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Antimicrobial Activity of Stem Bark of *Faidherbia albida*

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Authors' contributions

This work was carried out in collaboration between all authors. Author WAU designed the study and made the final copy. Author SMJ did the laboratory work, manage the literature searches and wrote the first draft of the manuscript. Author ZHA performed the statistical analysis and improved on the draft. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

This study was aimed at evaluating the antimicrobial activity of stem bark extract of *Faidherbia albida* on *E. Coli, Salmonella typhi* and *Shigella sp*. The study was conducted in laboratory of Biochemistry department of Modibbo Adama University of Technology, Yola, Adamawa, Nigeria, between July and October, 2012. The antimicrobial activity was carried out using agar disc diffusion method and the crude extract was separated using column chromatography. The phytochemical analysis of the crude methanolic stem bark extract of *Faidherbia albid*a revealed the presence of tannins, saponins and alkaloids. The *in vitro* antimicrobial activity of the crude methanolic extract of the stem bark showed highest activity on *Salmonella typhi* with zone of inhibition of 12.0±0.17mm compared to *E. coli* and *Shigella sp*. Column chromatography of the crude extract eluted with benzene/methanol, acetic acid/methanol and ethylacetate/methanol gave three fractions designated: I, II and III; of which fraction III showed highest activity against the test organisms. Minimum inhibitory concentration of the most active fraction is 60mg/ml on *E. coli* and *Salmonella typhi* and 80mg/ml on *Shigella sp.*The *in vitro* antimicrobial activity of the crude methanolic stem bark extract of *Faidherbia albida* was also compared to some standard antibiotics. The results showed that azithromycin had highest activity ranging from 17.0 ± 0.11mm to 23.0 ± 0.12mm against the test organisms with no significance difference *(P<0.05)* compared to the activity of fraction III. There was also no significant

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(p<0.05) difference between the effect of the crude methanolic stem bark extract and that of cirprofloxacin with zone of inhibition ranging between 12.0±0.08mm to 20.0± 0.15.mm These results reveal the antibacterial nature of the stem bark of *Faidherbia albida* and suggest that it could be exploited in the management of diseases caused by the tested organisms in human.

Keywords: Faidherbia albida; phytochemical analysis; antimicrobial activity; E. coli, Salmonella typhi and Shigella sp.

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1. INTRODUCTION

Faidherbia albida belong to the family; *Mimosaceae* and is widely used in folk medicine in Africa. It is common and widely distributed across Senegal to Northern Nigeria, and extending from Sub-Saharan Africa to Egypt and in East Africa Southward to the Transvaal [1]. The common names of the plant include winter thorn and apple-ring acacia. The Hausa people of Northern Nigeria called it “Gawo” while in Fulfulde it is called “Chaski”. In Nigeria, an infusion of the bark is taken for fever, cough and to assist in child birth [2,3]. The plant is added to a portion to treat chest pain by the Fulanis of Nigeria [4]. A decoction of the bark is used in cleansing fresh wounds, in a manner similar to that of potassium permanganate, used as an emetic in fevers by the Masai people of East Africa, taken for diarrhoea in Tanganyika [5] and for colds, haemorrhage, leprosy and ophthalmic in West Africa.

The aqueous extract of *F. albida* possesses potent anti-pyretic, anti-inflammatory and anti-diarrheal effects and this pharmacologically justifies its folkloric use in the management of fever, rheumatic inflammatory conditions and diarrhoea [6]. In Northern Nigeria, West Africa, the cattle-rearing nomads take a decoction of the stem bark orally for the management of sleeping sickness (trypanosomiasis).

Anti-malarial activity of ethanolic stem bark extract of *F.albida* against early infection, curative and prophylactic effect in mice at safe doses was also reported [7]. They also reported that its oral median lethal dose was greater than 5000 mg/kg body weight, which suggests that orally administered stem bark extract of *F. albida* is practically non-toxic. This high safety profile may have been responsible for its wide spread use in different ethno therapeutic interventions [7].

This study was therefore designed to evaluate the antimicrobial activity of the crude methanolic stem bark extract and partially separated extracts of the stem bark.

2. MATERIALS AND METHODS

2.1 Plant Material

The stem bark of *F. albida* was collected around Yolde Pate Ward in Yola South Local Government, Adamawa State in June, 2012. Identified and authenticated at the Department of Plant Sciences, Modibbo Adama University of Technology, Yola, Adamawa State. Nigeria.

2.2 Test Organisms

The microorganisms *Escherichia coli*, *Salmonella typhi* and *Shigella sp* used were collected from Microbiology laboratory of Federal Medical Centre Yola and the department of Microbiology Modibbo Adama University of Technology, Yola Adamawa State.

2.3 Extract Preparation

The stem bark of *Faidherbia albida* was removed from the plant, washed and air-dried for 5-days at room temperature. The stem bark was pounded using pestle and mortar and the powder (150g) macerated in methanol (1000 ml) and left overnight. The mixture was filtered and evaporated using rotary evaporator.

2.4 Separation of the Crude Methanolic Stem Bark Extract of *Faidherbia albida*

Slurry was prepared by dissolving 30g silica gel in 100ml methanol: water (1:1) and packed in a column (1.5 x 30cm). The column was loaded with 15ml of the crude extract and sequentially eluted with benzene/methanol (9:1), and acetic acid/methanol (1:1) ethylacetate/methanol (19:1). The fractions were collected separately, concentrated under pressure using rotary evaporator [11].

2.5 Phytochemical Analysis

Chemical tests were conducted on the methanolic crude extract and the most active fraction to identify the constituents as described by Trease and Evans [8], Sofowora [9].

a) Test for Tannins:
About 0.5g of the extract was boiled in 10ml of water in a test tube and then filtered. A few drops of 0.1% Ferric chloride was added and observed for brownish green or a blue black colouration.

b) Test for Saponins:
One gram of the extract was boiled in 10ml of distilled water in a water bath and filtered. About 5ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which the formation of emulsion was observed.

c) Test for Flavonoids:
Five milliliters of dilute ammonia solution was added to a portion of the extract followed by addition of Conc. H_2SO_4. Observation of yellow colouration in the extract indicates the presence of flavonoids.

d) Test for Steroids:
Two millilitres of acetic anhydride was added to 0.5ml of the extract and then followed with 2ml H_2SO_4. The change of colour from violet to blue or green indicates the presence of steroids.

e) Test for Alkaloids:
About 0.5g of the extract was stirred with 5ml of 1% Hcl on the steam bath. The solution was filtered and 1ml of the filtrate was treated with 2 drops of picric acid. The turbidity of the filtrate on addition of picric acid indicates the presence of alkaloids.

f) Test for Terperoids:
Five milliliters of methanolic extract was mixed with 2ml of chloroform, 3ml of conc. H_2SO_4 was carefully added to form a layer. Observation of reddish brown colouration of the interface that was formed indicates the presence of terpenoids.

g) Test for Cardiac Glycosides:
Five milliliters of methanolic extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. About 1ml of conc. H_2SO_4 was added to the mixture. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides.

h) Test for Anthraquinones:
One gram of the extract was mixed with 10ml benzene in a conical flask and the mixture was filtered. To the filtrate, 5ml of 10% Ammonia solution was added and mixed thoroughly. The observation of a pink red or violet colour in the ammonia phase (bottom of the test tube) indicates the presence of anthraquinones.

2.6 Antimicrobial Activity Testing

The effect of the crude methanolic stem bark extract of *Faidherbia albida* was tested according to the method described by Emeruwa [10]. Wells were made on the surface of 19ml nutrient agar plates which was seeded with 0.1ml of 10^{-6} test organisms. Exactly 0.5ml of crude extract was aseptically introduced into the wells made. The plates were allowed to stand on the working bench for 30 minutes after which the plates were incubated for 24 hours at 37ºC in an incubator. The presence of zone of inhibition was regarded as positive and was expressed in terms of average diameter of the zone of inhibition. Similarly, the same method of Emeruwa [10] was employed in the case of different fractions obtained by separating the crude through the column chromatography.

2.7 Dilution and Assay of Standard Antibiotics

Two hundred and fifty (250) milligrams of each drug was dissolved in 1000ml of distilled water. Exactly 1ml 0f each was taken and serially diluted to 10^{-3} to achieve 30 µg which was used for the assay. The method of Emeruwa [10] was employed for the assay.

2.8 Determination of Minimum Inhibitory Concentration (MIC)

MIC of the crude methanolic stem bark extract of *Faidherbia albida* was determined by dilution of the crude to various concentrations of 40, 60, 80, 100 and 120 mg/ml respectively. Equal volume of crude extract and nutrient broth were mixed in a test tube. Specifically, 0.1ml of standardized inoculum ($1.0x10^{-4}$cfu/ml) was added in each tube. The tubes were incubated aerobically at 37ºC for 18-24 hrs. Tube containing extract and growth media without inoculum was used as control. The lowest concentration of the extract that produced no visible bacterial growth (no turbidity) when compared with the control tube was regarded as MIC [26]. Similarly, the same treatment was made with the most active fraction.

2.9 Statistical Analysis

Results were expressed as the Mean $\pm$Standard error of mean. Statistical analysis of data was carried out using student's t-test. Differences in mean were considered to be significant when $p<0.05$.

3. RESULTS

3.1 Recovered Crude Extract

The recovered crude extract after evaporation weighed 14.03 g which was stored at 5ºC until when required for use.

3.2 Phytochemical Analysis

The phytochemical analysis of both the crude methanolic stem bark extract of *Faidherbia albida* and that of most active fraction of the crude revealed the presence of Tannins, alkaloids, and saponins.

3.3 Effects of the Crude methanolic stem bark Extract of *Faidherbia albida* On the Test Organisms

The effect of the crude methanolic stem bark extract of *Faidherbia albida* was tested on *E. coli*, *Salmonella typhi* and *Shigella sp*. The extract showed highest activity of 12.0±0.17 mm on *Salmonella typhi* and lowest activity of 10.0±0.34 mm on *Shigella sp*. The result is presented in Table 1.

Table 1. Effects of the crude methanolic stem bark extract of *Faidherbia albida* on the test organisms

Test Organisms	Zone of Inhibition (mm)
E. coli	11.0 ± 0.12
Salmonella typhi	12.0 ± 0.17
Shigella sp.	10.0 ± 0.34

Value are expressed as mean ± SEM, n = 3

3.4 Effects of Different Fractions of the Crude methanolic stem bark Extract of *Faidherbia albida* on Test Organisms

The activity of different fractions of the crude extract was tested on *E. coli*, *Salmonella typhi* and *Shigella sp*. Of all the fractions, fraction III showed highest activity on the test organisms ranging from 14.0±0.06 to 23±0.21 mm. The result is shown in Table 2.

Table 2. Effects of different fractions of the crude methanolic stem bark extract of *Faidherbia albida* extract on the test organisms

Test organisms	Zone of Inhibition (mm)		
	Fraction I	Fraction II	Fraction III
E. coli	11.0 ± 0.21	21.0 ± 0.31	23.0 ± 0.21
Salmonella typhi	18.0 ± 0.06	20.0 ± 0.06	21.0 ± 0.17
Shigella sp.	13.0 ± 0.12	11.0 ± 0.12	14.0 ± 0.06

Values are expressed as Mean ± SEM, n=3
Fraction I = Benzene/methanol
Fraction II = Acetic acid/methanol.
Fraction III = Ethylacetate/methanol.

3.5 Effect of Some Standard Antibiotics Used Against the Test Organisms

The Activities of Azithromycin, ciprofloxacin and chloramphenicol which are some of the standard antibiotics used against *E. coli*, *Salmonella typhi* and *Shigella sp* were carried out and the result showed azithromycin had highest activity (Table 3).

Table 3. Effects of some standard Antibiotics used against the test organisms

Test organisms	AZM	CPF	CHL
E. coli	23.0± 0.12	17.0 ± 0.23	21.0± 0.12
Salmonella typhi	17.0 ± 0.11	20.0 ± 0.15	15.0 ± 0.12
Shigella sp.	17.0 ± 0.20	12.0 ± 0.08	15.0 ± 0.25

Values are expressed as Mean± SEM, n=3
AZM = Azithromycin,; CPF = Ciprofloxacin; CHL = Chloramphenicol

3.6 Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration of crude extract is 80mg/ml on *E. coli* and *Salmonella typhi*, 100mg/ml on *Shigella sp.* While the MIC of most active fraction is 60mg/ml on *E. coli* and *Salmonella typhi* and 80mg/ml on *Shigella sp*. The result is presented in Tables 4 and 5.

Table 4. Minimum Inhibitory concentration (MIC) of the crude methanolic stem bark extract of *Faidherbia albida* on the test organisms

Test organisms	Concentration of extracts in mg/ml.				
	40mg/ml	60mg/ml	80mg/ml	100mg/ml	120mg/ml
E. coli	+	+	−	−	−
Salmonella typhi	+	+	−	−	−
Shigella sp.	+	+	+	−	−

+= Indicates growth; − = Indicates no growth

Table 5. Minimum inhibitoy concentration (MIC) of the active fraction of the crude methanolic stem bark extract of *Faidherbia albida* on the test organisms

Test organisms	Concentration of extracts in mg/ml.				
	40mg/ml	60mg/ml	80mg/ml	100mg/ml	120mg/ml
E. coli	+	−	−	−	−
Salmonella typhi	+	−	−	−	−
Shigella sp.	+	+	−	−	−

+= Indicates growth; − = Indicates no growth

4. DISCUSSION

The phytochemical analysis of the crude methanolic extract of stem bark of *Faidherbia albida* revealed the presence of tannins, saponins and alkaloids. This finding is in agreement with that of Tijani et al. [6]. These phytochemicals are also present in the most active fraction of the crude extract. The antimicrobial activity exhibited by plant could be due to the presence of potent phytoconstituents in the extract [12].

Tannins have been reported to hasten the healing of wound, inflamed mucus membrane and to arrest bleeding. Plants containing tannins are astringent in nature and are used in the treatment of intestinal disorders such as diarrhoea and dysentery therefore showing antibacterial activity [13].

The antibacterial effect of crude methanolic stem bark extract of *Faidherbia albida* is attributed to the presence of tannins, the phytochemical which is reported to inhibit bacterial growth and protease activity by damaging the cell and cytoplasm, causing rapid structural destruction [14,15].

The presence of alkaloids in the crude methanolic stem bark extract of *Faidherbia albida* have also contributed to its antimicrobial effect because alkaloids have been reported to have antimicrobial and antiparasitic properties [16]. About 300 alkaloids showing such activity was also reported [17]. The alkaloids berberine and palmatine are known to inhibit the multiplication of bacteria, fungi and viruses [18].

Fractionation in some cases may result in improved activity but in others may result in loss of activity [19]. In this case fractionation of the crude methanolic stem bark extract of *Faidhebia albida* extract resulted in improved activity because when the activity of the crude methanolic stem bark extract and that of the different fractions collected was compared, there was a significant improvement (Tables 1 and 2). The observation that fraction III had highest activity is a good indication that the active ingredient of the crude methanolic stem bark extract of *Faidhebia albida* is contained in fraction III. This is also in agreement with the view of Nwodo, *et al* [20] who stated that purification of crude extracts could produce loss or gain of activity depending on the nature of interaction between the constituent compounds of the extract.

The minimum inhibitory concentration of the crude methanolic stem bark extract of *Faidhebia albida* and most active fraction were determined (Tables 4 and 5), this observation further confirmed that the fractionation of the crude extract has increased the activity of the extract. MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism. Clinically, the minimum inhibitory concentrations are used not only to determine the amount of antibiotic that the patient will receive but also the type of antibiotic used, which in turn lowers the opportunity for microbial resistance to specific antimicrobial agents [21].

The zones of inhibition of fraction III and that of Azithromycin statistically have no significance difference (Tables 3 and 5). This observation is attributed to the presence of some bioactive compounds or groups in the fraction with similar mechanism of action to that of the standard drug.

5. CONCLUSION

In conclusion, the present studies clearly reveals the antibacterial nature of the stem bark of *Faidherbia albida* and suggests that it could be exploited in the management of diseases caused by the tested organisms in humans. The mechanism of action of constituents of stem bark *Faidherbia albida* is difficult to speculate: however, many antibacterial agents exhibit their action through inhibition of nucleic acid, protein and membrane phospholipids biosynthesis [22]. The antibacterial agents in the extract of stem bark of *Faidherbia albida* act via some of the above mentioned mechanisms. Further study on the structural elucidation of the components of the extract is therefore recommended

CONSENT

Not applicable.

ETHICAL APPROVAL

The authors hereby declare that all experiments have been examined and approved by the Appropriate ethics committee and have been performed in accordance with the ethical Standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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