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Phytochemical Screening and Antimicrobial Activity of Crude Stem Bark Extracts of *Anogeissus leiocarpus*

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Authors' contributions

This work was carried out in collaboration between all authors. Authors DAA and AIU designed the study and performed the statistical analysis. Authors DAA, AUM and YYM wrote the first draft of the manuscript. Authors DAA and AIU managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was aimed at screening of phytochemicals and evaluation of antimicrobial activity of stem bark of *Anogeissus leiocarpus*
Study Design: Stem bark of *Anogeissus leiocapus* was extracted using solvent extraction method; with ethanol, n-hexane, chloroform, ethylacetate and water soluble extracts. Each extract was screened for phytochemicals and tested for sensitivity against *E. coli and Shigella dysenteriae.*
Place and Duration of the Study: The study was carried out at the department of Chemistry, Bayero University, Kano, Nigeria. The duration of the study is 6 months, 2 weeks.
Methodology: The stem bark of the plant was dried, ground, sieved and stored at room temperature. The sieved powder (200 g) of *Anogeissus leiocarpus* was percolated with 95%

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Ethanol (1 l). Maceration was conducted using ethanol, n-hexane, chloroform, ethylacetate and water soluble extracts. Each extract was screened for phytochemicals and tested for sensitivity against *E. coli and Shigella dysenteriae.*

Results: Phytochemical screening of the extracts revealed the presence of alkaloid, tannins, flavonoids, steroids and sugars while antimicrobial activity test at various concentration showed that all the extracts had antimicrobial activity against *E. coli* (causative of diarrhea) and *Shigella dysenteriae* (causative of dysentery) at different concentrations with the exception of ethyl acetate soluble extract which showed no antimicrobial activity against *E. coli*.

Conclusion: Thus, the plant can be a potential source of bioactive compounds to be considered in the management of infectious diseases, notably diarrhea and dysentery.

Keywords: Anogeissus leiocarpus; stem bark; solvent extraction; phytochemical screening; optical activity; sensitivity test.

1. INTRODUCTION

Many of the plant materials used in traditional medicine are readily available in rural areas and this has made traditional medicine economically cheaper than the modern medicine [1]. Over sixty percent of Nigerian population depends on traditional medicine for health care need. Our forefathers were compelled to use any natural substance they could find to ease their sufferings caused by acute and chronic illnesses, physical discomforts, wounds and injuries, and even terminal illnesses. Since ancient times, plants with therapeutic properties have secured an important place in the healing practices and treatment of diseases [2]. Medicinal properties of the plants are generally dependent on the presence of certain phytochemicals, such as alkaloids, tannins, saponins, flavonoids, reducing sugar and anthraquinone with bioactive bases thought to be responsible for antimicrobial property [3].

Anogeissus leiocarpus is an elegant tree of Africa, commonly known as "Axle wood tree or African birch" because of the silvery cast of the foliage like the temperate birch. It extends from the Sahel to forest zones and Senegal to Sudan and Ethiopia with savanna regions as its habitats [4]. In Nigeria, *A. leiocarpus* is popularly known with these local names: Hausa: *marike*; Nupe: *shici*; Fulfulde: *galaldi, kojoli,* Yoruba: *ayin, pako ayin, orin-odan*, Igbo: *atara*. Ethnobotanically, the decoction and maceration of the stem bark are used against anorexia, constipation, malaria, jaundice, fatigue, itching, eczema, psoriasis, carbuncles, wounds, sores, boils, cysts and various forms of hepatitis and ulcers, including diabetic ulcers; helminthosis, schistosomiasis, leprosy and bacterial infections and as chewing sticks [5]; trypanosomiasis [6]; cough and tuberculosis [7,8] and treatment of sexually transmitted infections in Mali [9]. Moreover, the leaf and bark find application in the treatment of naso-pharyngeal infections. The roots are used as medicines for the management of diarrhea, dysentery; genital stimulants, depressants; leprosy and liver disorder, as painkillers, and for the treatment of skin eruptions and venereal diseases [3]. However, the bark of the plant has not yet been used for the management of diarrhea and dysentery to the best of our knowledge. The latter is a potential fast killer condition which needs urgent attention. Although several studies have been conducted to explore plants with such antimicrobial activities, yet multiple options can provide better alternatives. This study is aimed at screening of phytochemicals and conducting antimicrobial activity tests on crude stem bark extracts of *Anogeissus leiocarpus.*

2. MATERIALS AND METHODS

2.1 Plant Collection

The stem bark of *Anogeissus leiocarpus* was collected from Kiru local government area of Kano state, Nigeria. The plant was identified as Marke in Hausa by traditional herbalists and taxonomically identified and authentificated by a taxonomist in the department of Biological sciences, Bayero university Kano, Nigeria.

2.2 Methodology

2.2.1 Preparation of the crude aqueous ethanol extract of the plant's stem bark

The stem bark of the plant was dried, ground, sieved and stored at room temperature. The sieved powder (200 g) of *Anogeissus leiocarpus* was percolated with 95% Ethanol (1 l) for two weeks. The extract was decanted, filtered and

labeled. Repercolation was done with second crude of Ethanol (500 ml) for one week. Extract was concentrated on rotary evaporation (R110) at 40ºC, the two residues combined and labeled as clo1. Merceration was then conducted on the crude ethanol extract using n-hexane in order to remove the fatty acid and esters. The residues from n-hexane fraction was labeled as Clo2 further maceration was done with chloroform, the residues labeled as Clo3 and ethylacetate soluble fraction Clo4, last residue was labeled as Clo5.

2.2.2 Optical activity test

The five stem bark extracts from *Anogeissus leiocarpus* were measured for optical activity using polarimeters at the same concentration. 1 g each of the extract was taken and dissolved in 10 ml of their corresponding solvent extract. The solution was poured in the length tube of polarimeter for measurement of optical activity. The concentration of the extract solution was expressed as concentration in g/dm^3=g/1000 ml [10].

2.2.3 Phytochemical screening

2.2.3.1 Test for alkaloids

To the extract (1.0 ml) in two separate test tubes, 3 drops of Dragendoriff's and Mayer's reagents were separately added. An orange red precipitate turbidity with dragendoriff or white precipitate with Mayer's reagent indicated the presence of alkaloids [10,11].

2.2.3.2 Test for steroids

2 ml of the extracts were taken into separate test tubes and evaporated by dryness. The residues were dissolved in acetic anhydride, chloroform was then added by means of a pipette followed by the addition of concentrated sulphuric acid by the side of the test tube. A brown ring at the interface indicated the presence of steroids [11].

2.2.3.3 Test for flavonoids

To 2 ml of 4 gm/ml each of the fractions, pieces of Magnesium ribbon were added followed by Conc. $HCl_{(aq)}$ drop wise. A color ranging from crimson to magenta indicated the presence of flavonoids [11].

2.2.3.4 Test for tannins

2 ml of the fraction was diluted with distilled water in a test tube, and 3 drops of 5% ferric chloride solution was added. A green-black or blue-black coloration indicated the presence of tannins.

2.2.3.5 Test for reducing sugars

1 ml of each fraction was taken in separate test tubes. The fractions were diluted with 2 ml of distilled water followed by addition of Fehling's solution (A+B) the mixture warmed. Brick-red precipitates at the bottom of the test tubes, indicated the presence of reducing sugars.

2.2.4 Biological study of on the extracts

2.2.4.1 Preparation sensitivity discs

Filter paper discs of 6 mm diameter were punched out from whatman no.1 filter paper using a paper puncher and placed in a sterile bijou bottles. The paper discs were subsequently sterilized by autoclaving at 121ºC for 15 min and allowed to cooled [12].

2.2.4.2 Sensitivity test

2.2.4.2.1 Preparation of test extracts

1 g of each crude extracts were separately dissolved in 1 mL of dimethylsulfoxide (DMSO) to make a stock solution of 1,000,000 µg/mL.0.1 ml of each of the solution was dissolved with 0.9ml of DMSO to make standard solution (i) which give concentration of 1,000 µg/ml. 0.2 Ml each of the stock solution was dissolved with 0.8 ml of DMSO to make standard solution.(ii) which gave concentration of 2,000 µg/m, 0.5 m each of the stock solution was dissolved with 0.5 ml of the DMSO (iii) which gave concentration of 5,000 µg/ml. 0.1 ml of each of the stock solution was dissolved with 0.9 ml of DMSO to make the standard solution (iv) which gave concentration of 10,000 µg/ml. These several concentration were prepared in bijou bottles for each extracts which 100 discs filter paper were introduced, with exception of stock solution [12].

2.2.4.2.2 Medium preparation

14 g of nutrient agar was weighed into 1litre conical flask. The nutrient agar was dissolved in 500 cm^3 of distilled water with vigorous mixing. The solution obtained was autoclaved for sterilization at 121ºC for 15mins. The sterilized medium (solution) was allowed to cool to 45ºC, which was then introduced into petridishes.

2.2.4.2.3 Inoculation

The inoculum was distributed evenly over the test plate by spreading the plate with the bacterial suspension. The uninoculated gap 3-4 cm wide were left to separate the test disc. Thus, four discs were tested at 37ºC for 24 hrs. After 24 hrs, the plates were observed for determination of diameter of inhibition zone.

3. RESULTS AND DISCUSSION

The phytochemical screening of stem bark of *A. leiocarpus* revealed the presence of alkaloid, steroids, flavonoids, tannins and sugars (Table 1). Similar studies using other parts of the plant suggest the presence of these bioactive compounds in the plant [13-17].

Phytochemicals exert their antimicrobial activity through different mechanisms as reported in this result (Tables 4 and 5). The antibacterial activity of flavonoids had been reported to result from their ability to form complexes with bacterial cell walls, extracellular and soluble proteins [18]. This fact support the usefulness of *A. leiocarpus* in the remedies of diarrhea and dysentery and it is one of the reasons why this plant is widely used for the treatment of many diseases among tribes in Africa.

Tannins act by iron deprivation, hydrogen bonding or specific interaction with proteins such as enzymes, cell envelopes and complex formation with polysaccharides [18-20]. Herbs that have tannins as their component are prevalent in nature and are used for treating intestinal disorders such as diarrhea and dysentery [21]. Thus exhibiting antimicrobial activity *A. leiocarpus* extract inhibited the growth of *E. coli* and thus supports the usefulness of this plant in treating diarrhea and dysentery among Yoruba tribe of Southwestern Nigeria [22-24].

Alkaloids was also found to be present in the stem bark extract of *A. leiocarpus*. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms [25]. The antibacterial properties of alkaloids have been reported to be as a result of their ability to intercalate with DNA [26]. Some kinds of steroids have been reported to have immune-enhancing benefits [26,27].

Table 1. Result of phytochemical screening of the stem bark extract of *Anogeissus leiocarpus*

Plant extract	Reducing sugar	Alkaloids	Tannins	Steroids	Flavonoids
Ethanol	+	-	+	+	+
n-hexane	+	+	-	+	-
Chloroform	-	+	+	-	-
Ethyl acetate	-	-	+	+	-
Distilled water	+	-	+	+	+

Where + indicate the presence of natural product; - Indicate absence of natural product

Table 2. Result of the soluble extracts obtained from stem bark of *Anogeissus leiocarpus*

Extract	Solvent (ml)	Weight (g)	Color obtained
Ethanol	1500	20.11	Dark brown
n-hexane	100	2.21	Yellow brown
Chloroform	100	3.56	Brown
Ethyl acetate	100	2.22	Pink
D. water	150	6.2	Brown

Table 3. Optical activity measurement

Plant extract	Degree of optical rotation	Temperature of the optical rotation (ºC)	Concentration of extract (g/ml)
Ethanol fraction	0.0^0	28.2	0.01
n-hexane fraction	0.0^0	28.2	0.01
Chloroform fraction	0.0^0	28.3	0.01
Ethylacetate fraction	$+0.1^0$	28.3	0.01
Distilled water fraction	-0.1^0	28.3	0.01

Table 4. Sensitivity test against *Escherichia coli* bacteria using stem bark extract of *Anogeissus leiocarpus*

Extract	Concentration (µg/ml)/zone of inhibition (mm)				Test organism
	10,000	2,000	5,000	10,000	
Ethanol extract (clo1)	8.00	10.00	16.00	12.00	
n-hexane extract (clo2)	0.00	0.00	10.00	0.00	*Escherichia*
Chloroform (clo3)	7.00	9.00	11.00	0.00	*coli*
Ethyl acetate (clo4)	0.00	0.00	0.00	0.00	
Distilled water extract (clo5)	0.00	9.00	8.00	9.00	

Table 5. Sensitivity test against Shigella dysentriae bacteria using stem bark extract of *Anogeissus leiocarpus*

Extract	Concentration (µg/ml)/zone of inhibition (mm)				Test organism
	1,000	2,000	5,000	10,000	
Ethanol extract (clo1)	10.00	9.00	16.00	10.00	
n-hexane extract (clo2)	0.00	0.00	11.00	8.00	*Shigella*
Chloroform extract (clo3)	7.00	9.00	11.00	8.00	*dysenteriae*
Ethylacetate extract (clo4)	9.00	0.00	5.00	8.00	
D.water extract (clo5)	7.00	0.00	12.00	12.00	

Optical activity test showed that ethanol, n-hexane and chloroform extracts were optically inactive while ethylacetate and distilled water extracts were found to be optically active (Table 3), being dextrorotatory and levorotatory respectively. Optical activity has been used in the study of configuration of compounds, mechanism of various reactions and also to decide between alternative structures for the given compounds.

Moreover, the sensitivity test of the extracts against *E. coli* showed that the ethanol and chloroform extracts were more active followed by distilled water and n-hexane, ethylacetate extracts being inactive against *E. coli* but active against *Shigella dysenteriae* (Table 2).

Taken together all these facts support the utilization of *A. leiocarpus* in various African countries such as Nigeria, Mali and Cote d'Ivoire to prepare local medications for the treatment of diseases.

4. CONCLUSION

According to our findings stem bark of *Anogeissus leiocarpus* can be used for the management of diarrhea and dysentery by virtue of their antimicrobial activity against *Escherichia coli (causative of* diarrhea) and *Shigella dysentriae (causative of* dysentery) respectively. However, hygiene and dosage need to be carefully taken into consideration prior to using the plant to prepare medication.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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