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Evaluation of *In-vitro* Antibacterial and Anthelmintic Activities of *Sauropus androgynus (Phyllanthaceae)* Plant Extracts

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Research Article

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Abstract

The present study was carried out to evaluate the qualitative analysis of various phytochemicals and *in-vitro* evaluation of antibacterial and anthelmintic activities of Ethanolic leaf extracts of *Sauropus androgynus* (*Phyllanthaceae*). The extracts were subjected to preliminary phytochemical screening for the identification of different phytoconstituents, the leaves indicated the presence of proteins, resins, steroids, tannins, glycosides, reducing sugar, carbohydrates, saponins, sterols, terpenoids, acidic compounds, cardiac glycosides, catechol, phenols, alkaloids, flavanoids. The different concentrations of Ethanolic 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 *Sauropus androgynus* (Indian earthworm). The extracts was found to possess vermifuge and vermicidal activity and the results were compared with standard drug Albendazole.

Keywords: Phytochemical Screening; *Sauropus Androgynus*; Antibacterial Activity; *Sauropus Androgynus*; Anthelmintic Activity; Gentamycin; Albendazole

Introduction

Since the ancient time naturally occurring plants have played an important role in the discovery of new therapeutic agents. Almost all antibiotics are subjected to the problem of bacterial resistance. Therefore, newer herbal antibacterial compounds from plants to overcome the resistance are under investigation [1]. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties [2] *Sauropus androgynus* L. Merr., also known as katuk, star gooseberry, or sweet leaf, is a shrub grown in some tropical regions as a leaf vegetable. It is most popular in South Asia and Southeast Asia. In India it also known as Multivitamin Plant as it contains an excellent source of vitamins A,B, C carotenoid and also it has high nutritive value and contains phytochemicals which can act as antioxidant [3]. The leafy vegetable *Sauropus androgynus* is commonlyused as an

effective medicinal herb in the treatment of diabetics, cancer, inflammation, microbial infection, cholesterol and allergy due to its antioxidant effect [4].

Materials and Methods

Plant Material Collection and Preparation of Extract

The leaves of *Sauropus androgynus* were collected near Bharathinagar, Mandya district, Karnataka, India. The leaves were shade dried, powdered and sieved through 40mesh. The powdered leaves were successively extracted by maceration process using 90% ethanol as solvent. The extracts obtained were evaporated under drying to remove the solvent completely a paste form of drug is obtained these were taken for further studies.

Phytochemical Screening

Phytochemical analysis was performed using standard procedures: The freshly prepared extracts of *Sauropus androgynus* was qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extracts was performed using standard procedures [5,6].

Test for Carbohydrates by Molisch's Test

To the test solution add few drops of alcoholic a-naphthol, then add few drops of concentrated sulphuric acid through sides of test tube, purple to violet colour rings at the junction of two layers

Test for Reducing Sugar by Fehling's Test: About 0.5g each portion was dissolved in distilled water and filtered. The filtrate was heated with 5mlof equal volumes of Fehling's solution A and B. formation of a red precipitate of cuprous oxide was an indication of the presence of reducing sugars.

Test for Protein by Xanthoprotein Test: To the 5ml of test solution, add 1ml of concentrated nitric acid and boil, yellow precipitate is formed. After cooling it, add 40% Sodium hydroxide solution orange color is formed.

Test for Terpenoids by Salkowski Test: Treat the extract with few drops of concentrated sulphuric acid, red color at lower layer indicates presence of steroids and formation of yellow colored lower layer indicates presence of triterpenoids.

Test for Alkaloids by Mayer's Reagent: Alkaloids give cream color precipitate with Mayer's reagent (Potassium mercuric iodide solution)

Test for Glycosides by Borntrager's Test: Boil the test material with 1ml sulphuric acid in a test tube for five minutes. Filter while hot. Cool the filtrate and shake with equal volume of dichlomethane or chloroform. Separate the lower layer of dichlomethane or chloroform and shake it with half of its volume of dilute ammonia. A rose pink to red color is produced in the ammonical layer.

Test for Cardiac Glycosides by Keller-Killian Test: Extract the drug with chloroform and evaporate it to dryness. Add 0.4ml of glacial acetic acid containing trace amount of ferric chloride transfer to a small test tube, add carefully 0.5ml of concentrated sulphuric acid by the side of the test tube. Acetic acid layer shows blue color.

Antibacterial Activity

Test organism used

The various organisms like *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis, Escherichia coli* are procured from Department of Microbiology Bharathi College of pharmacy Bharathinagara, Mandya district, Karnataka, India

Antibacterial Assay

Leaf extracts of *Sauropus androgynus* was evaluated for antibacterial activity against several gram positive and gram negative organisms. The antibacterial activity of Leaf extracts of *Sauropus androgynus* was performed using Agar cup-plate method 20ml of sterile nutrient agar medium was poured into sterile Petri-dishes and allowed to solidify [7]. The Petri dishes were incubated at 37^0C for 24 hours to check for sterility. The medium was mixed with the organisms by pour plate method using sterile agar broth (4ml) contained 1ml culture. Bores were made on the medium using sterile borer. Leaf extracts of *Sauropus androgynus* was dissolved in water to obtained different concentration (100, 200 mg/ml) and sterilized by filtration through a whattman filter paper no. 1, and 0.5 ml of the different concentration of extraction were added to the respective bores. 0.5 ml of Gentamycin at a concentration of (40 µg/ml) was taken as standard reference. All the plates were kept in a incubator at 37^0C 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. Earth worms have been used widely for the evaluation of Anthelmintic compounds. All the earth worms were of approximately equal size (6cm). They were collected from local moist place of Bharathi College of pharmacy situated in Bharathinagara, Mandya district.

Anthelmintic Assay

Anthelmintic activity was evaluated by exposing the adult *Pheritima posthuma* to different concentration Leaf extracts of *Sauropus androgynus*. The anthelmintic activity was performed according to the method of Ghosh et al. [8] with slight modifications. The ethanolic extracts of *Sauropus androgynus* were dissolved in minimum amount of water and then volume was adjusted. 10 ml of formulation containing two different concentrations of each of the extract of extracts were prepared and 6 worms (same type) were placed in the petri dishes. 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 sometime that worms neither moved when shaken vigorously nor when dipped in hot water (50°C) followed by fading away of their body colours. Albendazole (20mg/ml) was used as reference standard.

Results

Preliminary Phytochemical Screening of *Sauropus androgynus* leaves indicated the presence of proteins, resins, steroids, tannins, glycosides, reducing sugar, carbohydrates, saponins, sterols, terpenoids, acidic compounds, cardiac glycosides, catechol, phenols, alkaloids, flavonoids. The results of the phytochemical screening are given in the following (Table 1).

Sl.no	Name of the test	Phytochemical analysis of *Sauropus androgynus*
1	Test for carbohydrates Molisch test	+
2	Test for reducing sugar Fehling's test	+
3	Test for Proteins Xanthoprotein test	+
4	Test for alkaloids Mayer's test	+
5	Test for Cardiac glycosides Keller killian's test	+
6	Test for glycosides Borntrager's test	+
7	Test for terpenoids Salkowski test	+

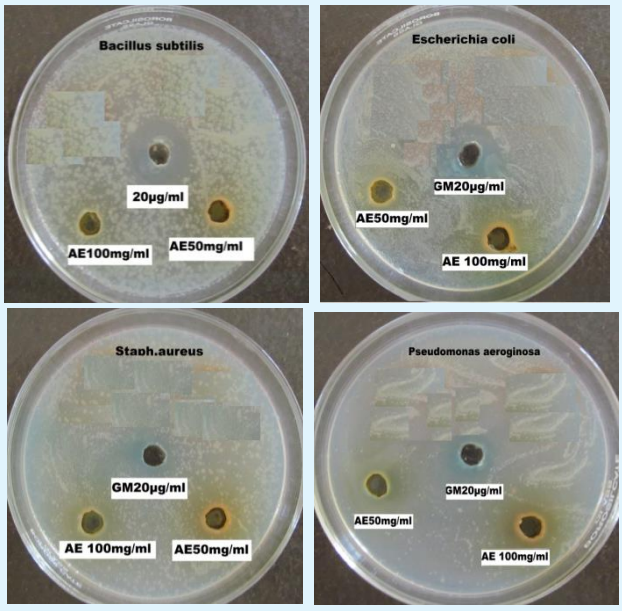
Table 1: Phytochemical constituents analysis of *Sauropus androgynus.*
+ = Prescence, - = Absence

Anti-Bacterial Activity

The leaf extracts of *Sauropus androgynus* was studied for antibacterial activity employing standard cylinder method. Microbes used were *Bacillus subtilis, Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*. Both gram-positive and gram-negative bacteria were sensitive to the extract (Table 2, Figure 1). The antibacterial activity of the leaf extracts of *Sauropus androgynus* was related to their chemical composition. The diameters of the inhibition zones were measured in millimeter [9].

S. No.		Zone of inhibition (in mm)			
		Ethanolic extract			
		50mg/ml	100mg/ml		
1.	*Bacillus subtilis ATCC11774*	8±0.2887**	10±0.2887**	15±0.5774**	6±0.2887
2.	*Escherichia coli ATCC10536*	12±0.2887**	15±0.5774**	10±0.2887**	6±0.2887
3.	*Staphylococcus aureus ATCCBAA1026*	10±0.2887**	16±0.7077**	18±0.7077**	6±0.2887
4.	*Pseudomonas aeruginosa ATCC10662*	9±0.2887**	10±0.2887**	12±0.2887**	6±0.2887

Table 2: Antibacterial activity of *Sauropus androgynus* leaf extract ** P< 0.01 as compared with control according to one-way ANOVA.



1. Bacillus subtilis 2. Escherichia coli 3. Staphylococcus aureus 4. Pseudomonas aeruginosa

Figure 1: Antibacterial activity of *Sauropus androgynus* leaf extract.

Anthelmintic Activity

The different concentrations of Leaf extracts of *Sauropus androgynus* 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 2) that ethanolic leaf extracts of *Sauropus androgynus* demonstrated paralysis as well as death of worms in less time compared to standard Albendazole (20mg/ml). The results indicate that extract possesses vermicidal activity and thus, may be useful as a anthelmintic.

Treatment group	Dose in mg/ml	Time taken for paralysis(min)	Time taken for death(min)
	100mg/ml	8± 1.03	15± 2.03
	200mg/ml	6± 1.23	10± 2.09
	10mg/ml	8± 1.03	16± 1.64
	20mg/ml	7± 1.23	13± 2.09
Control	-	-	-

Table 3: Anthelmintic activity of *Sauropus androgynus* leaf extract.
All values represent Mean ± SD; n= 6 in each group. ; - no activity

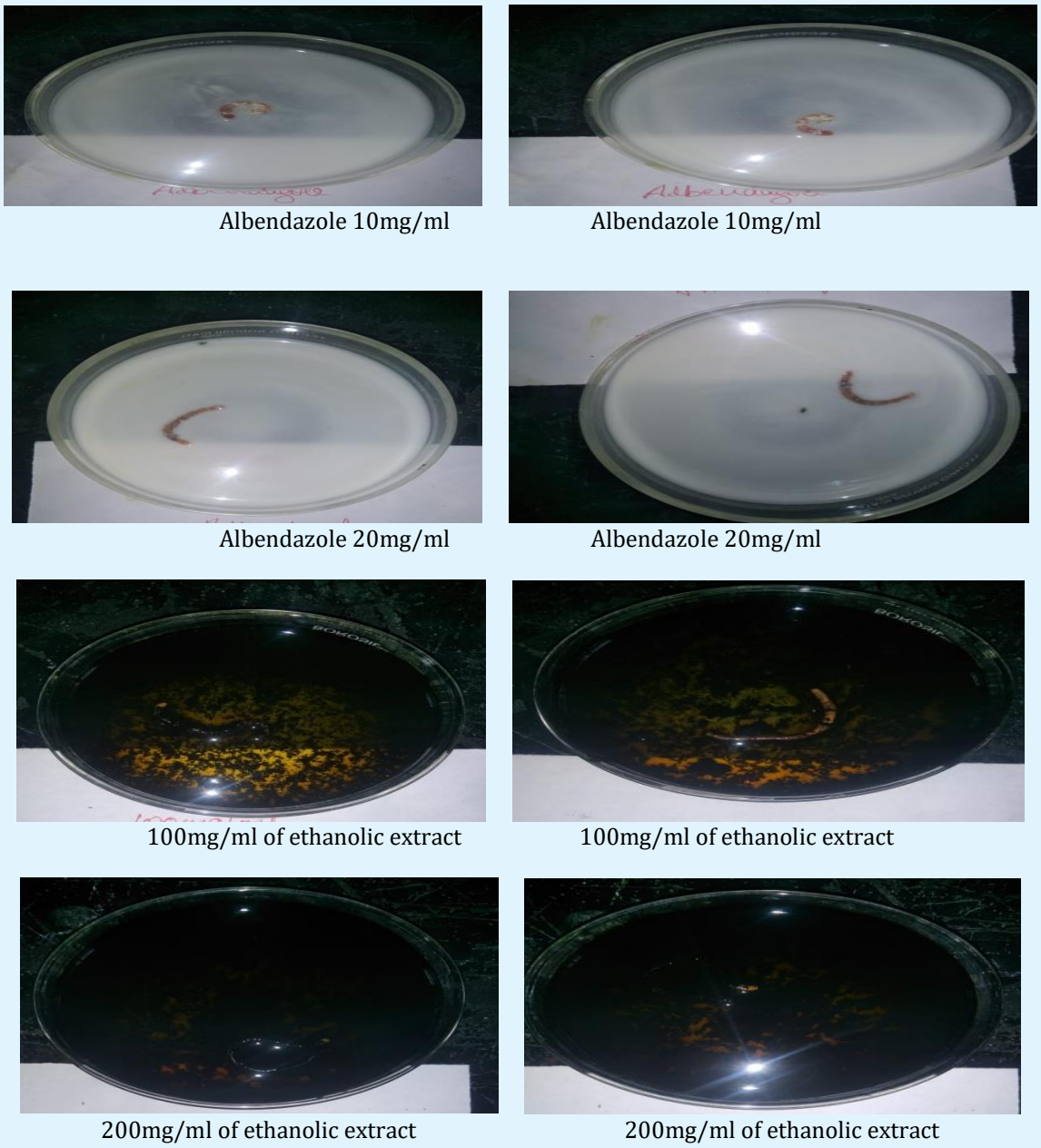


Figure 2: Anthelmintic activity of *Sauropus androgynus* leaf extract.

Discussion

Preliminary Phytochemical analysis detected presence of alkaloids, tannins, flavanoids, saponins, etc. several studies indicate the presence of these bioactive compounds in plant materials was related to antibacterial activity. Ethanolic leaf extract of *Sauropus androgynus* 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 ethanolic extract of the plant was comparable to that of reference standard drug Gentamycin (40µg). Ethanolic extracts of *Sauropus androgynus* exhibited good antimicrobial activity. Antimicrobial property of saponin is due to its ability to cause leakage of proteins and certain enzymes from the cell [10].

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 [11]. 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. 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. *Sauropus androgynus* Ethanolic 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 *Sauropus androgynus* 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.

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 medicnal plants to serve as alternate source of combating infections in human beings which may also be of lower cost and lesser toxicity.

Conclusion

The results of antibacterial and anthelmintic activities showed that the *Sauropus androgynus* leaf has significant antibacterial and anthelmintic activities. Further research work has to be carried out to isolate bioactive molecules responsible for their activity and to investigate and screen the compounds to evaluate other biological activities.

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