ORIGINAL ARTICLE

Extraction and pharmacological evaluation of flavonoids of *sida acuta* Burm. f.

Alka Jindal, Padma Kumar, Geeta Singh
Department of Botany, University of Rajasthan, Laboratory of Plant Tissue Culture and Secondary Metabolites, Jaipur, Rajasthan, India

Background: Flavonoids are group of polyphenolic compounds which are known to have medicinal properties and play a major role in the successful medicinal treatments from ancient times. **Aims:** The present study is conducted to evaluate the antimicrobial activity of free and bound flavonoids of *Sida acuta* Burm. f. **Materials and Methods:** The disc diffusion assay was performed against three bacteria (*Escherichia coli*, *Staphylococcus aureus* and *Proteus mirabilis*) and two fungi (*Aspergillus flavus* and *Aspergillus niger*). Minimum inhibitory concentrations were evaluated by the micro broth dilution method, while minimum bactericidal/fungicidal concentrations were carried out by subculturing the relevant samples. Total activity was also calculated for the extracts, to relate minimum inhibitory concentration of the extract with its amount isolated from 1 g of dried plant material. **Statistical Analysis:** All the values of the results of disc diffusion assay were expressed as means of three replicates ± standard error means. **Results:** The flavonoid extracts showed only antibacterial activity, but were inactive against the tested fungi. Free flavonoids from roots and bound flavonoid from stems exhibited antibacterial activity against all tested bacteria. *S. aureus* was the most susceptible microorganism which was sensitive toward 6 extracts out of 7 active extracts followed by *P. mirabilis* (5 extracts) and *E. coli* (3 extracts), respectively. **Conclusion:** The antibacterial flavonoids from *S. acuta* could be used in developing novel antibacterial drugs.

Key words: Antimicrobial activity, flavonoid, *sida acuta*, total activity

INTRODUCTION

Higher plants remain as an almost untapped reservoir of potentially useful chemical compounds not only as drugs but also as unique templates that could serve as a starting point for synthetic analogues. Many drugs have been developed with phytochemicals or taking phytochemicals as lead molecules. Some important mainline drugs include digitoxin, aspirin, taxol, ergotamine, morphine, cocaine and reserpine.[1] According to the World Health Organization, approximately 25% of modern drugs used in the United States have been derived from plants and it is estimated that at least 7,000 medical compounds in the modern pharmacopoeia are derived from plants. Today nearly 88% of the total global populations turn to plant-derived medicines as first line of defense to maintain health and combating ailments. People of Asia are utilizing plants as part of their routine health management and oriental world is also coming on the same way. In the direction of developing new antimicrobial agents from herbal resources, research has been geared toward screening of medicinal plants for exploring their antibiotic potential.[2] Many researchers have focused on the investigation of natural products and plant extracts as a source of new bioactive molecules.[3-5]

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Sida acuta (Burm. f.) (Family: Malvaceae) is a taproot and perennial shrub that grow well in many soils. The plant is frequently found in pastures, cultivated lands, roadsides and lawns. It has a variety of traditional uses. In Nicaragua, the decoction of the entire plant is taken orally for asthma, fever, aches and pains, ulcers and as an anti-worm medication; while decoction of the dried entire plant is taken orally for venereal diseases.[6,7] In India, the hot water extract of the dried entire plant is administered orally as a febrifuge, an abortifacient and diuretic.[8] Previously, antibacterial and antifungal activities of crude extracts of *S. acuta* have been reported.[9-11] The aim of the present investigation is to extract and screen flavonoids (free and bound) from different parts (root, stem, leaf and buds) of *S. acuta* for their antimicrobial activity.

MATERIALS AND METHODS

Plant Materials

Different parts of *Sida acuta* (root, stem, leaf and buds) were collected from different localities of Jaipur, in the month of June, 2008. The plant was identified at

Address for correspondence: Miss. Alka Jindal, Department of Botany, University of Rajasthan, Laboratory of Plant Tissue Culture and Secondary Metabolites, Jaipur - 302 055, Rajasthan, India. E-mail: jindal4@gmail.com
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Herbarium, Department of Botany, University of Rajasthan, Jaipur. All the parts of *S. acuta* were separately shade dried and were milled to a fine powder using a grinder.

Preliminary Detection of Flavonoids

Two methods were used to determine the presence of flavonoids in each sample (root, stem, leaf and buds) of *S. acuta*.[12,13] The aqueous extracts were prepared by soaking 50 g of dried powdered samples in 100 ml of distilled water for 12 h. The extracts were filtered using Whatman filter paper No. 1.

Dilute ammonia solution (5 ml) was added to a portion of the aqueous filtrate of each part of the plant, followed by addition of concentrated H_2SO_4. A yellow color observed in each extract indicated the presence of flavonoids. The yellow coloration disappeared on standing.

Few drops of NaOH solution was added to each test extracts. A yellow color observed in each extract. The color disappeared after addition of dilute acid, indicate the presence of flavonoids.

Extraction of Flavonoids

Different parts of *S. acuta* (root, stem, leaf and buds) were subjected for flavonoids extraction, following the well established method.[14] Hundred grams of finely powdered plant part was Soxhlet extracted with hot 80% methanol (500 ml) and filtered. Filtrate was re-extracted successively with petroleum ether (fraction I), ethyl ether (fraction II) and ethyl acetate (fraction III) using separating funnel. Petroleum ether fraction was discarded due to being rich in fatty substances, whereas ethyl ether and ethyl acetate fractions were analyzed for free and bound flavonoids, respectively. Ethyl acetate fraction was hydrolyzed by refluxing with 7% H_2SO_4 for 2 h (for removal of bound sugars from the flavonoids). Resulting mixture was filtered and filtrate was extracted with ethyl acetate in separating funnel. Ethyl acetate extract thus obtained was washed with distilled water till neutrality. Ethyl ether (free flavonoids) and ethyl acetate fraction (bound flavonoids) were dried in *vaccuo* and weighed.

Antimicrobial Activity

The disc diffusion assay[15] was used for antimicrobial activity of the flavonoid extracts against 3 pathogenic bacteria: Gram positive (*Staphylococcus aureus* MTCC 87) and Gram negative (*Escherichia coli* MTCC 46 and *Proteus mirabilis* MTCC 1425) and 2 pathogenic fungi: *Aspergillus flavus* MTCC 277 and *Aspergillus niger* MTCC 282. They were procured from IMTECH, Chandigarh, India. Bacterial strains were grown and maintained on Muller-Hinton Agar medium while fungal strains were kept on Sabouraud Dextrose Agar medium. For antimicrobial activity, standard size of microbial inoculums (1×10^8 CFU/ml for bacteria and 1×10^7 CFU/ml for fungi) were used with 1 mg/disc concentration of both the test extracts and standards (streptomycin for bacteria and itraconazole for fungi). Each extract was tested in triplicate. Antimicrobial activity was determined by measuring zone of inhibition (IZ) in mm. Activity Index (AI) for each extract was also calculated by using the following formula:

AI = IZ of the extract/IZ of the standard.

Minimum Inhibitory Concentration

Minimum inhibitory concentration (MIC) was determined for each plant extract showing activity against test pathogens in disc diffusion assay. Microbroth dilution method[16] was followed for determination of MIC values. Experiments were conducted three times and the mean values were recorded.

Minimum Bactericidal/Fungicidal Concentration

Minimum bactericidal/fungicidal concentration (MBC/MFC) was determined by subculturing 50 µl from each well showing no apparent growth. Least concentration of extract showing no visible growth on subculturing was taken as MBC/MFC.

Total Activity

Total activity (TA) for each active extract was also calculated, which is the volume at which the test extract can be diluted without loosing the ability to kill microorganisms.[17] It is calculated by dividing the amount of extract from 1 g plant material by the MIC of the same extract.

Total activity = Amount extracted from 1 g plant material/MIC of the extract.

RESULTS

All the parts (root, stem, leaf and buds) of *Sida acuta* showed positive response in preliminary detection test of flavonoids. Flavonoid content estimated in each gram of dried plant material was recorded [Table 1]. Content of free flavonoids were obtained maximum in leaves (8.15 mg/g.d.w) whereas bound flavonoids were maximum in bud (1.75 mg/g.d.w). Total flavonoids were found to be maximum in leaves (8.5 mg/g.d.w).

Table 1: Quantitative estimation of flavonoids of *S. acuta*

Part	Flavonoids (mg/g.d.w)		
	Free	Bound	Total
Root	4.25	0.5	4.75
Stem	5.0	0.45	5.45
Leaf	8.15	0.35	8.5
Bud	4.10	1.75	5.85

Most of the research has been carried out on screening i.e., determination of inhibition zone (IZ) of crude extracts but without activity index (AI), minimum inhibitory concentration (MIC), minimum bactericidal/fungicidal concentration (MBC/MFC) and total activity (TA) calculation. Antimicrobial activity (assessed in terms of IZ and AI) of the flavonoids, tested against selected microorganisms, was recorded [Table 2]. All the flavonoid extracts showed antibacterial activity against selected bacteria while the extracts showed no activity against the selected fungi at tested concentration. Total 8 extracts of different parts were tested against selected pathogens, among which 7 extracts showed bioactivity. However, 1 extract showed no activity against any selected microorganisms. Most susceptible organism in the investigation was *Staphylococcus aureus* against which most of the extracts showed inhibition zone, followed by *Proteus mirabilis* and *Escherichia coli*. Best activity against *S. aureus* (IZ 20 mm ± 0.234 and AI 0.8±0.002) and *P. mirabilis* (IZ 20 mm ± 0.234 and AI 0.83±0.041) was observed by bound and free flavonoids of root, respectively whereas best activity against *E. coli* (IZ 10.1 mm ± 0.333, AI 0.5±0.013) was shown by bound flavonoids of stem.

The range of MIC and MBC of extracts recorded was 0.019 to 0.625 mg/ml and 0.019 to 1.25 mg/ml, respectively [Table 3]. Same values of MIC and MBC was recorded against *E. coli* (0.625 mg/ml), *S. aureus* (0.156, 0.019 and 0.625 mg/ml) and *P. mirabilis* (0.019 mg/ml). Total activity was also calculated [Table 4]. Maximum TA values calculated were 6.8, 27.24 and 223.68 ml/g against *E. coli*, *S. aureus* and *P. mirabilis*, respectively.

DISCUSSION

Alkaloids, flavonoids, steroids, terpenoids, cardiac glycoside and phenolic compounds from *Sida acuta* have been reported.[18,19] Antimicrobial activity of various crude extracts of *S. acuta* have also been reported earlier but still meager work has been carried out as far as the antimicrobial activity of specific metabolite is concerned and no work has been carried out to extract and screen free and bound flavonoids of *S. acuta*.

The present study indicated that the flavonoid extracts of all the parts of *S. acuta* have got profound antibacterial potential. The extracts presents broad spectrum of antibacterial activity since the extracts were found to be active against both the Gram positive (*Staphylococcus aureus*) and Gram negative (*Escherichia coli* and *Proteus mirabilis*) bacteria. The results of the antibacterial activity of present study were in agreement with the findings of previous researchers.[20-24] Thus the observation could positively

Table 2: Inhibition zone and activity index of flavonoids of *S. acuta*

Part	Extract	Test microorganisms									
		E. coli		*S. aureus*		*P. mirabilis*		*A. flavus*		*A. niger*	
		IZ mm	AI	IZ mm	AI	IZ mm	AI	IZ mm	AI	IZ mm	AI
Root	F	9±0.577	0.45±0.003	13±0.167	0.52±0.001	20±0.234	0.83±0.041	-	-	-	-
	B	-	-	20±0.234	0.8±0.002	12.4±0.278	0.51±0.001	-	-	-	-
Stem	F	-	-	9.6±0.577	0.38±0.007	-	-	-	-	-	-
	B	10.1±0.333	0.5±0.013	10±0.333	0.4±0.029	8±0.882	0.33±0.007	-	-	-	-
Leaf	F	-	-	8±0.882	0.32±0.007	-	-	-	-	-	-
	B	-	-	9±0.577	0.36±0.013	10±0.333	0.41±0.029	-	-	-	-
Bud	F	8.5±0.882	0.42±0.029	-	-	8±0.882	0.33±0.003	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-
Standard		20		25		24		15		10	

F: Free flavonoids; B: Bound flavonoids; ±: SEM (Standard error mean), (–): No inhibition; Standards: Streptomycin for bacteria and Itraconazole for fungi; IZ – Inhibition zone; AI – Activity index

Table 3: Minimum inhibitory concentration and minimum bactericidal/fungicidal concentration of flavonoids of *S. acuta*

Part	Extract	Test microorganisms									
		E. coli		*S. aureus*		*P. mirabilis*		*A. flavus*		*A. niger*	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC	MIC	MFC
Root	F	0.625	1.25	0.156	0.156	0.019	0.019	-	-	-	-
	B	-	-	0.019	0.019	0.156	0.312	-	-	-	-
Stem	F	-	-	0.312	0.625	-	-	-	-	-	-
	B	0.625	0.625	0.625	1.25	0.625	1.25	-	-	-	-
Leaf	F	-	-	0.625	1.25	-	-	-	-	-	-
	B	-	-	0.625	0.625	0.312	0.625	-	-	-	-
Bud	F	0.625	1.25	-	-	0.625	1.25	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-

All figues are in mg/ml unit; F: Free flavonoids; B: Bound flavonoids; (–) : Not determined since there was no activity; MIC – Minimum inhibitory concentration; MBC – Minimum bactericidal concentration; MFC – Minimum fungicidal concentration

Table 4: Total activity of flavonoids of *S. acuta*

Part	Extract	Total activity (ml/g)				
		Test microorganisms				
		E. coli	*S. aureus*	*P. mirabilis*	*A. flavus*	*A. niger*
Root	F	6.8	27.24	223.68	-	-
	B	-	26.31	3.2	-	-
Stem	F	-	16.02	-	-	-
	B	0.72	0.72	0.72		
Leaf	F	-	13.04	-	-	-
	B	-	0.56	1.12	-	-
Bud	F	6.56	-	6.56	-	-
	B	-	-	-	-	-

F: Free flavonoids; B: Bound flavonoids; (–): Not determined since there was no activity

justify the usefulness of the plant.[6,7,25] Many of the tested extracts showed excellent antibacterial activity, which may be of use for formulating new, safer and ecofriendly drug. The results of the investigation do not reveal that which chemical compound is responsible for aforementioned activity. Therefore, further studies would focus on isolation of the bioactive compounds from this plant.

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