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Original Research Article

Antimicrobial Studies on *Desmostachya bipinnata* Rootstock

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ABSTRACT

The ethanolic extract of *Desmostachya bipinnata* rootstock 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.* Antimicrobial activity was determined by the well diffusion method. The ethanolic extract was found to inhibit *K. pneumoniae, E. coli, B. cereus, S. typhimurium* and *P. vulgaris.*

Keywords: *Desmostachya bipinnata;* rootstock; antimicrobial

INTRODUCTION

Desmostachya bipinnata (Linn.) belongs to the family, Poaceae (Graminaea). It is known as Sacrificial Grass or Saved Gram in English [1]. It is used for medicinal as well as holy purposes. It is known as Tharuppai and its kudineer is prescribed for any type of disorder, fevers, itching and diuretic problems in Siddha literatures [2, 3]. It is useful for curing urinary tract diseases and excessive vaginal discharges. Many secondary metabolites such as scopoletine, umbelliferone, sugars, amino acids, carbohydrates [4]; kaempferol, quercetin, quercetin-3-*O*-glucoside, trycin, trycin-7-*O*-glucoside from the aerial part [5]; 4′-methoxy quercetin-7-*O*-glucoside from the whole plant [6]; 2,6-dihydroxy-7-methoxy-3H-xanthen-3-one from leafy culms [7]; eseroline, camphene, caryophyllene diepoxide, β-

eudesmol, isobornyl acetate, tricyclene, calarene, endoborneol, (+, -) trans-2, 6- gamma- irone, caryophyllene oxide, diphenyliodinium bromide, L-limenone, 2-cyclohexene-1-one, caryophyllene oxide, 8-nitro-12-tridecanolide and caryophyllene oxide from the aerial part [8] were reported earlier. These essential oil constituents from aerial parts showed strong antibacterial activity in 50, 25, 10 & 4 µg/ml concentrations against *Staphylococcus epidermidis, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* by disc diffusion method and the minimum inhibitory concentration (MIC) of the essential oil for all the tested organisms was >4 µg/ml [8]. The previous studies on the roots showed considerable antibacterial activity against *Klebsiella pneumoniaee, Escherichia coli, Staphylococcus aureus* [9]. The ethanolic extract was found to exhibit antibacterial activity in the decreasing order against *Micrococcus luteus, Bacillus subtilis*, *Proteus merabiles*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Sarcina ventricull*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Serratia marcesens.* Similarly, it was active against *Candida tropicalis, Candida albicans*, *Aspergillus fumigates, Aspergillus flavus* and *Pencillium chrysogenum* [10]. In the present investigation, authors aim to screen the activity against *A. hydrophila, B. cereus, B. subtilis,* E. *aerogens, E. coli, K. pneumoniae, P. vulgaris, P. aeruginosa, S. typhimurium, S. aureus, S. pyrogens, V. fischeri* and *C. albicans.*

MATERIALS AND METHODS

Plant Material

The plant material was collected from Dharmapuri district in Tamil Nadu while in flowering during the month of August 2011. It was authenticated by Dr. R. Chelladurai, Botanist, Survey of Medicinal Plants Unit, Palayamkottai. Voucher specimen of the plant (No. ACC. No. 7320) has been deposited in the Pharmacognosy department of Siddha Central Research Institute, Arumbakkam, Chennai-106.

Stock and Working Solutions of Plant Extract

The ethanolic extract (1 g) of the plant *D. bipinnata* 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

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 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) were used for the study. 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 37°C for 18–24 hours. The cultures were stored under refrigerated condition.

All the test bacterial organisms were confirmed using specific biochemical tests [11] and fungal organism by staining technique [12].

Antimicrobial activity

Antimicrobial activity was determined by the well diffusion method [13,14,15]. A homogenous suspension of the bacteria were prepared in 6 ml of saline and shaken vigorously to compare with the McFarland's standards [11]. 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 Muller Hinton agar at an inoculum concentration of 1-5 x10^5 ml of the fungal culture and maintained at 37^{o}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. Wells of 6 mm diameter were made on the solidified Muller Hinton agar with the help of a sterile plunger. From each of the working solutions of 500, 250, 125, 62.5, 31.25, 15.625 mg/ml, 50 µl were loaded aseptically on the subsequent wells and labelled. Standard disc of ciproflaxin 10 µg was placed on the inoculated plate as positive control. The plates were left undisturbed 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 DISCUSSIONS

The results of the antimicrobial activity and the zone of inhibition are presented in the Table 1.

The ethanolic extract of *D. bipinnata* root stock showed maximum activity against *K. pneumoniae (*NCIM 2957), *E.coli* (NCIM 2931) followed by B*. cereus* (NCIM 2458), S*. typhimurium* (NCIM 2501) and *P. vulgaris* (NCIM 2857). The activity against *E. aerogens* (NCIM 5139) was observed with 500 mg/ml concentration and no activity was found in the lower concentrations. The growth of other bacteria and fungi were not found in the tested concentrations of the plant extract. The minimum inhibitory concentration against *B. cereus* and that of *E. coli* was found to be 31.25 (mg/ml); the MIC against *P. vulgaris* and S*. typhimurium* was 62.5 (mg/ml); against *E. aerogens* was 500 (mg/ml). As the extract inhibited the growth of *K. pneumoniae* at 15.625 (mg/ml) concentrations also, it was screened with further dilutions viz., 7.813, 3.906, 1.953, 0.977 and 0.488 mg/ml and was found no inhibition at 0.488 mg/ml concentrations and the MIC of the extract against *K. pneumoniae* was considered as 0.977 mg/ml. The extract was found to inhibit the growth of four gram negative bacteria and one gram positive bacteria. *K. pneueoniae,* the gram negative bacteria causes damage to human lungs; *E. coli* is a gram negative bacteria causing diarrheal and urinary tract infections; *B. cereus* is a gram positive bacteria responsible for food borne diseases such as nausea, vomiting and diarrhea.; *P. vulgaris* creates urinary tract infections; *S. typhimurium* is also a gram negative bacteria responsible for non-typhoidal fevers.

Table 1: Antimicrobial activity of ethanolic extract of *D. bipinnata*

Sl. No	Organism	Zone of Inhibition (in mm)						Standard
		500 (mg/ml)	250 (mg/ml)	125 (mg/ml)	62.5 (mg/ml)	31.25 (mg/ml)	15.625 (mg/ml)	
1	*A. hydrophila* (ATCC 7966)	-	-	-	-	-	-	32
2	*B. cereus* (NCIM 2458)	18	16	14	12	9	-	28
3	*B. subtilis* (NCIM 2197)	-	-	-	-	-	-	29
4	*E. aerogens* (NCIM 5139)	10	-	-	-	-	-	28
5	*E. coli* (NCIM 2931)	22	21	20	18	12	-	33
6	*K. pneumoniae* (NCIM 2957)	25	24	22	19	18	16	30
7	*P. vulgaris* (NCIM 2857)	13	12	11	10	-	-	26
8	*P. aeruginosa* (NCIM 2945)	-	-	-	-	-	-	30
9	*S. typhimurium* (NCIM 2501)	17	15	14	13	-	-	29
10	*S. aureus* (NCIM 5021)	-	-	-	-	-	-	28
11	*S. pyogenes* (ATCC 19615)	-	-	-	-	-	-	30
12	*V. fischeri* (ATCC 7744)	-	-	-	-	-	-	29
13	*C. albicans* (NCIM 3471)	-	-	-	-	-	-	26

CONCLUSION

D. bipinnata rootstock is used for treating asthma, urinary tract infections and fever which is supported by the activity against *K. pneumoniae, E. coli, B. cereus, P. vulgaris* and *S. typhimurium.*

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