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Research Article

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Phytochemical screening and evaluation of *in vitro* Antibacterial and Anthelmintic activities of *Sida acuta* leaf extracts

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ABSTRACT

The study was carried out to evaluate the qualitative analysis of various phytochemicals and invitro evaluation of antibacterial and anthelmintic activities of Ethanolic and chloroform leaf extracts of Sida acuta (Malvaceae). The extracts were subjected to preliminary Phytochemical screening for the identification of different Phytoconstituents and was found to have alkaloids, phytosterols, tannins, flavonoids, saponins, etc. The different concentrations of Ethanolic and chloroform extracts were subjected to antibacterial screening against various Gram positive and Gram negative bacteria and the results were compared with that of standard drug Gentamycin. Different concentrations of leaf extracts were tested to evaluate Anthelmintic activity using pherithema postuma (Indian earthworm).Both the extracts were found to possess vermifuge and vermicidal activity and the results were compared with standard drug Piperazine citrate.

Key words: Phytochemical Screening**,** *Sida acuta*, antibacterial activity, Pheretima posthuma, Anthelmintic activity, Piperazine citrate.

INTRODUCTION

Nature has been a source of medicinal agents since time immemorial. The importance of herbs in the management of human ailments cannot be over emphasized. It is clear that the plant kingdom harbours an inexhaustible source of active ingredients invaluable in the management of many intractable diseases.In India, medicinal plants form the back bone of several indigenous traditional systems of medicine. Phytochemicals from medicinal plants serve as lead compounds in drug discovery and design [1]. WHO report depicts that more than 80% of world's population rely on plants based products to meet their health care needs. Nearly 25 to 45% of modern prescriptions contain plant derived lead molecules as a basic source in drug formulations [2]. Sida acuta (Malvaceae), is an erect perennial shrub found throughout the hotter parts of India and Nepal. It is used for various medicinal purpose such as liver disorders diuretic & abortifacient in ayurvedic preparations, asthma, fever, headache (migrane), cough, cold, ulcer, anthelmintic, snake bite, urinary diseases, female disorders, antifertility agent and sedative [3,4]. The aerial part of the plant is most frequently used part. Phytochemical screening on *Sida acuta* resulted in the isolation of several alkaloids and steroidal compounds with the potential to induce quinine reductase and to inhibit7,12-dimethylbenz –(a) anthracene induced preneoplastic lesions in mouse mammary organ [5].

The present study was carried out to evaluate the qualitative analysis of various phytochemicals and *invitro* evaluation of antibacterial and Anthelmintic activities of Ethanolic and chloroform leaf extracts of *Sida acuta* .

EXPERIMENTAL SECTION

Plant material collection and Preparation of extract:
The leaves of *Sida acuta* were collected near East Godavari district, Andhra Pradesh, India. The leaves were shade dried, pulverized and sieved through 40 mesh. The powdered leaves were successively extracted with chloroform and Ethanol in soxhlet apparatus. The extracts obtained were evaporated under vacuum to remove the solvent completely. Then these were taken for further studies.

Phytochemical screening:
Qualitative assay for the presence of plant phytoconstituents such as carbohydrates, alkaloids, glycosides, flavonoids tannins and saponins were carried out on the powdered leaves following standard procedure [6].

Antibacterial Activity:
Test Organism Used
The various organisms like *Staphylococcus aureus ATCCBAA 1026, Bacillus subtilis ATCC 11774, Staphylococcus werneri ATCC 27836, Pseudomonas putida ATCC 700007, Pseudomonas aeruginosa ATCC 10662,, Escherichia coli ATCC 10536, Kleibsella neumonia ATCC33495* are procured from Microbes Speciality Lab Danavaipeta, Rajahmundry, East Godavari District 533103, Andhra Pradesh, India.

Antimicrobial Agent
The reference standard Gentamycin was procured from Pradeep Organics and chemicals Pvt. Ltd, Hyderabad.

Antibacterial Assay
Leaf extracts of *Sida acuta* was evaluated for antibacterial activity against several gram positive and gram negative organisms. The antibacterial activity of Leaf extracts of *Sida acuta* was performed using Agar cup-plate method. 20ml of sterile nutrient agar medium was poured into sterile Petri-dishes and allowed to solidify. The Petri dishes were incubated at 37^{o}C for 24 hours to check for sterility. The medium was seeded with the organisms by pour plate method using sterile agar broth (4 ml) contained 1 ml culture. Bores were made on the medium using sterile borer. Leaf extracts of *sida acuta* was dissolved in water to obtained different concentration (100 , 200 mg/ml) and sterilized by filtration through a Whattman filter paper no.1, and 0.05 ml of the different concentrations of extract were added to the respective bores.0.05ml of Gentamycin at a concentration of (25µg/ml) was taken as standard reference. All the plates were kept in a refrigerator at 2 to 8 ^{o} c for a period of 2 hours for effective diffusion of test compounds and standards. Later, they were incubated at 37ºc for 24 hours. The presence of definite zone of inhibition of any size around the cup indicated antibacterial activity. The diameter of the zone of inhibition was measured and recorded.

Anthelmintic activity
Worm Collection and Authentication
The antihelminthic assay was performed on healthy adult Indian earth-worms *Pheritima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings were used in the present study. Because of easy availability, earthworms have been used widely for the evaluation of Anthelmintic compounds. All the earth worms were of approximately equal size (6cm). They were collected from collected from local moist place of Aditya nagar gardens situated in Surampalem.

Anthelmintic Assay
Anthelmintic activity was evaluated by exposing the adult *Pheritima posthuma* to different concentration Leaf extracts of *Sida acuta* .The Anthelmintic activity was performed according to the method of Ghosh *et al*. with slight modifications. The Ethanolic and chloroform extracts of *Sida acuta* were dissolved in minimum amount [7] of Water and then volume was adjusted. 50 ml of formulation containing two different concentrations of each of the extract (50 and 100 mg / ml) of both Extracts were prepared and 6 worms (same type) were placed in the petridishes. The standard drug and extract solutions were prepared freshly before starting the experiment. Time for paralysis was noted when no movement could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50 °C) followed by fading away of their body colours. Piperazine citrate (20 mg / ml) was used as reference standard.

RESULTS AND DISCUSSION

Preliminary Phytochemical screening

Phytochemical screening of the extracts of Sida acuta revealed, the presence of alkaloids, phytosterols, tannins, flavonoids, saponins, etc.(table 1)

Table 1 : Preliminary Phytochemical screening of leaf extracts of *Sida acuta*

S.No	Phytoconstituents	Chloroform extract	Ethanolic extract
1	Carbohydrates	+	+
2	Alkaloids	+	+
3	Phytosterols	-	+
4	Saponins	+	+
5	Fixed oils	+	+
6	Tannins	+	+
7	Flavonoids	+	+

Antibacterial Activity

The Leaf extracts of *Sida acuta* was studied for antibacterial activity employing standard cylinder method. Microbes used were *Bacillus subtilis, Staphylococcus aureus, Staphylococcus werneri, Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Kleibsella pneumonia, Pseudomonas putida.* Both gram-positive and gram-negative bacteria were sensitive to the extract. The antibacterial activity of the leaf extracts of *Sida acuta* was related to their chemical composition. The diameters of the inhibition zones were measured in millimeter [8]. Preliminary Phytochemical analysis detected presence of alkaloids, phytosterols, tannins, flavonoids ,saponins,etc. Several studies indicates the presence of these bioactive compounds in plant materials was related to antibacterial activity. *Chloroform Leaf Extract Of Sida acuta* has shown excellent antibacterial activity against gram negative organism compared to that of gram positive which is evident from the Table 2 . The susceptibility pattern exhibited by the test organism to the seed extracts could be exploited for probable medicinal purposes in chemotherapy among humans with the current spread of antibiotic resistance almost at geometric scale [9] .The zone of inhibition for various organisms were recorded. Activity of chloroform extract of the plant was comparable to that of reference Standard drug Gentamycin(25µg). Chloroform Leaf extracts of S*ida acuta* exhibited good antimicrobial activity

Table 2 : Antibacterial activity of *Sida acuta* leaf extracts

Microorganism	Zone of inhibition(mm)				Gentamycin 25µg/ml
	Chloroform Ext		Ethanolic Ext		
	100mg/ml	200mg/ml	100mg/ml	200mg/ml	
Gram positive					
Staphylococcus aureus	13± 1.02	18 ± 2.36	13± 1.02	18 ± 2.36	20± 1.39
Bacillus subtilis	18 ± 2.09	21 ± 3.96	11 ± 1.09	13 ± 2.96	22 ± 1.52
Staphylococcus werneri	16 ± 3.68	18 ± 2.87	10 ± 3.68	12 ± 2.87	19 ± 1.23
Acinobacterium	11 ± 1.20	20 ± 1.42	12 ± 2.87	14 ± 2.87	16 ± 2.20
Gram negative					
Pseudomonas putida	20 ± 5.20	25 ± 2.87	11 ± 1.20	13 ± 0.87	21 ± 2.31
Pseudomonas aeruginosa	20 ± 4.02	26 ± 2.39	13 ± 1.02	15 ± 1.39	23 ± 3.21
Enterobacter cloacae	18 ± 3.06	24 ± 1.26	11 ± 2.06	14 ± 1.86	14 ± 2.63
Salmonella typhora	18 ± 1.06	20± 2.26	13 ± 1.06	15 ± 0.26	22 ± 5.63
Klebsiella pneumonia	18 ± 2.80	20 ± 1.29	12 ± 2.80	15 ± 1.29	20 ± 1.28
Escherichia coli	16 ± 2.29	19 ± 1.68	11 ± 2.69	15± 1.28	20 ± 1.36

Values are expressed as Mean±SEM, n=3

Anthelmintic Assay*:*

The different concentrations of Leaf extracts of S*ida acuta* were evaluated for Anthelmintic activity using adult Indian earthworm model. The extracts exhibited a dose-dependent inhibition of spontaneous motility (paralysis). It is evident from (Table 3, figure 1) that chloroform leaf extracts of S*ida acuta* demonstrated paralysis as well as death of worms in less time compared to Ethanolic leaf extracts of S*ida acuta*. With higher doses (100 mg/ml) the effects were comparable with the that of piperazine citrate (20mg /ml) . The results indicate that extract possesses vermicidal activity and thus, may be useful as an anthelmintic.

Helminthes are recognized as a major problem to livestock production throughout the tropics. Parasitic helminthes affect human being and animals by causing considerable hardship and stunted growth. Most diseases caused by helminthes are of a chronic and debilitating in nature [10]. Preliminary Phytochemical analysis detected presence of alkaloids, phytosterols, tannins, flavonoids ,saponins,etc and these constituents may be responsible for Anthelmintic activity. The origin of many effective drugs is found in the traditional medicine practices and in view of this several studies have undertaken pertaining to testing of natural compounds for their proclaimed Anthelmintic activity. *Sida*

acuta chloroform leaf extract has shown significant Anthelmintic activity than Ethanolic leaf extract evident from Table 3 .In light of this, the results of the present study suggest that the extract of *Sida acuta* could be used in the control of helminthic infections namely Ascariasis, hookworm infections etc as the worms used in the study are in resemblance with the intestinal parasitic worms.

Fig 1 : Anthelmintic activity of leaf Extracts of *Sida acuta*

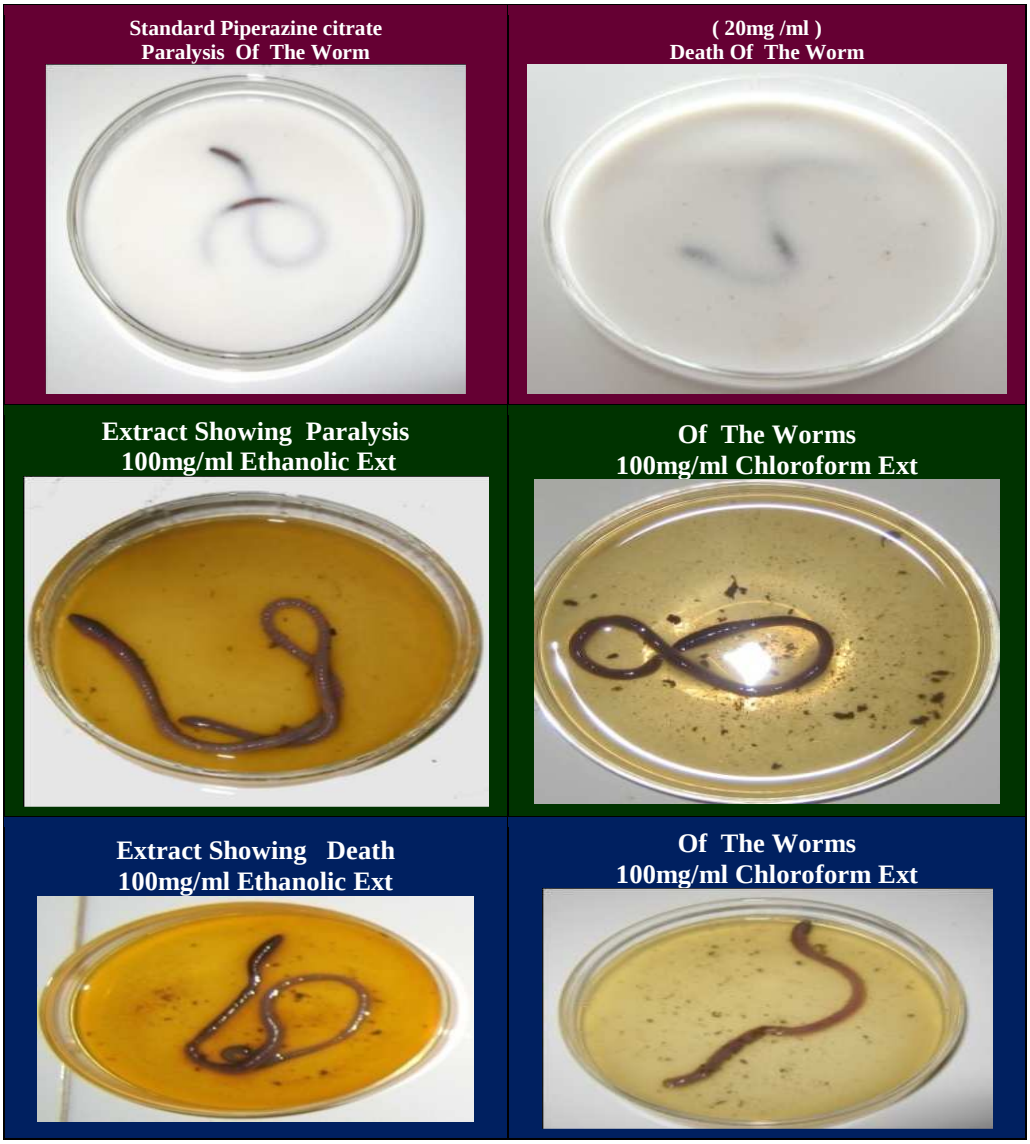

Table 3: Anthelmintic activity of leaf Extracts of *Sida acuta*

Treatment Group	Dose in mg/ ml	Time taken for paralysis(min)	Time taken for death(min)
Ethanolic leaf Extract Of Sida acuta	50 100	21 ± 1.03 15.40 ±2.36	50 ±1.23 30 ±2.05
chloroform leaf Extract Of Sida acuta	50 100	13.50 ± 1.03 6.40 ±2.36	20 ±1.23 9 ±2.05
Standard (Piperazine citrate)	20	30 ±2.09	55 ±1.23
DMSO	-	-	-

All values represent Mean ±SD; n= 6 in each group. ; - no activity

CONCLUSION

It is very necessary to introduce new and biologically safe and active drugs eco-friendly in nature and effective as antibacterial agents and Anthelmintic agents. Usually medicinal plants contain several phytochemical compounds, which are very much necessary to control the growth of the microorganisms, helminthes. Scientists have realized an immense potential in natural products from medicinal plants to serve as alternate source of combating infections in

human beings which may also be of lower cost and lesser toxicity. Further investigations are required in order to isolate more new compounds from the plant extracts of *Sida acuta* and to test their bioactivities with the aim of increasing the drug arsenal currently used in the treatment and prophylaxis of human and animal diseases

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