



PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL EFFICACY OF *ALTERNANTHERA NODIFLORA* EXTRACTS

¹Feka, P.D., ¹Mohammed, S.Y., ²Shaibu, M.A. and ¹Solomon, R.S.

¹Nigerian Institute of Leather and Science Technology, Zaria, Nigeria

²Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria

Correspondence Author: fekapaschal@yahoo.com

ABSTRACT

The phytochemical constituents and antimicrobial activities of *Alternanthera nodiflora* extracts were analyzed. Plant sample was extracted using methanol and water. Qualitative phytochemical screening revealed the presence of alkaloids, carotenoid, flavonoids, terpenoids, cardiac glycosides, phenols and saponins while tannins were absent in both extracts. The antimicrobial potential of the extracts was tested against *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*, *Candida albicans* and *Aspergillus niger*. The Susceptibility patterns of the test organisms to varying concentrations (100mg/ml, 75mg/ml, 50mg/ml and 25mg/ml) of both extracts were determined by Kirby Bauer method. From this study, antimicrobial activity of the plant extracts was highest at 100mg/ml with Methanolic extract having more antimicrobial activity than aqueous extract. The extracts showed high activity against *Candida albicans* but no activity was observed against *Aspergillus niger* while the highest antibacterial activity of the extract was observed against *Staphylococcus aureus*. The higher antimicrobial activity in methanolic extract than aqueous extracts could be attributed to the degree of polarity of the extraction solvent.

Key words: *Alternanthera nodiflora*, Phytochemicals, Antimicrobial activity, Extracts.

INTRODUCTION

Over the years plants and their extracts have been applied as herbal remedy for diverse human ailments. Presently plants are still being utilized in numerous developing countries as source of therapeutic agents because they believe medicinal plants are readily available, accessible, affordable, potent and relatively low incidences of adverse reactions compared to modern conventional drugs (Ogu *et al.*; 2012). Plants have been and are still the rich source of many natural products. Most of the plants used by the rural communities have biologically active compounds that have been shown by generations to be effective against specific disorders. The global demand for herbal medicine is not only large, but also growing (Srivastava, 2000).

The potential of higher plants as source for new drugs is still largely unexplored. Among the estimated 250,000 – 500,000 plants species, only a small percentage has been investigated phytochemically and the fraction submitted for biological or pharmacological screening is even smaller. Thus, any phytochemical investigation of a given plant will reveal only a very narrow spectrum of its constituents (Gerhartz *et al.*; 1985).

Alternanthera nodiflora belongs to the family *Amaranthaceae*. Its common names are: common joy weed, native carpetweed, *Mai kai dubu* in Hausa, while the common names in Yoruba and Igbo are unknown. Other varieties of *Alternanthera* serve both as food and medicine in Asia and African countries, treating an astonishing range of external and internal conditions. Every part of this plant has found a medicinal use (Uniyal *et al.*; 2006, Balandrin *et al.*; 1985). The approximately 200 species of *Alternanthera* are highly versatile. They are colorful garden accents with foliage in shades of green, red, pink, purple and yellow. They have been used for

centuries in formal garden designs and also perform well in containers and as houseplants. Some varieties are used as water garden and aquarium plants. Other varieties of *Alternanthera* serve as both food and medicine in Asian and African countries, treating an astonishing range of external and internal conditions. Phytochemicals are biologically active substances found in plants in small amounts, which are not established nutrients but which nevertheless seem to contribute significantly to protection against degenerative disease (Ivor, 2002). Phytochemicals are basically divided into two groups, i.e. primary and secondary constituents according to their functions in the plant metabolism. Primary constituents comprise common sugars, amino acids, proteins and chlorophyll while secondary constituents consist of alkaloids, flavonoids, saponin, phenolics and so on (Khandare, 2012).

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are source of many potent and powerful drugs (Vocks, 1996). A wide range of plant parts are used for extract as raw drugs and they possess varied medicinal properties. The different parts used include roots, stem, flower, fruits, and twigs and modified plant organs. Considering the vast potentiality of plants as sources for antimicrobial drugs with reference to antibacterial and antifungal agents, a systematic investigation was undertaken to screen the local flora for antibacterial and antifungal activity from *Alternanthera nodiflora*.

MATERIALS AND METHODS

Sample Collection and Preparation

The plant *Alternanthera nodiflora* was collected from a residential area in Zangon-Shanu along Samaru road Zaria, Kaduna State, Nigeria.

The plant specimen was identified in the Department of Biological Sciences, Ahmadu Bello University Zaria, air dried on a laboratory work bench at room temperature for two weeks and ground into powder using laboratory pestle and mortar.

Extraction of Plant Material

Aqueous Extraction

The aqueous extract of the plant was prepared by soaking 10g of powdered sample in 200ml distilled water for 24hours. The extracts were then filtered using whatman filter paper no 1. The extract was then concentrated by heating on water bath to 50ml of the original volume of the extract.

Methanol Extraction

Ten grams of the powdered plant sample was soaked in 100ml of methanol for the same 24hours at room temperature with occasional stirring. The content was then filtered using filter paper. The extract was then concentrated to 50ml of the extract and stored in an air tight container in a refrigerator at 4⁰C until it is required for analysis.

DETERMINATION OF ANTIMICROBIAL ACTIVITY

Test Organisms

The following micro organisms; (*Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Aspergillus niger* and *Candida albicans*) were collected based on their clinical and pharmacological importance. The micro organisms were obtained from Microbiology Department, Ahmadu Bello University Zaria. The bacterial stock culture was inoculated for 24hours at 37°C on nutrient agar and sabouraud dextrose agar at 28°C following refrigeration storage at 4°C to maintain the stock culture.

Antimicrobial Susceptibility Testing

The agar well diffusion method as described by Bauer *et al.*, (1966) with slight modification was adopted in this assay. A loopful of the standardized (0.5 McFarland) bacterial and fungal cell suspended was inoculated into well dried sterile Nutrient agar and Sabouraud Dextrose agar. The plant extract was reconstituted in Dimethylsulfoxide (DMSO) to obtain the working concentration of 100mg/ml, 75mg/ml, 50mg/ml and 25mg/ml. A quantity (0.1ml) of each extract was inoculated into well earlier bored with a sterile borer in each plate. The plates were allowed to stand for 30minutes on the work bench for pre – diffusion of extracts. The NA and SDA plates were incubated at 37°C and room temperature for 24 – 48 hours. The antimicrobial activity of the extracts were determined after the incubation period by measurement of mean diameter zones of inhibition produced by the extracts against the test organisms and results were recorded in millimeter (mm) using a transparent ruler (Ogu *et al.*; 2012).

PHYTOCHEMICAL SCREENING OF THE EXTRACTS

The extracts were tested for the presence of phytochemicals such as alkaloids, flavonoids, carotenoids, saponins, steroid, cardiac glycosides, tannins, terpenoids, phlobatannins and phenols using

the standard procedures described by Treese and Evans (1989), Harborne (1973) and Sofowora (1993).

Test for Alkaloids

One gram of powdered sample was boiled with water and 10ml hydrochloric acid on a water bath and then filtered while hot. The pH of the filtrate was adjusted with ammonia to 6.7. Very small quantities of the following reagents were added separately to about 0.5ml of the filtrate in a different test tube and were observed.

- i. Few drops of Mayer's reagent were added to the filtrate in a test tube and a cream precipitation formed indicating the presence of alkaloids.
- ii. Dragendoff's reagent was added to extract forming rose – red precipitation.
- iii. Picric acid solution was added to extract the test tube was observed for turbidity indicating the presence of alkaloids.
- iv. About 10% tannic solutions were added to extract, a yellow precipitation was observed.

Test for Carotenoids

One gram of specimen sample was extracted with 10ml of chloroform in a test tube with vigorous shaking. The reacting mixture was filtered using what man filter paper No. 1 and 85% sulphuric acid was added. There was a blue color at the interface of the mixture which showed the presence of carotenoids.

Test for Flavonoids

One gram of the powdered dried plant was boiled with 10ml of distilled water for 5minutes and filtered while hot. Few drops of 20% sodium hydroxide solution were added to 1ml of the cool filtrate. A change to yellow color was observed which on addition of acid changed to colorless solution indicating the presence of flavonoids.

Test for Terpenoids

A quantity (5ml) of extract was mixed in 2ml of chloroform and 3ml of concentrated H₂SO₄ was then added and a layer was formed. A reddish brown precipitate coloration at the interface was formed indicating the presence of terpenoids.

Test for Cardiac glycosides

Five ml of extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with about 1ml of concentrated sulphuric acid. A brown ring at the interface was observed indicating the deoxy sugar characteristics of cardenolides. A violet ring appeared below the ring while in acetic acid layer, a greenish ring was formed.

Test for Phlobatannins

An aqueous extract of plant sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins when a deposition of a red precipitate is formed.

Test for Tannins

One gram of powdered sample was boiled with 20ml distilled water for 5minutes in a water bath and was filtered while hot. One ml of cool filtrate was added to 5ml distilled water and a few drops of 10% ferric chloride were added. It was observed for any formation of precipitates and any color change; a bluish – black or brownish – green precipitate indicated the presence of tannins.

Test for Steroids

Two ml of acetic aldehyde was added to 0.5ml methanol extract of sample with 2ml H₂SO₄. The color changes from violet to blue or green indicating the presence of steroids.

Test for Phenols

Two ml of the extract was added to 2ml of ferric chloride solution. A deep bluish – green solution formed shows the presence of phenols.

Test for Saponins

One gram of powdered sample was boiled with 10ml distilled water in a water bath for 10minutes. The mixture was then carried out to observe persistent froth (bubbles/foaming).

A portion of the filtrate (2.5ml) was diluted to 10ml with distilled water and shaken vigorously for 2minutes, frothing indicated the presence of saponins. To the above solution 2 drops of olive oil was added and shaken vigorously for a few minutes. Formation of a fairly stable emulsion indicated the presence of saponins.

RESULTS

The results of this study are presented in table 1 and 2. The results of phytochemical analysis of *Alternanthera nodiflora* showed that tannins and steroids were absent in the aqueous extract of the plant while in the methanol extract phlobatannins and tannins were absent (Table 1).

The antimicrobial activity of the extracts of *Alternanthera nodiflora* were studied in different

concentration (100, 75, 50 and 25mg/ml) against three pathogenic bacterial strains (*Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*) and two fungal strains (*Aspergillus niger* and *Candida albicans*). The plant extract posses' potential antibacterial effect against *S. aureus*, *S. typhi* and *E. coli*, and antifungal activity against *C. albicans*(Table 2.0).

The methanol extract showed significant activity against *S. aureus* (15mm) and *S. typhi* (10mm) and least zone of inhibition observed in *E. coli* (5mm) at 100mg/ml concentration. The highest antifungal activity of 20mm in *C. albicans* at 100mg/ml concentration and no zone of inhibition observed against *A. niger* (0mm) even at 100mg/ml concentration of the plant extract. The aqueous extract showed maximum antibacterial activity against *S. aureus* and *S. typhi*, and least observed in *E. coli* at different concentration. Significant antifungal activity was observed against *C. albicans* and no activity observed against *A. niger*. From the study, *Alternanthera nodiflora* have no antimicrobial activity on *A.niger* highest activity on *Candida albicans* and most antibacterial activity on *S.aureus*. In this study, the plant extracts were found to inhibit the growth of all the test bacteria and a fungus, indicating that this plant possesses antimicrobial properties and an increasing antimicrobial activity was observed with increasing concentrations of the extracts.

Table 1: Phytochemical Constituents of *Alternanthera nodiflora* Extracts

Phytochemical	Aqueous extract	Methanol extract
Alkaloids	+	+
Carotenoids	+	+
Flavonoids	+	+
Terpenoids	+	+
Cardiac glycosides	+	+
Phlobatannins	+	-
Steroids	-	+
Phenols	+	+
Saponins	+	+
Tannins	-	-

Key: + = Presence of phytochemical, - = Absence of phytochemical

Table 2: Antimicrobial Activity of *Alternanthera nodiflora* Extracts

Extract	Concentration (mg/ml)	Zone of inhibition (mm)				
		<i>S. aureus</i>	<i>S. typhi</i>	<i>E. coli</i>	<i>C. albicans</i>	<i>A. niger</i>
Methanol extract	100	15.00	10.00	5.00	20.00	0.00
	75	13.00	4.50	3.00	16.00	0.00
	50	10.00	4.00	1.50	11.50	0.00
	25	6.00	2.50	0.00	10.00	0.00
Aqueous extract	100	10.00	8.00	4.00	14.00	0.00
	75	6.00	5.50	2.50	10.00	0.00
	50	4.50	4.00	1.00	4.50	0.00
	25	3.00	2.50	0.00	2.50	0.00

DISCUSSION

The antimicrobial activity of medicinal plants extracts have been attributed to the presence of phytochemical compounds in them and the presence of these compounds usually justifies the use of the plants for treatment of infections caused by susceptible pathogens (Hassan *et al.*, 2009). The extracts of

Alternanthera nodiflora have shown significant antimicrobial activities against the isolates tested and the slight variation exhibited by the methanolic extract suggests that this solvent dissolved a greater percentage of the bioactive ingredients of this plant than the aqueous extract mostly due to polarity.

The inhibition of *S. aureus*, suggests that this plant possesses broad spectrum antimicrobial properties which could be used in the treatment of skin diseases and food poisoning of which the pathogen is commonly implicated. Also the inhibition of *C. albicans* by this plant extract suggests that it possesses antifungal property and can thus be tried as antifungal agent for the treatment of refractory candidiasis (oral) that has a major global challenge with HIV/AIDS patients (Ogu *et al.*, 2012).

The significant antimicrobial properties of the plant extract of *Alternanthera nodiflora* could be attributed to the presence of the bioactive compounds detected in this study. Alkaloids, saponins, flavonoids, phenols, terpenoids, cardiac glycosides, carotenoids, phlobatannins and steroids have been documented in this study. These principles have been known for many years to exhibit biological activity, such as effect on the central nervous system, antibacterial, antitumor and anthelmintics activity (Harborne, 1973). Many alkaloids are known to have effect on the

central nervous system and some act as antipyretic such as morphine, a painkiller. Analgesia is another property of many alkaloids containing plants used in traditional medicine.

Saponins however, are a special class of glycosides which possesses antifungal activity. The significant activity of the extract against *C. albicans* in this sample might be attributed to the action of this bioactive ingredient. The presence of cardiac glycosides, steroids and carotenoids has been documented to inhibit the growth of bacteria and found to possess antioxidant potentials (Ogu *et al.*, 2012).

CONCLUSION

This study has demonstrated that the plant extract of *Alternanthera nodiflora* possesses bioactive ingredients that have antibacterial and antifungal activities against some human pathogens, this justifies their uses in traditional herbal medicine for treating infectious diseases as claimed by the traditional healers.

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