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Antimicrobial Activity of *Senna alata* Linn.

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The antimicrobial activity of aqueous and organic extracts of the roots and leaves of *Senna alata* were studied using the cup plate agar diffusion method. All the extracts demonstrated considerable activity against both Gram negative and Gram positive bacteria and some fungi with the organic extracts showing higher activity than the aqueous extracts. The minimum inhibitory concentration and minimum bactericidal concentration of the methanol extracts ranged between 6-20 mg/ml and 25-100 mg/ml for bacteria and fungi respectively. Preliminary phytochemical analysis showed that the extracts contained tannins, saponins, glycosides, flavonoids and phenols. The results obtained show the basis for the local usage of *S. alata* Linn as an antimicrobial.

Key words: *Senna alata*, antimicrobial activity, phytochemical analysis, antibiotics

INTRODUCTION

Infectious diseases are the world's leading human and animal killers. The situation is further complicated by the rapid development of multi-drug resistance to available antimicrobial agents. In recent years, pharmaceutical companies have focused on developing drugs from natural products. Plants still remain the most effective and cheapest alternative sources of drugs [1]. Drug discovery must be a continuing process if effective chemotherapeutic agents against the rapidly increasing drug resistant bacteria and fungi are to be obtained. The local use of natural plants as primary remedies due to their pharmacological properties is quite common in Asia, Latin America and Africa [2].

Senna alata (*Cassia alata*) Linn (Fabaceae) is an ornamental shrub which grows well in forested areas of West Africa. The plant has widely been employed for combating dysentery, helminthic infections and stomach disorders. In Ghana and Nigeria, the decoctions of the fresh leaves, roots and seeds has been used for the treatment of wound infections, bronchitis and asthma as well as ring worm and other infectious skin diseases [3]. The leaves have been reported to be useful in the treatment of convulsions, gonorrhoea, heart failure, abdominal pains, oedema and also as a purgative [4]. *Cassia alata* has been reported to contain anthraquinones and the methanol fractions were found to be active against *Aspergilus flavus* [4-5]. This study was therefore carried out to investigate the antimicrobial activity of the root and leaf extracts of the plant against some infectious bacteria and fungi.

MATERIALS AND METHODS

Plant materials were collected from the wild in Yola North local government area of Adamawa state, Nigeria and were identified and authenticated at the Biological Sciences Department, Federal University of Technology, Yola, Adamawa State, Nigeria.

Preparation of Crude and Organic Extracts

The preparation was carried out to simulate the common traditional practice where herbalists often carry out percolation with the application of heat in order to obtain decoctions containing active principles for the desired treatment. Organic solvents (acetone and methanol) were also used for possible enhancement of the extraction process. The freshly collected fresh mature leaves and roots were chopped into

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pieces and shade dried at 32-35 ºC to constant weight for 5 days. Subsequently, 50 g of each of the plant parts was coarsely powdered using a mortar and pestle and finely powdered using an electric blender. The powder was transferred into closed containers. Each of the powdered air-dried plant material was extracted with water, acetone and methanol. For this purpose, 25 g of each powdered sample was mixed with 100 ml of deionised distilled water or organic solvent in a conical flask, plugged, shaken at 120 rpm for 30 min and extracted by warming at 60 ºC in a shaker water bath at 50 rpm for 2 h. This was allowed to cool, and then filtered rapidly through four layers of gauze. The debris was extracted one more time with 100 ml of solvent at 60 ºC in the shaker water bath at 50 rpm for 2 h and filtered again. The two filtrates were then pooled and a more refined filtration was carried out using Whatman no. 1 filter paper. The resulting filtrates were then concentrated in a rotary evaporator and subsequently lyophilized to dryness for aqueous filtrates. The yield of powder was 48 % (aqueous extracts), 32 % (acetone extracts) and 20 % (methanol extracts) for the stem bark and 46 % (aqueous extracts), 30 % (acetone extracts) and 24 % (methanol extracts) for the leaves.

Test Organisms

Bacterial and fungal isolates used for this work included the Gram negative bacteria *Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi* and *Shigella flexnerri* as well as the Gram positive bacteria *Staphylococcus aureus* and *Streptococcus pyogenes*, all clinical isolates obtained from the Specialist Hospital Yola, Adamawa State, Nigeria. Fungal isolates used were laboratory isolates which included *Aspergillus flavus, A. niger* and the yeasts *Candida albicans* and *Cryptococcus neoformans* obtained from the Microbiology Laboratory of the Department of Microbiology, Federal University of Technology, Yola, Adamawa State, Nigeria. All the bacterial strains were suspended in nutrient broth and incubated at 37 ºC for 8 h. Mueller Hinton agar (MHA) and Nutrient agar (Oxoid, Basingstoke, United Kingdom) were used in the test for antibacterial activity while potato dextrose agar (PDA) (Difco, Detroit, Michigan, USA) was used in the test for antifungal activity.

Determination of phytochemical constituents

The freshly prepared extracts were subjected to standard phytochemical analysis for different constituents such as tannins, alkaloids, flavonoids, glycosides, saponins and phenols as described in the literature [6].

Determination of antimicrobial activity

Antimicrobial activity of the aqueous and organic extracts of the plant sample was evaluated by the cup plate agar diffusion method [7]. Bacterial cultures were adjusted to 0.5 McFarland turbidity standard and inoculated onto MHA plates (diameter 15 cm). Prior to the determination of antimycotic activity, all the fungal isolates including *Candida albicans* were first adjusted to a concentration of 10^6 spores/ml. Cultures of *C. albicans* were suspended in sterile solution of 0.9 % normal saline and the spores of the other filamentous fungi were suspended in Tanquay buffer and then inoculated onto PDA plates. A sterile cork borer was used to make wells of 6 mm diameter on the MHA and of 13 mm diameter on PDA. The width of the zones of inhibition was recorded after incubation. In each of the wells in the culture plates previously seeded with the test organisms, 100 µl aliquots of extract dilutions reconstituted in minimum amount of solvent at concentrations of 50 and 100 mg/ml were applied. Sterile glycerol was used as a negative control. Wells containing 20 µl aliquots of ciprofloxacin (10 µg/ml), streptomycin (30 µg/ml), nystatin (10 mg/ml) and amphotericin B (10 mg/ml) served as positive controls. Bacterial and *C. albicans* cultures were incubated at 37 ºC for 18 to 24 h while the filamentous fungal cultures were incubated at 30 ºC for 36 to 48 h. After incubation, antimicrobial activity was determined by measurement of the width of the zones of inhibition. For all the extracts, the tests were carried out in triplicate against each organism.

Determination of minimum inhibitory concentration and minimum microbicidal concentration

The minimum inhibitory concentration (MIC) of the methanol extracts was determined for each of the test organisms in triplicate at concentrations of 3, 6, 9, 12, 15, 20, 25, 50 and 100 mg/ml. To obtain these concentrations, 1 ml aliquots of the extracts at double strength concentrations (6, 12, 18, 24, 30, 40, 50 and 200 mg/ml) were added to test tubes containing 1 ml of nutrient broth for the bacteria and potato dextrose broth for the fungi. A loopful of the test organisms previously diluted to 0.5 McFarland turbidity standard for bacterial isolates and 10^6 spores/ml for fungal isolates was introduced to the respective tubes. The procedure was repeated using the standard antimicrobials ciprofloxacin, streptomycin, nystatin and amphotericin B instead of the extracts. A tube containing nutrient broth only was seeded with the test organisms to serve as a negative control. Tubes containing bacterial cultures were then incubated at 37 ºC for 24 h while tubes containing fungal spore cultures were incubated at 30 ºC for 36 h. After incubation the tubes were examined for microbial growth by observing for turbidity.

To determine the minimum microbicidal concentration (MMC), which includes minimum bactericidal (MBC) and minimum fungicidal concentrations (MFC), a loopful of broth was collected from those tubes which did not show any growth in the MIC determination and inoculated on sterile nutrient agar (NA) for bacteria and Sabourauds Dextrose agar (SDA) for fungi by streaking. To serve as a control, NA and SDA only were streaked with the respective test organisms. Plates inoculated with bacteria were then incubated at 37 ºC for 24 h while those inoculated with fungi were incubated at 30 ºC for 48 h. After incubation, the concentration at which no visible growth was seen was recorded as the MBC and MFC respectively.

RESULTS AND DISCUSSION

Preliminary phytochemical analyses revealed that *Senna alata* (*C. alata*) contained saponins, alkaloids, flavonoids, tannins, phenols and glycosides as shown in Table 1. These bioactive compounds have been reported to be used by plants for protection against bacterial, fungal and pesticidal infections and are responsible for antimicrobial activity [8]. In antimicrobial susceptibility testing, all the extracts demonstrated significant activity against Gram positive bacteria and fungi (Table 2). The highest activity was demonstrated by methanol extracts of both the roots and the leaves.

Table 1. Results of Phytochemical Analysis of *Senna Alata* Extracts.

Phytoconstituent	Plant part/Extract			
	Roots		Leaves	
	ME	WE	ME	WE
Saponins	+	+	+	+
Alkaloids	+	-	-	-
Flavonoids	-	-	+	-
Tannins	+	-	-	-
Glycosides	+	+	+	+
Phenols	-	-	+	+

Key: ME = methanol extract, WE = water extract, + = present; - = absent

Table 2. Antimicrobial Activities of Extracts of *Senna alata*

Organisms	Zone of inhibition (mm)									
	Root extracts			Leaf extracts			Antimicrobial agents			
	WE	ME	AE	WE	ME	AE	CIP (10 µg/ml)	STN (30 µg/ml)	AMP B (10 mg/ml)	NYS (10 mg/ml)
E. coli	8	8	6	6	8	6	18	16	NA	NA
P. mirabilis	6	6	4	4	6	4	16	8	NA	NA
Ps. aeruginosa	6	6	6	6	6	6	12	6	NA	NA
S. typhi	8	8	8	6	8	8	18	8	NA	NA
S. flexnerri	6	8	6	8	8	8	22	18	NA	NA
S. aureus	8	8	8	10	10	10	28	22	NA	NA
Str. pyogenes	10	10	10	12	12	10	20	18	NA	NA
A. flavus	4	4	4	4	6	4	NA	NA	8	10
A. niger	4	6	6	4	6	4	NA	NA	10	12
C. albicans	6	8	8	6	8	6	NA	NA	12	16
Cr. neoformans	6	8	8	6	8	6	NA	NA	12	14

WE = water extract, ME = methanol extract, AE = acetone extract, - = no measurable zone, NYS = Nystatin, CIP = ciprofloxacin, STN = streptomycin, AMP B = amphotericin B and NA = not applicable.

Different solvents have different solubility capacities for different phytoconstituents, hence the differences in the activities of the various extracts [9]. Of the bacteria tested *Str. pyogenes* and *S. aureus* were the most susceptible to all the extracts followed by *Salmonella typhi* and *Escherichia coli*. Of the fungal species tested, the most susceptible were *Cryptococcus neoformans* and *C. albicans* while the least susceptible was *A. flavus*.

The results of MIC and MMC determination showed that the MIC and MMC of the extracts ranged between 6-20 mg/ml and 25-100 mg/ml for the bacteria and fungi respectively while those for the antimicrobial agents ranged between 3-20 mg/ml and 12-100 mg/ml for the bacteria and fungi respectively (Table 3).

Table 3. Minimum Inhibitory Concentration (Mg/Ml) and Minimum Microbicidal Concentration (Mg/Ml) of Methanol Extracts of *Senna Alata*

Organisms	Root extracts		Leave extracts		Antimicrobial agents			
	MIC	MBC	MIC	MBC	CIP	STN	AMP B	NYS
E. coli	12	12	15	15	3	12	NA	NA
P. mirabilis	15	12	20	20	3	20	NA	NA
Ps. aeruginosa	15	15	20	20	3	12	NA	NA
S. typhi	12	15	15	15	6	15	NA	NA
S. flexnerri	9	9	15	15	3	20	NA	NA
S. aureus	9	9	12	12	3	15	NA	NA
Str. pyogenes	6	6	12	12	6	12	NA	NA
A. flavus	100	100	100	100	NA	NA	50	100
A. niger	100	100	100	100	NA	NA	50	100
C. albicans	50	50	50	50	NA	NA	20	100
Cr. neoformans	12	12	25	25	NA	NA	12	25

CIP = ciprofloxacin, STN = streptomycin, AMP B = amphotericin B and NYS = Nystatin.

Low MIC is an indication of high efficacy of the plant extract while high MIC may indicate low efficacy or possible development of resistance by the microorganisms to the antimicrobial [9].

CONCLUSION

The demonstration of the antimicrobial activity of *S. alata* in this work provides scientific basis for its use as a local health remedy. The plant can therefore be used in the treatment of gastrointestinal, urinary tract and wound infections as well as some mycotic infections. There is need for more research on the activity of the extracts against a wider range of bacteria and fungi and on the toxicology and further purification of the extracts for isolation of the pure active constituents.

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