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Research Article

ANTIMICROBIAL ACTIVITY OF LEAF EXTRACTS OF *SIDA CORDIFOLIA*

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ABSTRACT

The antimicrobial activity of *Sida cordifolia* leaf extracts (aqueous and methanolic) on pathogenic bacteria (*Staphylococcus aureus, Enterococcus fecalis, Pseudomonas aeruginosa,* and *Proteus mirabilis*) and fungi (*Candida albicans* and *Cryptococcus neoformans*) was tested by disc diffusion method. The aqueous extract was found to be moderately effective against bacteria and exhibited high antifungal activity at a concentration of 2mg/disc. The methanolic extract was effective on bacteria and did not show any antifungal activity. Acetone fraction did not contain any antimicrobial activity. Increasing concentrations of the extracts (aqueous and methanolic) exhibited increased zone of inhibition against the bacteria and fungi (7-20mm). Our results suggest that aqueous extracts of *Sida cordifolia* leaves show high antimicrobial activity and contains phytochemicals that are responsible for such an activity. Further analysis of the extracts to isolate and identify the phytochemicals from *Sida cordifolia* will be helpful to test the therapeutic potential and industrial applications.

Keywords: Antibacterial, Antifungal, Disc diffusion, McFarland standard and *Sida cordifolia*

INTRODUCTION

Medicinal plants with antimicrobial activity are known to offer protection against various bacterial, viral and other diseases [1, 2] and also find industrial applications. Antibiotic resistance is a serious health problem with significant mortality and morbidity from treatment failures and lead to increased health care costs. Continuing efforts to investigate new agents from natural sources offers clues to discover new antimicrobial agents. Testing the antibacterial and antifungal activity of plant extracts is beneficial to the food, dairy and bakery industries; as such extracts are safe and offer inexpensive and effective alternative methods of preventing microbial contamination. Indian plants constitute a rich untapped pool of medicinal plants due to semi-arid tropical climate and different plant parts (e.g. fruits, seeds, leaves, stem, bark and roots) are used to cure a variety of human diseases [3].

Aqueous or solvent extracts of different plant parts have been tested for pharmacological and therapeutic activities, such as antimicrobial, hepatoprotective, hypoglycaemic, hypolipidemic and other activities. Testing plant extracts for antimicrobial activity could be a good source to identify new antimicrobial drugs[4]. Considering the high potential of plants as a source of antimicrobial drugs a systematic investigation was undertaken to test the antibacterial and antifungal activity of *Sida cordifolia* [4].

Sida cordifolia (Family: Malvaceae) commonly known as Bala (Sanskrit) is a herb that is extensively used as a common herbal healing agent in the Indian subcontinent[5]. It is used in the folk medicine for several purposes: antirheumatic, antipyretic, laxative, diuretic, antiinflammatory, analgesic, hypoglycaemic, antiasthmatic, aphrodisiac and to relieve nasal congestion. The leaf, bark, seeds and roots are known to possess medicinal properties. Oils from *Sida cordifolia* seeds are applied to the sore muscles and joints in rheumatism and arthritis. Crushed leaves can be used to alleviate local pains and for the cure of external wounds and skin diseases[6]. A preliminary phytochemical screening of the hydroalcoholic extract of the leaves of *Sida cordifolia* demonstrated the presence of alkaloids, steroids, flavonoids and saponins. The leaves contain ephedrine, pseudoephedrine (vasoconscrictor), vasicinone, vasicine and vasicinol[7]. Methanolic extract of *Sida cordifolia* has been tested for antimicrobial activity and found to be effective against *Mycobacterium species, Bacillus subtilis, Klebsiella pneumonia, Micrococcus variance*[8]. But the antimicrobial activity of *Sida cordifolia* on the human pathogenic microorganisms has not been investigated. In the current paper we tested methanol, acetone and aqueous derived leaf extract of *Sida cordifolia* on two Gram positive and two Gram negative bacteria and two fungi.

MATERIALS AND METHODS

Plant material

Sida cordifolia leaves were collected during the months of mid April to May, 2012 in local area, Tirupati and authenticated by Department of Botany, Sri Venkateswara University, Tirupati, India. The leaves were washed thoroughly with tap water, blotted with filter paper and dried under shade for one week. Dried material was grounded to coarse powder and stored in desiccators. It was then extracted with the required solvents.

Chemicals

All the solvents used in this study were purchased from Merck Chemicals, India.

Preparation of leaf extracts

Coarse leaf powder (50g) was taken in a glass stoppered conical flask and appropriate (~ 200 ml) quantities of analytical grade solvents were added. The extraction was performed with different solvents in increasing order of polarity, acetone, methanol and water. The solvent powder mixtures were kept at room temperature for 48 hours with intermittent stirring every eight hours using a glass rod. After 48 hours, the extract was filtered through Whatman No.1 filter paper and the filtrate was concentrated using vacuum evaporator. The extracts were transferred to sterile screw cap bottles, labeled and stored in refrigerator (4^0c) until use.

Preparation of culture media

Dehydrated media, standard antimicrobial drugs (disc) and sterile discs were purchased from Hi-Media laboratories Ltd. All the media were prepared in sterilized glass petri-plates according to the manufacturer's instructions.

Growth and maintenance of micro-organisms

Bacterial strains *Staphylococcus aureus, Enterococcus fecalis, Proteus mirabilis, Pseudomonas aeruginosa, and* fungal strains *Candida albicans* and *Cryptococcus neoformans*, were obtained from the IMTECH, Chandigarh, India. The bacterial strains were grown in Muller-Hinton agar (MHA- pH 7.2) at 37±1^{0}C and fungi were grown in Sabouraud dextrose agar (SDA- pH 5.4) at 25±1^{0}C. The stock culture slants were maintained at 4^{0}C.

Preparation of inoculums

The bacteria were grown in nutrient broth (NB) at 37±1^{o}C and fungi were grown in Sabouraud dextrose broth (SDB) at 25±1^{o}C for 4 to 6 hours. The turbidity of the broth culture was adjusted to turbidity optically comparable to that of the 0.5 McFarland standards. This results in a suspension containing approximately 1 - 2 x 10^8 CFU/ml[9].

Reference and Control

Ciprofloxacin (5µg) and fluconazole (25µg) were used as standard antibacterial and antifungal drugs respectively. Equal volume of solvent was tested to determine the effect of solvent on antimicrobial activity of the extracts.

Determination of Antimicrobial activity (Disc diffusion method)

The Kirby-Bauer disc diffusion technique, recommended by the Clinical Laboratories Standards Institute (CLSI), was used for testing antimicrobial activity. A sterile cotton swab was dipped into the inoculum and streaked on the dried surface of sterile MHA/SDA plate.

Extracts were dissolved in dimethyl sulphoxide (DMSO) and required concentrations were prepared. Sterile discs impregnated with 20µl of various concentrations (175µg to 2mg) of acetone, methanolic and aqueous leaf extracts of *Sida cordifolia* were placed on the agar surface with gentle pressure to ensure complete contact with the agar surface. Negative controls (DMSO) and standards including ciprofloxacin and fluconazole were also tested. The MHA plates were incubated at 37±1ºC for 24 hours and SDA plates at 25±1ºC for 48 hours[10, 11]. The antimicrobial activity was determined by measuring the diameter of the inhibition zone (in 'mm'). Each assay was repeated three times and mean values were taken.

Table 1. ANTIMICROBIAL ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF *SIDA CORDIFOLIA* LEAVES.

Extract/Drug	Concentration	Zone of inhibition (mm)					
		SA	EF	PM	PA	CA	CN
Methanol	175 µg	0	0	0	9	0	0
	250 µg	0	0	0	10	0	0
	500 µg	7	0	0	11	0	0
	1 mg	15	0	14	14	0	0
	2 mg	17	0	15	15	0	0
Aqueous	175 µg	0	0	0	0	15	11
	250 µg	0	0	6	0	15	13
	500 µg	0	8	7	12	18	16
	1 mg	12	12	12	15	20	19
	2 mg	13	14	12	16	20	20
Ciprofloxacin	5 µg	29	24	29	20	-	-
Fluconazole	25 µg	-	-	-	-	25	20
DMSO	99.9%	0	0	0	0	0	0

SA: *Staphylococcus aureus*; EF: *Enterococcus fecalis*; PM: *Proteus mirabilis*; PA: *Pseudomonas aeruginosa*; CA: *Candida albicans*; CN: *Cryptococcus neoformans*; 0: No zone of inhibition; - : Not tested; mm: millimeter

Table 2. ANTIMICROBIAL ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF *SIDA CORDIFOLIA* LEAVES.

S.No.	Micro-organisms	Extracts	
		Methanolic	Aqueous
1	*Staphylococcus aureus*	+++	++
2	*Enterococcus fecalis*	-	++
3	*Pseudomonas aeruginosa*	+++++	+++
4	*Proteus mirabilis*	++	++++
5	*Candida albicans*	-	+++++
6	*Cryptococcus neoformans*	-	+++++

Table 3. LIST OF PHYTOCHEMICAL CONSTITUENTS

S.No.	Phytochemicals	Inference	References
1	Tannins	-	Koman et al., 1921
2	Mucins	+	Koman et al., 1921
3	Potassium nitrate	+	Koman et al., 1921
4	Resins	+	Koman et al., 1921
5	Resins acid	+	Koman et al., 1921
6	Flavones (5,7 – dihydrixy-3-isoprenyl flavones, 5-hydroxy -3-ispreny flavones)	+	Ranajit K et al., 1982
7	Essential oils	+	Ghosal et al., 1975
8	Alkaloids (ephedrine, pseudoephedrine, vasicinone, vasicine and vasicinol)	+++	Ghosal et al., 1975

RESULTS

Antimicrobial activity of different extracts against Gram positive bacteria (*Staphylococcus aureus*, *Enterococcus fecalis*), Gram negative bacteria (*Proteus mirabilis, Pseudomonas aeruginosa*) and fungi (*Candida albicans, Cryptococcus neoformans*) was evaluated by the disc diffusion method. All test strains of bacteria were sensitive to ciprofloxacin and fungal strains were sensitive to fluconazole. The solvent control (DMSO) showed no effect against tested bacteria and fungi.

Results of the antimicrobial activity of *Sida cordifolia* leaf extracts are shown in table 1. Aqueous extract exhibited highest inhibitory activity against tested microorganisms compared to other extracts. The aqueous extract exhibited strong antifungal activity on *Cryptococcus neoformans* and *Candida albicans* with inhibition zone of 20 mm diameter at highest concentration of 2mg/disc. Moderate antibacterial

activity on both Gram positive bacteria with zone diameter of 13mm and in Gram negative bacteria zone diameter of 12mm (*P. mirabilis*) and 16mm *(P. aeruginosa*) was observed at 2mg/disc concentration.

The methanolic extract of *Sida cordifolia* showed moderate antibacterial activity against both Gram negative bacteria with zone diameter of 15mm and Gram positive bacteria with zone diameter of 17mm (*S. aureus*) at 2mg/disc concentration, whereas *Enterococcus fecalis* was found to be more resistant at all the concentrations tested. But no antifungal activity was observed in any of the concentrations tested. Acetone faction did not show inhibitory activity against the microorganisms tested. Most of the antimicrobial compounds that have been identified were found to be soluble in polar solvents such as methanol and water [12,13,14,15].

DISCUSSION

Phytochemicals including alkaloids, flavonoids, triterpenoids, saponins, thymol and other compounds of phenolic nature were known to possess antimicrobial activity and better extracted using methanol and aqueous based solvents. This may be the reason for the strong antimicrobial activity of the methanolic and aqueous extracts compared to acetone extract. Our results indicate that the methanolic and aqueous extracts exhibited more inhibitory activity on Gram negative bacteria than Gram positive bacteria (Table 2). Methanolic extract inhibited *P.aerugiosa,*at low concentrations (zone diameter of 9mm at 175 µg/disc) indicating the presence of key compounds that are more selective against *P.aerugiosa* (the most resistant bacteria). Better antifungal activity was observed with aqueous extract even at low concentrations (zone diameter of 11 to 15mm at 175µg/disc) and at high concentrations, the activity was equivalent to fluconazole (20mm at 2mg/disc). The list of phytochemicals present in *Sida cordifolia* are given in table 3. Phytochemicals including vasicinol, ephedrine, vasicinone, hsypaphorine are known to show high antimicrobial activity and this could be the reason for the observed antibacterial and antifungal activity of extracts of *Sida cordifolia*.

Among the tested extracts, aqueous extract was found to be more effective than other extracts. Differences among the antimicrobial activity of the extracts could be due to the variable phytochemical composition among various extracts.

The results of the present study indicate that leaf extracts of *Sida cordifolia* plant exhibit antibacterial as well as antifungal activity against pathogenic microorganisms. Further studies to identify the newer phytochemicals that are responsible for antimicrobial activity of *Sida cordifolia* leaf extracts and determination of its toxicity are necessary before the extracts could be used.

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