Academic Sciences Asian Journal of Pharmaceutical and Clinical Research
Vol 5, Suppl 1, 2012 ISSN - 0974-2441

Research Article

ANTIBACTERIAL ACTIVITY OF SIDA ACUTA BURM. F. AGAINST HUMAN PATHOGENS

ALKA JINDAL*, PADMA KUMAR

Laboratory of Plant Tissue Culture and Secondary Metabolites, Department of Botany, University of Rajasthan, Jaipur-302055, India,
Email: jindal4@gmail.com

Received: 1 January 2012, Revised and Accepted: 7 February 2012

ABSTRACT

Alkaloids are well known for their antimicrobial properties. The present study was designed to evaluate the antibacterial activity of alkaloids of *Sida acuta* (Malvaceae) Burm. f. Alkaloids from different parts (root, stem, leaf and buds) of the plant were extracted and were screened for antibacterial activity by 'Disc Diffusion Assay' against two Gram negative bacteria (*Escherichia coli* and *Proteus mirabilis*) and one Gram positive bacteria (*Staphylococcus aureus*). Minimum inhibitory concentration, minimum bactericidal concentration and total activity of extracts against each sensitive pathogen have also been evaluated. Results of the present study indicate that all the tested alkaloid extracts of *S. acuta* have potent antibacterial activity against *P. mirabilis* and *S. aureus* whereas the extracts was found to be inactive against *E. coli*.

Keywords: Alkaloid, *Sida acuta*, Antibacterial activity, Disc diffusion assay, Total activity.

INTRODUCTION

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs in microorganisms has gradually increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized frequently as therapeutic agents [1]. This fact is the cause for concern, as numbers of patients are not responding positively towards existing antibiotics. Further emerging new multi-resistant bacterial strains, aggravate the problem.

The problem of microbial resistance is continuously growing hence the use of existing antimicrobial drugs in future is still uncertain. Therefore, immediate action is required to combat the problem, by encouraging research to develop new drugs; more so of herbal origin as synthetic drugs are known to cause side effects. The ultimate goal is to offer appropriate and efficient antimicrobial and present study is an effort in this direction.

Sida acuta Burm. f. a pantropical weed, native to Mexico and Central America but has spread throughout the tropics and subtropics [2] and frequently dominates pastures [3]. All the plant parts exert various pharmacological properties includes antiplasmodial, antimicrobial, antioxidant and cytotoxic activities. In Kenya, the whole plant is used to prepare "Bundugo", a supplementary strength magically added to a person [4]. In Western Colombia, whole plant is used for snakebites [5] and in India, it is used for fever, bronchitis, ulcer, diarrhea, dysentery and skin diseases [6]. The leaves are used for the treatment of eczema, kidney stone, headache, malaria, gonorrhea, breast cancer, poisoning, inflammation, stops bleeding, sores and wounds [7-9]. The paste of leaves is mixed with coconut oil and applied on head regularly for killing dandruffs and also for strengthening hair [10].

In earlier studies, selected pathogens have been proved to be the major causal organisms of various human infections viz. *Eschirichia coli* and *Proteus mirabilis* are the culprits of human urinary tract infections [11] and most of the human intestinal infections are due to *E. coli*. *S. aureus* causes a variety of suppurative, wound infections and food poisoning in human beings. *S. aureus* is a major causative agent of nosocomial infections [12]. Hence in the present study screening for antibacterial potential of the alkaloid extracts of *S. acuta* has been undertaken.

MATERIALS AND METHODS

Collection and identification of plant material

Sida acuta Burm. f. was collected from different localities of Jaipur in the month of June, 2008 and was identified at Department of Botany, UOR. A voucher specimen (RUBL-20428) was also submitted to the Herbarium of Botany Department, UOR.

Extraction of alkaloids

Different parts of the plant (root, stem, leaves and buds) were separated and washed thoroughly; shade dried and finely powdered using a blender. Alkaloids were extracted with all the separated parts of the plant following the well established method of Harborne [13]. Hundred grams of each finely powdered sample was extracted with 10% acetic acid in ethanol (final volume 500 ml) for 4 h. Extract were then concentrated to ¼ of the original volume and made alkaline by NH_4OH. Precipitates collected after centrifugation were washed with 1% NH_4OH, filtered, dried in *vaccuo* and weighed.

Test microorganisms

Bacterial strains of *E.coli* (MTCC 46), *S. aureus* (MTCC 87) and *P. mirabilis* (MTCC 1425) were procured from IMTECH, Chandigarh, India. These strains were grown and maintained on Muller Hinton Agar medium (Beef extract 2.0 g; Peptone 17.5 g; Starch 1.5 g; Agar 17.0 g; in 1000 ml of distilled water; Final pH 7.4 $\pm$ 0.2) at 37±2^0 C at 4^{0}C in the deep freezer until required for use.

Antibacterial activity of extracts

Disc diffusion assay (DDA)

Antibacterial screening of test extracts was carried out by the disc diffusion assay (DDA) method [14]. Muller-Hinton agar media plates were seeded with the prepared culture suspensions (1×10^8 cfu/ml). Sterilized filter paper discs of 6 mm diameter (Whatman no. 1) were impregnated with 100 µl of extract of 10 mg/ml concentration to give a final concentration of 1 mg/disc, left to dry in *vaccuo* to remove residual solvent, which might interfere with the determination. The extract discs were placed on the seeded media plates along with discs impregnated with of standard drug streptomycin in the same (1 mg/disc) concentration. These plates were kept at 4°C for 1 h for the diffusion of extracts into the media and thereafter were incubated at 37°C±2°C for 24 h. Zone of inhibitions (IZ) produced by the extracts around the discs were measured and the 'Activity Indices' (AI) was calculated by the well established formula. The experiment was performed three times to minimize the error and the mean values were recorded.

$$\text{Activity Index (AI)} = \frac{\text{Inhibition zone of the sample}}{\text{Inhibition zone of the standard}}$$

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)

Extracts that showed positive results in the disc diffusion assay against test pathogens were further evaluated for minimum inhibitory concentration. The microbroth dilution method [15] was performed for the determination of MIC values. A series of solutions of extracts ranging from 1-10 mg/ml concentrations were prepared by re-suspending the extracts in acetone (Inactive against test pathogens). Thereafter test solutions were made by two fold serial dilution of stock solution and were added to MH broth media in wells of 96-wells microtiter plates. Hundred microlitre inoculum suspension of standard size (1×10^8 cfu/ml) was added to each well.

Cultures containing standard drug were used as positive control while culture suspensions were used as negative control. Each time two sets were prepared, one was kept for incubation while another was kept at 4^0C for comparing the turbidity in the microtitre wells. MIC values were taken as the lowest concentration of the extracts in the well of the microtitre plate that showed no visible growth or turbidity after incubation. The turbidity of the wells in the microtitre plate was interpreted as the visible growth of microorganisms. Experiments were conducted three times and the mean values were recorded.

Minimum bactericidal concentration was carried out by subculturing 50 µl aliquots from each well. Least concentration of extract showing no visible growth on subculturing was taken as MBC value.

Total Activity (TA)

Total activity for each active extract was calculated by the well established formula [16]. TA value (ml/g) is the volume to which the extract can be diluted retaining the ability to inhibit the growth of microorganisms.

$$\text{Total Activity (TA)} = \frac{\text{Amount extracted from 1 g plant material (mg/g.d.w)}}{\text{MIC of the extract (mg/ml)}}$$

Table1: Alkaloid content different parts of *S. acuta*.

Plant Part	Quantity (mg/g.d.w)
Root	0.15
Stem	1.10
Leaf	3.35
Bud	7.00

Table 2: Inhibition zone (IZ) and Activity index (AI) of alkaloids of *S. acuta*.

Plant Part	Test Micoorganisms					
	E. coli		*S. aureus*		*P. mirabilis*	
	IZ	AI	IZ	AI	IZ	AI
Root	-	-	9	0.36	10	0.41
Stem	-	-	12	0.48	10	0.41
Leaf	-	-	14.8	0.59	12.6	0.52
Bud	-	-	15.5	0.62	9	0.37
Streptomycin	20		25		24	

Table 3: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of alkaloids of *S. acuta*.

Plant Part	Test Microorganism					
	E. coli		*S . aureus*		*P. mirabilis*	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
Root	-	-	0.625	1.25	0.625	0.625
Stem	-	-	0.156	0.312	0.312	0.625
Leaf	-	-	0.078	0.156	0.156	0.312
Bud	-	-	0.078	0.078	0.625	1.25

Table 4: Total activity (TA) of alkaloids of *S. acuta*.

Plant Part	Total activity (ml/g)		
	Test micorganism		
	E. coli	*S . aureus*	*P. mirabilis*
Root	-	0.24	0.24
Stem	-	7.05	3.52
Leaf	-	42.94	21.47
Bud	-	89.74	11.2

RESULTS

Alkaloids were extracted from each part of *S. acuta*. Alkaloid content estimated in each gram of dried plant material was recorded (Table1). Buds of the plant showed maximum amount of alkaloid content (7 mg/g.d.w), followed by leaves (3.35 mg/g.d.w), stem (1.1 mg/g.d.w) and roots (0.15 mg/g.d.w). Alkaloid extracts were screened for antibacterial potential against selected pathogens. All the extracts showed antibacterial potential against *S. aureus* and *P. mirabilis* while the extracts were found inactive against *E. coli*. The significant activity against *S. aureus* was shown by alkaloid extracts of buds (IZ-15.5 mm and AI-0.62), with same (0.078 mg/ml) values of MIC and MBC, followed by leaves (IZ-14.8 mm, AI-0.59, MIC- 0.078 mg/ml and MBC-0.156 mg/ml), stem (IZ-12 mm, AI- 0.48, MIC-0.156 mg/ml and MBC-0.312 mg/ml) and roots (IZ-9 mm, AI-0.36, MIC-0.625 mg/ml and MBC-1.25 mg/ml) whereas good activity against *P. mirabilis* was shown by alkaloid extract of leaves (IZ-12.6 mm, AI-0.52, MIC-0.156 mg/ml and MBC-0.312 mg/ml), followed by stem (IZ-10 mm, AI-0.41, MIC- 0.312 mg/ml and MBC-0.625 mg/ml), roots (IZ-10 mm, AI-0.41) with same (0.625 mg/ml) values of MIC and MBC and buds (IZ-9 mm, AI-0.37, MIC- 0.625 mg/ml and MBC-1.25 mg/ml; Table 2 and 3). Alkaloid extracts of buds and roots were found to be bactericidal in nature against *S. aureus* and *P. mirabilis*, respectively, showing same values of MIC and MBC. All the other active extracts were observed to be bacteriostatic in nature against *S. aureus* and *P. mirabilis* where the MBC values were greater than MIC values. TA was also calculated and recorded (Table 4). TA was highest for alkaloid extracts of buds (89.74 ml/g) and leaf (21.47 ml/g) against *S. aureus* and *P. mirabilis*, respectively.

Figure 1: Alkaloid content of different parts of *S. acuta*.

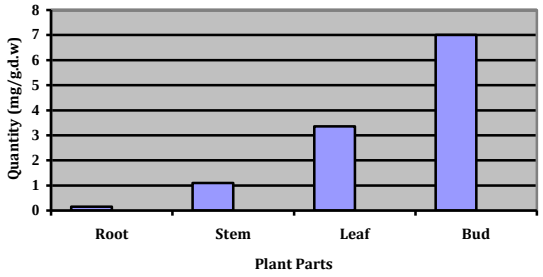
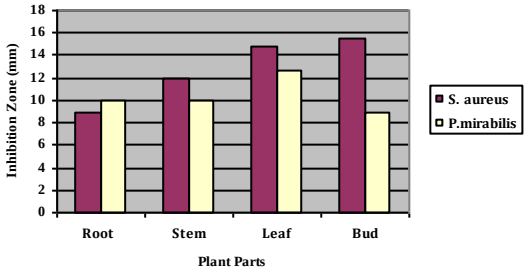


Figure 2: Inhibition zone of alkaloids of *S. acuta*.



DISCUSSION

The development of bacterial super resistant strains is resulting in failing of currently used antibiotic agents to end many bacterial infections. Hence a continuous research for getting new antimicrobial agents is the need of the present scenario, either by designing and synthesising new agents, chemically or through the search of new natural sources for antimicrobial agents [17]. The present study is an effort to explore new sources of natural and potent antibacterial agent.

Several reports have shown the antimicrobial properties of plant extracts under laboratory conditions [18-23]. In the present investigation *Sida acuta* has shown antibacterial potential against two out of three test pathogens which are the major causative organisms of various human diseases. Although the plant (*S. acuta*) has been studied previously for its antimicrobial activity but only restricted to the determination of IZ and that too without AI, MIC, MBC and TA evaluation. Hence could not explore for the preparation of antibiotics. However in the present study *S. acuta* has been evaluated for alkaloid

extracts using various parameters viz. IZ, AI, MIC, MBC and TA. It is worth mentioning that most of the extracts exhibit very low MIC values (a desirable features). Two active extracts were found to be bactericidal whereas remaining were found bacteriostatic in nature against *S. aureus* and *P. mirabilis* which indicates the significant potentiality of the tested extracts of *S. acuta.*

Acknowledgement

Authors are thankful to the Head of Botany Department, University of Rajasthan, Jaipur, India for providing all necessary facilities to carry out the work. Financial assistance provided by UGC is gratefully acknowledged.

REFERENCES

1. Cohen ML. Epidemiology of drug resistance: Implications for post- antimicrobial era. Science 1992; 257: 1050-5.
2. Holm LG, Plucknett DL, Pancho JV, Herberger JP. The worlds worst weeds: Distribution and biology. University Press of Hawaii, USA, Honolulu; 1977.
3. Mann A, Gbate M, Umar AN. *Sida acuta* subspecie *acuta*. Medicinal and economic plant of Nupeland, Jube Evans Books and Publication; 2003. p. 241.
4. Pakia, M. African Traditional Plant Knowledge Today: An Ethnobotanical Study of the Digo at the Kenya Coast. Doctorate Thesis, University of Bayreuth Germany. 2005.
5. Otero R, Nunez V, Jimenez SL, Fonnegra R, Osorio RG, Garcia ME, Diaz A. Snakebites and ethnobotany in the northwest region of Colombia: Part II: neutralisation of lethal and enzymatic effects of *Bothrops atrox* venom. J Ethnopharmacol 2000; 71: 505-11.
6. Ignacimuthu S, Ayyanar M, Sankara-Sivaramann K. Ethnobotanical investigations among tribes in Madurai District of Tamil Nadu (India). J Ethnobiol Ethnomed 2006; 2: 25.
7. Anani K, Hudson JB, De Souza C, Akpkagana K, Tower GHN, Amason JT, Gbeassor M. Investigation of medicinal plants of Togo for antiviral and antimicrobial activities. Pharma Biol 2000; 38: 40-5.
8. Kayode J. Conservation of indigenous medicinal botanicals in Ekiti State, Nigeria. J Zhejiang University Sci B 2006; 7: 713-8.
9. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotech 2005; 4: 685-8.
10. Malairajan P, Gopalakrishnan G , Narasimhan S , Veni KJK. Analgesic activity of some Indian medicinal plants. J Ethnopharmacol 2006; 106: 425-8.
11. Venier AG, Talon D, Party I, Mercier-Girard D Bertrandx. Patient and bacterial determinants involved in symptomatic urinary tract infections caused by *E. coli* with and without bacteraemia. Clin Microbial Infect 2007; 13(2): 205-8.
12. Alvarez LF, Paloman M, Insausti J, Olaechea P, Cerda F, Sanchez GJ, De La Torre MV. *Staphylococcus aureus* nosocomial infections in critically ill patients admitted in intensive care units. Med Clin (Barc) 2006; 126(17): 641-6.
13. Harborne JC. Phytochemical Methods: A guide to modern techniques of plant analysis, 2nd ed: London: Chapman and Hall Ltd., 1984.
14. Andrews JM. BSAC standardized disc susceptibility testing method. J Antimicrob Chemother 2001; 4: 43-57.
15. Basri DF, Fan SH. The potential of aqueous and acetone extracts of galls of *Quercus infectoria* as antibacterial agents. Indian J Pharmacol 2005; 37(1): 26-9.
16. Eloff JN. Quantifying the bioactivity of the plant extracts during screening and bioassay-guided fractionation. Phytomedicine 2004; 11(4): 370-1.
17. Bhavnani, SM, Ballow, CH. New agents for Gram-positive bacteria. Current Opinions in Microbiology 2000; 3: 528-34.
18. Okigbo RN, Ogbonnaya UO. Antifungal effects of two tropical plant leaf extracts (*Ocimum gratissimum* and *Aframomum melegueta*) on post harvest yam (*Dioscorea* spp.) rot. Afr J Biotech 2006; 5: 727-31.
19. Shariff N, Sudarshana MS, Umesha S, Hariprasad P. Antimicrobial activity of *Rauvolfia tetraphylla* and *Physalis minima* leaf and callus extracts. Afr J Biotech 2006; 5: 946-50.
20. Bouamama H, Noel T, Villard J, Benharref A, Jana M. Antimicrobial activities of the leaf extracts of two Moroccan *cistus* L. species. J Ethnopharmacol 2006; 104 1-2: 104-7.
21. Ergene A, Guler P, Tan S, Mirici S, Hamzaoglu E, Duran A. Antibacterial and antifungal activity of *Heracleum sphondylium* subsp. *artvinense*. Afr J Biotech 2006; 5: 1087-9.
22. Kiran B, Raveesha KA. Antifungal activity of seed extract of *Psoralea corylifolia* L. Plant Disease Res 2006; 20: 213-15.
23. Mohana DC, Raveesha KA. Anti-bacterial activity of *Caesalpinia coriaria* (Jacq.)Wild against plant pathogenic *Xanthomonas* pathovars: an eco-friendly approach. J Agri Tech 2006; 2: 317-27.