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RESEARCH ARTICLE

Evaluation of *Sida acuta* leaf extract for antibacterial activity

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SUMMARY

The aqueous and ethanolic leaf extracts of *Sida acuta* were evaluated for the antibacterial activity. *Klebsiella pneumoniae* was inhibited by both extracts while *Pseudomonas aeruginosa* by aqueous extract only. Other bacteria *E.coli, Staphylococcus aureus* and *Salmonella typhimurium* did not show any sensitivity to either of the extracts. Ethanolic extract proved to be better than the other in inhibiting the bacterial growth.

Key Words : Aqueous extract, Ethanolic extract, Bacteria, Antibacterial activity, Zone of inhibition

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Higher plants are capable of producing a variety of secondary metabolites of high structural diversity which include alkaloids, phenols, tannins, terpenoids, flavonoids, essential oils etc. (Evans *et al.,* 1986). Many of these biologically active compounds are the potential antimicrobial agents (Cowan, 1999) and can form the lead molecules in the development of new antimicrobial drugs. All plant parts are good source of antibacterial compounds (Dorman and Deans, 2000). The plant parts have been used in the preparation of number of new antibiotics (Iwu *et al.,*1999). According to WHO, most of the drugs available today contain atleast one active ingredient derived from plants. Thus plants can be the best source for designing and developing new antibiotics to counter the resistance being acquired in microorganisms due to the arbitrary use of drugs.

In recent years, interest in the investigation of natural materials as sources of new antibacterial agents has been renewed.There is a need to identify novel substances that are active towards pathogens with high resistance (Recio *et al.*,1989; Cragg *et al.*,1997).

Several plants have been screened worldwide to understand the antibacterial activities in different parts of plants (Pongpan *et al.*,1982; Vlietinck *et al.*,1995; Gislene *et al.*, 2000; Nair *et al.*, 2005; Parekh *et al.*, 2005; Nair and Chanda, 2007; Negi and Sharma, 2010).

Thus, in the present investigation, aqueous and ethanolic extracts of *Sida acuta* leaves were evaluated for antibacterial activity against five bacteria, *Escherichia coli*ATCC25922, K*lebsiella pneumoniae* ATCC10031, *Pseudomonas aeruginosa* ATCC27853, S*almonella typhimurium* ATCC 23564, *Staphylococcus aureus* ATCC 25923.

Sida acuta Burm. f.belonging to Malvaceae is a perennial shrub found growing, grow well in many soils. The plant is frequently found in pastures, cultivated lands and along the roadsides. It has a variety of traditional uses. In Nicaragua, the decoction of the entire plant is taken orally for curing asthma, fever, aches and pains, ulcers and also venereal disease (Barret, 1994; Coee and Anderson, 1996).

Malairajan *et al. (*2006), Saganuwa and Gulimbe (2006), Oboh *et al.* (2007), Ekpo and Etim (2009), Wake (2011) and Ibrahim *et al.* (2012) tested the antimicrobial activities of crude extracts of *S. acuta*. Jindal *et al.*(2012) evaluated the antibacterial activity of flavonoids extracted from *S.acuta.*

MATERIAL AND METHODS

Required plant material collected from the Kalsubai

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regions of Western Ghats were washed under running tap water and surface sterilized by 0.1% w/v $HgCl_2$, followed by rinsing twice in distilled water so as to remove the traces of $HgCl_2$.These leaves were then dried in shade at room temperature, homogenized to fine powder and stored in airtight bottles for further use.

Aqueous extraction :

Ten g of leaf powder was extracted in 100 ml of distilled water for 6 hrs on hot water bath. After every 2hrs, it was filtered through eight layers of muslin cloth and centrifuged at 5000g for 15min.The supernatant was collected and condensed in boiling water bath until the water was evaporated to get the residue which was stored in brown bottles at 4^0 C for further experiments.

Solvent extraction :

Ten g of dry leaf powder was extracted in 50 ml of ethanol on rotary shaker at 150 rpm for 24hrs, filtered through eight layers of muslin cloth and centrifuged at 5000g for 15min. The supernatant was collected and the solvent allowed to evaporate. The residue thus, obtained was stored in brown bottles at 4^0C for further experiments.

Bacterial cultures :

Five strains of bacteria *viz., Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 10031, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 23564 and *Staphylococcus aureus* ATCC 25923 were procured from the National Chemical Laboratory (NCL), Pune,India and maintained on Muller Hinton Agar slants at 4^0C. The bacteria were revived on sterilized MHA medium in Petri plates at 37^0C.

Inoculum preparation :

Bacterial strains were grown to exponential phase in saline medium (0.85% NaCl) at 37^0C for 24hrs and adjusted to final density of 10^4cfu/ml to obtain a turbidity visually compared to 0.5 McFarland standards (Andrews, 2001).

Antibacterial activity testing :

The antibacterial activity of leaf extract was evaluated by agar well diffusion assay (Perez *et al.,* 1990). Aqueous and ethanol leaf extracts were dissolved in distilled water and dimethyl sulfoxide (DMSO), respectively to get a concentration of 10mg/ml. 100µl inoculum (10^4cfu / ml; 0.5 MacFarland standards) of each test bacterium was spread with the help of sterile glass spreader on sterile MHA medium in Petri plates so as to achieve a confluent growth. Wells of 8mm diameter were made in the seeded agar plates with the cork borer. Each well was then filled with 50µl (10mg/ml concentration) of plant extract. The plates were allowed to stand for atleast half an hour for diffusion to take place and then incubated at 37^0C for 24hrs. DMSO and sterile distilled water was used as a negative control for the ethanolic and aqueous extracts, respectively. Different concentrations of tetracycline were tested for inhibitory effect on bacteria. The lowest concentration of 1mg/ ml and 25 µg/ ml of tetracycline required to inhibit *P.aeruginosa* and other bacteria, respectively was used in positive control sets. Antimicrobial activity was evaluated by measuring the diameter of zone of inhibition excluding the diameter of well against the test organism. Activity index (AI) for each extract was also calculated by using the following formula AI = ZoI of the extract/ ZoI of the standard.

RESULTS AND DISCUSSION

The findings of the present study are presented in Table 1.

Results from Table 1 indicate that only *K.pneumoniae* was inhibited by both aqueous and ethanolic extracts while *P. aeruginosa* by the ethanolic extract. Other bacteria did not show sensitivity to the extracts. However, Jindal *et al.* (2012) reported that the flavonoids from *S.acuta* leaves inhibited only *S. aureus* and not *E.coli.* It may be inferred that the sensitivity in bacterial strain is specific to a phyto-compound. Comparatively ethanolic extract showed better results. The results do not reveal any chemical compound responsible for the antibacterial activity and provides scope for further research on isolation and evaluation of different bioactive compounds from this plant.

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Table 1: Effect of *Sida acuta* leaf extract on the growth of bacteria

	Bacterial zone of Inhibition (in mm)									
	E. coli		*S. aureus*		*K. pneumoniae*		*P. aeruginosa*		*S. typhimurium*	
	A	E	A	E	A	E	A	E	A	E
Leaf extract	-	-	-	-	8.00 (0.52)	9.67 (63.07)	-	6.33 (84.4)	-	-
+ ve control	7.5		14.33		15.33		7.5 #		12.66	
-ve control	-		-		-		-		-	

A= Aqueous extract E= Ethanolic extract # 1mg/ml

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