

Potential Antibacterial Activity of the extracts of Local Chewing Sticks “*Fagara zanthoxyloides* and *Distemonanthus benthamianus*” against Gastrointestinal, Respiratory and Urinary Pathogens

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ABSTRACT

Background: The development of new antibacterial that offer significant activities over existing drugs is serious challenge to the health managers.

Objective: This study aims at investigating the possible activity of *Fagara zanthoxyloides* and *Distemonanthus benthamianus* roots extract against selected microorganism.

Methods: 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.

Results: 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. *Fagara 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 *Distemonanthus 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) which were tested against. Combinations of the extracts in ratios 50:50 and 75:25 yielded no increase in activity. The MIC and MBC for *Distemonanthus benthamianus* ranged from 6.25 mg/mL to 100 mg/mL and 25 to 200 mg/mL respectively while that of *Fagara zanthoxyloides* ranged from 1.56 mg/mL to 12.5 mg/mL and 50 to 200 mg/mL respectively.

Conclusion: The antimicrobial activity demonstrated by *Fagara zanthoxyloides* and *Distemonanthus 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; chief among them are the flavonoids which have potent antioxidant activities were found in these plant materials.

Keywords: Antimicrobial, Chewing sticks, Gastrointestinal, Respiratory, Urinary Pathogens

INTRODUCTION

The development of resistance by infectious agents to antibiotics has been a continuous challenge.¹ Alarming increase in the incidence of new and re-emerging infectious diseases and the resistance of microorganisms to existing antibiotics is a major health concern.² Resistance limits antibiotic choice available to prescribers, and sometimes leads to the 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 difficult to treat because they possess an additional outer membrane permeability barrier with multiple efflux pumps and antibiotic modifying enzymes.

There is a need for the discovery of new antimicrobial compounds with novel mechanisms of action and increased therapeutic and prophylactic activities in order to combat with the ever increasing cases of multi drug resistance worldwide.

Plants are major sources of medicines, as they have been used throughout human history. Medicinal plants are known to possess healing properties due to the presence of diverse secondary metabolites which are present in them.¹ It has been documented that about three-quarters of the world population relies upon traditional method of healing using mainly herbs for the health care of its people and 80% of the African population are documented to use some form of traditional herbal medicine.^{4,5} 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.⁶

Fagara zanthoxyloides Lam also known as *Zanthoxylum zanthoxyloides* Lam, is an indigenous plant used widely as chewing stick for tooth cleaning in West Africa.⁷ 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 5.⁸

Distemonanthus 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.⁹ 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 and it is used as chewing stick for oral 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.¹ A good number of researches have been documented in Nigeria, especially in the southwestern part on the antifungal, antibacterial and anti-plasmodium activities of these plants and their extracts. However, not much work have been documented in the North central part, therefore the need for this study.

METHODS

Collection of Plant Materials

Roots of *Fagara zanthoxyloides* and *Distemonanthus 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 and his team.¹² A 1.0 kg of the fine powder of *Fagara zanthoxyloides* was macerated with 4 liters of methanol while 0.6 kg of *Distemonanthus 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), Nutrient Broth (NB) and Mueller Hinton Agar (MHA). Each medium was prepared according to the manufacturer's instructions. The media were then sterilized using an autoclave at 121 °C for 15 minutes. They were then kept in a refrigerator at 4 °C 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 Odebiyi and his partner.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 pneumonia* and *Proteus mirabilis* were collected from Department of Microbiology and Parasitology, University of Ilorin Teaching Hospital. Standard strains of *Salmonella typhi* 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 *Fagara zanthoxyloides* and *Distemonanthus 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 *Fagara zanthoxyloides* and *Distemonanthus 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 the negative control (dimethyl-sulfoxide). The plates were allowed to stand for 1 hour to allow for pre-diffusion of the extracts before incubating for 18 hours 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 *Fagara zanthoxyloides* and *Distemonanthus 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. A suspension of each test organism from nutrient agar plates in sterile normal saline was standardized to match 0.5 McFarland turbidity standards. 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. The antibiotic discs namely: Ciprofloxacin (5 µg), Gentamicin (10 µg), Ofloxacin (5 µg) and Cefuroxime (30 µg) were placed on the seeded agar plates. The plates were then left to stand for 1 hour before they were incubated at 37 °C for 18 hours. After incubation, the plates were examined for zones of inhibition which were measured in millimeters using a meter rule. The experiment was carried out in triplicates and results obtained were then interpreted using the European Committee on Antimicrobial Susceptibility Testing.^{14,15}

Evaluation of the Activity 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 partner.¹⁶ 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 *Fagarazanthoxyloides* 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 μ L of the inoculum was added to all tubes except for tube 12 using a micropipette. Incubation was carried out at 37 °C for 24 hours. 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 *Distemonanthus 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.¹⁷ 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 hours 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 on each extract with Folin-Ciocalteu's phenol reagent.^{18,19} 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% Na₂CO₃ 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 spectrophotometer. 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 and his team with some modifications.²⁰ A 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 (Sigma Aldrich) was used as standard. All tests were performed in triplicates. Total flavonoid content were 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 co-worker with some modifications.²¹ 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:

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

Alkaloids determination

The method of Obadoni and Ochuko²² 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 crude alkaloid was weighed and calculated according to the equation:

$$\% \text{ amount of alkaloid (mg/g)} = \frac{\text{weight of precipitate}}{\text{weight of sample}} \times 100$$

• Assay of DPPH scavenging activity

The DPPH radical-scavenging activity of the test extracts was examined as described by Ebrahimzadehand his team with some modifications.²⁰ Different concentrations (0.03-0.1µg/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 IC₅₀ 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%) formethanolic extract of Fagara zanthoxyloides and 77.00 g (12.83%) for methanolic extract of Distemonanthus benthamianus. Qualitative phytochemical screening of the extracts revealed the presence of tannins, saponins, flavonoids and alkaloids for both the methanol extracts of Distemonanthus benthamianus and Fagarazanthoxyloides, and anthraquinone is absent in the plants as presented in Table 1.

The susceptibility test of the organisms used against the extracts is presented in Tables 2. 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 ATCC14028 while for 500 mg/mL extract of FZ gave much higher zones against all the organisms 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. Combining the extracts at different ratio does not give better zones of inhibition as compared to when used alone rather most of the zones produced were lower than with individual extracts as shown in Table 3.

Table 1: Qualitative Phytochemical Screening of the Methanolic Extracts of *Distemonanthus benthamianus* and *Fagarazanthoxyloides*

Pytochemicals	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	+	+

KEY

+ = Present

DB: *Distemonanthus benthamianus*

- = Absent

FZ: *Fagarazanthoxyloides***Table 2: Antimicrobial Activity of the Methanol Extracts of *Distemonanthus benthamianus* and *Fagarazanthoxyloides***

Test Organism	Zone of Inhibition of the Plant Extracts (mm)			
	DB (200 mg/mL)	DB (500 mg/mL)	FZ (200 mg/mL)	FZ (500 mg/mL)
<i>Staphylococcus aureus</i>	23.3 ± 0.6	24.7 ± 1.2	23.3 ± 0.6	25.7 ± 0.6
<i>Staphylococcus aureus</i> ATCC 25923	18.7 ± 1.2	21.3 ± 2.5	21.0 ± 1.0	24.3 ± 1.5
<i>Pseudomonas aeruginosa</i>	23.0 ± 1.0	25.7 ± 0.6	26.0 ± 2.6	29.3 ± 0.6
<i>Pseudomonas aeruginosa</i> ATCC 27853	19.3 ± 0.6	21.3 ± 0.6	24.0 ± 1.0	25.7 ± 0.6
<i>Escherichia coli</i>	23.0 ± 1.0	25.3 ± 0.6	25.3 ± 2.5	29.0 ± 1.0
<i>Escherichia coli</i> ATCC 25922	19.0 ± 1.0	21.7 ± 0.6	25.0 ± 2.0	27.3 ± 1.2
<i>Citrobacter freundii</i> ATCC 8090	18.0 ± 1.0	19.0 ± 1.0	20.7 ± 1.2	27.0 ± 2.6
<i>Salmonella typhi</i> ATCC 14028	22.0 ± 2.6	23.7 ± 2.3	21.0 ± 1.0	26.0 ± 2.0
<i>Klebsiella pneumoniae</i>	17.7 ± 1.2	22.3 ± 1.2	22.3 ± 0.6	24.3 ± 1.2
<i>Proteus mirabilis</i>	26.7 ± 1.5	29.0 ± 1.0	24.3 ± 0.6	28.0 ± 0.0

KEYDB: *Distemonanthus benthamianus*FZ: *Fagarazanthoxyloides*

Table 3: Combined Effect of the Methanol Extracts of *Distemonanthus benthamianus* and *Fagarazanthoxyloides* on Test Microorganisms

Test Organism			Zone of Inhibition of the			
Extracts (mm)						
			DB:FZ	DB:FZ	FZ:DB	FZ:DB
			DB:FZ	DB:FZ	FZ:DB	FZ:DB
			(200 mg/mL)	(500 mg/mL)	(200mg/mL)	(500mg/mL)
			(500mg/mL)	(200 mg/mL)	(500mg/mL)	(500mg/mL)
			50:50	50:50	75:25	75:25
75:25	75:25					
<i>Staphylococcus aureus</i>			19.0 ± 1.0	21.3 ± 1.2	20.3 ± 0.6	22.3 ±
0.6	19.3 ± 0.6	21.3 ± 1.2				
<i>Staphylococcus aureus</i> ATCC 25923			20.3 ± 0.6	25.7 ± 0.6	21.0 ± 1.0	24.3 ±
3.2	22.7 ± 2.5	24.7 ± 3.8				
<i>Pseudomonas aeruginosa</i>			20.7 ± 0.6	22.7 ± 0.6	16.3 ± 1.5	17.7 ±
0.6	21.3 ± 0.6	23.3 ± 0.6				
<i>Pseudomonas aeruginosa</i>			16.3 ± 2.1	19.3 ± 0.6	15.7 ± 0.6	17.0 ±
1.0	18.0 ± 1.0	20.3 ± 1.5				
ATCC 27853						
<i>Escherichia coli</i>			18.7 ± 2.1	20.3 ± 1.5	19.7 ± 0.6	24.0 ±
0.0	20.3 ± 1.2	24.0 ± 2.0				
<i>Escherichia coli</i> ATCC 25922			17.3 ± 0.6	20.0 ± 0.0	16.3 ± 1.2	17.0 ± 1.0
	18.3 ± 0.6	20.0 ± 1.0				
<i>Citrobacter freundii</i> ATCC 8090			17.7 ± 2.1	21.7 ± 0.6	21.3 ± 1.2	24.0 ±
1.7	20.0 ± 1.0	23.0 ± 1.7				
<i>Salmonella typhi</i> ATCC 14028			17.3 ± 1.2	22.0 ± 2.0	18.0 ± 4.4	24.3 ± 2.1
	20.0 ± 2.0	22.0 ± 2.0				
<i>Klebsiella pneumoniae</i>			15.7 ± 0.6	17.3 ± 1.2	20.7 ± 0.6	25.3 ± 2.5
	17.0 ± 1.0	19.3 ± 2.1				
<i>Proteus mirabilis</i>			16.7 ± 1.2	18.3 ± 1.2	16.7 ± 3.5	20.3 ±
6.7	17.0 ± 3.6	20.7 ± 0.6				

KEY

DB: *Distemonanthus benthamianus*

FZ: *Fagarazanthoxyloides*

The antibacterial activity of standard antibiotics against test organisms is presented in Table 4. Ofloxacin produced zones of inhibition with *Staphylococcus aureus* and had highest zones of inhibition of 31.0 ± 4.4 mm against *Citrobacter freundii* ATCC 8090. Generally, Ofloxacin gave better zones 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 Tables 6 and 7 respectively. 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 *Proteus 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 7.

Table 4: Activity of Standard Antibiotics against Test Microorganisms

Test organism	Zone of Inhibition (mm)			
	CXM (30µg)	OFX (5µg)	CIP (5µg)	CN (10µg)
<i>Staphylococcus aureus</i>	0.0 ± 0.0	24.3 ± 0.6	NT	NT
<i>Staphylococcus aureus</i> ATCC 25923	10.0 ± 1.0	22.3 ± 5.5	NT	NT
<i>Pseudomonas aeruginosa</i>	NT	NT	16.7 ± 2.1	0.0 ± 0.0
<i>Pseudomonas aeruginosa</i> ATCC 27853	NT	NT	31.0 ± 1.0	23.3 ± 0.6
<i>Escherichia coli</i>	9.7 ± 0.6	0.0 ± 0.0	NT	NT
<i>Escherichia coli</i> ATCC 25922	16.0 ± 1.0	28.3 ± 0.6	NT	NT
<i>Citrobacterfreundii</i> ATCC 8090	17.7 ± 0.6	31.0 ± 4.4	NT	NT
<i>Salmonella typhii</i> ATCC 14028	10.7 ± 1.2	29.3 ± 1.5	NT	NT
<i>Klebsiella pneumoniae</i>	0.0 ± 0.0	0.0 ± 0.0	NT	NT
<i>Proteus mirabilis</i>	20.7 ± 0.6	22.7 ± 2.3	NT	NT

KEY

CXM – Cefuroxime

CN – Gentamicin

NT – No zone of inhibition taken

CIP – Ciprofloxacin

OFX – Ofloxacin

Table 5: The Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) for *Distemonanthus benthamianus*

Test organism	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC
<i>Staphylococcus aureus</i>	12.5		
<i>Staphylococcus aureus</i> ATCC 25923	12.5	25.0	2
<i>Pseudomonas aeruginosa</i>	25.0	>200.0	8
<i>Pseudomonas aeruginosa</i> ATCC 27853	25.0	>200.0	8
<i>Escherichia coli</i>	12.5	100.0	8
<i>Escherichia coli</i> ATCC 25922	6.25	>200.0	32
<i>Citrobacterfreundii</i> ATCC 8090	6.25	25.0	4
<i>Salmonella typhii</i> ATCC 14028	100.0	100.0	1
<i>Klebsiella pneumoniae</i>	12.5	50.0	4
<i>Proteus mirabilis</i>	25.0	25.0	1

Table 6: The Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) for *Fagarazanthoxyloides*

Test organism	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC
<i>Staphylococcus aureus</i>	6.25		
<i>Staphylococcus aureus</i> ATCC 25923	3.12	>200.0	64
<i>Pseudomonas aeruginosa</i>	3.12		
<i>Pseudomonas aeruginosa</i> ATCC 27853	12.5	>200.0	16
<i>Escherichia coli</i>	3.12	>200.0	64
<i>Escherichia coli</i> ATCC 25922	1.56		
<i>Citrobacterfreundii</i> ATCC 8090	1.56	100.0	64
<i>Salmonella typhii</i> ATCC 14028	3.12	>200.0	64
<i>Klebsiellapneumoniae</i>	6.25	100.0	16
<i>Proteus mirabilis</i>	6.25	50.0	8

Table 7: Activity Index of the extracts of *Distemonanthusbenthamianus* and *Fagarazanthoxyloides*

Test Microorganisms	DB Extract	FZ Extract
<i>Staphylococcus aureus</i>	1.02	1.06
<i>Staphylococcus aureus</i> ATCC 25923	0.96	1.09
<i>Pseudomonas aeruginosa</i>	1.54	1.75
<i>Pseudomonas aeruginosa</i> ATCC 27853	0.69	0.83
<i>Escherichia coli</i>	2.61	2.99
<i>Escherichia coli</i> ATCC 25922	0.77	0.96
<i>Citrobacterfreundii</i> ATCC 8090	0.61	0.87
<i>Salmonella typhii</i> ATCC 14028	0.81	0.89
<i>Proteus mirabilis</i>	1.28	1.23

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

Table 8: Total saponins, alkaloid, flavonoid and phenol present in Fagara and Distemonanthus

Plant	% Saponins	% Alkaloid	Flavonoid (mg/g Quercetin)	Phenol (mg/g Galic acid)
Fagara	15.095	14.53	0.2905	2.4265
Distemonanthus	3.65	7.88	0.196	2.592

The plant Fagara (Table 8) showed considerable levels of saponins, alkaloids, flavonoids as well as phenols.

The percentage saponins in Distemonanthus (Table 8) is 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.²⁰ Phenolic compounds of plants fall into several categories; chief among these are the flavonoids which have potent antioxidant activities.²¹

Table 9: Scavenging activity of Fagara and Distemonanthus extracts

	Fagara mg/ml	Distemonanthus mg/ml
IC ₅₀	0.03965	0.05328
R square	0.9488	0.9646

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

R² is coefficient of determination; values obtained from regression lines with 95% confidence level.

The IC₅₀ measures the antioxidant activity. The smaller the IC₅₀, the greater the antioxidant activity. Thus fagara has a very high antioxidant activity. This correlates with the high flavonoids present in the extracts as shown in figure 2 and table 8.

DISCUSSION

The prevalence of antibiotic resistance which is an ever increasing threat to public health due to the development of various defence mechanisms against antimicrobials has brought the need for the exploitation and development of novel inhibitory agents against pathogenic resistant strains. This study further highlights the therapeutic values of the methanol extracts of *Fagara zanthoxyloides* and *Distemonanthus benthamianus* as possible antibacterial agents with wide spectrum of activity. The antibacterial activities could be attributed to the presence of some bioactive constituents which include phenols, unsaturated lactones, saponins, cyanogenic glycosides, glucosinates, alkaloids, tannins, linoleic and stearic acids in the plants.¹ Previous studies have documented the antifungal, antibacterial, anti-oxidant and antiinflammatory properties of these plant extracts.^{1,10,23,}

^{24,25,26} Studies have also shown the use of these plant extracts in the treatment of wide range of disorders including toothache, urinary infections, venereal diseases, rheumatism, lumbago, stomach aches and cough.²⁵ Flavonoids mechanism of activity have been shown to reduce free radicals by up-regulating, quenching and chelating radical intermediate compounds.²⁴ Phenolic

compounds of plants are grouped into several categories; most important among them are the flavonoids characterized by potent antioxidant activities.²⁷

The presence of alkaloids in *Fagara zanthoxyloides* is in accordance to the report of Elujoba and co-worker which described the main active ingredients in *F. zanthoxyloides* as alkaloids namely; berberine, fagaronine, chelerythrine, canthin-6-one and benzoic acid derivatives.⁸ It has also been documented that the presence of alkaloid in *Fagara zanthoxyloides* may contribute to its anti-inflammatory property due to the ortho-hydroxymethyl benzoic acid which also has made the plant useful in the management of sickle cell crisis.¹ *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli* were susceptible to the methanolic extract of *Fagara zanthoxyloides* which is in agreement with the work of Agbulu's team.¹² Statistical analysis showed that there is a significant difference in antimicrobial activity between the two concentrations (200 mg/mL and 500 mg/mL) of *Fagara zanthoxyloides* used.

Distemonanthus benthamianus was observed to show antimicrobial activity against all the pathogenic organisms tested in this study, some of the organisms tested 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*.^{8,28,29}

Distemonanthus 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 showed that there is no significant difference between the two concentrations of *Distemonanthus benthamianus* used in this study. This therefore further highlight the therapeutic property of the plant.

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 antibiogram 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 *Fagarazanthoxyloides* 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.

Hagerman and Butler 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.³⁰ Parekh and Chanda 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.³¹ It is believed that the tannin present in African chewing stick (such as *Fagara zanthoxyloides* and *Distemonanthus benthamianus*) may contribute to their antibacterial effect.³² Isolation of the pure active components of these two plants has been a problem in Nigeria due to poor funding of our institutions.

CONCLUSION

The methanol extracts of the roots of *Fagara zanthoxyloides* and *Distemonanthus 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 especially with the increasing incidence of resistance to the existing antibiotics.

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