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Phytochemical and antimicrobial investigations on various parts of *Sida acuta* Burm. f.

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ABSTRACT

Sida acuta Burm. f. belongs to the mallow family, Malvaceae. The study investigated the quantitative phytochemical contents and *in vitro* antimicrobial activities of *S. acuta* at different concentrations using standard techniques. Antimicrobial activity was evaluated by disc diffusion method and Minimum Inhibitory Concentrations for the absolute concentrations were determined by Agar well diffusion method. Test of significance was measured using Duncan's Multiple Range Test. There were presence of alkaloid, flavonoid, saponin, tannin, cardiac glycosides, terpenoid, anthraquinone and steroid in the leaf, stem and root of the plant at varying levels. Alkaloid at 2.31±0.03mg/100g was the highest and it was detected in the leaf. All the plant parts exhibited inhibitory activities against all the test organisms but were dose-dependent. At 500 mg/ml, the leaf extract gave highest inhibition of *Pseudomonas aeruginosa*, *Micrococcus varians*, *Candida albicans* while the root extract gave highest inhibition of *Escherichia coli*, *Salmonella typhi* and *Aspergillus flavus*. Findings of this study, therefore, showed that all parts of *S. acuta*, particularly the leaf and the root, possessed antimicrobial properties which can be pharmaceutically harnessed.

Keywords: Alkaloid, antibacteria, antifungi, *Candida albicans*, *Escherichia coli*, *Aspergillus flavus*.

INTRODUCTION

Sida acuta Burm. f. belongs to the genus *Sida*, of the mallow family, Malvaceae. It is commonly known as stubborn weed. It is an erect, branched, small perennial shrub with a woody tap root, and hairy branches up to 1 m high [1]. It is predominant in roadsides, waste areas, grazing land, disturbed land and abandoned farmlands.

Traditional medicine has started gaining credence over the last decade. Different parts as well as whole plants are used in folk medicine for treatment of different afflictions. A wide range of medicinal uses of *S. acuta* have been extensively documented. The use of *S. acuta* in treatment of asthma, renal inflammation, colds, fever, headache, ulcer and worm infections in regions around Central America has been reported [2]. The leaves are used for their diuretic, demulcent, anthelmintic and wound healing properties [3]. Moreover, it is used as a medicine in treatment of liver disorders, urinary disease, nervous disorder, blood disorder, biliary disease [4]. In Nigeria, the leaves of *S. acuta* are chewed for treating gonorrhea [5].

Any substance that kills or inhibits the growth of microorganisms with negligible side effects on the host is considered as an antimicrobial [8]. In addition, it could be natural or man-made. The most common fatal bacterial diseases are respiratory infections [7]. Fungal infection normally happens when the immune system is weak or the microbes are too much for it to handle. Earlier work in antimicrobial actions of this plant were based on aerial parts, mainly leaf [8-10], hence, there is need to investigate the underground parts. The objectives of this study therefore, were to determine the quantitative phytochemical compositions of various parts of *S. acuta* as well as its antimicrobial properties.

MATERIALS AND METHODS

Collection of Sample

Matured *S. acuta* was collected from an abandoned farm land in Nibo, Anambra State in the month of May. The plant specimen was identified by Dr. C.A. Ezeabara, of the Department of Botany, Nnamdi Azikiwe University, Awka, Anambra State, where the voucher specimen was deposited.

Preparation of Samples for Analysis

The fresh plant parts were washed with clean water and oven dried at a temperature 65^{o}C for 12 hours. The leaves, stems and roots of *S. acuta* were later cut into bits with knife and then oven-dried at a

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temperature of 70°C for 12 hours to remove all moisture. The samples were grounded in a mortar with a pestle, and then in a blender (Omega, USA) into powdered form.

Extraction of Plant Materials

Methanol Extraction

The ethanol extract of the plant was prepared by soaking the powdered sample of the leaf, stem and root in 100ml of ethanol. The whole set up was left for 72 hours at room temperature and thereafter filtered using Whatman filter paper. The extract was then concentrated using rotary evaporator and allowed the solvent to evaporate. The concentrated extract was stored in an air tight container in a refrigerator at 20°C until it is required for analysis.

Quantitative phytochemical analysis

Alkaloid and flavonoid determinations in the test samples were determined by the gravimetric and acid hydrolysis gravimetric method of Harborne [11] respectively. The method used was described by AOAC [12] was used to determine saponin content. Tannin determination was done with Folin-Denis spectrophotometric method of Pearson [13]. Determination of anthraquinone was done with the method described by Ezeabara and Okonkwo [14]. Cardiac glycoside was determined by alkaline picarate colorimeter method as outlined by Trease and Evans [15].

Microbial Analysis

The pure cultures of the microorganisms were obtained from Department of Microbiology, IITA Ibadan, Oyo State. The bacteria isolates include gram positive and the gram negative bacteria which include *Escherichia coli, Salmonella typhi, Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa* and *Micrococcus varians.* The fungi were *Aspergillus niger, Candida albicans, Fusarium solani* and *Rhizopus sp.*

Preparation of Media

The various media used were prepared according to manufacturers' instructions as indicated on the product label. The quantities required were measured using a weighing balance (in grams) into a conical flask and dissolved in the appropriate volume of water using a measuring cylinder. The media were properly mixed and sterilized by autoclaving at 121° C for 15 minutes at 760mmHg.

Isolation of the Test Organisms

Using a wire loop, a colony of the test organisms were collected from pure cultures at soil Microbiology Unit, IITA Ibadan, Oyo State, Nigeria.

Antimicrobial Test Procedures

Preparation of Stock Solution

Stock solutions of the methanolic extracts were prepared by weighing 2.0g of each methanolic extracts using electronic weighing machine in the department. This was then dissolved completely in 2.0ml of Dimethyl sulfoxide (DMSO) in sterile test tubes giving a stock solution concentration of 1000mg/1ml (that is, 1000mg/ml or 1g/ml) per extract. The stock solution was then labeled approximately and stored in room temperature till required for use.

Determination of Inhibitory Activity

Inhibitory activity of methanol extract of leaf, stem and root of *S. acuta* antibiotics (positive control) and Dimethyl sulfoxide (negative control) were determined using disc diffusion method. 5mm discs were impregnated with concentration of 1000mg/ml of the methanolic extract, 100mg/ml of antibiotic (Streptomycin) and 1000mg/ml of DMSO.

After the disc had taken up the methanolic extractions, and the controls, the disks were removed, dried and placed on the media which the test microorganisms were freshly inoculated, then incubated at 37°C within 24 hours for bacteria and 25°C within 72 hours for fungi. Antimicrobial activity was determined after 24 hours (for bacteria) of incubation and 72 hours (for fungi) of incubation by measuring the zone of inhibition around each paper disc in millimeters (mm) [16].

Determination of Minimum Inhibitory Concentration

Minimum inhibitory concentrations (MIC) for the absolute (stock) concentrations were determined by agar well diffusion method; 5-sterile plates were prepared, and nutrient broth poured into each of plates, and then allowed drying. With some Standardized inoculums (106cfu/ml), a loop full of the different test organisms were inoculated and streaked onto each of the 5-plates when dried. Then, 10-holes were dug using an agar borer for the varying concentrations of methanolic extracts of root, leaf and stem. A marker was used to rule each plate to separate 5-holes each for the methanolic extracts.

Varying concentrations of the different extracts were done by serial dilution method using DMSO as diluent. The absolute/stock concentrations of extracts used were 1000mg/ml which is 100% respectively. Five test tubes per extract were prepared on test tube rack. The dilutions were for the following ranges: 50% (500mg/ml), 25% (250mg/ml), 12.5% (125mg/ml) and 6.25% (62.5mg/ml) in each of the test tubes. DMSO served as the negative control. A measured 50ul volume of each dilution was added aseptically into the holes seeded with the test organisms in the nutrient agar plate using a syringe which measures in ul and allowed in the incubator for 35°C in 24 hours. The lowest concentration of methanolic extracts showing a clear zone of inhibition was considered as the MIC [17].

Determination of Minimum Bactericidal/Fungicidal Concentration

The plates with the Minimum Inhibitory Concentrations (MIC) were further incubated for another 24 hours at 35°C to test which organism would grow on the zones of inhibition. Those plates after 24 hours' organisms were completely killed and clear zones remained were referred to as bactericidal for bacteria and fungicidal for fungi [18].

Statistical analysis

Analysis of Variance (ANOVA) using SPSS version 21 was employed in analyzing the data collected from the study. Test of significance was measured using Duncan's multiple range test (DMRT).

RESULTS

The result of the quantitative phytochemical compositions of methanol extracts of the leaf, stem and root of *S. acuta* was shown in Table 1. The table revealed that the leaf extract had the highest compositions of alkaloids, flavonoids and steroids being 0.55±0.02, 2.31±0.03 and 1.85±0.04 mg/100g respectively. Highest content of saponins at 0.81±0.05 mg/100g was detected in the stem extract, while highest values of tannins, anthraquinone and cardiac glycosides being 1.67±0.03, 1.91±0.08 and 1.48±0.05 mg/100g were found in the root extract respectively.

The leaf had the highest inhibition against *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* being 7.11±0.04, 6.89±0.02 and 7.63±0.04mm respectively at 62.5 mg/ml. At 125 mg/ml, the leaf showed the highest inhibition against *S. aureus*, *Salmonella typhi* and *E.*

coli being 8.19±0.05, 7.93±0.05 and 8.67±0.03mm respectively. The leaf also exhibited the highest level of inhibition against *S. aureus*, *S. typhi* and *P. aeruginosa* being 9.40±0.21, 10.77±0.14 and 9.68±0.41mm respectively, at 250 mg/ml. At 500mg/ml, the leaf had highest inhibition against *P. aeruginosa* and *M. varians* being 13.34±1.82 and 13.06±1.42mm respectively. The stem had highest inhibition against *S. aureus* and *Bacillus cereus* at 12.61±1.86 and 12.61±0.47mm respectively. The root restrained the growth of *Salmonella typhi* and *E. coli* being 13.96±0.78 and 13.77±1.15mm respectively. In comparison among the control and the three plant extracts, the control gave the highest inhibition of all the pathogens. There was a significant difference in the inhibitory activities of the leaf, stem and root extracts of *S. acuta* against all the bacterial pathogens assayed ($p<0.05$) (Table 2).

For fungal pathogens, the leaf extract gave the highest inhibition of *Aspergillus flavus* and *Candida albicans* being 8.15±0.05 and 8.23±0.02mm respectively; the stem extract gave the highest inhibition of *Rhizopus sp.* at 7.44±0.03mm while the root extract gave highest inhibition of *Fusarium solani* being 6.58±0.04mm at 62.5 mg/ml concentration. At 125 mg/ml concentration, the leaf extract gave the highest inhibition of *A. flavus* and *C. albicans* being 10.24±0.05 and 10.30±0.08mm respectively, while the stem extract gave the highest inhibition of *F. solani* and *Rhizopus sp.* at 8.67±0.021 and 9.55±0.07mm respectively. At 250 mg/ml concentration, the leaf extract gave highest inhibition of *A. flavus* being 12.95±0.92 mm and *C. albicans* being 13.18±0.22 mm, while the stem extract gave highest inhibition of *F. solani* and *Rhizopus sp.* being 12.16±0.33 and 10.56±1.01mm respectively. At 500 mg/ml concentration, the leaf extract gave highest inhibition of *C. albicans* being 14.46±0.49 mm, while the root extract gave highest inhibition of *A. flavus*, *F. solani* mm and *Rhizopus sp.* at 14.82±0.22, 13.49±0.29 and 12.87±0.57mm respectively. In comparison between the control and plant extracts, the control gave highest inhibition of all the pathogens. There was a significant difference in the inhibitory activities of the leaf, stem and root extracts of *S. acuta* against all the fungi pathogens assayed ($p<0.05$) (Table 3).

DISCUSSION

Sida acuta parts are loaded with bioactive compounds that have powerful health benefits. Alkaloids occurred highest in the leaf which is thought to be the major active component, with others being detected in the lesser extent. The therapeutic values of plants depend on their chemical components which produce specific pharmacological activities on the human and animal body. Hence, leaf of this plant could be regarded as rich source of alkaloid.

There were considerable antibacterial and antifungal activities against all the selected bacteria and fungi in all the concentrations with maximum activity at 500 mg/ml. This indicated that plant extract hinder the growth of microorganisms at a higher level. The root extract gave the highest inhibition against *E. coli* and *Salmonella typhi* while the leaf caused the greatest growth retardation of *P. aeruginosa and M. varians*. This might be due to high values of cardiac glucosides, anthraquinone and tannin in the root. It has been reported that tannin exhibit antimicrobial activity through a variety of mechanisms [19]. These implied that the methanol root extracts of *S. acuta* could be applied for formulation of novel antibacterial drugs targeted against *E. coli* and *Salmonella typhi* while the leaf extract could be used for manufacture of drugs against *P. aeruginosa and M. varians*. These properties could be attributable to the highest level of alkaloids present in the leaf. It was reported that alkaloids extracted from *Sanguisorba officianalis* had antimicrobial properties against *P. aeruginosa* and *E. coli* [20].

For fungal pathogens, the highest zone of inhibition was observed in *A. flavus* and *C. albicans* at concentrations of 62.5, 125, and 250 mg/ml; which was exhibited by the leaf extracts. At 500 mg/ml, the leaf extract also gave the highest inhibition of *C. albicans* being 14.46±0.50 mm, while the root extract gave highest inhibition of *A. flavus* being 14.82±0.22 mm. This finding disagrees with the report of Oboh *et al*. [21], who stated that ethanol extracts of *S. acuta* aerial parts had no inhibitory activity against *E. coli, P. aeruginosa* and *C. albicans*. These showed that *A. flavus* and *C. albicans* are susceptible to methanol extract of *S. acuta*. This might be due to high values of cardiac glucosides, anthraquinone and tannin in the root or either of the phytochemical. This property is probably the reason behind the oral use of a decoction of the dried entire *S. acuta* plant for venereal diseases in Nicaragua [22]. This demonstrated that it can be used for synthesis of antifungal drugs for treatment of diseases caused by these organisms.

In conclusion, considering the level of alkaloid in the leaf of this plant, it could be used as an analgesic. Moreover, the phytochemicals present in leaf, stem and root of *S. acuta* exhibited antibacterial and antifungal properties presenting it as a potent plant in treatment of bacterial and fungal infectious diseases. Hence, the bioactive agents could be isolated and incorporated in synthesis of new drugs.

Table 1: Mean quantitative phytochemical compositions of the methanol extracts of *Sida acuta* leaf, stem and root

Compositions (mg/100g)	Plant Parts		
	Leaf	Stem	Root
Alkaloid	2.31±0.03^{c}	0.96±0.04^{a}	0.34±0.00^{b}
Cardiac glucosides	1.20±0.01^{a}	1.36±0.02^{b}	1.48±0.05^{c}
Steroid Anthraquinone	1.85±0.04^{c}	1.66±0.03^{b}	0.70±0.05^{a}
	1.62±0.02^{b}	1.50±0.04^{a}	1.48±0.05^{c}
Saponin	0.28±0.05^{a}	0.81±0.05^{b}	0.26±0.01^{a}
Tannin	1.51±0.02^{b}	1.34±0.01^{a}	1.67±0.03^{c}
Flavonoid	0.55±0.02^{b}	0.23±0.01^{a}	0.25±0.07^{a}

Results are in Mean± Std of triplicate determinations. Means with the same letter in a column is not significantly different ($p>0.05$)

Table 2: Effects of methanol extracts of leaf, stem and root of Sida acuta on bacterial pathogens

Concentrations (Mg/ml)	Bacterial Strains	Mean Zone of Inhibition (mm) ± SD			
		Control	Leaf	Stem	Root
62.5	*Staphylococcus aureus*	15.79±0.09^{d}	7.11±0.04^{c}	6.34±0.05^{a}	6.48±0.03^{b}
	Salmonella typhi	15.23±0.02^{d}	6.89±0.02^{c}	6.36±0.50^{b}	6.08±0.02^{a}
	Escherichia coli	15.52±0.010^{d}	7.63±0.04^{c}	7.21±0.06^{b}	6.87±0.05^{a}
	Pseudomonas aeruginosa	17.23±0.00^{d}	6.69±0.01^{b}	4.98±0.00^{a}	6.73±0.09^{c}
	Bacillus cereus	16.12±0.06^{d}	5.43±0.09^{c}	4.66±0.54^{a}	5.25±0.07^{b}
	Micrococcus varians	18.27±0.06^{d}	7.32±0.01^{b}	5.64±0.02^{a}	7.33±0.04^{c}

125	*Staphylococcus aureus*	15.79±0.09^{d}	8.19±0.05^{c}	6.40±0.06^{a}	7.52±0.03^{b}
	Salmonella typhi	15.23±0.02^{d}	7.93±0.05^{c}	6.08±0.11^{a}	7.12±0.05^{b}
	Escherichia coli	15.52±0.01^{d}	8.67±0.03^{c}	6.27±0.06^{a}	7.89±0.05^{b}
	Pseudomonas aeruginosa	17.23±0.01^{d}	7.72±0.02^{b}	6.01±0.01^{a}	7.79±0.06^{c}
	Bacillus cereus	16.12±0.06^{d}	6.49±0.06^{c}	5.06±0.04^{a}	6.29±0.09^{b}
	Micrococcus varians	18.27±0.06^{c}	8.39±0.04^{b}	6.68±0.05^{a}	8.35±0.03^{b}
250	*Staphylococcus aureus*	15.79±0.09^{c}	9.40±0.21^{b}	8.57±0.17^{a}	9.19±0.60^{b}
	Salmonella typhi	15.23±0.02^{d}	10.77±0.14^{c}	8.01±0.45^{a}	10.00±0.22^{b}
	Escherichia coli	15.52±0.01^{c}	11.12±1.04^{b}	9.44±0.34^{a}	11.49±0.00^{b}
	Pseudomonas aeruginosa	17.23±0.00^{c}	9.68±0.41^{b}	8.85±0.59^{a}	8.44±0.30^{a}
	Bacillus cereus	16.12±0.06^{d}	7.47±0.36^{b}	6.42±0.26^{a}	8.04±0.30^{c}
	Micrococcus varians	18.27±0.06^{c}	10.28±0.61^{b}	9.38±0.28^{a}	10.35±0.39^{b}
500	*Staphylococcus aureus*	15.79±0.09^{b}	11.70±1.38^{a}	12.61±1.86^{a}	11.16±1.31^{a}
	Salmonella typhi	15.23±0.02^{d}	12.91±0.77^{b}	10.06±0.15^{a}	13.96±0.78^{c}
	Escherichia coli	15.52±0.01^{c}	11.66±0.70^{a}	10.72±0.59^{a}	13.77±1.15^{b}
	Pseudomonas aeruginosa	17.23±0.00^{c}	13.34±1.82^{b}	11.07±1.25^{a}	11.59±0.29^{a}
	Bacillus cereus	16.12±0.05^{c}	11.59±0.40^{a}	12.61±0.47^{b}	10.61±1.14^{a}
	Micrococcus varians	18.27±0.06^{b}	13.06±1.42^{a}	11.67±1.50^{a}	12.16±0.08^{a}

Results are in Mean± Std of triplicate determinations. Means with the same letter in a column is not significantly different (p>0.05)

Table 3: Effects of methanol extracts of leaf, stem and root of *Sida acuta* on fungal pathogens

Concentrations(Mg/ml)	**Fungal Strains**	**Mean Zone of Inhibition (mm) ± SD**			
		Control	**Leaf**	**Stem**	**Root**
62.5	*Aspergillus flavus*	16.83±0.06^{d}	8.15±0.05^{c}	7.34±0.08b	7.21±0.00^{a}
	Candida albicans	17.32±0.09^{d}	8.23±0.02^{c}	6.98±0.08^{b}	6.69±0.01a
	Rhizopus sp	16.76±0.05^{c}	6.47±0.00^{a}	7.44±0.03^{b}	6.47±0.03^{a}
	Fusarium solani	17.02±0.05^{b}	6.56±0.06^{a}	6.55±0.00^{a}	6.58±0.04^{a}
125	*Aspergillus flavus*	16.83±0.06^{d}	10.24±0.05^{c}	9.46±0.09^{b}	9.26±0.03^{a}
	Candida albicans	17.32±0.09^{d}	10.30±0.08^{c}	9.09±0.07^{b}	8.75±0.06^{a}
	Rhizopus sp	16.76±0.05^{c}	8.58±0.02^{a}	9.55±0.07^{b}	8.55±0.05^{a}
	Fusarium solani	17.02±0.05^{b}	8.66±0.04^{a}	8.67±0.02^{a}	8.64±0.08^{a}
250	*Aspergillus flavus*	16.83±0.06^{b}	12.95±0.92^{a}	11.71±0.74^{a}	11.30±1.23^{a}
	Candida albicans	17.32±0.09^{b}	13.18±0.22^{a}	12.92±0.09^{a}	13.07±0.32^{a}
	Rhizopus sp	16.76±0.05^{b}	10.06±0.07^{a}	10.56±1.01^{a}	10.54±0.67^{a}
	Fusarium solani	17.02±0.05^{c}	11.07±0.91^{a}	12.16±0.33^{b}	10.98±0.25$_{a}$
500	*Aspergillus flavus*	16.83±0.06^{d}	13.29±0.26^{b}	12.70±0.13^{a}	14.82±0.22^{c}
	Candida albicans	17.32±0.09^{d}	14.46±0.49^{c}	12.23±0.30^{b}	11.38±0.10^{a}
	Rhizopus sp	16.76±0.05^{c}	12.75±0.73^{b}	11.76±0.46^{a}	12.87±0.57^{b}
	Fusarium solani	17.02±0.05^{c}	13.18±0.24^{b}	12.75±0.34^{a}	13.49±0.29^{b}

Results are in Mean± Std of three different determinations. The same letter in a column is not significantly different (p>0.05).

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