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Antimicrobial studies on *Coldenia procumbens* Linn. whole plant

Shakila R[1], Ganesan R[1], Meeradevi Sri P[2], Arul Antony S[3], Sathiyarajeswaran P[1]

[1]Siddha Central Research Institute (Central Council for Research in Siddha), Anna Hospital Campus, Arumbakkam, Chennai-600 106, India.
[2]Regional Research Institute for Unani Medicine, Royapuram, Chennai-600 013, India.
[3]PG & Research Department of Chemistry, Presidency College, Chepauk, Chennai-600 005, India.

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ABSTRACT

The ethanolic extract was screened for growth inhibition of microbes such as *Aeromonas hydrophila, Bacillus cereus, Bacillus subtilis,* Enterobacter *aerogens, Escherichia coli, Klebsiela pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus, Streptococcus pyrogens, Vibrio fischeri* and *Candida albicans.* The ethanolic extract was found to inhibit *K. pneumoniae, E. coli, B. cereus, S. typhimurium* and *P. vulgaris.*

Key words: Tripaksi, Serupadai, Serupadi, Borginaceae, Well diffusion method.

INTRODUCTION

Coldenia procumbens Linn. is procumbent herb found wild in fields, dried lakes and roadsides in warmer parts of India [1]. It is a member of Boraginaceae family. *C. procumbens* is described as Tripakṣī in Sanskrit as per the Ayurvedic literatures and is meant for rheumatism and abscess [2]. Powder form of the plant is prescribed in the dose of 3-6 g [3]. *C. procumbens* is described as Serupadai or Serupadi in Siddha literatures. It is consumed either as kudineer or in the form of powder [3]. *C. procumbens* Linn. finds place in many medicinal purposes [4]. The paste of fresh leaves of *C. procumbens* Linn. are applied to rheumatic swelling [5]. Dried plant and equal part of fenugreek seeds are powdered and applied for causing suppuration of boils [6]. It is used to relieve fever, piles, leucorrhoea and menorrhagia [7]. The methanolic extract of *C. procumbens* Linn. has been used as antidote for snake poison by Yanadi tribes in South India [8] Previous workers reported the *in-vitro* antibacterial activity of aqueous and ethanolic extract of leaf of *C. procumbens* against *Staphylococcus aureus*, *Streptococcus pyrogenus*, *Salmonella typhi*, *Escherichia coli* and a fungus *Candida albicans* [9]. Other *in vitro* studies are available for antioxidant activity of methanolic extract of *C. procumbens* Linn. [10,11] and *in vitro* anthelmintic activity [12]. In this communication, authors aim to investigate the inhibiting capacity of ethanolic extract of *C. procumbens* whole plant against some selected organisms.

MATERIALS AND METHODS

Plant Material: The whole plant of *Coldenia procumbens* Linn was collected during March 2011 from Pudukkottai district, Tamil Nadu. It was authenticated by Dr. Sasikala Ethirajulu, Department of Pharmacognosy, Siddha Central Research Institute, Chennai. A voucher specimen (ACC.No.7311) has been deposited in the herbarium of the institute.

Stock and Working Solutions of Plant Extract: The ethanolic extract (1 g) of the plant *C. procumbens* Linn was weighed accurately and dissolved in 1 ml of dimethyl sulphoxide to make the stock solution of concentration 1000 mg/ml. From this stock solution, serial dilutions of 500, 250, 125, 62.5, 31.25, 15.625 mg/ml. Further dilutions such as 7.813, 3.906, 1.953, 0.977 and 0.488 mg/ml were made for finding out the minimum inhibitory concentration.

Test Organisms: Inhibition of organisms such as *A. hydrophila* (ATCC 7966), *B. cereus* (NCIM 2458), *B. subtilis (*MTCC 441), *E. aerogens* (NCIM 5139), *E. coli* (ATCC 25922), *K. pneumonia* (NCIM 2957), *P. vulgaris* (NCIM

***Corresponding Author Address:** Dr. Arul Antony S, PG & Research Department of Chemistry, Presidency College, Chepauk, Chennai-600 005, India. Email:smaapresidency@gmail.com*

2857), *P. aeruginosa* (NCIM 2945), *S. typhimurium* (NCIM 2501), *S. aureus* (NCIM 5021), *S. pyrogens* (ATCC 19615), *V. fischeri* (ATCC 7744) and *C. albicans* (MTCC 227) was studied. The ATCC cultures were procured from Christian Medical College; MTCC cultures from Institute of Microbial Technology, Chandigarh and NCIM cultures from National Chemical Laboratory, Pune and were maintained by serial sub-culturing every month on nutrient agar slants and incubating at 37ºC for 18–24 hours. The cultures were stored under refrigeration.

All the test bacterial organisms were confirmed using specific biochemical tests [13] and fungal organism by staining technique [14].

Antimicrobial activity: Well diffusion method was followed for determining antibacterial activity [15-17]. A homogenous suspension of the bacteria was prepared in 6 ml of saline and shaken vigorously to compare with the McFarland's standards [13]. The suspension was diluted with saline to a density equivalent to barium sulphate standard, 0.5 McFarland's unit. The plates were inoculated within 15 minutes of the preparation of suspension before the occurrence of any difference in density of bacterial cultures. Similarly, the 7 days old culture of *Candida albicans* was grown on Mullen Hinton agar at an inoculum concentration of 1-5 x10^5 ml of the fungal culture and maintained at 37^{0}C. Required quantity of Muller Hinton agar was prepared and 20 ml was transferred into the plates and allowed to solidify. The bacterial cultures of 0.5 McFarland unit equivalent concentrations and 0.1 ml of the fungal inoculums were uniformly swabbed on the solidified agar by rotating the plates in all the directions. Sterile plungers were used for making wells of 6 mm diameter on the solidified Muller Hinton agar. 50 µl of all the working solutions of 500, 250, 125, 62.5, 31.25, 15.625 mg/ml were loaded aseptically on the subsequent wells and properly labelled. Standard disc of ciproflaxin 10 µg, the positive control was placed on the inoculated plate. The plates were not disturbed for 15 min at room temperature and then the plates were incubated at 37ºC, 24 h for bacterial cultures and 48 h for fungal culture. The zone of inhibition was measured in millimeters.

RESULTS AND DISCUSSION

The ethanolic extract of *C. procumbens* whole plant was found to inhibit five bacteria and one fungal strain. It showed maximum activity against *B. subtilis*, *E. coli*, *K. pneumonia* followed by *S. typhimurium* and *P. aeruginosa*. The ethanolic extract also inhibited the growth of *Candida albicans.*

Table 1. Antimicrobial activity of ethanolic extract of *C. procumbens*

Organism	Zone of Inhibition (in mm)						
	500 (mg/ml)	250 (mg/ml)	125 (mg/ml)	62.5 (mg/ml)	31.25 (mg/ml)	15.62(mg/ml)	Standard
A. hydrophila (ATCC 7966)	-	-	-	-	-	-	32
B. cereus (NCIM 2458)	-	-	-	-	-	-	28
B. subtilis (NCIM 2197)	27	25	20	17	13	10	29
E. aerogens (NCIM 5139)	-	-	-	-	-	-	28
E. coli (NCIM 2931)	25	23	20	18	13	10	33
K. pneumonia (NCIM 2957)	22	19	18	16	-	-	30
P. vulgaris (NCIM 2857)	-	-	-	-	-	-	26
P. aeruginosa (NCIM 2945)	13	12	11	10	8	-	30
S. typhimurium (NCIM 2501)	18	15	11	7	6	-	29
S. aureus (NCIM 5021)	-	-	-		-	-	28
S. pyogenes (ATCC 19615)	-	-	-	-	-	-	30
V. fischeri (ATCC 7744)	-	-	-	-	-	-	29
C. albicans (NCIM 3471)	20	16	13	10	5	-	26

The minimum inhibitory concentration against *B. subtilis* and *E. coli* were < 15.6 mg/ml; that of *S. typhimurium*, *P. aeruginosa* and *C. albicans* were found to be 31.25 (mg/ml); the MIC against *K. pneumonia* was 62.5 (mg/ml). As the extract inhibited the growth of *B. subtilis* and *E. coli* the lowest tested concentration, ie., 15.62 mg/ml, the extract was further diluted to 7.813, 3.906, 1.953, 0.977 and 0.488 mg/ml. Growth of *B. subtilis* was found in the concentration of 0.488 mg/ml and hence the MIC against *B. subtilis* was considered as 0.977 mg/ml. In case of *E. coli*, the growth was observed in the concentration of 3.906 mg/ml and therefore the minimum concentration required to inhibit the growth of *E. coli was* considered as 7.813 mg/ml. *B. subtilis* is a rod shaped gram positive bacteria which use its flagella for a swarming motility. It is commonly found in the upper layers of the soil. Before the introduction of antibiotics, *B. subtilis* was popular as an immuno stimulatory agent to support treatment of gastrointestinal and urinary tract diseases. *E. coli*, *K. pneumonia* and *S. typhimurium* are rod shaped gram negative bacteria causing gastrointestinal, UTI and non-typhoidal fever. P. *aeruginosa* is a rod shaped gram negative bacteria with unipolar motility which cause disease in plants, animals and humans. It causes infections in the airway, urinary tract, burns, wounds and other blood infections. *C. albicans* is a fungus which can grow as yeast and as filamentous cell. It causes opportunistic oral and genital infections. It is found in human mouth and gastro intestinal tract. It's over growth causes candidosis.

From the above results, it is evident that the ethanolic extract of *C. procumbens* is effective against gram positive as well as gram negative bacteria and fungus. In the earlier phytochemical investigation of this plant, two cyanoglucosides viz., ehretioside A1 and lithospermoside have been isolated. The cyanoglucoside, Ehretioside A1 was reported to have histamine inhibiting activity [18]. Wedelolactone, a coumastane compound earlier reported [4] from this plant was proven activity against *E. coli*, *B. subtilis*, *S. typhimurium*, *S. aureus S. epidermidis*, *Shigella flexneri* and *Pseudomonas aeruginosa* [19,20]. Hence these phytochemicals present in *C. procumbens* can be considered as the lead molecules to exhibit antibacterial activity.

CONCLUSION

The ethanolic extract of whole plant of *C. procumbens* showed activity against *B. subtilis*, *E. coli*, *K. pneumonia*, *K. pneumonia*, *S. typhimurium* and *P. aeruginosa*.

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