

Phytochemical Compositions and Antibacterial Activities of Some Medicinal Plants Found in Aliero Area, Kebbi State, Nigeria

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ABSTRACT

In this research, hexane and acetone extracts of *Bauhinia rufences*, *Securidaca longepedunculata* and *Ziziphus abyssinica* were investigated for antibacterial activity. The phytochemical screening of the plants leaves extracts was conducted using standard methods of analyses. The effects of the two solvent extracts on the bacterial species (*Escherichia coli*, *Psuedomonas aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus*) were determined using agar well diffusion method. The most susceptible microorganisms were *P. aeruginosa*, *S. typhi* while the least susceptible was *E.coli*. Highest antibacterial activity was observed with hexane extract of *B. rufences* against *S. typhi* (12.75mm). While minimum activity was observed with hexane extract of *S. longepedunculata* and acetone leaves extract of *B. rufences* against *E. coli* (6.00mm and 6.00mm respectively). Hexane extracts had more inhibitory effects compared to acetone extracts, but were less potent when compared to ampiclox used as standard control. It can be concluded that these plants can be useful in the treatment of bacterial infections especially of the tested species which are common in our communities and the plants can be of easy access compared to synthetic medicines especially in our rural communities.

Keywords: Phytochemicals, Antibacterial activity, Medicinal plants, Hexane extracts, acetone extracts.

INTRODUCTION

Plants are plagued with diseases which may have devastating consequences on their population, therefore in order to serve as defense; plants manufacture secondary metabolites which help them fight diseases. These metabolites found in plants are the main sources of medicinal properties of the plants and therefore serve as the main sources of new pharmaceuticals and health care products for the benefit of mankind [1].

Medicinal plants generally contain a number of compounds which may be potential natural antibacterial for the treatment of common bacterial infections. It is estimated that today, plant materials are present in or have provided models for 50% of western drugs [2]. Plant derived medicines are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment [3]. Therefore there is urgent and continuous need to discover new antimicrobial compounds from plant resources with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases [4].

Widespread of diseases such as tuberculosis, typhoid fever coupled with high poverty level in developing countries like Nigeria has made investment and investigations on herbal plants an attractive endeavor in human healthcare. This is because most of the available synthetic medicines are too expensive for most patients. In the last decade, there had been renewed attention and interest in the use of traditional medicine globally [5] as source of many important medical pharmaceuticals.

For centuries, plants have been used throughout the world as drugs and remedies for various diseases. These drugs serve as prototype to develop more effective and less toxic medicines. Hence, an attempt has been made to evaluate antibacterial activity of the folk's medicinal plants used in North-western region of Nigeria against some bacterial pathogens.

Antibacterial activity is the ability of a substance to inhibit bacterial growth or kill the cells. Different types of antibiotics and chemotherapeutic agents are being used in the treatment of one form of disease or the other. Most of these antibiotics were originally derived from micro-organisms while the chemotherapeutic agents are from plants. However, nowadays these antibiotics and chemotherapeutic agents are obtained by various synthetic processes. Most countries in West Africa especially Nigeria are richly blessed with forests containing arrays of different herbs, shrubs and trees [6].

Securidaca longepedunculata is a shrub of about 10 cm high, 2 to 9cm long and 0.5 to 2.5cm broad leaves commonly found in the entire North-western zone of Nigeria. The plant belongs to the family *Polygalaceae*. In northern Nigeria, the Nupe and the Hausa tribes utilize *S. longepedunculata* ethnomedicinally as a remedy for

numerous human and animal ailments [7]. This plant is also used for the treatment of every conceivable ailment such as headache, rheumatism, tuberculosis, cancer, venereal diseases, diabetes as well as abortifacient [8] and probably that is why the Hausas refer to it as “*uwar magunguna*” (the mother of all medicines).

Bauhinia rufescens is a scandent shrub or small tree belonging to the giant family *Leguminosae*, subfamily *Leguminosae caesalpiniodeae*, usually 1-3 m high, sometimes reaching 8 m, often scraggy, stunted and multi stemmed. The plant has wide array of medicinal and socio-cultural uses [9]. It is used in treatment of cholera and the Hausas people called it “*Jirga*”.

The *Z. abyssinica* or *Magarya* (local Hausa name) belongs to the family *Rhamnaceae* which consists of small trees that are indigenous to tropical Africa. In Nigeria, it is used for charcoal, medicine, bees forage and as live fence [10].

In spite of the ever increasing efforts by researchers to discover medicinal potentials of plants, the potentials of many higher plants as source for new drugs is still largely unexplored. Bacterial infections can be treated with a wide range of antibiotics. Most of these antibiotics are either too expensive or not readily accessible by many people particularly those living in rural communities. Further to this, the development of bacterial resistance to many existing antibiotics is a major concern. These concerns justify the urgent need to discover novel drugs for the treatment of bacterial infections more especially from plant resources.

MATERIALS AND METHODS

Collection and Preparation of the Plants Materials

The three plants (*Bauhinia rufences*, *Securidaca longepedunculata* and *Ziziphus abyssinica*) were collected in March, 2014 within Aliero Local Government Area, Kebbi State, Nigeria. The plants were identified and authenticated by a plant taxonomist in the Department of Biological Sciences, Kebbi State University of Science and Technology, Aliero, where voucher specimens of the plants (0258, 073 and 012 respectively) were deposited. The leaves of the plants were dried under shade and ground into powders. The powders were then put into clean cellophane bags and kept in a cool dry place for further use.

Sample Extraction

Fifty gram (50g) of each powdered plant material was weighed and soaked in 200 mL of n-hexane or acetone and allowed to stand for 72 hours. Then filtered using muslin cloth and Whatman filter paper No. 1. The filtrates obtained were evaporated to dryness at 40°C using rotary evaporator. The extracts were weighed, kept in well labeled sterile bottles and stored in a refrigerator for further analyses.

Qualitative Phytochemical Screening

Five grams (5g) from each of the hexane and acetone residues of the plants leaves were each dissolved in 40 mL of distilled water and then subjected to the phytochemical screening using standard methods [11, 12] to test for the presence of flavonoids, phenols, tannins, saponins, alkaloids, terpenoids and glycosides.

Test for Flavonoids

Two milliliters (2 mL) of 10% Sodium chloride was added to 2 mL of the extract in a test tube. A yellow color formation which turns colorless upon addition of 2 mL of dilute hydrochloric acid indicates a positive result.

Test for Phenols

Two milliliters (2 mL) of the extract was mixed with few drops of 10% ferric chloride solution. The formation of greenish-blue, violet or blue-black coloration indicates a positive result.

Test for Tannins

Five (5) drops of 0.1% ferric chloride (FeCl_3) was added to 2 mL of the extract. Formation of a brownish green or blue-black coloration indicates a positive result.

3.11.1.4 Test for Saponins

Three gram (3g) of the powered extract were placed into a beaker and 15 mL of distilled water was added and then heated for 3 minutes, filtered hot, Cooled and the following tests were carried out on the filtrate:

Frothing test: The extract (1 mL) was placed into a test tube and then shaken vigorously. Formation of froth that last for up to 1 minute indicates the presence of saponins.

Emulsifying test: A 5 mL of the extract was diluted with 10mL of distilled water. Five milliliters (5 mL) of the mixture was then taken into a test tube and 5 mL of olive oil was added. The formation of an emulsion when shaken vigorously for 30 seconds indicates the presence of saponins.

Test for Alkaloids

The extract (2 mL) was added to 2 mL of 10% hydrochloric acid. A 1 mL of the mixture was then treated with few drops of Wagner’s reagent and another 1 mL was treated with few drops of Mayer’s reagent. Formation of an orange precipitate indicates a positive result.

Test for Terpenoids

Two milliliters (2 mL) of the extract was mixed with 2 mL of chloroform and 1 mL of concentrated sulphuric acid was carefully added to form a layer. A clean upper and lower layer with a reddish brown interphase indicates a positive result.

Test for Glycosides

Two milliliter (2 mL) of acetic acid was added to 2 mL of the extract. The mixture was cooled in a cold water bath, and then 2 mL concentrated H₂SO₄ was added, colour development from blue to bluish green indicates the presence of glycosides.

Bacterial Species

The test bacterial species used were *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Staphylococcus aureus*. They were obtained from Microbiology unit, Department of Biological Sciences, Kebbi State University of Science and Technology, Aliero.

Media Preparation

A 28g of nutrient agar was dissolved in 1 L of distilled water in a conical flask. The mixture was heated using a hot plate until all the contents is completely dissolved. It was then sterilized using autoclave at 121°C for 15 minutes then allowed to cool at room temperature and poured on to petri dishes and allowed to stand for 40 minutes to solidify.

Antibacterial Activity Screening

The standard Agar well diffusion method [13] was used to screen the antibacterial activity of the extracts. The bacterial isolates were separately inoculated into the nutrient agar (NA) plates using sterile cotton swabs. Well of 6.0 mm in diameter were cut in the agar matrix and equal amounts of the reconstituted extract were introduced into each well at concentration of 100 mg/mL, 150 mg/mL and 200 mg/mL. Wells containing hexane and acetone alone were used as negative controls. The extracts were allowed to diffuse into the agar matrix for 1 hour before incubating at 37°C for 24 hours. The diameter of the zone of inhibition which is a measure of antibacterial activity of the plants extracts was measured in millimeter using transparent ruler. The standard antibacterial drug (Ampiclox) at concentration of 100 mg/mL was used as standard control.

RESULTS

The results of the phytochemical screening are presented in Table 1, while those of the antibacterial activity are presented in Table 2 and 3.

Table 1: Phytochemical Composition of the Hexane and Acetone Leaves Extracts

Phytochemical	<i>Ziziphus abyssinica</i>		<i>Bauhinia rufences</i>		<i>S. longepedunculata</i>	
	Hexane	Acetone	Hexane	Acetone	Hexane	Acetone
Alkaloids	++	-	++	+	++	+
Phenols	-	++	++	++	+	++
Saponins	+	-	+	++	-	+
Tannins	+	++	++	++	++	++
Glycosides	++	+	+	++	++	-
Flavonoids	+	-	++	-	++	-
Terpenoids	++	++	-	-	++	++

+ = Moderately Present, ++ = Highly present, - = Not detected

Table 2: Antibacterial Activities of the Hexane Leave Extracts

Plant	Extracts (mg/mL)	Zone of inhibition diameter (mm)			
		<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. typhi</i>	<i>S. aureus</i>
<i>Z. abyssinica</i>	200	–	8.00 ± 0.71	8.25 ± 1.06	–
	150	–	7.00 ± 0.00	7.25 ± 1.06	–
	100	–	7.75 ± 1.77	8.75 ± 1.06	–
	C	–	16.25 ± 1.77	15.25 ± 0.35	–
<i>B. rufences</i>	200	10.00 ± 1.41	11.75 ± 1.06	12.75 ± 1.06	11.50 ± 0.71
	150	8.50 ± 1.41	11.00 ± 1.41	11.25 ± 1.06	12.25 ± 1.06
	100	9.50 ± 1.41	9.75 ± 0.35	11.75 ± 1.06	10.50 ± 0.71
	C	15.25 ± 1.06	15.50 ± 0.71	15.25 ± 0.35	15.50 ± 0.71
<i>S. longepedunculata</i>	200	6.50 ± 0.00	7.25 ± 1.61	7.50 ± 1.41	–
	150	6.00 ± 0.71	8.00 ± 1.41	8.75 ± 0.35	–
	100	7.50 ± 0.71	8.25 ± 1.06	9.00 ± 0.71	–
	C	16.5 ± 1.41	16.5 ± 0.71	17.5 ± 1.06	–

Values are presented as Mean ± SD of triplicates. C = Control (Ampiclox), - = No inhibition

Table 3: Antibacterial Activities of the Acetone Leaves Extracts

Plant	Extract (mg/mL)	Zone of inhibition diameter (mm)			
		<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. typhi</i>	<i>S. aureus</i>
<i>Z. abyssinica</i>	200	9.25 ± 3.89	8.00 ± 5.66	–	11.00 ± 2.12
	150	9.00 ± 4.95	8.50 ± 5.66	–	10.75 ± 3.89
	100	9.25 ± 1.77	6.50 ± 2.83	–	6.75 ± 4.60
	C	15.75 ± 1.77	15.50 ± 0.71	–	13.75 ± 1.06
<i>B. rufences</i>	200	8.25 ± 1.77	9.50 ± 0.00	7.25 ± 0.35	10.50 ± 0.71
	150	8.00 ± 1.41	9.75 ± 0.35	8.25 ± 0.35	7.50 ± 0.00
	100	6.00 ± 0.71	11.25 ± 3.89	6.50 ± 1.41	7.25 ± 2.47
	C	13.00 ± 0.71	15.75 ± 1.77	14.75 ± 0.35	15.00 ± 1.41
<i>S. longepedunculata</i>	200	8.75 ± 0.35	8.75 ± 1.06	7.50 ± 0.00	–
	150	8.25 ± 1.06	8.50 ± 1.41	9.00 ± 0.71	–
	100	8.75 ± 0.35	11.00 ± 2.83	9.00 ± 1.41	–
	C	15.75 ± 1.06	15.25 ± 1.77	18.75 ± 0.35	–

Values are presented as Mean ± SD of triplicates. C = Control (Ampiclox), - = No inhibition

DISCUSSION

Table 1 presented the results of the qualitative phytochemical screening of the hexane and acetone leaves extracts of the plants. The results indicated the presence of alkaloids, phenols, saponins, tannins, glycosides, flavonoids and terpenoids in all the three plants tested. The presence of alkaloids and flavonoids in both hexane and acetone extracts (Table 1) proved the efficacy of the plants against the infectious diseases that they are used locally for [14]. Likewise, the presence of saponins and glycosides in all the plants justified the traditional uses of the plants in the treatment of tuberculosis and typhoid [15]. The glycosides detected are non-toxic but can get hydrolyzed to release phenolic compounds which are toxic to microbial pathogens [16].

Plant based antibacterial agents have enormous therapeutic potentials as they can serve the purpose with lesser side effects that are often associated with synthetic antibacterial drugs [17]. Alkaloids which are found to be high in the three plants could also make these plants to be effective against diarrhea as a result of bacterial infection. Tannins bind to proline rich proteins and interfere with protein synthesis of cell wall in microorganism.

Table 2 shows the results of zone of inhibition of hexane leaves extracts against the tested organisms. *B. rufences* has the highest inhibitory activity of 12.75 mm at concentration of 200 mg/mL against *S. typhi*, while, *S. longepedunculata* has the lowest zone of inhibition of 6.00 mm at concentration of 150 mg/mL against *E. coli*.

Table 3 shows the result of zone of inhibition of acetone leaves extracts against all the tested organisms. *B. rufences* indicated inhibitory activity against all the tested organisms and has the highest activity of 11.25 mm at concentration of 100 mg/mL against *P. aeruginosa*, and lowest activity of 6.00 mm at concentration of 100 mg/mL against *E. coli*.

In general comparing the results in table 2 and that of 3, hexane extracts have more antibacterial activity than acetone extracts. This may be due to high efficiency of extraction compared to acetone [18]. Malu *et al.*, [19] reported antibacterial activity of various extracts of *B. rufences* against *C. bacillus*, *S. epidermidis* and *S. viridians*. Bele *et al.*, [20] also confirmed that the methanol extract of *B. rufences* showed a significant zone of inhibition against *E. coli*, *S. aureus* and *B. rufences* is known to contain resins and volatile oils such as borneol, camphene, citral, eucalyptol, linalool, phenllandrene, and phenols [21] which may be responsible for its potent antimicrobial activities.

The extracts of *S. longepedunculata* were active against the three (3) bacterial species showing maximum zone of inhibition (11 mm) against *P. aeruginosa* from acetone extract. This may be due to the presence of tannins (Table 1). Tannins are known for their astringent property and antimicrobial activity [22].

Alkaloids and saponins detected in *Z. abyssinica* may be responsible for the antibacterial activity of the plant. Among all the extracts, *Z. abyssinica* was found to have least antibacterial activity which has also been reported in previous study by Akhtar *et al.*, [23].

Medicinal and healing properties of herbs are closely related to their chemical components which are classified into some major groups like alkaloids, acids, essential oils, steroids, saponins, tannins etc., and getting these chemicals out into the herbal remedy depends upon the solubility of these compounds in various solvents. Against all the tested bacterial strain, we observed that hexane extract of all the samples showing much better antibacterial activities in contrast to acetone extract, which may be because of organic nature of hexane and also for the reason of its high capacity to dissolve more organic and active antimicrobial compounds [22]. The antimicrobial action of the acetone extracts could be ascribed to the anionic components such as thiocyanate, nitrate, chlorides and sulfates besides other water soluble components which are naturally occurring in the plant material [24]. These results confirmed the substantiation of previous studies which have reported that hexane is a better solvent for more consistent extraction of antimicrobial substances from medical plants compared to other solvents, such as water [25].

The use of plant extracts with known antimicrobial properties can be of great significance in therapeutic treatments but several studies have also reported various types of contamination of herbal medicines which include microorganisms and toxins produced by microorganisms, pesticides and toxic heavy metals [26]. As a result, sterilization is needed especially for aqueous extracts before use to get rid of these contaminations.

CONCLUSION

This research work revealed the antimicrobial properties of the tested medicinal plants. The antibacterial activities of these plants were due to the presence of some phytochemical constituents like alkaloids, tannins, flavonoids, saponins, etc. These plants have high antibacterial activity which suggested that they can be used traditionally for the treatment of such infectious diseases and can also be used as a source of new antibacterial agents.

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