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ANTIBACTERIAL ACTIVITY OF ALKALOIDS FROM SIDA ACUTA

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ABSTRACT

Sida acuta is a shrub indigenous to pantropical regions. The plant is widely used for its various pharmacological properties. Among compounds of pharmacological interest occurring in the plant, are indoloquinoline alkaloids. The aim of the present study was to investigate the antibacterial activity of alkaloids of S. acuta. The alkaloids had a good antibacterial activity against the test microorganisms. In the agar-well diffusion assay, highest inhibition zone diameters were recorded with Gram-positive bacteria.

KEYWORDS: Quindoline, indoloquinolines, antibacterial, Sida acuta.

INTRODUCTION

This traditional medicine uses numerous plants, among them, Sida Acuta Burm f. (Malvaceae). S. Acuta is a shrub indigenous to pantropical areas, widely distributed in these regions and widely used in traditional medicine. The aerial part of the plant is the most frequently used part. In central America, the plant is used to treat asthma, renal inflammation, colds, fever, headache, ulcers and worms. In Colombia the plant is known to treat snake bites. Otero et al. demonstrated that the ethanolic extract of the plant had an effective moderate activity against the venom of Bothrox athrox. The plant is traditionally used in the treatment of malaria, diarrhoea and many other diseases. Research focussed on malaria led to the identification of alkaloids, principally cryptolepine the major alkaloid of the plant, as its antimalarial agent. More recently, we found that polyphenol extract of the plant had a weak antioxidant activity through in vitro free radicals scavenging assays, on the other hand the extract was very active on pathogenic bacteria and this activity may be influenced by the

polymerisation size of the phenolic compounds. Phytochemical screening on S. acuta resulted in the isolation of several alkaloids and steroidal compounds.

Among the compounds isolated from S. acuta, its alkaloids appeared to be of great interest in pharmacological studies. These alkaloids belong to the family of indoloquinolines. The purpose of the present study was to investigate the antimicrobial activity of alkaloids from S. acuta against Gram-positive and Gram-negative bacteria.

MATERIALS AND METHODS

Collection of Plant Material

The root of sida acuta were collected from kollam district in kerala in month of april 2016. After washing with water the root were dried for 10 days in shade. Then they are weighed and kept in airtight container and stored in refrigerator for future use.

PREPARATION OF EXTRACT

Maceration (Steady State Extraction)

In maceration (for fluid extract), whole or coarsely powdered plant-drug is kept in contact with the solvent in a stoppered container for a defined period with frequent agitation until soluble matter is dissolved. This method is best suitable for use in case of the thermolabile drugs.

PROCEDURE

About 25g of shade dried powdered material was added with 250ml ethanol. The container was shaked for every half an hour for period of 24 hours. The extract was filtered, concentrated and dried. This dried viscous material obtained was used for the analysis.

MICROORGANISMS AND ANTIBIOTICS

Clinically isolated microorganisms were obtained from Department of Microbiology. The bacterial strains were grown in the nutrient broth and maintained on nutrient agar slants at 4°C. Antibiotic Ciprofloxacin (10mcg) were used for antibiotic susceptibility study on identified and clinically isolated microorganisms.

QUALITATIVE PHYTOCHEMICAL SCREENING

The different qualitative chemical tests were performed on the methanolic extracts of S. acuta root for establishing chemical profile and detected various phytoconstituents are given table1 on discussion.

ANTIBACTERIAL ASSAYS

Antibacterial activity of the extract of Sida acuta was studied using the disc diffusion method. Petri plates containing 10 ml of Muller Hinton agar medium were seeded with 24h old culture of a selected bacterial strain. Sterile filter paper discs (6mm) containing 100mg/disc of a plant extract residue dissolved in acetone were placed on the surface of the medium. Acetone and water alone served as negative controls. A standard disc containing reference antibiotics (ciprofloxacin) were used as a positive control. Incubation was done for 24h at 37^0C. The assessment of anitbacteial activity was based on the measurement of diameter of zone of inhibition formed around the disc. Six determinations were conducted for the extract.

TIME-KILL ASSAY

Escherichia coli CIP 105182 and Shigella dysenteriae CIP 54051 were chosen arbitrary to perform time-kill assay. Thus, 0.5 Mac Farland standards suspensions of the microorganisms were diluted to have 50ml of approximately 105 cfu/ml in nutrient broth, then 160 and 480 µg/ml alkaloids that corresponded to 2 MIC of Escherichia coli CIP 105182 and Shigella dysenteriae CIP 54051, respectively, were added to the corresponding culture. The cultures were incubated in air at 37°C in incubator shaker. At 0, 1,2,3,4,5 and 6 h, analiquot of 100µl was removed and diluted with 10ml sterile broth. The obtained suspension was used to inoculate 90mm diameter Petri plates with a sterile non toxic cotton swab on a wooden applicator as indicated before in the agar-well diffusion assay. After 48 h incubation at 37°C, the viability of the microorganisms was evaluated by the presence of colonies on the plates. The experiment was carried out twice.

RESULT AND DISCUSSION

Phytochemical Screening

The chemical tests indicates the presence of alkaloids in the root extract of sida acuta.

ANTIBACTERIAL ACTIVITY

The anti-bacterial assays in this study were performed by using the disc diffusion method.

In order to follow the reduction of the amount of microorganisms in an inoculum as a function of the time, time-kill assay was performed with E. coli CIP 105182 and Sh. dysenteriae CIP 54051, arbitrarily selected among the test microorganisms. The results showed that after 5 h exposition there was no viable microorganism in the initial inoculum (Table 2) and the effect of alkaloids was faster on E. coli than it was on Sh. dysenteriae.

DISCUSSION

Sida acuta, the common wireweed is a species of flowering plant in the mallow family, Malvaceae. The present study was conducted to find out the antibacterial activity of root extract of sida acuta. The phytochemical studies shows the presence of alkaloidal content in root extract of sida acuta.

Table: 1 phytochemical screening of methanolic extract of sida acuta.

Phytochemical test	ethanol extract
Alkaloids	
Mayer's test	++
Wagner's test	++
Hager's test	++
Dragendroff's test	+
Carbohydrates & Glycosides	
Molish's test	+
Fehling's test	-
Barfoid's test	-
Benidict's test	-
Borntrager's test	+
Legal's test	+
Saponins Foam test	-
Proteins & amino acid	
Millon's test	+
Ninhydrin test	+
Phenolic compounds & flavonoids	
Ferric chloride test	++
Lead acetate test	++
Gelatin test	+

-absent, +present, ++abundant

Table: 2 Antibacterial activity (zone of inhibition diameter) of ethanol extract of *sida acuta* against clinically isolated and standard strains of Gram positive and Negative bacteria

Microorganism	Gram(+/-)	Ciprofloxacin	Ethanol extract
Staphylococcus aureus ATCC25923	+	15	25±05
Staphylococcus aureus ATCC29737	+	14	23±07
B.cereusLMG 13569	+	19	20
E. coli (n= 4)	-	26	18±04
E. coli CIP 105182.	-	20	20

Table: 3 Viability of microorganisms after 6 hours exposure to the Sida acuta alkaloid extract.

Time (h)	0	1	2	3	4	5	6
E.coli CIP105182	+ (uc)	+ (uc)	21±05	06±02	-	-	-
Sh. dysenteriae CIP 54051	+ (uc)	+ (uc)	+ (uc)	18±04	04±02	-	-

+: for the presence of the colonies.

-: for absence of colonies,

UC: uncountable.

The results are the means of number of the colonies ± standard deviations.

CONCLUSION

In conclusion, the alkaloids displayed good antimicrobial activity against several test microorganisms. The results of the present study support the traditional medicinal use of S. acuta and suggest that a great attention should be paid to this plant which is found to have many pharmacological properties.

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