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Cytotoxic Activities and Antibacterial Screening of Extract from the Leaves of *Sarcocephalus latifolus* (J.E Smith) E.A Bruce (Rubiaceae)

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ABSTRACT

The powdered leaves of *Sarcocephalus latifolius* was extracted using 95% ethanol and partitioned into five soluble solvent fractions of varying polarity. The crude ethanol extract and solvent fractions were screened for cytotoxicity against the larvae of *Artemia saline* using Brine Shrimp Test (BST) method. The crude extract and five solvent fractions were further tested for antimicrobial activity using agar diffusion method. The ethanol and ethyl acetate extracts were found to be moderately active on BST at LC_{50} value of 705.48 and 914.99 µg/ml respectively. The results for antimicrobial activity tests of crude extract and five solvent fractions revealed the highest zone of inhibition 18.0 mm at 10,000 µg/ml was exhibited by the chloroform soluble fraction of the leaves of *Sarcocephalus latifolius* on *Salmonella typhi*. The ethanol, chloroform and methanol fractions of *S. latifolius* exhibited zone of growth inhibition 15 mm, 16 mm, and 15 mm against *S. aureus* at 10,000 µg/ml. The potency was comparable to the standard antibiotics (i.e Ciprofloxin) used.

Key words: Antibacterial activity, Cytotoxicity and *Sarcocephalus latifolius* (J.E Smith).

INTRODUCTION

Medicinal plants are used locally in the treatment of infectious diseases caused by bacteria, fungi, viruses and parasites. Fossil records reveal that the use of plants in medicine dated back to middle Paleolithic age, approximately 60, 000 years ago [1]. Over 60% of people in Nigerian rural areas depend on the traditional medicine for the treatment of their ailments [2]. Plant materials have been used as essential part of human society since the civilization started. Medicinal plants are boon of nature to cure a number of ailments of human beings [3]. The plants were reported to be used as foods, flavours, insect deterrents, ornamentals, fumigants, spices, and cosmetics [4].

Natural products were reported to represent over 50% of all drugs in clinical use, while the products derived from higher plants represent approximately 25% of the total [5]. Plant derived medicines are widely used because they are relatively safer than the synthetic alternatives, easily available and affordable. Many plants species have been evaluated for their anti-microbial activity in the past 20 years and since then efficacy of many diseases have been put to test in many laboratories [6].

Typhoid fever has been associated with man since close to the first appearance of mankind and it may have first infected human ancestors anywhere from 200,000 to 2 million years, ago [7]. The bacterium can survive in contaminated water, but does not have any host other than man. There are over 1,000 different strains of the bacterium of which only a few cause typhoid. A closely related bacterium *Salmonella typhimurium* causes a similar disease in mouse, but does not affect man and the infestation of the pathogen in the mouse is used as a model to study the disease [7]. *S. typhi* attacks the tissues on the inner surface of the intestine which constitute the first line of defense against food and water-borne infections. It does not have the cell surface features that normally trigger a defensive response but it can fool the cells of the patches to take it in without attacking it. The infected tissue becomes inflamed resulting in diarrhea or constipation. There may be bleeding of the intestine leading to bloody stools. In severe cases, the disease may punch holes in the intestine leading to peritonitis infection of the abdominal cavity and death. Even if the patient recovers from the infection, it may leave residual damage, such as the formation of attachments of the damaged areas of the intestine to the abdominal wall, or the development of a chronic infection that leads to the patient becoming a carrier [7].

Sarcocephalus latifolius (J.E Smith) E.A Bruce (Rubiaceae), known as ‘‘Tafashiya’’ in Hausa, is a shrub of about 4 m high. The leaves are broadly elliptic to oval-rounded, roots are yellowish in colour and shortly acuminated, cuneate to round at base. The leaves are 10-25 cm long and 7-15 cm broad. Inflorescence are in capitate, globose, and densely flowered. The fruits are globase, alveolate, fawn-yellow or reddish at maturity, the seeds are small, brownish and numerous. *S. latifolus* is a Sudano-gunean species that is widely spread in all-intertropical Africa [8]. The plant was reported to be used in the treatment of malaria, hypertension [9], and as a chewing stick [10]. The root of *S. latifolius* has various medicinal purposes ranging from, tooth decay, jaundice, constipation, indigestion, ascites, hernia and leukaemia. The leaves and the roots are used to treat heamostatic, male sterility, wounds, swellings, leprosy, syphilis sores. The leaves of the plant are used for the treatment of cancer [11], gastrointenstinal tract disorders, prolonged menstrual flow [12].

Biological activity on leaf extracts of *S. latifolius* showed antibacterial activity against *Enterobacter sp*., *Escherichia coli* and *S. aureus* [8]. Ethanol extracts of *S. latifolious* decreased the level of parasitaemia in a dose-dependent manner in mice experimentally infected with *Trypanosoma brucie-brucei* [13]. Aqueous ethanol extracts of the plant exhibited activity against *S. typhi*, *E. coli*, *Shigella flexneri*, and *staphylococcus aureus.* Phytochemical analysis of the root extract of *S. latifolius* led to the isolation of new indole alkaloids, 21-O-methylstrictosamide aglycone and 21-O-ethylstrictosamide agycone together with angustoline. Strictosamine was also reported to be isolated from the roots, leaves and stem bark and reported to displayed moderate antiplasmodial activity against *P falciparum* [14].

This study screened the extract obtained from the leaves of *Sarcocephalus latifolius* against some selected microbes and *Artemmia saline* for their antimicrobial and cytotoxicity potentials.

MATERIALS AND METHODS

Collection of Plant Materials

The plant materials used in this study were collected in June, 2007, from Yako village in Kiru Local Government Area of Kano State, Nigeria. The plants were identified at the Bayero University, Kano, and authenticated at the Department of Biological Sciences, Ahmadu Bello University Zaria, Nigeria. A voucher specimen (No1268) has been deposited at the Herbarium.

Extraction of Plant Materials

The air-dried and ground plant sample (200 g) were extracted with absolute ethanol (700 mL) at room temperature for two weeks. The percolates were evaporated to dryness *in vacuo* to afford a residue coded: F001 [15].

Fractionation of Crude Extract

The crude extract (F001) was solvent partitioned to give chloroform (F002), water (F003) and ethylacetate (F004) soluble fractions. The chloroform soluble fraction (F002) was further partitioned between n-hexane and methanol to give n-hexane (F005) and methanol (F006) soluble fractions (Table 1). All the fractions were concentrated *in vacuo*, weight of the fractions was recorded and stored in freezer [15, 16].

Brine Shrimp Lethality Bioassay

A brine shrimp lethality (BST) bioassay is capable of detecting a broad spectrum of bioactivity present in crude extracts. The technique is easy to learn, cheaper and utilizes small

amount of test materials. The bioassay provides a front-line screening that can be backed up by more specific and more expensive bioassays once the activity has been detected [17]. The plant extract was screened against brine shrimp larvae of *Artemia saline* according to the method previously described [15, 18]. In this test, sea water obtained from Lagos Beach, Nigeria was used to culture the *Artemia larvae*. To enhance the solubility of the test, dimethylsulphoxide was added to test materials and control vials. The results are presented in Table 2. The extent of toxicity of a plant extract bioactivity is estimated using the Lethal concentration LC_{50} value of the mortality of the brine shrimp larvae using Finney program. The lower LC_{50} values in µg/ml the more efficacious as drug it could be [21].

Antimicrobial Bioassay

Sources of Microorganisms

Pure cultures of *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* were obtained from the Microbiology Laboratory, Aminu Kano Teaching Hospital, Kano, Nigeria. The three bacterial cultures were maintained in nutrient agar slant at 4 °C before use.

Preparation of Inocula

The inoculum was prepared from the stock cultures which were maintained on nutrient agar slant at 37 °C overnight and sub-cultured in nutrient broth using a sterilized wire loop and incubated at 37 °C for 24 hours. The density of suspension to be inoculated was determined by comparison with 0.5 McFarland standard of Barium sulphate solution (1% v/v).

Preparation of Sensitivity Disc

A paper puncher was used to prepare discs of about 6 mm diameter from whatman's No 1 filter paper. Batches of 100 discs were transferred into Bijou bottles and sterilized in the oven at 110 °C for 24 hours. The stock solution of 10 mg/ml of the plant extract for the bioassay was prepared by dissolving 0.01 g of each fraction of *S. latifolius* in 1 ml Dimethyl sulfoxide (DMSO) (i.e 10,000 µg/ml). Three concentrations of 5000, 2000, and 1000 µg/ml were prepared by dissolving 0.5 ml. 0.2 ml and 0.1 ml of the stock solution into 0.5 ml, 0.8 ml and 0.9 ml of DMSO, respectively. One milliliter (1 ml) of the extract from 10,000 µg/ml, 5000 µg/ml, 2000 µg/ml and 1000 µg/ml concentrations was transferred into separate bottles containing 100 discs. Since each disc can absorb 0.01 ml, the four bottles yielded discs of 100 µg/disc, 50 µg/disc, 20 µg/disc, and 10 µg/disc, respectively.

Antibacterial Susceptibility Test

Disc agar diffusion method described by Kirby-Bauer [19] and demonstrated by Mukhtar and Tukur [20] was employed for antibacterial assay. Then four concentrations 100 µg/disc,

50 µg/disc, 20 µg/disc, and 10 µg/disc for each fraction of *S. latifolius* extract were prepared. A sterile wire loop loaded with standard culture was used in streaking agar plates distributed evenly and aseptically in an inoculation chamber. A standard antibiotic disc Ciprofloxacin (30 µg, control disc) was aseptically pressed firmly at the center using sterile forceps unto the inoculated plates. The zone of inhibition (in diameter, mm) was measured to the nearest whole number using a transparent meter ruler (Table 3).

RESULTS AND DISCUSSION

The physical parameters (weight, colour and texture) of the crude extract and various factions from the leaves of *S. latifolius* are presented in Table 1. The results of the preliminary BST screening of solvent partitioned extracts of the leaves of *S. latifolius* showed that about 67% of the plant extracts tested were moderately active in BST (Table 2). The bioassay showed that ethanol extract (F001) of *S. latifolius* LC_{50} = 705.48 µg/ml (Table 2) exerted highest lethal activity. The lowest activity was found in the ethyl acetate soluble fraction (F004) LC_{50} = 914.99 µg/ml. The water soluble fraction (F003) have BST at LC_{50} values greater than 1000 µg/ml and therefore not active on brine shrimp. This may be partly the reason why some shrimps prefer to associate with the plants in their sea environment. These results suggest potency in the plants extracts under investigation.

Table 1: Some physical parameters of the crude and various fractions of *S latifolius* Smith leaves extracts

Fraction	Weight (g)	Colour	Texture
F001	4.70	Pale yellow	Gummy
F002	2.30	Brownish	Gummy
F003	0.25	Dark green	Solid
F004	0.40	Brownish	Gummy
F005	0.60	Brownish	Gummy
F006	0.80	Brownish	Gummy

Key: F001 = crude ethanol extract; F002 = chloroform soluble fraction; F003 = aqueous fraction; F004 = ethyl acetate soluble fraction; F005 = n-hexane soluble fraction; F006 = methanol soluble fraction.

Table 2: The activity in (BST) of the crude and various fractions of *Sarcocephalus latifolius* leaves

Fractions	LC_{50} µg/mL	Remarks
F001	705.48	Active
F002	NT	NT
F003	>1000	Inactive
F004	914.99	Active
F005	NT	NT
F006	NT	NT

LC_{50} is determined at 95% confidence interval. NT= Not tested

The antibacterial activities tests were carried out on all the fractions obtained as shown in the Tables 3 to 8. The susceptibility of bacterial culture to extract was determined by measurement in the following ranges: 0-7 mm indicates inactive; 8-7 mm indicates weak activity while 12 mm and above indicates strong activity.

The zone of inhibition, 18.0 mm at 10,000 µg/ml was exhibited by the chloroform soluble fraction of the leaves of *S. latifolius* on *S. typhi*. The potency of the chloroform fraction was comparable to the standard antibiotics (i.e Ciprofloxin) used. Ciprofloxin has a universal activity against the three test organisms *S. aureus, S. typhi* and *E.coli* with zone of inhibition ranging from 12.0 to 40.0 mm. Ethanol, chloroform and methanol fractions of *Sarcocephalus latifolius* exhibited zone of growth inhibition 15 mm, 16 mm and 15 mm against *S. aureus*, at 10,000 µg/ml while ethyl acetate soluble fraction showed no activity against *S. aureus* but strongly active against E. coli (16 mm at 10,000 µg/ml).

Table 3: Antimicrobial activity of the ethanol extract (F001) of *Sarcocephalus latifolius* leaves

	Diameter of zone of inhibition (mm) / Extract concentration (µg/ml)			
Isolates	1000	2000	5000	10000
control				
Staphylococcus aureus	00	09	11	15
24				
Salmonella typhi	00	07	11	13
20				
Escherichia coli	08	11	12	14
36				

Table 4: Antimicrobial activity of the chloroform fraction (F002) of *Sarcocephalus latifolius* leaves

	Diameter of zone of inhibition (mm) / Extract concentration (µg/ml)				
Isolates	1000	2000	5000	10000	
control					
Staphylococcus aureus	08	10	13	16	29
Salmonella typhi	00	07	09	18	25
Escherichia coli	07	11	12	15	32

Table 5: Antimicrobial activity of the aqueous fraction (F003) of *Sarcocephalus latifolius* leaves

	Diameter of zone of inhibition (mm) / Extract concentration (µg/ml)				
Isolates	1000	2000	5000	10000	
control					
Staphylococcus aureus	00	08	10	12	30
Salmonella typhi	00	09	12	15	27
Escherichia coli	00	08	11	13	29

Table 6: Antimicrobial activity of the ethyl acetate fraction (F004) of *Sarcocephalus latifolius* leaves

	Diameter of zone of inhibition (mm) / Extract concentration (µg/ml)				
Isolates	1000	2000	5000	10000	control
Staphylococcus aureus	00	00	00	00	32
Salmonella typhi	00	00	00	00	32
Escherichia coli	00	10	12	16	32

Table 7: Antimicrobial activity of the methanol fraction (F005) of *Sarcocephalus latifolius* leaves

	Diameter of zone of inhibition (mm) / Extract concentration (µg/ml)				
Isolates	1000	2000	5000	10000	control
Staphylococcus aureus	00	09	13	15	38
Salmonella typhi	00	00	09	12	30
Escherichia coli	00	00	08	16	37

Table 8: Antimicrobial activity of the n-hexane fraction (F006) of *Sarcocephalus latifolius* leaves

	Diameter of zone of inhibition (mm) / Extract concentration (µg/ml)				
Isolates	1000	2000	5000	10000	control
Staphylococcus aureus	00	00	09	11	29
Salmonella typhi	00	08	10	13	26
Escherichia coli	00	10	12	14	37

Key: Zone of inhibition for disc = 6mm

CONCLUSION

Based on this study, the leaves of *S. latifolius* provides a scientific basis for the ethnomedicinal uses of the plant in the Northern region of Nigeria to cure typhoid fever. The zones of inhibition exhibited by ethanol, methanol and chloroform fractions of *S. latifolius* on *staphylococcus aureus* justified their uses by traditional medicinal practitioners in the treatment of sores, bores, and open wounds. The cytotoxic activity observed on the ethanol,

and ethyl acetate fractions of *S. latifolius* may lead to the discovery of new cytotoxic compounds. The extracts should also be evaluated for the pesticide activity. Further research to detect and characterize bioactive compounds of the leaves of *Sarcocephalus latifolius* needs to be carried out.

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