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IN VITRO PLANT REGENERATION AND ANTIBACTERIAL ACTIVITY STUDIES ON THREE ENDEMIC SPECIES OF *CEROPEGIA*

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ABSTRACT

Plants are important sources of potentially useful structures for the development of new chemotherapeutic agents. To identify the antibacterial activity of *Ceropegia juncea, Ceropegia candelabrum* var. *candelabrum* and *Ceropegia spiralis in vitro* plant extracts of the plant for its antibacterial activity was performed with different solvents (ethanol, aqueous and chloroform) against various human pathogens viz., *Staphylococcus aureus, Escherichia coli, Klebsiella pnemoniae* and *Pseudomonas,* by agar well diffusion method. The ethanol extract from the *in-vitro* plant showed a higher inhibitory effect, a maximum zone of inhibition 1.6 cm (100µl).On the basis of these experimental results, *in vitro* plants of these *Ceropegia* species could be considered for further isolation and evaluation as therapeutic antimicrobial.

KEYWORDS: ***Ceropegia juncea*, *Ceropegia candelabrum* var. *candelabrum* and *Ceropegia spiralis*, *in vitro* plant extract, antibacterial activity.**

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INTRODUCTION

Nature has bestowed a large number of diverse types of plants, the richest source of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs[1]. Large number of plant is being constantly screened for their possible antimicrobial activity. Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs[2]. *Ceropegia* L. (Asclepiadaceae) is a pantropical Old World genus of over 200 species occurring around much of the perimeter of the Indian Ocean [3]. Of the 48 *Ceropegia* species found in India, 28 are endemic to the Peninsular India and many species of this genus have been added to the list of Indian endangered plants [4-5]. Most of this *Ceropegia* species became critically endangered and threatened because of their small population size, narrow range of distribution, over exploitation, rapid urbanization, tubers are prone to fungal infection and thus decay of tubers etc. *Ceropegia juncea* is an endemic, succulent tuberous and medicinal herb found in India occupying dry areas. Crassulacean acid metabolism is reported in this species [6]. This is the only Indian *ceropegia* species with reduced leaves or leaves are absent. The pharmacological importance of *Ceropegia juncea* is mainly due to the presence of a pyridine alkaloid '*Ceropegin*' a potential antipyretic, analgesic, local anaesthetic, antiulcer, mast-cell stabilizing, hepato-protective, tranquilizing, and hypotensive [7]. The fleshy stem is used as a raw material for traditional and folk medicines for the treatments of stomach and gastric disorders[8]. The fleshy stem is crushed with milk taken orally for three days to cure the decease Ulcer [9]. *Ceropegia candelabrum* var. *candelabrum* known as the "glabrous goglet flower" is a perennial medicinal herb found at the edges of moist deciduous forests [10]. *Ceropegia spiralis* Wight is an annual herb growing wildly in South India and is in endangered category [11]. The *Ceropegia spiralis* root tubers contain many nutritional elements. The starchy tubers are used as a nutritive tonic [12]. So, far there was no report on antibacterial activity of these three endangered *Ceropegia* species. In the present study, a protocol on *in vitro* propagation as well as the antibacterial activity of *Ceropegia juncea*, *Ceropegia candelabrum* and *Ceropegia spiralis,* against different bacterial strains was reported.

MATERIALS AND METHODS

(i)Preparation of Explants for In Vitro Establishment

Whole plants (*C. juncea, C. spiralis, C. candelabrum* var.*candelabrum*) was collected from the western Ghats of Kanyakumari District, Tamilnadu for the continuous source of explants for *in vitro* culture. Young, healthy and disease free portion of the branches was selected and used as explants. The explants (1-1.5 cm.) were thoroughly washed under running tap water for 10 min and then treated with a few drops of Tween -20 (v/v) for 1 min. The surface sterilization was carried out with 0.1 % (w/v) $Hgcl_2$ for 2 min followed by gentle shaking. After surface sterilization the explants were rinsed thoroughly seven times with sterile distilled water. The sterile nodal explants were cultured on MS medium fortified with different concentrations of various growth regulators for multiple shoot bud induction (Fig: 1, 2 &3).

(ii) Preparation of Plant Extracts

For the preparation of plant extract to test antibacterial activity, the plants parts were collected from 30 days old *in vitro* shoots, and thoroughly washed with tap water for 3 times. The washed plants were dried in shade at room temperature, powdered and stored in air-tight sterile containers. The weighed quantity (20 gm) of dry powder was subjected to successive solvent extraction method by using aqueous, Chloroform, and Ethanol. The

extracts were passed through double-layered muslin cloth and filtered through filter paper. The filtrates were pooled and evaporated in the air at room temperature, to obtain a final residue. The extracts were dissolved in Dimethyl-sulphoxide to make the final concentrations and refrigerated for further use.

(iii)ANTIBACTERIAL ASSAY- AGAR WELL DIFFUSION METHOD

The antimicrobials present in the plant extract are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in centimetres.

REAGENTS

1. Muller Hinton Agar Medium (1 L)

The medium was prepared by dissolving 33.9 g of the commercially available Muller Hinton Agar Medium[13] (HiMedia) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.

2. Nutrient broth (1L)

One litre of the nutrient broth was prepared by dissolving 13 g of commercially available nutrient medium (HiMedia) in 1000ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes.

3. Gentamycin (standard antibacterial agent, concentration: 20mg / ml)

(iv) PROCEDURE

Petriplates containing 20ml Muller Hinton medium were seeded with the 24hr culture of bacterial strains such as*, Staphylococcus aureus , E coli , Klebsiella pnemoniae* and *Pseudomonas* .Wells of approximately 10 mm was bored using a well cutter and sample of 25, 50, and 100 µl concentration were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well [13]. Gentamycin was used as a positive control.

RESULTS AND DISCUSSION

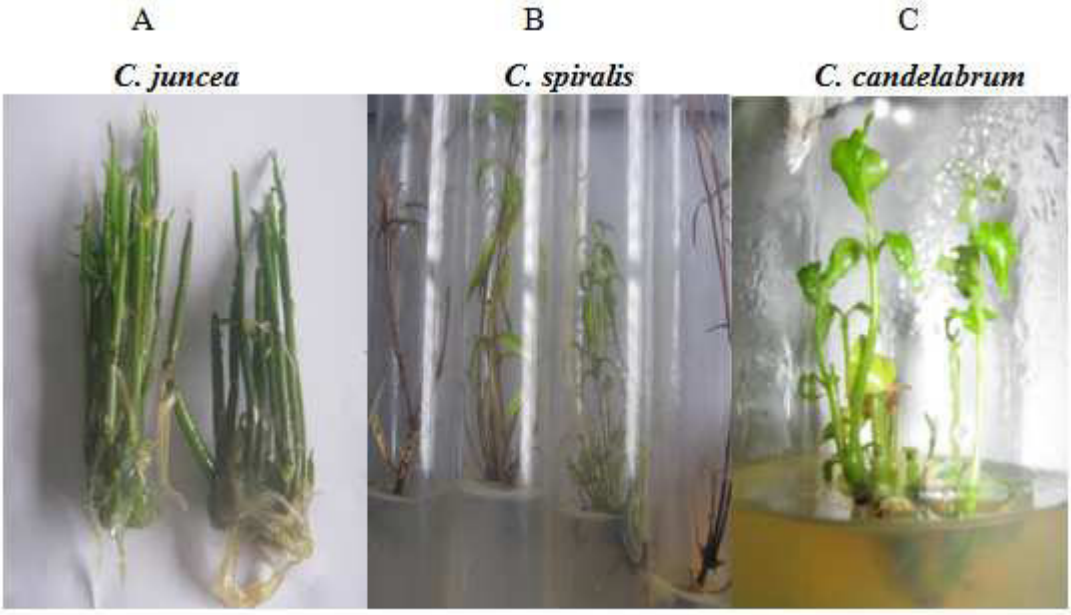

Figure 1
Multiple shoots bud induction of C. juncea (A), C. spiralis (B) and C. candelabrum var.candelabrum (C).

In the present study nodal segment of *C. spiralis, C. candelabrum* and *C. juncea* was cultured in the MS Medium (1962) containing different growth hormones. Maximum shoots proliferation and shoot elongation of *C. juncea* was noticed in MS medium containing 1.5 mg/l BAP and1.0 mg/l Kn (Fig.1 A). However, shoot regeneration from *C. spiralis* nodal explants were observed in 1.25 mg/l BAP and1.0 mg/l Kn(Fig.1 B). Maximum shoot proliferation and shoot elongation of *C. candelabrum* was observed in MS medium fortified with 1.5 mg/l BAP alone (Fig.1 C).The antibacterial activity of aqueous, chloroform and ethanol extracts of *C. spiralis, C. candelabrum* and *C. juncea* at different concentration displayed effective zone of inhibition against different bacterial strains. The tested chloroform extract of *Ceropegia* species posses potential antibacterial activity against, *Staphylococcus aureus, Escherichia coli, Klebsiella pnemoniae* and *Pseudomonas* (Table-1)

Table – 1

Test microorganisms	Zone of inhibition(cm)											
	Chloroform(Vol.µl) Zone of inhibition(cm)				Aqueous(Vol.µl) Zone of inhibition(cm)				Ethanol(Vol.µl) Zone of inhibition(cm)			
	12.5	25	50	100	12.5	25	50	100	12.5	25	50	100
Ceropegia juncea												
S. aureus	---	---	1.0	1.4	---	---	---	---	---	---	1.1	1.3
E. coli	---	---	----	----	---	---	0.5	1.0	---	---	1.0	1.2
K. pnemoniae	---	0.5	1.0	1.5	---	---	0.9	1.4	---	1.0	1.2	1.3
Pseudomonas	---	---	1.0	1.5	---	---	1.0	1.5	---	---	1.0	1.5
Ceropegia candelabrum												
S. aureus	---	0.7	1.2	1.5	---	0.6	1.0	1.4	---	1.0	1.2	1.3
E. coli	---	---	0.4	0.9	---	---	0.4	0.7	---	1.0	1.2	1.6
K. pnemoniae	---	---	---	---	---	---	---	---	---	---	0.9	1.1
Pseudomonas	---	---	---	0.9	---	---	---	---	---	---	0.9	1.1
Ceropegia spiralis												
S. aureus	---	---	---	---	---	---	---	---	---	1.0	1.2	1.4
E. coli	---	---	---	---	---	---		---	---	---	1.1	1.4
K. pnemoniae	---	---	---	---	---	---	---	---	---	---	0.9	1.0
Pseudomonas	---	---	---	---	---	---	---	---	---	---	---	1.0

The chloroform extract of *Ceropegia juncea* showed antibacterial activity against the bacterial strains with 1.5cm inhibitory zone formation. The highest antibacterial activity possessed has shown zone of inhibition 1.5cm (100µl) against *Klebsiella pnemoniae and Pseudomonas,* and least activity recorded the zone of inhibition at 0.5cm (25µl) against *Klebsiella pnemoniae.* On the other hand chloroform extract of *C. candilabrum* exhibit antibacterial activity against*, Staphylococcus aureus, Escherichia coli* and *Pseudomonas* with 1.5 cm. The highest antibacterial activity showed the zone of inhibition 1.5cm (100µl) against *Staphylococcus aureus* and least activity recorded 0.4cm (50µl) against *Escherichia coli.* In general the chloroform extract of C.juncea, failed to inhibit the growth of *Escherichia coli.* similarly *Ceropegia candelabrum* against *Klebsiella pnemoniae* and *C. spiralis* against *Staphylococcus aureus, Escherichia coli, Klebsiella pnemoniae and Pseudomonas*. Similar results were reported in antibacterial activity of *in vitro grown C. pusilla* against different bacterial strains[14] The ethanol extract of *Ceropegia juncea* reported significant antibacterial activity against *Staphylococcus aureus, Escherichia coli , Klebsiella pnemoniae* and *Pseudomonas* with the zone of inhibition of 1.5cm. The highest antibacterial activity showed the zone of inhibition 1.5cm (100µl) against *Pseudomonas*, and least activity 1.0cm (25µl) against *Klebsiella pnemoniae,* 1.0cm (50µl) against *Escherichia coli and Pseudomonas.* On the other hand *C. candelabrum* reported antibacterial activity against*, Staphylococcus aureus, Escherichia coli, Klebsiella pnemoniae and Pseudomonas* showed the zone of inhibition around 1.6 cm. The highest antibacterial activity showed the zone of inhibition 1.6cm (100µl) against *Escherichia coli* and least activity recorded 0.9cm (50µl) against *Klebsiella pnemoniae* and *Pseudomonas.* In general ethanol extract of *C. Spiralis* are highly sensitive against *Staphylococcus aureus, Escherichia coli, Klebsiella pnemoniae and Pseudomonas*. The present data coincides with the findings of the concentration of 0.5mg/ml ethanol extract of *Riccinus communis* effectively inhibit the

growth of *Staphylococcus aureus* [15], whereas the same concentration of *C. pusilla* inhibit the growth of *Escherichia coli, Klebsiella pnemoniae, Bacillus* sp. and *Staphylococcus aureus*[16]. The aqueous extract of *C. juncea* reported significant antibacterial activity against *Staphylococcus aureus, Escherichia coli, Klebsiella pnemoniae and Pseudomonas* with a zone of inhibition around 1.5cm. The highest antibacterial activity with a zone of inhibition 1.5cm (100µl) against *Pseudomonas*, and least activity was recorded against *Escherichia coli* with 0.5cm (50µl). On the other hand, aqueous extract of *C. candelabrum* exhibit highest activity against *S. aureus* and *E.coli* showed the zone of inhibition 1.4cm (100µl). The highest antibacterial activity with a zone of inhibition 1.4cm (100µl) against *Staphylococcus aureus* and least activity were recorded against *Escherichia coli* with a zone of inhibition 0.4cm (50µl). In general the aqueous extract failed to inhibit the growth of *C. juncea* against *Staphylococcus aureus. C.candelabrum* against *Klebsiella pnemoniae* and *Pseudomonas. C. spiralis* showed negative result against *Staphylococcus aureus, Escherichia coli, Klebsiella pnemoniae and Pseudomonas*. The obtained antibacterial report of *Ceropegia* species are in close agreement with the aqueous extracts of plant which do not have much activity against selected bacteria when compare to other extracts[17]. Activities vary with the species of the plants and plant material used. Thus the study ascertains the values for further attention to identify the active compounds responsible for this activity with the required minimum inhibitory concentration.

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