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Comparative Studies on Antimicrobial Activity of *Sida acuta* (Malvaceae) Leaf Extracts

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The antimicrobial activity of 90% ethanol, methanol, ethyl acetate and aqueous extracts of leaves of *Sida acuta* was investigated to verify its claimed ethno medicinal use as an antimicrobial agent and comparative study of different extracts was carried out. The antimicrobial activity of the different extracts was tested against standard strains and clinical samples of some bacteria and fungi using agar well diffusion method. Commercial antibiotics were used as positive standard references to determine the sensitivity of the strains. All the extracts showed significant inhibitory activity against gram positive bacteria (*Bacillus subtilis, Staphylococcus aureus*), gram negative bacteria (*Escherichia coli, and Proteus vulgaris*), fungi (*Candida albicans* and *Aspergillus niger*) and clinical isolates of *Staphylococcus faecalis, Staphylococcus aureus* and some gram positive bacteria. The range of zone of inhibition was found to be 10-30 mm and minimum inhibitory activity was as low as 2 μg/ml. The zone of inhibition was highest with ethanol extract followed methanol, ethyl acetate and aqueous extracts for all the microorganisms used. Aqueous extract was effective only on *P. vulgaris*.

Key words: *Sida acuta,* Antimicrobial activity, ethno medicinal use.

Sida acuta (Malvaceae) common wire weed is an erect, branched small perennial herb or sub shrub which grows abundantly on cultivated fields, waste areas, road sides and open clearings of tropics and sub tropics[1]. In traditional therapeutics, the plant is used as oral medicine for fever, asthma, aches, pains, ulcers, diarrhea, dysentery, as an anti worm medication and the decoction of dried plant is taken orally for venereal diseases[2, 3]. The paste of leaves is mixed with coconut oil and applied on head regularly for killing dandruffs and also for strengthening hair in ghat regions of India[4]. Another study showed that the ethanolic extract of plant has a moderate activity against the lethal effect of *Bothrops atrox* venom and the analgesic properties of the whole plant extract were demonstrated in animal model[5]. The plant has been screened for its cancer chemo preventive properties[6]. The *in vitro* anti plasmodial activity of the plant was reported[7]. The described pharmacological properties of the plant involve antiplasmodial, antimicrobial, antioxidant, cytotoxic activities and many other properties.

The antibacterial activity of chloroform extract of dried leaves of *S. acuta* on *Mycobacterium smegmatis* has been reported[8]. The steroidal fraction was active against *E.coli, K. pneumoniae, P. vulgaris, P. pyocyanae, S.albus* [9]. The seed extract was reported to show

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antibacterial activity on *E,coli, P. circhorii and S. typhimurium*[10]. Multidrug resistance by various bacterial species has become a major problem in pharmacothereupatics[11]. Studies on *S. acuta* have revealed the presence of medicinally active constituents[12]. The present study investigates antimicrobial activity of *S. acuta* aqueous and organic extracts against gram+ve and gram-ve bacteria and few fungi. Also a comparison is made with respect to different extracts.

MATERIAL AND METHODS

Plant material

The aerial parts of *Sida acuta* were collected in April 2008 from roadsides of GITAM university campus, Visakhapatnam and is authenticated by Department of Botany, Andhra University.

Preparation of extracts

The leaves are collected and air dried for five days and is then ground to powder using an electric mill. 15gms of the powdered leaves of the plant material is subjected to soxhlet extraction for 18hrs using double distilled water, methanol, ethanol and ethyl acetate separately. The extracts were evaporated to dryness under reduced pressure at 40°C and weighed. 3.10gms of methanol extract, 1.4gms of ethyl acetate, and 2.4gms of ethanol extracts were dissolved in dimethyl sulphoxide (DMSO) to give a final concentration of 1mg/ml which were used for antimicrobial tests.

Test organisms

The following test organisms were used in this study. *Bacillus subtilis* (NCIM 2063), *Staphylococcus aureus* (NCIM 3021), *Escherichia coli* (NCIM 2066), *Klebseilla pneumoniae* (NCIM 2957), *Proteus vulgaris* (NCIM 2027), *Candida albicans* (NCIM 3557), *Aspergillus niger* (NCIM 1054). The bacterial and fungal strains are obtained from National collection of Industrial Microorgainsms, National Chemical Laboratory, Pune. Clinical strains are obtained using selective media like mannitol salt (*Staphylococcus*), blood agar (*Streptococcus faecalis*), Mac conkey agar (gram positive bacteria), Sabourand dextrose (fungi).

Microbiological studies

The bacteria were grown in Muller-Hinton media and fungi in Sabourand dextrose agar (Himedia Pvt Ltd., Mumbai, India) at 37°C and maintained on nutrient agar slants at 4°C and stored at -20°C. Inoculum of test organisms was prepared by growing pure isolate in nutrient broth at 37°C for overnight. The overnight broth cultures was subcultured in fresh nutrient broth and grown for 3 hrs to obtain log phase culture. The agar plates were prepared by pour plate method using 20ml nutrient medium. The molten sterile nutrient agar medium is cooled to 45°C and mixed thoroughly with 1ml of growth culture of concerned test organism (1×10^8 cells) and then poured into the sterile pertidishes (9.0 ×1.5cm diameter) and allowed to solidify. *C. albicans* and *A. niger* are grown on Sabourand dextrose agar and suspension in one fourth strength Ringers solution was used to prepare the seeded Sabourand dextrose agar plates. Wells of 6mm size were made with sterile cork borer and 25ìl of extract (1.0mg/ml) was added to each well aseptically. Ampicillin (10µg/disc), tetracycline (20µg/disc) and fucanozole (20µg/disc) were included as standard antimicrobial agents and tested along with the extract. DMSO served as negative control. The agar plates were incubated at 37°C for 24hrs while Sabourand dextrose agar plates were incubated at 25°C for 48hrs. The diameter of zones of inhibition was measured in mm using Himedia zone reader. Results presented are the average values of triplicates.

Determination of minimum inhibitor concentration (MIC)

The minimum inhibitor concentration was determined by agar diffusion method. The extracts were incorporated into Muller-Hinton medium and Sabourand dextrose agar at concentrations of 5 µg to 40 µg. A control without the extract was also set up. The lowest concentration of extract that inhibited the growth of microorganisms was considered as MIC.

RESULTS

The antimicrobial activity of *Sida acuta* extracts are presented in Table 1, It was found that the aqueous extract of *S. acuta* did not exhibit any activity against the microorganisms tested except for *B. subtilis,* whereas all the organic extracts studied exhibited different levels of antimicrobial activity against gram +ve,

gram –ve, fungal strains and clinical isolates. Most significant inhibitory activity was observed with ethanol extract against all bacterial species studied with zone of inhibition ranging from 30mm with *E.coli* to 33 mm with *S. aureus*. Methanol and ethyl acetate exhibited moderate activity, with zone of inhibition ranging from 20-22mm and 17-20mm respectively. Interestingly the organic extracts exhibited antifungal activity with zone of inhibition ranging from 30mm-32mm with *C. albicans* and *A.niger*. The clinical isolates *S.aureus, S. faecalis* and a gram +ve bacteria also showed zone of inhibition of 30mm, 25mm and 22mm respectively.

The minimum inhibitory concentration of the extract against the test organisms are shown in Table.2. The minimum inhibitory concentration was 6 µg against both *S. aureus* & *K. pneumoniae* 5 µg against *E.coli*, 4µg against *B subtilis* & *P. Vulgaris* and 3µg against *C.albicans* lastly 2 µg against *A.niger*. The control did not produce any inhibitory activity against the organisms. The minimum inhibitory concentration of the aqueous extract for all organisms was not determined since there was no significant inhibitory activity. These results showed that *Sida acuta* exhibited good antimicrobial activity especially against fungal and a few bacterial strains. They justify the use of *S.acuta* as a traditional medicinal plant for various ailments.

Table 1. Antimicrobial activity of *Sida acuta* extracts on different microbial strains

Microorganism	Zone of growth inhibition in mm			
	Ethanol extract (40µg/well)	MethanolExtract (40µg/well)	Ethyl acetate extract (40µg/well)	AqueousExtract (40µg/well)
Bacteria				
B. Subtilis	28 ± 2	20 ± 1	17 ± 1	15 ± 2
S. aureus	30 ± 2	20 ± 1	10 ± 1	ND
E. coli	29 ± 2	20 ± 1	18 ± 1	ND
P. vulgaris	30 ± 2	22 ± 2	17 ± 1	ND
K. pneumoniae	ND	ND	ND	ND
Fungi				
C. albicans	21 ± 2	27 ± 2	20 ± 2	ND
A. niger	30 ± 1	22 ± 1	ND	ND
Clinical isolates				
S. aureus	30 ± 2	17 ± 1	ND	ND
Strept. faecalis	25 ± 1	15 ± 1	ND	ND
Gram +ve	22 ± 2	14 ± 1	ND	ND

Table 2. Minimum growth inhibitory concentration of ethanol extract of *S.acuta*

Organisms	MIC (µg / well)
B. Subtilis	4
S. aureus	6
E. coli	5
P. vulgaris	4
K. pneumoniae	ND
C. albicans	2
A. niger	3

Table 3. Concentration of standard antibiotics used as positive control

Antibiotic	Concentration used (µg / disc)
Tetracycline	20
Ampicillin	10
Fucanozole	20

DISCUSSION

The ethanol extract of *S.acuta* exhibited a broad spectrum of antimicrobial activity ranging from gram +ve to a few gram –ve and common fungi. The other organic extracts exhibited

moderate inhibitory activity. Akerele[13] reported inhibitory activity against gram +ve organisms and none against gram –ve bacteria with ethanol crude extract. But all the organic extracts in this study showed inhibition against gram +ve, gram –ve and fungi which may be due to extraction by soxhlet apparatus. Presence of various natural alkaloids like crytolepine and scopoletin in the organic extracts of *S.acuta* makes it a potent ethnomedicinal source.

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