Potential Antibacterial Activity of Two Important Local Chewing Sticks "*Fagara zanthoxyloides* and *Distemonanthus benthamianus*" along with Antioxidant Capacities

A.O. Shittu[1], A. Aliyu[2], M.S David[2], N.S Njinga[3] and H.I Ishaq[2]

[1]Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmaceutical Sciences University of Ilorin
[2]Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences University of Ilorin
[3]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences University of Ilorin

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ABSTRACT: In order to authenticate and ascertain the various claim by the rural dwellers that depend on chewing sticks for their oral hygiene and protection against innumerable diseases, the antibacterial activity of the roots of *Fagara zanthoxyloides* and *Distemonanthus benthamianus* were evaluated. Preliminary phytochemical screening was carried out using standard methods. *In-vitro* antibacterial activity of the methanol extracts of both plants was also carried out using the agar well diffusion method against standard strains of *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Salmonella typhii* ATCC 14028, *Citrobacter freundii* ATCC 8090 and also some clinical isolates of *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae* and *Proteus mirabilis* at different concentrations and ratios. Preliminary phytochemical screening showed that the extracts contained alkaloids, saponin, tannins, steroids, flavonoids, anthraquinones, cardiac glycosides and phenol. All the test microorganisms were susceptible to the inhibitory effect of the extracts at concentrations of 200 mg/ml and 500 mg/ml. *F. zanthoxyloides* had better antimicrobial activity with zones of inhibition ranging from 21.0 to 26.0 mm at 200 mg/ml and 24.3 to 29.3 mm at 500 mg/ml while *D. benthamianus* had zones of inhibition ranging from 17.7 to 26.7 mm at 200 mg/mL and 19.0 to 26.7 mm at 500 mg/mL. Some of the test microorganisms were resistant to the standard antibiotics (cefuroxime, ciprofloxacin, ofloxacin and gentamicin). Combinations of the extracts in ratios 50:50 and 75:25 yielded no increase in activity. The MIC and MBC for *D. benthamianus* ranged from 6.25 mg/ml to 100 mg/mL and 25 to 200 mg/ml, respectively while that of *F. zanthoxyloides* ranged from 1.56 mg/ml to 12.5 mg/mL and 50 to 200 mg/ml, respectively. The antimicrobial activity demonstrated by *F. zanthoxyloides* and *D. benthamianus* indicates that they would be valuable in the management of urinary, respiratory and gastrointestinal tracts infections while at the same time helping to mitigate the problem of antimicrobial resistance. Phenolic compounds of plants, among them flavonoids are the chief constituents, which have potent antioxidant activities.

Key words: Antimicrobial, chewing sticks, gastrointestinal, respiratory, urinary, oro-dental pathogens

INTRODUCTION

The development of resistance by microorganisms to chemical agents remains a serious health problem.[1] The continuous increase in the incidence of new and re-emerging infectious diseases and the resistance of microorganisms to existing antimicrobial agents has become a major health concern.[2] Resistance limits antibiotic choice available to prescribers, and sometimes leads to the choice more toxic or more expensive drugs being used. Furthermore, the development of new antibiotics that offer significant benefits over existing drugs is lacking.[3] The gram-negative bacteria infections are

Correspondence to: A.O Shittu
E-mail, neobiogate@yahoo.com

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difficult to treat due to the fact that they possess an additional outer membrane that constitutes permeability barrier with multiple efflux pumps and antibiotic modifying enzymes. At present the clinical development of potential drugs against gram-negative nosocomial infections that are significantly better than the existing drugs are still limited.[3]

There is a need to discover new antimicrobial compounds with novel mechanisms of action and increased therapeutic and prophylactic activity. Since the discovery of multidrug resistance in bacteria, the efficacy of some common antimicrobials is falling but some medicinal plants can offer the alternatives.

Plants are a major source of medicines, as they have been used throughout human history. According to the world health organization (WHO), about three-quarters of the world population relies upon traditional method of healing using mainly herbs for the health care of its people.[4] Many traditional healing herbs and their parts have been shown to have medicinal value and can be used to prevent, alleviate, or cure several human diseases.[5]

F. zanthoxyloides Lam also known as *Z. zanthoxyloides* Lam, is an indigenous plant used widely as chewing stick for tooth cleaning in West Africa.[6] It imparts a tingling, peppery taste and numbness when used to brush the teeth. Its antimicrobial activity has been shown to be due to the alkaloids (berberine, chelerythrine and canthin-6-one) which are most active at pH 7.5 (or during tooth decay) and simple benzoic acid derivatives which are most active at pH 5.[7]

D. benthamianus Baill is one of the perennial trees of the evergreen, semi-deciduous and secondary forest of West Africa tropics mainly in the Cameroon, Ghana and Nigeria.[8] It is used traditionally for the treatment of bronchitis, rheumatism, fever, as a laxative and the dye is used as a pain killer. *D. benthamianus* is used in traditional African medicine to treat bacterial, fungal and viral infections,[9] and it is used as chewing stick for oro-dental hygiene.[10,11]

The need for newer, novel and effective antimicrobial agents that would combat the burden of antimicrobial resistance and treat infectious diseases has necessitated the need to turn to medicinal plants which are readily abundant, cheap and efficacious as possible sources of antimicrobial agents. Recent research on these plants and their extracts has focused on their effect on bacterial organisms *Streptococcus mutans*, *Streptococcus mitis* and oral anaerobes, which are the organisms commonly implicated in dental caries and orodental infections. Not much work have been published on their effect on bacterial pathogens that are implicated in urinary tract, respiratory tract and gastrointestinal tract infections, which are the organisms rapidly developing resistance to existing antibiotics.

METHODS

Collection of plant materials. Roots of *F. zanthoxyloides* and *D. benthamianus* used in this study were obtained from Lafenwa Market, Abeokuta, Nigeria. Identification and authentication of the plants were carried out at the Herbarium Unit of the Department of Plant Biology, University of Ilorin, Nigeria with voucher numbers UILH/001/1110 and UILH/001/1241 assigned respectively to the plants. The dried samples were then grinded well into a fine powder with a grinder. The powders were then stored in air tight containers at room temperature for further use.

Extraction of plant materials. Extraction was carried out using a modification of the method described by Agbulu *et al.*[12] 1.0 kg of the fine powder of *F. zanthoxyloides* was macerated with 4 liters of methanol while 0.6 kg of *D. benthamianus* was macerated with 2.4 liters of methanol at room temperature for 72 hours with occasional agitation. Filtration using a Whatman No 1 filter paper was carried out and the solvent evaporated using rotary evaporator (BUCHI Rotavapor R-124, Switzerland), to obtain the methanol crude extracts of both plants. The extracts were freeze dried (U-Therm International (H.K.) Limited), weighed and stored appropriately.

Preparation of culture media. The culture media used include Nutrient Agar (NA-Oxoid), Nutrient Broth (NB-Oxoid) and Mueller Hinton Agar (MHA- Oxoid). Each medium was prepared according to the manufacturer's instructions. The media were then sterilized using an autoclave at 121ºC for 15 minutes. They were then kept in a refrigerator at 4ºC until needed**.**

Phytochemical screening. Qualitative phytochemical screening of the extracts was carried out for the presence of saponins, tannins, flavonoids, glycosides, steroids, anthraquinones, phenols and alkaloids using the methods described by Adebiyi and Sofowora.[13]

Typed and clinical isolates. The test microorganisms used which comprised of both standard strains of *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and clinical isolates of *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae* and *Proteus mirabilis* were collected from Department of Microbiology and Parasitology, University of Ilorin Teaching Hospital. Standard strains of *Salmonella typhii* ATCC 14028 and *Citrobacter freundii* ATCC 8090 were obtained from the Department of Pharmaceutical Microbiology, University of Ibadan, Nigeria.

Preparation of plant extracts. Two grams (2.0 g) and five grams (5.0 g) each of the methanolic extracts of *F. zanthoxyloides* and *D. benthamianus,* were weighed using an analytical weighing balance and dissolved in 10 mL of Dimethyl-sulfoxide (DMSO) to obtain concentrations of 200 mg/mL and 500 mg/mL respectively.

Antimicrobial screening of the extracts against test microorganisms. The antimicrobial activity of the methanol extracts of *F. zanthoxyloides* and *D. benthamianus* were determined using the agar diffusion method. A suspension of an overnight culture of each test microorganism in normal saline was standardized to 0.5 McFarland turbidity. One milliliter (1 ml) of the standardized inoculum was added to 19 ml of molten Mueller Hinton Agar, transferred into sterile petri dishes and allowed to set. Wells were made using a sterile number 4 cork borer into the seeded Mueller Hinton agar plates. The base of each well was sealed with two drops of molten Mueller Hinton agar and allowed to set. Each well was filled with 100 µl of the different concentrations of the plant extracts and DMSO as the negative control. The plates were allowed to stand for 1 hr to allow for pre-diffusion of the extracts before incubating for 18 hrs at 37°C. The diameter of zone of inhibition was observed and measured in millimeter using a calibrated meter rule. Experiment was carried out in triplicates for each test organism and the mean values were computed.

The above process was repeated but with the wells filled with a combination of the *F. zanthoxyloides* and *D. benthamianus* extracts combined in ratios 50:50 and 75:25 at concentrations of 200 mg/mL and 500 mg/ml.

Standard antibiotics susceptibility testing. The test organisms were screened for antibiotic susceptibility using a modified Kirby-Bauer agar disc diffusion method. The antibiotic discs namely Ciprofloxacin (5 µg), gentamicin (10 µg), ofloxacin (5 µg) and Cefuroxime (30 µg) were used. The experiment was carried out in triplicates and results obtained were then interpreted using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) Breakpoint tables for interpretation of MICs and zone diameters, 2016.

MIC AND MBC determination of the extracts. The minimum inhibitory concentration (MIC) was carried out using a modification of the classical method of serial dilution described by Mazzola and his associates.[14] In twelve (12) sterile screw tubes, 1 mL of nutrient broth (NB) was distributed in to every tube except for tube number 1. For the first and second tubes of the series, 1 ml of the methanol extract of *F. zanthoxyloides* at 200 mg/ml was added; tube 2 was stirred and 1 ml was withdrawn and transferred into tube 3. This successive transference was repeated until tube 11. As much as 100 µl of the inoculum was added to all tubes except for tube 12 using a micropipette.

Incubation was carried out at 37°C for 24 hrs. Controls were made using tubes 11 and 12 as positive (Nutrient broth and inoculum) and negative (Nutrient broth and antimicrobial) controls. After this period, reading was carried out and the tube with the least concentration that does not permit any growth when compared with the control was considered as the minimum inhibitory concentration (MIC). The above was repeated for the methanol extract of *D. benthamianus* at 200 mg/ml.

The minimum bactericidal concentration (MBC) of the plant extracts was determined by a modification of the method of Spencer and Spencer.[15] Samples were taken from the tubes with no visible turbidity (growth) and the first tube showing growth in the MIC assay and subcultured onto freshly prepared Mueller Hinton agar plates. After incubation for 24 hrs at 37°C, the minimum bactericidal concentration was taken as the lowest concentration of the extract that prevents growth on the surface of the agar plates.

Quantitative analysis

Determination of total phenolics. Total phenolic contents of the methanolic extracts were evaluated with Folin-Ciocalteu's phenol reagent.[16,17] 2.0 mg/ml of the extract solution in methanol was mixed with 2.0 ml Folin-Ciocalteu reagent previously diluted with water (1:9 v/v). After 5 minutes, 1.6 mL of 7 % Na_2CO_3 solution was added with mixing. The tubes were shaken for 5 seconds and allowed to stand for 30 min at 40°C in an oven for color development. Absorbance was then measured at 765 nm using UV-vis spectrophoto-meter. Samples of extract were evaluated at a final concentration of 0.01 mg/ml. Gallic acid at different concentrations of 0.01 to 0.07 mg/ml was used as standard. All tests were performed in triplicates.

Total phenolic content was expressed as mg/g gallic acid equivalent using the following equation based on the calibration curve: $y = 1.6232x$, $R^2 = 0.6658$, where y was the absorbance and x was the concentration.

Determination of total flavonoids. Colorimetric aluminum chloride method was used for flavonoid determination of each extract according to the methods of Ebrahimzadeh associates[18] and Nabavi associates[17] with some modifications. 3.0 mL solution of each extract dissolved in methanol was separately mixed with 3.0 mL of 2.0% aluminum chloride. After one hour at room temperature, the absorbance was measured at 420 nm. Extracts were evaluated at a final concentration of 0.1 mg/ml. Quercetin was used as standard. All tests were performed in triplicates. Total flavonoid content was calculated as quercetin equivalents (mg/g) using the following equation based on the calibration curve: $y = 0.0.23x$, $R^2 = 0.9424$, where y was the absorbance and x was the concentration.

Saponin determination. The saponin content in each plant extract was estimated as described by Kim and his co-workers[19] with some modifications. Each of the extracts (50.0 mg) was separately dissolved in 1 ml of methanol and separated in two equal halves in two different separating funnel. In each test tube, 5 ml of distilled water was added, and then 2 ml of diethyl ether was also added and shaken vigorously. The ether layer was discarded, while the purification process was repeated. 2.0 ml of n-butanol was added and shaken, then allowed to stand for separation after which the *n*-butanol layer was then collected. This process was repeated exhaustively. The n-butanol extracts were washed twice with 2.0 ml of 5.0 % aqueous NaCl after which it was dried over a water bath at 40°C. After evaporation, the samples were dried in the oven to obtain a constant weight. The test was carried out in duplicates. The saponin content was calculated according to the equation:

$$\text{Amount of saponin } (\text{mg/g}) = \frac{\text{Weight of residue}}{\text{Weight of sample}}$$

Alkaloids determination. The method of Obadoni and Ochuko[20] was used in which 3 g of the powdered sample was weighed into 150 ml of 20% acetic acid in ethanol and allowed for 4 h. The mixture was filtered and the filtrate concentrated using a water bath at 55°C to one-quarter of its original volume. Concentrated NH_4OH was added drop wise into the extract until precipitation was

complete. The whole solution was allowed to settle and then filtered. The precipitate which is the crude alkaloid was washed with dilute NH_4OH solution. The crude alkaloid was weighed and calculated according to the equation:

$$\% \text{ amount of alkaloid (mg/g)} = \frac{\text{Weight of precipitate}}{\text{Weight of sample}} \text{ x100}$$

Antioxidant assay

Assay of DPPH scavenging activity. The DPPH radical-scavenging activity of the test extracts was examined as described by Ebrahimzadeh associates[18] with some modifications. Different concentrations (0.03 - 0.1 µg/ml) of each extract were separately added, to an equal volume of methanolic solution of DPPH (100 µM). The mixture was allowed to react at room temperature in the dark for 30 minutes. Vitamin C was used as standard control while a mixture without the extract was taken as blank. After 30 minutes, the absorbance (A) was measured at 518 nm. Each test was carried out in duplicates converted into the percentage antioxidant activity using the following equation:

$$\% \text{ scavenged} = \frac{\text{Absorbance (DPPH)} - \text{Absorbance (Extract)}}{\text{Absorbance (DPPH)}} \times 100$$

The IC_{50} values of each fractions were calculated by nonlinear regression using graph pad prism. Where the abscissa represented the concentration of fractionated extract and the ordinate represented the average percent of scavenging capacity from the triplicates.

RESULTS

Extraction of the plant materials yielded 22.49 g (2.25%) for methanolic extract of *F. zanthoxyloides* and 77.00 g (12.83%) for methanolic extract of *D. benthamianus.* Qualitative phytochemical screening of the extracts revealed the presence of tannins, saponins, flavonoids and alkaloids for both the methanol extracts of *D. benthamianus* and *F. zanthoxyloides,* and anthraquinone is absent in the plants as presented in table 1.

The susceptibility test of the organisms used against the extracts and standard antibiotics are presented in tables 2. There were no zones of inhibition with DMSO against all the organisms tested. Higher zones of inhibition were recorded for higher concentrations of the extract against the organisms tested. At 200 mg/ml, FZ extracts gave slightly increased zones as compared with DB extract except for *Staphylococcus aureus* and *Salmonella typhi* ATCC 14028 while for 500 mg/ml extract of FZ gave much higher zones against all the organisms (except in *P. miribilis*) as against the DB extract. DB extract exhibited highest inhibition against *Proteus mirabilis* at both concentrations while *Pseudomonas aeruginosa* was most susceptible to the inhibitory activity of FZ extracts.

The antibacterial activities of standard antibiotics against test organisms are also presented in table 2. Ofloxacin produces zone of inhibition of *Staphylococcus aureus* and had highest zones of inhibition of 31.0 ± 4.4 mm against *Citrobacter freundii* ATCC 8090. Generally, Ofloxacin gave better zone inhibition than cefuroxime against all the organisms tested except on clinical sample of *Escherichia coli* where cefuroxime had better zone of inhibition. The zones produced by these standard antibiotics are however lower than those produced by the extracts except in the activity of Ofloxacin against *Citrobacter freundii* and *Escherichia coli* ATCC 25923. Ciprofloxacin exhibited better activity against *Pseudomonas aeruginosa* ATCC 27853 than the clinical isolate *Pseudomonas aeruginosa* and is slightly higher than 29.3 ± 6 mm inhibition zone produced by FZ (500 mg/ml).

The ratios of MBC and MIC of the DB and FZ are presented in table 3. The MBC: MIC ratio of DB is very high against *Escherichia coli* ATCC 25922 while that of other organisms used are low. Activity against *Salmonella typhii* ATCC 14028 and P*roteus mirabilis* had same MBC and MIC. In the case of FZ, the MBC is very high when compared with the MIC

and would be more of a bacteriostatic agent at the concentrations used. The activity index of the DB extracts is greater than 1 in 40% of the organisms tested while 50% of the organisms exhibited higher zones of inhibition when compared with the standard organisms as presented in table 3.

Table 1. Qualitative phytochemical screening of the methanolic extracts of *D. benthamianus* and *F. zanthoxyloides.*

Active principles	Test	DB	FZ
Alkaloids	Hager reagent	+	+
	Wagner reagent	+	+
Saponin	Frothing test	+	+
Tannins	Ferric chloride test	+	+
Steroids	Salkowski test	+	+
Flavonoids	Shinoda test	+	+
Anthraquinones	Borntrager test	-	-
Cardiac glycosides	Keller killiani test	+	+
Phenol	Ferric chloride test	+	+

+ = Present, DB: *Distemonanthus benthamianus*, - = Absent, FZ: *Fagara zanthoxyloides*

Table 2. Antimicrobial activity of the methanol extracts of *D. benthamianus* (DB), *F. zanthoxyloides* (FZ) and standard antibiotics.

Test organism	Zone of inhibition of the plant extracts and standard antibiotics (mm)							
	DB (200 mg/ml)	DB (500 mg/ml)	FZ (200 mg/ml)	FZ (500 mg/ml)	CXM (30 µg)	OFX (5 µg)	CIP (5 µg)	GEN (10 µg)
Staphylococcus aureus	23.3 ± 0.6	24.7 ± 1.2	23.3 ± 0.6	25.7 ± 0.6	0.0 ± 0.0	24.3 ± 0.6	NT	NT
S. aureus ATCC 25923	18.7 ± 1.2	21.3 ± 2.5	21.0 ± 1.0	24.3 ± 1.5	10.0 ± 1.0	22.3 ± 5.5	NT	NT
Pseudomonas aeruginosa	23.0 ± 1.0	25.7 ± 0.6	26.0 ± 2.6	29.3 ± 0.6	NT	NT	16.7± 2.1	0.0 ± 0.0
P. aeruginosa ATCC 27853	19.3 ± 0.6	21.3 ± 0.6	24.0 ± 1.0	25.7 ± 0.6	NT	NT	31.0 ± 1.0	23.3± 0.6
Escherichia coli	23.0 ± 1.0	25.3 ± 0.6	25.3 ± 2.5	29.0 ± 1.0	9.7 ± 0.6	0.0 ± 0.0	NT	NT
E. coli ATCC 25922	19.0 ± 1.0	21.7 ± 0.6	25.0 ± 2.0	27.3 ± 1.21	6.0 ± 1.0	28.3 ± 0.6	NT	NT
Citrobacter freundii ATCC 8090	18.0 ± 1.0	19.0 ± 1.0	20.7 ± 1.2	27.0 ± 2.61	7.7 ± 0.6	31.0 ± 4.4	NT	NT
Salmonella typhii ATCC 14028	22.0 ± 2.6	23.7 ± 2.3	21.0 ± 1.0	26.0 ± 2.0	10.7 ± 1.2	29.3 ± 1.5	NT	NT
Klebsiella pneumoniae	17.7 ± 1.2	22.3 ± 1.2	22.3 ± 0.6	24.3 ± 1.2	0.0 ± 0.0	0.0 ± 0.0	NT	NT
Proteus mirabilis	26.7 ± 1.5	29.0 ± 1.0	24.3 ± 0.6	28.0 ± 0.0	20.7 ± 0.6	22.7 ± 2.3	NT	NT

DMSO: as control shows no zone of inhibition.
CXM – Cefuroxime; CIP – Ciprofloxacin; GN – Gentamicin; OFX – Ofloxacin; NT – No zone of inhibition.

The plant FZ (Table 4) showed considerable levels of saponins, alkaloids, flavonoids as well as phenols.

The percentage saponins in DB (Table 4) was very low as well as the percentage alkaloids. The total phenolic content was considerable.

Flavonoids have been shown to reduce free radicals by up-regulating, quenching and chelating radical intermediate compounds.[21] Phenolic compounds of plants fall into several categories; chief among these are the flavonoids which have potent antioxidant activities.[22]

IC50 is defined as the concentration (µg/ml) sufficient to obtain 50% of a maximum scavenging capacity.

R^2 is coefficient of determination; values obtained from regression lines with 95% confidence level.

The smaller the IC_{50}, the greater the antioxidant activity. Thus FZ has a very high antioxidant activity. This correlates with the high flavonoids present in the extracts as shown in table 5.

Table 3. The Minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) for *D. benthamianus*, *F. zanthoxyloides* and activity index of their extracts.

Test organism	*D. benthamianus*			*F. zanthoxyloids*		Activity index of the extracts		
	MIC (mg/ml)	MBC (mg/ml)	MBC/MIC (mg/ml)	MIC (mg/ml)	MBC	MBC/MIC	DB Extract	FZ Extract
Staphylococcus aureus	12.5			6.25			1.02	1.06
S. aureus ATCC 25923	12.5	25.0	2	3.12	>200.0	64	0.96	1.09
Pseudomonas aeruginosa	25.0	>200.0	8	3.12			1.54	1.75
P. aeruginosa ATCC 27853	25.0	>200.0	8	12.5	>200.0	16	0.69	0.83
Escherichia coli	12.5	100.0	8	3.12	>200.0	642.61	2.99	
E. coli ATCC 25922	6.25	>200.0	32	1.56			0.77	0.96
Citrobacter freundii ATCC 8090	6.25	25.0	4	1.56	100.0	64	0.61	0.87
Salmonella typhii ATCC 14028	100.0	100.0	1	3.12	>200.0	64	0.81	0.89
Klebsiella pneumoniae	12.5	50.0	4	6.25	100.0	16	-	-
Proteus mirabilis	25.0	25.0	1	6.25	50.0	8	1.28	1.23

Note: The highest mean zones of inhibition of the plant extracts and standard antibiotics were used in the activity index.

Table 4. Total saponin, alkaloid, flavonoid and phenol present in FZ and DB.

Plant extract	% Saponins	% Alkaloid	Flavonoid (mg/g Quercetin)	Phenol (mg/g Galic acid)
FZ	15.095	14.53	0.290	2.426
DB	3.65	7.88	0.196	2.592

Table 5. Scavenging activity of FZ and DM methanol extracts.

	FZ mg/ml	DM mg/ml
IC_{50}	0.03965	0.05328
R square	0.9488	0.9646

DISCUSSION

Well known examples of plant active principles with antimicrobial properties include phenols, unsaturated lactones, saponins, cyanogenic glycosides, glucosinates, alkaloids, tannins, linoleic and stearic acids.[23] Phytochemical screening of *F. zanthoxyloides* indicated the presence of alkaloids, tannins, saponins and glycosides which is in line with the work of Banso and Ngbede.[23] The presence of alkaloids in *F. zanthoxyloides* confirmed the report of Elujoba and associates.[7] which described the main active ingredients in *F. zanthoxyloides* as alkaloids: berberine, fagaronine, chelerythrine, canthin-6-one and benzoic acid derivatives. The antimicrobial activities of the methanol extracts of *D. benthamianus* and *F. zanthoxyloides* could be attributed to the presence of some of these active constituents in the plants.

The organisms tested in this study have been implicated to cause gastrointestinal, respiratory tract and urinary tract infections. A few earlier reports have also demonstrated the antimicrobial properties of *D. benthamianus.*[24,8,9] *D. benthamianus* was observed to inhibit the growth of *Pseudomonas aeruginosa* ATCC 27853 which is in conformation with the work of Bankole and his co-workers.[25] The antibacterial activity of the extract was shown to be greater at higher concentration. This finding is in line with a previous report by Christiana and associates[26] where the higher concentrations showed appreciable growth inhibition. The Minimum Inhibitory Concentration (MIC) for *Pseudomonas aeruginosa* ATCC 27853 was 25 mg/ml which is in contrast to the report of Kareem and associates.[24] where the MIC was 50 mg/mL although the ethanol extract was used. *D. benthamianus* appeared to have a better antimicrobial activity compared to standard cefuroxime which is an indication of its great potential as a novel antibacterial agent with a broad

spectrum of activity against both Gram positive and Gram negative bacteria.

Statistical analysis (T-test) showed that there was no significant difference between the two concentrations of *D. benthamianus* used in this study.

Staphylococcus aureus, Pseudmonas aeruginosa, Klebsiella pneumoniae and Escherichia coli were susceptible to the methanolic extract of *F. zantho-xyloides* which is in agreement with the work of Agbulu and his team.[12] Statistical analysis showed that there was a significant difference in antimicrobial activity between the two concentrations (200 mg/ml and 500 mg/ml) of *F. zanthoxyloides* used.

The combination of the concentrations of the extracts (50:50 and 75:25) did not show any appreciable increase in antimicrobial activity, rather it showed slight inhibitory effect on all the pathogens tested which may be due to some antagonistic effects.

The susceptibility profiles of the test microorganisms showed that there is a high degree of resistance to the standard antibiotics (cefuroxime, ofloxacin, ciprofloxacin and gentamicin) especially cefuroxime as 80% organisms tested were resistant to it.

The methanol extract of *F. zanthoxyloides* showed the highest activity index of 2.99 against *Escherichia coli*, suggesting that its antimicrobial activity is almost thrice that of the standard antibiotic (cefuroxime) for the strain under consideration as it is not so for *Escherichia coli* ATCC 25922 with an activity index of 1.70 for cefuroxime and 0.96 for ofloxacin.

A ratio of MBC to MIC greater than 4 defines bacteriostatic activity.[27] For *D. benthamianus*, the ratio of MBC to MIC indicates that the extract has bacteriostatic activity against *Pseudomonas aeruginosa*, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* and *Escherichia coli* ATCC 25922. The extract had the best bactericidal activity against *Staphylococcus aureus* ATCC 25923, *Citrobacter freundii* ATCC 8090 and *Proteus mirabilis* as the extract inhibited growth and caused death of the organisms at relatively low concentrations ranging from 6.25 to 25.0 mg/ml.

F. zanthoxyloides had low MIC values but high MBC values and for some organisms the extract failed to cause death within the range of concentrations tested.

Hagerman and Butler.[28] reported that tannins have been shown to form irreversible complexes with proline-rich proteins which would lead to inhibition of cell-wall-protein synthesis, a property that may explain the mode of action of these two extracts. Parekh and Chanda.[29] also reported that tannins possess broad antimicrobial properties by means of different mechanisms that include enzyme inhibition, oxidative phosphorylation reduction and iron deprivation, among others. It is believed that the tannin present in African chewing stick (such as *F. zanthoxyloides* and *D. benthamianus*) is responsible for the antibacterial effect. Many plants contain non-toxic glycosides which can get hydrolyzed to release phenolics which are toxic to microbial pathogen.[30]

In this present work, the plant extract, *F. zanthoxyloides* (Table 4) showed considerable levels of saponins, alkaloids, flavonoids as well as phenols.

The percentage saponins and alkaloids in *D. benthamianus* (Table 4) were found to be lower, while the total phenolic content was considerable.

Phenolic compounds of plants are grouped into several categories, e.g., saponn, flavonoids, and tannins. Of these categories, flavonoids are the most important due to their potent antioxidant activities.[22] The flavonoids exert their antioxidant activity by quenching, regulating, and chelating radical intermediate compounds.[21] FZ and DM both show high antioxidant activities which may also exert their antioxidant activities through same routes.

CONCLUSION

The methanol extracts of the roots of *Fagara zanthoxyloides* and *D. benthamianus* have exhibited significant antibacterial activity against some of the gram-positive and gram-negative microorganisms implicated in the pathogenesis of respiratory tract, gastrointestinal tract and urinary tract infections. Hence, this may give rise to a therapeutic opportunity in the management and treatment of respiratory tract,

gastrointestinal tract and urinary tract infections and even oro-dental infections especially with the increasing incidence of resistance to the existing antibiotics.

CONFLICT OF INTEREST

There was no any conflict of interest whatsoever, and every sited phrase and reference has been dully acknowledged.

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