important role in the health care of about 80 percent of world population in developing countries and depend largely on traditional medicines, of which herbal medicines constitutes the most prominent part¹. The rest of the 20% also depend substantially on the plant-based medicines. In India, high species richness in plants enhancing the scope of the medicinal plant research. The use of which for human and veterinary health care has probably continued in an unbroken tradition for well over two millennium, decades. The number of plants having confirmed therapeutic properties or yielding a clinically useful chemical compound thus lies around 700 species. In addition, almost 25% of the entire compounds of current prescription drugs were derived originally from plant sources². Of the estimated 25,000 flowering plant species in the world today, only about 10% have been scientifically examined for their medical application, mostly in rudimentary way. Undoubtedly, many more plant-derived medicinal substances await discovery³. The information on the medical wealth of these examined 10% of plant species is scattered in different sources. The Western Ghats is very rich in its medicinal wealth. The forests and the vegetation's of this region is a treasure house of about 700 medicinal plants. Out of which some are used for traditional and folk medicinal practices. Many are exploited commercially for their active enzymes and their commercial value.

Caralluma is a xerophytic genus includes about 120 taxa, with a wide African, Asian, South African and Southeast European distribution⁴. It belongs to subtribe stapeliinae (tribe *Ceropegiae*, sub family Asclepiadoideae and family Apocyanaceae), which has its center of origin in East Africa⁵. About 13 species and seven varieties of *Caralluma* occur in India. Out of the thirteen species,

eleven species are solely endemic to south India⁶. Rare and endangered species are *C. bhupenderiana*, *C. sarkariae*, *C. nilagiriana*, *Boucerosiatruncato-coronata*, *B. procumbens* and *B. pauciflora*⁷. Ethanobotanically, it is being used either to cure diabetes, fat accumulation or as significant anti-inflammatory and antitumor activity^{8, 9&10}. The photochemistry of genus *Caralluma* is characterized by many pregnane glycosides. While recently megastimane glycosides also have been isolated¹¹ *Caralluma* extracts have also been found to be appetite suppressant a property which is well known to Indian tribal and hunters. Indian folk lore records its use as a potent appetite suppressant and weight loss promoter¹². The juicy stem of *C. hiberculata* is bitter tonic febrifuge stomachic and carminative and it is use full in rheumatism and consume as vegetable especially when cooked with minced meat¹³. *C. sinaica* can cause significant decrease in glucose level in healthy animal¹⁰. In another report, it was observed that *C. fimbriata* can be used in weight reduction¹⁴ *Caralluma* species is known for its antihyperglycemic activity¹⁵ and *C. edulis* for antidiabetic properties¹⁶. The extract of *C. attenuate* and *C. edulis* had hypoglycemic properties and provide synergistic effect in combination with the phlorizin extract which beneficially modified glucose transport, blood and urine glucose levels, blood insulin levels and helps in weight loss¹⁷. The indiscriminate and destructive harvesting of these plants continues unable despite increased governmental regulation, resulting in many species becoming endangered. *C. nilagiriana* is a succulent medicinal plant depleted due to over exploitation, lack of organized cultivation and completely eaten by sheep and goats, the wild population has become restricted to Nilgiris, Tamilnadu. Regrettably, this species has now been added to the list of Indian endemic plants¹⁸. To our knowledge, there is no report on

antibacterial activity of *C. nilagiriana*. Hence the present study was attempted to know the level of antibacterial activity against certain human pathogenic bacteria

MATERIALS AND METHODS

Plant Material: The species, *C. nilagiriana* was collected from foot hills of Ooty, Nilgiri District, Tamil Nadu and confirmed the binomial with the voucher specimen was deposited in the Department of Botany, Government Arts College (Autonomous) Coimbatore.

SAMPLE PREPARATION

(i) Extraction of Dried Plant material

The fresh plant was carefully washed with tap water, rinsed with distilled water, and air-dried for one hour. Then it was cut into small pieces and dried in room temperature for two weeks. Then it was grounded into powder with the help of hand mill and stored in room temperature. About 20g of the powdered plant was successively extracted with, chloroform methanol and aqueous using Soxhlet apparatus till exhaustion and finally aqueous extract was prepared by using chloroform: water (1:99) by simple maceration at room temperature. All the extracts were carefully evaporated in a rotary evaporator under controlled temperature and reduced pressure to get the extract^{11, 20}.

(ii) Bacterial Strains and Culture Conditions

Bacterial strains was obtained from the Department of Microbiology, Hindusthan College of Arts and Science, Coimbatore, and cultured on Mueller-Hinton agar medium with regular intervals for subculture and stored at 20°±2°C. Stock cultures containing 1X 10⁷CFU/ ml (0.5 MacFarland) of each bacterial strains were frozen a 20°C, thawed when required to perform the test and cultured for two days in nutrient broth. The cultures obtained were vortexed, large agglomerates allowed to sediment

completely and the supernatant further diluted 1:5 in complete minimal broth.

(iii) Test Microorganisms

Salmonella typhi, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* were used in the study. Eighteen hour broth culture of the test bacterial isolates was suspended in sterile nutrient broth. They were standardized according to National Committee for Clinical Laboratory Standards (NCCLS) by gradually adding normal saline to compare their turbidity to McFarland standard of 0.5 which is approximately 1.0 × 10⁶CFU/ml.

(iv) Antimicrobial Assay- Well diffusion method

The modified agar well diffusion method was employed to determine the antimicrobial activities²¹. About 0.2 ml of the standardized 24 hour old culture of the tested organisms in nutrients broth was spread onto sterile prepared Muller Hinton Agar plates. Different types of the extracts chloroform, ethanol and methanol (30 µg/ml) were prepared and compared with tetracycline (30 µg /ml,) as a standard. With the aid of a sterile cork borer, wells of about 6 mm in diameter were bored onto the plates. About 0.5 ml of each concentration of the extracts was dispensed into the wells and then allowed to stand for about 15 minutes for prediffusion of the extracts to occur. These were then incubated at 37⁰C for 24 hours. At the end of the incubation period, inhibition zones formed on the agar were evaluated in millimeter (mm). The diameter of the zones of inhibition in the plates was measured by calculating the diameters of inhibition²².

RESULTS AND 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 flavonoids are reported to have major group of phenolic compounds for their antiviral properties²³, antimicrobial²⁴ and

tannins²⁵ which inhibit the bacterial growth by damaging the cell membrane²⁶.

The data on antimicrobial activity are given in the Table -I .which clearly showed that all the extracts have antimicrobial activity almost equivalent to that of control (tetracycline). Methanol, chloroform and aqueous extracts shown better activity against *K. pneumonia*, *P.aeruginosa* and *S. aureus* than the control. Aqueous extracts were more effective against *K. pneumonia* and *S. aureus*. Methanol extract was more effective against *K. pneumonia* and *P.aeruginosa*. Chloroform extracts were shown more activity against *K. pneumonia* 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.aeruginosa* with about (30± 1.84 mm) inhibition zone.

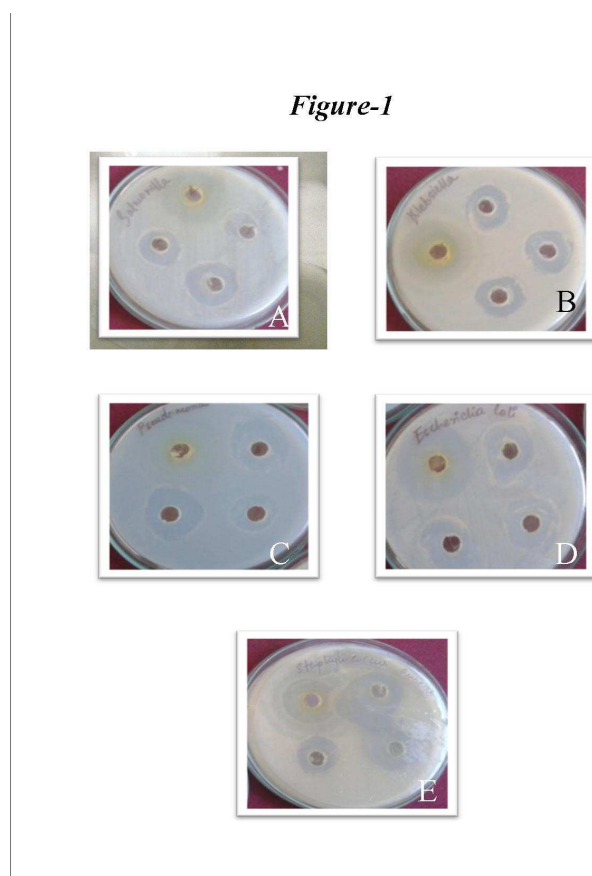
P.aeruginosa and *Bacillus subtilis*, especially those with multi drug resistance, are among the most difficult to treat with conventional antibiotics²⁷. In the present study the growth of *P. aeruginosa* was remarkably inhibited by the methanol extracts of *C. nilagiriana*(30 ± 1.84mm) (Table-I). However the methanol extract showed low activity against another bacterium *S. typhi* (15 ± 1.56 mm) in comparison to that of aqueous extract and methanol extract. The aqueous extracts showed more activity in *K. pneumonia* and *S. aureus* (26 ± 1.44mm) and (22 ± 1.68mm) that the methanol extract and chloroform extract showed low activities. The chloroform extract showed high activity on

the pathogen *K. pneumonia* (22 ± 1.41mm) inhibition zone. Both aqueous extracts and methanol extract showed high antibacterial activity than the chloroform extract. Among the five selected pathogens, against *C. nilagiriana*, three different extracts showed better antibacterial activity than the standard, tetracycline. The inhibitory activity of plant extracts generally depends on the concentration, type of parts used and microbes tested.² The accumulation and concentration of secondary metabolites which are responsible for inhibitory activity varies according to the plant parts²⁸. It may be the reason for the variation in the inhibitory activity of extracts of *C. nilagiriana* due to the presences of alkaloids, tannins, flavonoids, terpenoids, glycosides, amino acids and carbohydrates, ect in this plant. Further studies are needed to isolate and characterize the bioactive principle compounds to develop new antibacterial drug.

CONCLUSION

Plant extracts have great potential as antimicrobial compounds against microorganisms. Thus, they can be traditionally used in the treatment of infectious diseases caused by resistant microbes. The synergistic effect from the association of antibiotic with plant extracts against resistant bacteria leads to new choices for the treatment of infectious diseases. This effect enables the use of the respective antibiotic when it is no longer effective by itself during therapeutic treatment.

Figure 1
Antimicrobial activity of various extracts of *Caralluma nilagiriana*



- A. *Salmonella typhi*
 B. *Klebsiella pneumonia*
 C. *Pseudomonas aeruginosa*
 D. *Escherichia coli*
 E. *Staphylococcus aureus*

Table: 1
Antimicrobial activity of various extracts of *Caralluma nilagiriana*

S.No	Organism	Tetracycline (Control)	Zone of inhibition (in mm)		
			Methanol Extract	Aqueous extract	Chloroform Extract
1	<i>Salmonella typhi</i>	28 ± 1.74	15 ± 1.56**	20 ± 1.68**	25 ± 1.55**
2	<i>Klebsiella pneumonia</i>	20 ± 1.65	25 ± 1.38**	26 ± 1.44**	22 ± 1.41*
3	<i>Pseudomonas aeruginosa</i>	28 ± 1.81	30 ± 1.84*	25 ± 1.57**	15 ± 1.39**
4	<i>Escherichia coli</i>	32 ± 2.34	25 ± 1.52**	23 ± 1.26**	28 ± 1.96**
5	<i>Staphylococcus aureus</i>	20 ± 1.93	18 ± 1.79*	22 ± 1.68*	16 ± 1.35**

Values are mean ± SD of six samples * - $p < 0.05$ ** - $p < 0.01$ ns – Not significant

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