September 2009 Volume 5 Issue 3

Trade Science Inc.

Natural Products

An Indian Journal

Short Communication

NPAIJ, 5(3), 2009 [143-145]

In vitro studies in *Caralluma adscendens* R.Br. (Roxb) var. *adscendens* against intestinal pathogens

Dakshayani Malkapur, G.M.Vidyasagar*

Department of Postgraduate Studies and Research in Botany Gulbarga University,Gulbarga-585106, Karnataka-585106, (INDIA)

E-mail : gmvidyasagar@rediffmail.com

Received: 20th June, 2009 ; Accepted: 30th June, 2009

ABSTRACT

Based on the prevailing traditional knowledge at Hyderabad Karnataka region, *Caralluma adscendens var.adscendens* was selected for the present studies and extracted the plant materials in different solvents like, methanol, ethanol, ethyl acetate, hexane and water. These different solvent extracts were tested against seven clinical bacterial isolates viz, *Vibrio cholerae*, *Shigella dysentriae* Type1, *Salmonella paratyphi* B, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Klebsiella pneumoniae* and *Escherichia coli*. Ethyl acetate extract showed significant inhibitory activity among the solvent extracts tested, while water extract has negligible activity against the pathogens. The root extracts in all the solvents including aerial part extract in hexane did not show any activity. The ethyl acetate aerial part extract at 80mg/ml concentration showed higher activity against most of the bacterial isolates and similar and even higher activity than penicillin and ampicillin at 10mg/ml. The standard ciprofloxacin had more significant inhibitory activity compared to other extract and standards.

KEYWORDS

Antimicrobial activity;
Caralluma adscendens var.*adscensens*;
Intestinal pathogens.

INTRODUCTION

Caralluma, a small, erect, fleshy and soft thorn herb with four grooved stem round in shape, locally known as manganakodu. Stem is generally devoid of leaves and form small flowers in a variety of dark colours. Their pods are erect, linear and about 2.5 cm in length with velvety touch. The *Caralluma* belongs to family Asclepiadaceae (cacti) is listed as edible by numerous natives of India over many centuries[1]. The species of *Caralluma* found in India are edible and serve as traditional medicine. In Hyderabad Karnataka region, *Caralluma. adscendens* var.*adscendens* is commonly grown during rainy season within the thorny shrubs. The tribal communities use the plant as food as well as medicine for some stomach disorders. The available literature reveals no scientific report on the use of this plant as antimicrobial agent. Therefore, the present studies were carried out to record its effectiveness against the pathogens responsible for stomach disorders in the region.

MATERIALS AND METHODS

Fresh plant material was collected from different places of Gulbarga, washed under running tap water, air dried, homogenized to fine powder and used for extraction in five different solvents.

Short Communication

Aqueous extraction: 10g of air dried powder was placed in distilled water and boiled for 6h. At an interval of 2h, it was filtered through muslin cloth and centrifuged at 5000rpm for 15 min. The supernatant thus obtained was evaporated and stored at 4^0C. Finally, 40mg/ml and 80mg/ml concentrations were prepared using 25% DMSO (Dimethylsulphoxide).

Solvent extraction: 10g of air dried powder was placed in 100ml of each solvent in a conical flask, plugged with cotton and then kept on a rotary shaker for 24h. After 24h, the content was filtered through muslin cloth and the solvent was evaporated. The extract was stored at 4°C for further use.

Screening for antibacterial activity: Seven clinical bacterial isolates such as, *V. cholerae, S. paratyphi B, P. vulgaris, S. dysentriae, K. pneumoniae, E. coli* and *P. aeruginosa,* were collected from M.R. Medical College, Gulbarga and confirmed their identity using literature. Antibacterial activity of plant extracts was tested using modified agar well diffusion method[2]. Culture plates were prepared by pouring nutrient agar medium into sterile petri plates. The inoculums containing 10^8/ ml bacterial cells was spread over agar medium using sterile cotton swabs to get uniform distribution of bacterial cells. The dried plant extract was dissolved in 25% aqueous DMSO to a final concentration of 40mg/ml and 80mg/ml. Wells were made in the petri plate with the help of 8 mm cork-borer and test compound was introduced into the wells. The Petri plates were kept for diffusion for half an hour in cold and incubated overnight at 37^0C. Microbial growth was determined by measuring the diameter of inhibition zone. 25% DMSO was used as control.

Statistical analysis: Analysis of variance (ANNOVA) and Duncan's test were carried out using SPSS package and presented the results in table.

RESULT AND DISCUSSION

The antimicrobial activity of *Caralluma adscendens* aerial parts studied against seven intestinal bacterial pathogens revealed maximum growth inhibition in ethyl acetate, followed by ethanol, methanol and water and the results are presented in the table.

Table : Antibacterial activity of different solvent extracts of *caralluma adscendens .var. adscendens* and standards

Organisms	Ethyl acetate		Methanol.		Ethanol.		Water		Penicillin	Ampicillin	Chloremp--henicol	Ciprofloxacin
	40%	80%	40%	80%	40%	80%	40%	80%				
V.c	12.00^{d}±1.15	15.00^{e}±0.57	5.00^{b}±0.57	6.00^{bc}±0.57	5.00^{b}±1.52	8.00^{c}±0.57	0.00^{a}±0.00	0.00^{a}±0.00	13.00^{de}±0.57	13.33^{de}±1.20	15.00^{e}±1.15	20.00^{f}±0.57
S.p	3.00^{ab}±0.57	5.00^{c}±0.00	2.00^{a}±0.57	4.00^{bc}±0.57	3.00^{ab}±0.57	2.33^{ab}±0.33	3.00^{ab}±0.57	3.33^{ab}±0.33	5.00^{c}±0.57	10.00^{d}±0.57	9.00^{d}±0.57	10.00^{d}±0.57
P.v	8.00^{cd}±1.00	12.00^{ef}±1.15	2.33^{ab}±0.88	6.00^{c}±0.57	3.33^{b}±0.33	10.00^{dc}±0.57	0.00^{a}±0.00	0.00^{a}±0.00	20.00^{g}±0.57	25.00^{h}±1.15	13.00^{f}±0.57	20.00^{g}±1.15
S.d	7.67^{cd}±1.20	12.00^{e}±0.57	3.00^{ab}±0..57	5.00^{bc}±0.57	5.00^{bc}±1.15	10.33^{de}±1.20	2.00^{a}±0.57	2.33^{a}±0.33	12.00^{e}±1.00	13.00^{e}±1.15	17.00^{f}±1.15	20.00^{g}±1.00
K.p	5.00^{bc}±0.57	10.00^{d}±1.15	3.00^{b}±0.57	6.00^{c}±0.57	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	5.00^{bc}±0.57	12.00^{d}±0.57	11.00^{d}±1.00	18.00^{e}±1.52
E.c	5.33^{ab}±1.20	9.00^{c}±1.52	5.00^{ab}±0.57	6.00^{ab}±1.15	5.00^{ab}±1.00	8.00^{bc}±1.00	3.33^{a}±0.33	5.67^{ab}±0.33	5.00^{ab}±1.15	7.00^{bc}±0.57	22.00^{e}±0.57	18.00^{d}±0.57
P.a	2.00^{bc}±0.00	2.00^{bc}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	2.33^{bc}±0.33	2.00^{bc}±1.00	0.00^{a}±0.00	0.00^{a}±0.00	1.00^{a}±0.00	3.00^{bc}±0.57	3.00^{c}±0. 57	12.67^{d}±1.45

A.–Aerial part, R- Root, V..c – Vibrio cholerae, S. p - Salmonella paratyphi B, P. v – Proteus vulgaris, S. d –Shigella dysentriae Type1, K.p - Klebsiella pneumoniae, E.c - Escherichia coli, P. a - Pseudomonas aeruginosa.

Values are the mean of three sets ± SEM

*** ANNOVA Results**

Indicate Significant difference ($p<0.05$)

$^{a-g}$ Mean value with the same superscript within a row do not differ significantly.

Ethyl acetate extract at 80% concentration showed a maximum of 15.00^{e}±0.57mm, 12.00^{ef}±1.15mm, 12.00^{e}±0.57mm and 10.00^{d}±1.15mm inhibition in *V. cholerae*, *P. vulgaris*, *S. dysentriae* Type1 and *K. pnemoniae* respectively. Similarly, the ethanol extract showed maximum growth inhibition in P. *vulgaris* (10.00^{dc} ±0.57 mm) and *S. dysentriae* Type1 (10.33^{de} ±1.20mm) at 80% concentration. The methanol and water extracts were comparatively less effective against all the bacteria tested. Among the bacteria, *P. aeruginosa* was found to be resistant to all the solvent extracts including standards such as, penicillin, ampicillin and chloremphenicol, however a considerable growth inhibition of 12.67^{d}±1.45 mm was seen in ciprofloxacin. Both the concentrations of ethyl acetate, methanol, ethanol and water extracts showed no or very less growth inhibition in *P. aeruginosa, P. aeruginosa,* and *S. paratyphi* B, *P. aeruginosa, K. pnemoniae* and *S. paratyphi* B, *V. cholera, P. vulgaris, K. pnemoniae, P. aeruginosa, S. paratyphi* B, and *S. dysentriae* Type1 respectively.

The results reveal that the sensitivity of the organisms to all the four extract is concentration dependent. All the extracts have shown significant difference against *V. cholera, P. vulgaris and K. pneumoniae.* However, among the extracts, ethyl acetate extract showed significantly ($p<0.05$) higher activity. All the four extracts were not significantly different in activity against *S. paratyphi* B and *P. aeruginosa*.

The literature survey indicated wide occurrence of *Caralluma* species, but most of its varieties are listed as endangered ones. They are known for carminative, febrifugal, anthelmintic, anti-rheumatic, anti-diabetic and anti-hyperglycaemic, anti-pyretic, anti-inflammatory, anti-nociceptive, and anti-oxidant properties. The *Caralluma* is also kown as appetite-suppressants and CNS stimulant[3]

In the present study, the ethyl acetate extract of *C.adscendens var.adscendens* showed significant antibacterial activity against six clinical intestinal bacteria except *P. aeruginosa.* Maximum inhibition was recorded at 80% concentration against *V.cholerae,* which was better than penicillin and ampicillin and similar to chloremphenicol. The ethyl acetate extract was also better for *S. dysentriae,* which was comparable with penicillin and ampicillin. Among the organisms, *P. aeruginosa* was found to be resistant to antibacterial agents tested; however the crude ethyl acetate extract found inhibitory indicating its effectiveness in pure form.

The aqueous extract appears to have less antibacterial activity than methanol, ethanol and ethyl acetate extract unlike that of traditional practice, where the bacterial intestinal infection is treated by administering water decoction. The ethyl acetate extracts in some plants found better than other solvent extracts[4]. Ethyl acetate extract of *Combretum racemosum* is active against *S. typhii, P. aeruginosa and E. coli* causing gastrointestinal infections[5]. Similar report is on *Chrozophora senegalensis* ethyl acetate extracts against bacteria responsible for diarrhea[6]. In another report on the aerial parts of *Bacopa monnieri* Linn., ethyl acetate fraction was found more potent than n-butanol fraction against *S. faecalis, P. aeruginosa, S. typhii, V. cholerae, S. dysenteriae* and *E. coli* causing stomach disorders[7]. *C. arabica* has been shown to possess anti-gastric ulcer and cytoprotective properties against damage produced by phenylbutazone, indomethacin, ethanol, sodium hydroxide, and/or cold restraint stress[8] and Evalvation of *Caralluma tuberculata* pretreatment for the protection of rat gastric mucosa against toxic damage[9]. The result of the present study supports the use of *C.adscendens* var. *adscendens* traditionally for the treatment of stomach disorders.

ACKNOWLEDGEMENT

First author is thankful to Gulbarga University, Gulbarga for providing merit fellowship and also express gratitude towards Prof. Maheshwarappa, Department of Library science, Gulbarga University, Gulbarga for helping in statistical analysis.

REFERENCES

[1] Reports of K.S.Ladha; Medicinal Natural Products Research Laboratory, Universty of Mumbai, Matunga, Mumbai, India.

[2] D.Onkar, Dhingra, B.James; Basic plant pathology methods, 287-305 **(1995)**.

[3] Reports of Rajendra Ramaswamy; *Caralluma* extract products and processes for making the same-Patent 7060308, **(2004)**.

[4] P.Guven, Çelik Keymet, Uysal Sezgin, Ismet; Pharmaceutical Biology., **43(1)**, 67-71 **(2005)**.

[5] A.Onocha, E.O.Audu, O.Ekundayo, O.O.Dosumu; Bioprospecting and Ethnopharmacology, **1**, **(2005)**.

[6] H.Usman, Y.M.Musa, A.A.Ahmadu, M.A.Tijjani; African journal of Traditional, Complimentary and Alternative medicine., **4(4)**, 488-494 **(2007)**.

[7] T.Ghosh, T.K.Maity, A.Bose, G.K.Dash, M.Das; Indian J.Pharm.Sci., **69(2)**, 312-314 **(2007)**.

[8] M.N.M.Zakaria, M.W.Islam, R.Radhakrishman, X.M.Liu, A.Ismail, M.Kamil, K.Chan, A.Al-Attas; Pharmaceutical Biology., **40**, 225-230 **(2002)**.

[9] M.D.Al-Herbi, S.Qureshi, M.Raza, M.M.Ahmed, M.Afzal, A.H.Shah; Toxicol.Appl.Pharmacol., **128**,1-8 **(1994)**.