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Research Article

PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTIMICROBIAL ACTIVITY OF METHANOLIC EXTRACT OF *CISSUS SETOSA* ROXB.

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ABSTRACT

Preliminary phytochemical screening and evaluation of *in vitro* antimicrobial activity of aerial parts of methanolic extract of the traditional medicinal herb, *Cissus setosa* were carried out. The results of the study report the presence of almost all major secondary metabolites except alkaloids in the extract. The strong antibacterial inhibition was noted against the gram positive bacteria, *Micrococcus luteus* (10 mm / 500 µg of sample) and strong antifungal inhibition was noted against the species, *Candida parapsilopsis* (10 mm / 1500 µg of sample).

Keywords: *Cissus setosa,* phytochemical screening, antimicrobial activity.

INTRODUCTION

India is one of the mega biodiversity countries in the world in regard to genetic resources of medicinal plants. Many of them represent a rich source of antimicrobial agents due to its phytochemical constituents[1-3]. The knowledge of chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies. These plant – derived compounds are likely to provide a valuable source of new medicinal agents[4,5]. Despite the huge plant diversity, still a large number of species has to be explored pharmacologically to bring out their medicinal uses particularly for antimicrobial properties. *Cissus setosa* is a prostate herb belongs to the family, Vitaceae. It harbors many medicinal uses and it is prescribed for various ailments in traditional medical practices of Tamil Nadu[6]. The macerated leaves are used as a poultice to promote suppuration and to aid in the extraction of the guinea worm[7]. Leaf extract is given to drink for two days to expel the intestinal worms[8] and used for washing cattle and vessels also[9]. The leaves of this species along with the leaves of *Caryratia pedata* are roasted and oiled and then applied to boils for healing[10]. However, no scientific validation has been made for its antimicrobial property. To address this lacuna, antimicrobial assay was made in this species to confirm the traditional knowledge on antimicrobial property of *Cissus setosa.*

MATERIALS AND METHODS

Plant collection and extraction

The aerial parts of the study species, *Cissus setosa* were collected from Palani hills, Tamil Nadu, India. The plant materials were shade dried, pulverized and extracted with pure methanol by a soxhlet apparatus. The obtained extracts were filtered and concentrated in a rotary vacuum evaporator at 45°C under reduced pressure. Then the concentrated extract was stored at 4°C until use.

Preliminary phytochemical analysis

Qualitative phytochemical screening of methanolic extract was carried out by following the methods of Horbone (1984)[11], Kokate *et al*., (1995)[12] and Prabhakaran, (1996)[13].

Microbial strains

Bacterial strains used for the assay were as followings: Gram positive bacteria: *Staphylococcus aureus, Bacillus cereus* and *Micrococcus luteus.* Gram negative bacteria: *Escherichia coli, Pseudomonas aeruginosa, P. fluorescens, Shigella sonnei, Aeromonas hydrophila, Klebsiella pneumonia* and *Salmonella paratyphi.* The fungal strains used are *Candida albicans, C. parapsilopsis, Aspergillus flavus, A. fumigates, A. terreus, Fusarium oxysporum, Microphomina phaseolina, Alternaria alternate, Rhizoctonia solani* and *Penicillium* sp. Both microbes were obtained from the Department of Microbiology, Hindustan College of Arts and Science, Coimbatore, India. The bacterial stock cultures were maintained on nutrient agar slants at 4°C and the fungal cultures were maintained on potato dextrose agar medium at 4°C.

Antimicrobial assay

The antimicrobial activities were performed using the agar well diffusion method[14,15]. The methanolic extract of aerial parts of *Cissus setosa* was dissolved in the Dimethyl sulfoxide (DMSO) to a final concentration of 100 mg/ml. Each bacterial strain was suspended in nutrient broth and each fungal strain was suspended in potato dextrose broth and incubated for 8 h at 37°C. Nutrient agar (NA) plates and potato dextrose agar (PDA) plates were seeded with bacterial strains for 8h and fungal strains for 16h respectively. In each of these plates, wells were cut out using sterile cork borer. Using sterilized dropping pipettes, different concentrations of plant extract such as 500, 1000, 1500 and 2000 µg/ml were carefully added into the wells separately and allowed to diffuse at room temperature for 2 h. The plates were then incubated at 37°C for 18 – 24 h. Gentamycin (10 µg) and Ketaconazole (10 µg) were used as positive controls and the solvent DMSO was used as negative control. The antimicrobial activity was evaluated by measuring the diameter of inhibition zone.

Table 1: Preliminary phytochemical screening in aerial parts of *Cissus setosa* by using methanolic extract

Phytochemical constituent	Name of the test	Presence / absence
Alkaloids	Wagner's test	-
Flavonoids	Shinoda	+
	Lead acetate	+
Phenolics and tannins	Lead acetate	+
	Ferric chloride	-
	Sodium hydroxide	-
Steroids and sterols	Salkowski's test	+
Carbohydrates	Fehling's test	+
	Benedict's test	+
Saponins	Honey comb test	+
	Foam test	-
Glycosides	Glycoside test	+
Protein	Biuret test	+
	Ninhydrin test	+

'+'– presence of compounds; '-'– absence of compounds

Table 2: *In vitro* antibacterial activity of aerial parts of *Cissus setosa* by agar well diffusion method

Organisms	Zone of inhibition (mm)				
	Methanolic extract of *Cissus setosa*				Standard Gentamycin
	500 µg/mL	1000 µg/mL	1500 µg/mL	2000 µg/mL	10 µg/mL
Gram Positive Bacteria					
Staphylococcus aureus	-	-	-	-	-
Bacillus cereus	-	-	10.5 ± 0.71	11.5 ± 0.71	21.0 ± 0.0
Micrococcus luteus	10.0 ± 0.0	11.0 ± 0.0	12.0 ± 0.0	13.0 ± 1.41	23.0 ± 0.0
Gram Negative Bacteria					
Escherichia coli	-	-	10.5 ± 0.71	11.5 ± 0.71	24.5 ± 0.71
Pseudomonas aeruginosa	-	-	-	-	17.5 ± 0.71
P. fluorescens	-	-	-	-	21.0 ± 0.0
Shigella sonnei	-	-	-	-	21.0 ± 0.0
Aeromonas hydrophila	-	-	-	-	20.0 ± 0.0
Klebsiella pneumoniae	-	-	-	-	18.0 ± 0.0
Salmonella paratyphi	-	-	10.5 ± 0.71	11.0 ± 0.0	24.5 ± 0.71

Values are means of three independent determinations ± standard deviation, (–) - No inhibition

Table 3: *In vitro* antifungal activity of aerial parts of *Cissus setosa* by agar well diffusion method

Organisms	Zone of inhibition (mm)				
	Methanolic extract of *Cissus setosa*				Standard Ketaconazole
	500 µg/mL	1000 µg/mL	1500 µg/mL	2000 µg/mL	10 µg/mL
Candida albicans	-	-	-	-	10.0 ± 0.0
Candida parapsilopsis	-	-	10.0 ± 0.0	11.5 ± 0.71	10.5 ± 0.71
Aspergillus flavus	-	-	-	-	15.0 ± 1.41
Aspergillus fumigatus	-	-	-	-	-
Aspergillus terreus	-	-	-	-	13.0 ± 0.0
Fusarium oxysporum	-	-	-	-	-
Microphomina phaseolina	-	-	-	-	-
Alternaria alternate	-	-	-	-	-
Rhizoctonia solani	-	-	-	-	-
Penicillium sp.	-	-	-	-	-

Values are means of three independent determinations ± standard deviation, (–) - No inhibition

Statistical analysis

All the analyses were done in triplicate and results were expressed as mean ± SD. The data were subjected to one way analysis of variance (ANOVA) and the significance of the difference between mean was determined by Duncan's Multiple Range Test with significance level, $P < 0.05$. ANOVA was performed using the statistical software SPSS (SPSS Inc. Chicago, USA).

RESULTS AND DISCUSSION

The results of the preliminary phytochemical analysis are presented in Table 1. The aerial parts of methanolic extract showed the presence of all most all major secondary metabolites except alkaloids. As it is a species of dry habitat, the water stress may induce the plant to produce large variety of secondary metabolites for its defense mechanism. It is of common fact that plants of semi-arid and arid habitats can produce huge variety of secondary metabolites[16,17]. The methanolic extract of the plant showed high antibacterial activity against the Gram positive bacteria, *Micrococcus luteus* (10 mm / 500 µg of sample). However, for inhibition high concentration of methanolic extract was needed against the bacteria, *Bacillus cereus* (10.5 mm / 1500 µg of sample), *Escherichia coli* (10.5 mm / 1500 µg of sample) and *Salmonella paratyphi* (10.5 mm / 1500 µg of sample). No inhibition effect was found against the remaining bacteria tested. This activity was compared with synthetic standard, Gentamycin (Table 2). The response of methanolic extract of the aerial parts of *Cissus setosa* to the control of growth of various bacteria showed that it was species specific. The variation in membrane nature and hence the degree of mechanism of action of bioactive compounds in the extract of

plant species studied are the factors responsible for this fact[18,19]. Further, the varying degree of sensitivity of test organisms of bacteria may be due to the intrinsic tolerance of microorganisms and the nature and combinations of phytocompounds present in the crude extract[20]. In addition it was found that *Cissus setosa* is not gram positive or gram negative specific and generally both kinds of bacteria were influenced. It may be due to the presence of specific ingredients present in the extract to act upon the cell membrane of both types of bacteria[21]. The antifungal activity showed that *Candida parapsilopsis* (10 mm / 1500 µg of sample) was highly sensitive to the methanolic extract of *Cissus setosa.* The zone of inhibition is almost nearer to the standard, Ketaconazole (10.5 mm / 10 µg of sample). The other fungi were resistant to the extract of the study species (Table 3). It indicates that the extract of *Cissus setosa* is narrow range of inhibitory activity over the broad spectrum of fungal species tried. Quality and quantity of chemical ingredients in the extract and degree of resistance of fungal specie due to membrane nature may influence the inhibitory activity[22]. The overall study suggests that among the bacteria, *Micrococcus luteus* and among the fungi, *Candida parapsilopsis* were effectively controlled by the methanolic extract of aerial parts of *Cissus setosa.* The above mentioned bacterium is an opportunist pathogen causes septic shock, pneumonia and urinary tract infections in an immune deficient person[23]. Similarly, the fungal species *Candida parapsilopsis* causes wound and tissue infections in immune-compromised patients[24]. Therefore, in order to control the diseases/problems caused by the bacterium, *Micrococcus luteus* and fungus, *Candida parapsilopsis* the study species, *Cissus setosa* can be used as a source for the preparation of drugs.

CONCLUSION

The results of the present work indicates that the plant species, *Cissus setosa* posses antimicrobial property. Further studies aimed to elucidate the structure of active principles responsible for the antimicrobial activity.

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