Int J Pharm Bio Sci 2013 Jan; 4(1): (B) 42 - 48

Research Article **Microbiology**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

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

R.PRABAKARAN AND K.KALIMUTHU*

Plant Tissue Culture Division, PG and Research Department of Botany, Government Arts College (Autonomous), Coimbatore-641018, India

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(30µg/ml). The antibacterial activity justifies its use in traditional medicine. In *Salmonella typhi. Escherichia. coli*, and *Staphylococcus aureus* all the three extracts(30µg/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 aeroginosa* 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.**

K.KALIMUTHU
Plant Tissue Culture Division, PG and Research Department of Botany, Government Arts College (Autonomous), Coimbatore-641018, India

Corresponding author

INTRODUCTION

Plants have been used as healers and health rejuvenators since time immemorial. Even now, WHO recognizes that medicinal plants plays 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 part[1]. 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 sources[2]. 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 discovery [3]. 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 distribution[4]. It belongs to subtribe stapeliinae (tribe *Ceropegiae*, sub family Asclepiadoideae and family Apocyanaceae), which has its center of origin in East Africa [5]. About 13 species and seven varieties of *Caralluma* occur in India. Out of the thirteen species, eleven species are solely endemic to south India[6]. 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 isolated [11] *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 promoter [12]. 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 meat [13].*C.sinaica* can cause significant decrease in glucose level in healthy animal[10]. In another report, it was observed that *C.fimbriata* can be used in weight reduction [14]*Caralluma* species is known for its antihyperglycemic activity[15]and *C.edulis*is for antdiabetic properties[16].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 loss [17]. 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 plants [18]. 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 10^7CFU/ 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 aeroginosa, 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 × 10^6CFU/ml.

(iv) Antimicrobial Assay- Well diffusion method

The modified agar well diffusion method was employed to determine the antimicrobial activities[21]. 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 37^0C 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 inhibition[22].

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 properties[23],antimicrobial[24] and

tannins[25] which inhibit the bacterial growth by damaging the cell membrane[26].

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.aeroginosa* 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.aeroginosa*. *C*hloroform 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.aeroginosa* with about (30± 1.84 mm) inhibition zone.

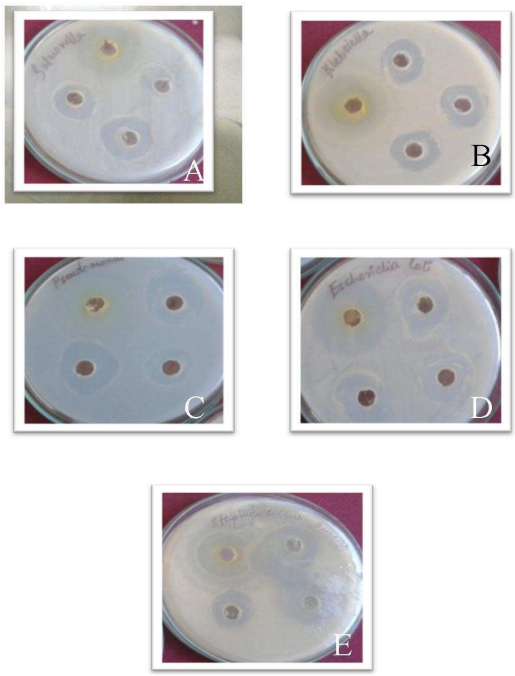
P.aeroginosa and *Bacillus subtilis*, especially those with multi drug resistance, are among the most difficult to treat with conventional antibiotics [27]. In the present study the growth of *P. aeroginosa* was remarkably inhibited by the methanol extracts of *C. nilagiriana*(30 ± 1.84mm) (Table-I). 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 parts [28].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

Figure-1



A. Salmonella typhi
B. Klebsiella pneumonia
C. Pseudomonas aeroginosa
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	*Salmonella typhi*	28 ± 1.74	15 ± 1.56**	20 ± 1.68**	25 ± 1.55**
2	*Klebsiella pneumonia*	20 ± 1.65	25 ± 1.38**	26 ± 1.44**	22 ± 1.41*
3	*Pseudomonas aeroginosa*	28 ± 1.81	30 ± 1.84*	25 ± 1.57**	15 ± 1.39**
4	*Escherichia coli*	32 ± 2.34	25 ± 1.52**	23 ± 1.26**	28 ± 1.96**
5	*Staphylococcus aureus*	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

REFERENCES

1. Farnsworth,Global Importance of Medicinal Plants, Conservation of Medicinal Plants - Proc. International Consultation, 21-27 March, Chian Mai University, Thailand (1988).
2. Balandrin,M.F., Jocke, Wurtele,E. Natural plant chemicas: source of industrial and echanical materials. Science 228: 1154-1160.(1985).
3. Akerale, Global Importance of Medicinal Plants, Conservation of Medicinal Plants; (1991).
4. Mabberley DJ The plant book.Cambridge University press, Cambridge. (1993).
5. Meve U, Liede S Subtribal division of Ceropegieae (Apocynaceae – Asclepiadaceae). Taxon. 53:61-72. (2004).
6. Jagatap AP, Singh NP Fascicles of Flora of India. Fascicle 24.Botanical Survey of India.Culcutta, (1999).
7. Nayar MP Hot spots of endemic plants of India, Nepal and Bhutan. Tropical Botanical Garden Research Institute. Trivandrum. India,(1996).
8. Ramesh, M. Rao,Y.N. Kumar, M.R.Rao, A.V.N.A. Prabhakar,M.C.and Reddy, B.M. Actinociceptive and and-inflammatory activity of carumbelloside-I isolated from *Caralluma umbellate*, J.Ethanopharmacol. 68: 349-352. (1999).
9. Zakaria NMM, Islam MW,Radha Krishnan R,Chen HB, KamilM, Al-GifrilAN,ChanK,Al-Attas A Antinociceptive and anti-inflammatory properties of *Caralluma arabica*.J.Ethnopharmacoal., 76:155-158. (2001).
10. HabibuddinM,Daghriri HA, Humaira T, Quhtani MSA, Hefzi AAH Antidiabetic effects of alcoholic extracts of *Caralluma sinaicaL.*onsteptozotocine-induced diabetics rabbits. J. Ethnopharmacol.117:215-220, (2008).
11. Jayakar, B.Rajkapoor, B ,Efforts of *Caralluma altenuata* in normal and alloxen induced diabetic rate.J. Herb.Phamacother. 4:35-40. (2004).
12. Raja Sreelatha, and Pullaiah, JInduction of somatic embryogenesis and plant regeneration from internodal explants of Caralluma stalagnifera. Botamy Res. Internatinal.3(1):17-20. (2010).
13. Shinwari ZK, Watanabe T, Rehman M, Youshikawa T Apictorial guide of medicinal plants of Pakistan. Kohat University of Science Technology.Kohat, Pakistan. p.91,(2006).
14. Lawrence RM, Choudhary S,*Caralluma fimbriata* in the treatment of obesity .12th annual congress on anti aging medicine. Winter session December 2-5. Las vegasNv USA. (2004).
15. Venkatesh S, Reddy GD, Reddy BM, Ramesh M, Rao AVNA Antihyperglycemic activity of*Caralluma attenuate*. Fitoterapia, 74: 274-279. (2003).
16. WadoodA,WadoodN,Shah SA ,Effect of *Acacia arabica* and *Caralluma edulis* on blood glucose levels of normal and alloxan diabetic rabbits. J.Pak. Med. Assoc.9: 208-212. (1989).
17. Khan AW, Khatoon S Ethnobotanical studies on some useful herbs of Haramosh andBugrote valleys in Gilgit, northern areas of Pakistan. Pak. J. Bot. 40:43-58. (2008).
18. Botanical Survey of India. Studied on rare and endangered species. http://.envfor.nic.in/bsi/reseaach.html.(2002).
19. Paech K, Tracey MV. Modern methods of plant analysis.Vol-I. Germany: Springer-Verlag,Berlin-Gottingen-Heidelberg, 78-79. 1956).
20. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy, 29th ed. NiraliPrakashan, Pune.; 318-320. (2004)
21. Collins, C. H., Lynes, P. M., Grange, J. M. *Microbiological Methods* (7th edition). Butter wont-Heinemann Ltd, Britain pp. 175-190. (1995).

22. Hewitt W, Vincent S In: Theory and application of microbiological assay. (1989).
23. Mehrangiaz KK, Seyed AE, Masoud SG, Esmaeel AS, Amirhossein .S.Antiviral activity of aerial subsets of *Artemisia* species against Herpes Simple virus type 1(HSV1)*in vitro.Asian Biomed*;5(1): 63-68 (2011).
24. Maria Lysete AB, Maria Raquel FL, et al. Studies on the antimicrobial activity brine shrimptoxicity of *Z. tuberculosa* extract and their main constituents. *Annels of CliMicrobAntimic; 8*: (2009).
25. JulianeliTDl., Jackson RGS, Kelly S Ana Silvia SC, et al. selective spasmolytic effect of a new furanoflavoquinone derivative from diplotrobine on guinea-pig trachea. *J Che Pharm Res*; 3 (1):249-258 (2011).
26. Mohammed Sham Shihabudeen H, Hansi Priscilla D, Kavitha T. Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants. *Int J of PharmaSci Res*; 1(10):430-434 (2010).
27. Chandler, R.F., S.N. Hooper and M.J. Harvey, Ethnobotany and phytochemistry of yarrow, *Achilleamillefolium*, Compositae. Econ. Bot., 36: 203-223. (1982).
28. Essawi ., T and Srours, M. Screening some Palestinian medicinal plants for antibacterial activity. J. Ethanopharmacol . 70: 343-349,(2000).