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Zanthoxylum zanthoxyloides leaf extracts demonstrate antimicrobial effect on selected oral pathogens

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ABSTRACT

Background: Oral infections continue to be a major health problem and may be associated with conditions such as colorectal cancer, gum bleeding, toothache, preterm birth among pregnant mothers, arterial hardening, myocardial infarction and stroke. Yet available conventional treatments for oral infections are not only expensive and sometimes harmful but also ineffective compared to cheap and readily available plant-based ethnodentistry therapies because of increased resistance by microorganisms. This has necessitated the need to explore plant-based medicine for the purposes of controlling oral infection. Objective: The study assessed anti-microbial activity of three extract fractions of *Zanthoxylum zanthoxyloides* (*Z. zanthoxyloides*) leaf, which has long been used as chewing stick to maintain oral hygiene, against three selected oral pathogens: *Lactobacillus acidophilus* (*L. acidophilus*), *Aggregatibacter actinomycetemcomitans* (A. *actinomycetemcomitans*), and *Candida albicans* (*C. albicans*). Materials and Methods: The crude extract of *Z. zanthoxyloides* leaf was prepared, and increasing concentrations of each extract fraction and respective standard antibiotics were tested against the growth of each selected oral pathogen by using serial broth dilution. After 24 hours culturing, absorbance of each test broth was measured spectrophotometrically at 450 nm. Percentage inhibition by each extract fraction, vehicle and standard antibiotics: Fluconazole (FLUCO), amoxicillin and metronidazole (AMOX&MET), and ciprofloxacin (CIPRO) against *C. albicans*, *A. actinomycetemcomitans* and *L. acidophilus* respectively, were determined indirectly from absorbance values by proportions relative to an untreated standard broth. The IC_{50} of each drug was determined from log concentration - response curve whilst minimum inhibitory concentration (MIC) of each fraction was determined using scatter plot with line of best fit. Results: The extract fractions produced concentration-dependent inhibition of growth. Except in the case of *Lactobacillus acidophilus* where growth inhibitory effect of the extract fractions was lower compared to standard antibiotic (ciprofloxacin), for the remaining pathogens, the extract fractions produced comparable growth inhibitory effects at higher (in the case of *Candida albicans*) and lower (in the case of *Aggregatibacter actinomycetemcomitans*) concentrations relative to standard antibiotics (Amoxacillin & Metronidazole and Fluconazole). Conclusion: *Z. zanthoxyloides* leaf extracts have anti-microbial activity against the three studied oral pathogens; thus, it can be explored for active anti-microbial agents against oral infections in which the studied pathogens may be implicated.

Keywords: Antibacterial activity, *Candida albicans*, Oral pathogens, Serial broth dilution, *Zanthoxylum zanthoxyloides*.

INTRODUCTION

Despite advancements in dental science, oral diseases like dental caries, periodontitis, oral tissue lesions and oral cavity cancers are major health problems worldwide [1,2]. Globally, it is estimated that 2.3 billion people suffer from caries of permanent teeth and more than 530 million children suffer from caries of primary teeth [3]. Prevalence of dental caries in school aged children is up to 90% and the majority of adults are also affected [2]. Globally, about 30% of people aged 65–74 have no natural teeth. Evidence abounds linking risk of colorectal cancer [4], gum bleeding, toothache, preterm birth among pregnant mothers [5,6], chronic kidney disease [7], myocardial infarction [8] and stroke [9,10] and many other medical conditions to oral infections. It has been shown that most forms of periodontal disease such as plaque, tooth decay and toothache are caused by bacterial pathogens with over 600 bacterial species and a considerable number of fungal species inhabiting the oral cavity of humans [11]. Common among these oral pathogens are Gram-positive bacteria such as *Streptococcus mutans, Lactobacillus* species and some non-mutans streptococci which are associated with caries formation [12], Gram-negative bacteria such as *A. actinomycetemcomitans* which is associated with aggressive periodontitis [13] and commensal yeasts such as *C. albicans* which is associated with oral candidiasis (oral thrush) [14].

Although there are several conventional drugs used in treating oral infections, these drugs are not in most cases affordable to ordinary individual and worse of all they come with adverse effects such as changes on oral microbiota, teeth staining, bad taste, dryness and burning sensation [15]. Oral bacteria have been reported to show increased resistance towards common antibiotics such as penicillin, cephalosporin, erythromycin, tetracycline and metronidazole which are commonly used for the treatment of oral infection [16]. With the rise in bacterial resistance to antibiotics, there has been an increased interest in the development of other classes of antimicrobials and antifungals for the control of infection particularly from indigenous plants [17,18] which are mostly affordable, relatively safe, readily available and widely accepted. The natural products derived from medicinal plants are believed to produce biologically active compounds which are responsible for their chemotherapeutic activity [19]. This trend has necessitated that the bioactivity of many of these medicinal plants against common oral pathogens be scientifically established.

One of the commonest plants with widespread ethnobotanical usage as herbal remedy in Africa is *Z. zanthoxyloides.* The plant (from the family Rutaceae) is a tree or shrub up to 8–12m high, widely distributed in West Africa as a well-known medicinal tree with its roots, root bark, stem bark, fruits and leaves being used for various pharmaceutical purposes [20]. Studies have shown that extracts from the plant may have anti-plasmodial [20], gastro-protective [21], antioxidant [22], Anti-trypanosomal [23], antidiabetic [24], antimicrobial [25,26], insecticidal [27] and many other chemotherapeutic properties. The woody stem of *Z. zanthoxyloides* has long been used in ethnodentistry as a chewing stick to maintain oral hygiene, particularly among rural folks in Ghana and many African countries [28,29]. Although there are a number of studies on the antimicrobial activity of *Z. zanthoxyloides* root, stem and leaf against oral pathogens [1,25,30,31], to the best of our knowledge, little is known about studies assessing anti-microbial activity of three solvent fractions (petroleum ether, ethanol and ethyl acetate) of the leaves of *Z. zanthoxyloides* against isolated oral pathogens. The present study therefore focused on providing further scientific knowledge on the anti-microbial activity of extracts of *Z. zanthoxyloides* on *L. acidophilus, A. actinomycetemcomitans* and *C. albicans*.

MATERIALS AND METHODS

Chemicals and Reagents

The materials used included media such as Sabouraud Dextrose agar (SDA) and Blood agar for culturing bacteria, positive control antibiotics, Metronidazole and Amoxicillin (Letap Pharmaceuticals Ltd, Ghana), Ciprofloxacin (Maxheal Laboratories Pvt. Ltd., India) and Fluconazole (Belco Pharma, India). Chemicals such as petroleum ether, ethyl acetate, ethanol, methanol, hydrochloric acid, sulphuric acid, chloroform, sodium hydroxide (VWR Prolab Chemicals, France), hydrogen peroxide, Wagner's reagent, Mayer's reagent, ferric chloride and gelatin solution were also used. All other solvents and chemicals used in the study were of analytical grade. Equipment such as a gas bath oscillator, dry air oven, aerobic incubator and other apparatus such as petri dishes, beakers, pipettes and a spectrophotometer among others were also used in the study.

Ethical Clearance

The study was approved by the University of Cape Coast Institutional Review Board (UCCIRB), University of Cape Coast (UCCIRB/CHAS/2016/42) and approval given by management of the Dental Clinic. Also, patients' consent was sought for by means of verbal or written agreement after the aim of the study was clearly explained to the participants.

Experimental Design

This was a laboratory experimental study to determine the anti-bacterial activity of the petroleum ether, ethanol and ethyl acetate extracts of *Z. zanthoxyloides* against selected oral pathogens. The test bacteria were acquired from the University of Cape Coast Dental Clinic. The research was carried out in the laboratories of Department of Biomedical Sciences, University of Cape Coast and Cape Coast Teaching Hospital. The plant extracts were screened for phytochemicals present and then assayed for their anti-bacterial activity using serial broth dilution method.

Plant collection, identification and authentication

The fresh leaves of *Z. zanthoxyloides* were collected from North Ola, a suburb of Cape Coast Metropolis in the Central region of Ghana. Figures 1 shows the branches, leaves and fruits of the plant. The samples were examined and authenticated by Mr. Francis Otoo, the curator at the Herbarium Unit of University of Cape Coast.

Figure 1: Aerial part of *Z. zanthoxyloides* showing branches, leaves and fruits

Preparation of *Z. zanthoxyloides* crude extracts

The leaves were thoroughly washed and shade-dried for 6 weeks and mechanically milled into powder. 100 g of the powdered leaf was extracted successively to obtain the extract. The dried powdered sample of the leaf was first defatted using 700 mL of petroleum ether at 60 – 80 ºC. The defatted sample was air dried to remove any traces of solvent and was exhaustively extracted using 600 mL of 95 % ethanol in a Soxhlet apparatus. The resultant extract was concentrated by steam drying using a rotary evaporator to obtain 7.5 g of a dark greenish paste which was labelled *Z. zanthoxyloides* extract (ZZE). Three different solvents (ethanol, ethyl acetate and petroleum ether) were used for the extraction procedure. The extracts were filtered separately through Whatman filter paper and concentrated using steam drying by rotary evaporator and dried in a desiccator to obtain semi solid products. Prior to use, five different concentrations (2500, 1250, 625, 312.5 and 156.25 ug/mL) were prepared from each of the extracts by serial dilution.

Phytochemical analysis

Phytochemical analysis of the crude extracts of leaves were performed according to methods described in previous studies [32–34] with slight modifications. The phytochemicals screened for included alkaloids, flavonoids, tannins, terpenoids, saponins and phenols as follows:

Test for alkaloids

To 0.5 g of the dried extracts, 5 mL of 10% HCl was added and stirred whiles heating. From the resulting mixture, 1 mL each of the filtrates was pipetted into test tubes. Dragendorff, Mayer and Wagner reagents were added. In each case a sample test tube of each filtrate was reserved as reference. Formation of a white precipitate indicated the presence of alkaloid.

Test for saponins

This was screened by shaking 0.5 g of the dried extracts with water in a test tube. Frothing which persists on warming was used as evidence for the presence of saponins.

Test for Terpenoids

A 1 mL of crude extracts was slowly added to 400µL of chloroform, followed by careful addition of 400µL of concentrated sulphuric acid. Formation of a reddish-brown color at the interface indicated the presence of terpenoids.

Test for Flavonoids

A 1.5 mL of a 50% aqueous methanol was added to 4 ml of the crude extracts. The solution was warmed and magnesium turning added. 5 to 6 drops of concentrated HCl were added to the solution and observed for red coloration.

Test for Tannins

Tannins were detected using a modified version of the Ferric chloride test. Briefly, 2 drops of 1% aqueous ferric chloride reagent were added to 0.5 mL of crude extract and observed for the formation of blue-black, green or green-black coloration.

Test for Phenols

Ethyl alcohol was added to 2 mL of the crude extract and few drops of ferric chloride solution added. A dark green color indicated the presence of phenol.

Isolation and Identification of Test Pathogens

Fungal pathogen (*C. albicans)* was obtained from the University of Cape Coast Dental Clinic. These species were cultured on Sabouraud Dextrose Agar (SDA) plates and incubated at 25 °C for 3 days. Candida species were sub-cultured on SDA supplemented with chloramphenicol and incubated at 37°C for 72 hours to obtain pure isolates. Identification of *C. albicans* was done using germ tube test. Three (3) drops of human serum were pipetted into a test tube. A yeast colony was inoculated into the serum in the test tube and incubated at 37 °C for 2.5 hours. A drop of the suspension was placed on a clean glass slide and a cover glass placed over the suspension. Sprouting yeast cells, that is tube-like outgrowths from the cells under microscope were observed after 2 - 3 hours of incubation at 37 °C. Bacterial Species used were *A. actinomycetemcomitans* and *L. acidophilus*. The micro-organisms were sub-cultured on Blood agar and incubated aerobically at 37°C. *A. actinomycetemcomitans* was identified by a positive indole test. Microscopy showed shaped inner structures that were seen in colonies on selective media. Identification of the isolate of *L. acidophilus* was performed using gram staining, motility, biochemical tests and morphological features. The isolated strain was Gram positive cocci-bacilli, non-motile and catalase positive. It fermented maltose, lactose, sucrose and glucose, but unable to ferment arabinose and sorbitol. A summary of results of biochemical tests for the bacterial isolates are represented in Table 1.

Table 1: Results of biochemical tests to confirm *L. acidophilus* and *A. actinomycetemcomitans*

Test	Reaction
L. acidophilus	
Gram reaction	+
2 % NaCl tolerance	+
2 % Bile tolerance	+
Glucose fermentation	+
Galactose	+
Sucrose	+
TH ELactose	+
Mannose	+
Catalase	-
Motility	-
Indole	-
TSI	-
Citrate	-
A. actinomycetemcomitans	
Gram reaction	-
Glucose fermentation	+
Motility	-
Fructose	+
Maltose	+
Indole	-
Catalase	+
Oxidase	-

(+) indicates positive reaction, (-) indicates negative reaction

Antibacterial Assay, Determination of Minimum Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC)

After crude preparation of extract fractions (Petroleum ether extract, PEEZZ; ethanol extract, EEZZ; and ethyl acetate extract, EAEZZ) and confirmation (biochemical test, colony morphology, Gram-staining and biotyping in the case of bacteria isolates and germ tube in the case of *C. albicans*) of isolates (*C. albicans, L. acidophilus* and *A. actinomycetemcomitans*). Increasing concentrations of each extract fraction (156.25, 312.5, 625, 1250 and 2500 µg/mL) and respective standard antibiotics were tested against the growth of each selected oral pathogen by using serial broth dilution (as described in a previous study [35] with slight modification). Briefly, fresh colonies of pure cultures were obtained by subculture on blood agar plate after 24 hours aerobic incubation at 35 ± 2ºC. Inoculates equivalent to 0.5 McFarland standard (1 to 2×10^8 colony-forming units (CFU)/mL) were prepared for each of the three isolates. A final inoculum of 5×10^5 CFU/mL required for the broth micro-dilution was achieved first by 1:150 dilution in Mueller Hinton broth (MHB) medium for each isolate. Subsequently, a 1:2 dilution with the final drugs dilutions above brought the final required inoculum to 5×10^5 CFU/ml. A 1 ml of each drug dilution in broth (the double concentrations) was added to the respective tubes. Aliquots of 0.5 ml of the inoculum suspension of each isolate was then added to the contents of the tubes and thoroughly mixed. After 24 hours incubation, absorbance of each test broth was measured spectrophotometrically at 450 nm. Percentage inhibition by each extract fraction and standard antibiotics (Fluconazole, FLUCO; amoxicillin and metronidazole, AMOX&MET; and ciprofloxacin, CIPRO for *C. albicans*, *A. actinomycetemcomitans* and *L. acidophilus* respectively) were determined indirectly from absorbance values by proportions relative to an untreated standard broth. The MIC is the lowest concentration of

antimicrobial agent that completely inhibits growth of the organism in tubes as detected by the unaided eye. The IC_{50} of each drug was determined from log concentration- response curve whilst MIC values for each fraction was determined using scatter plot with line of best fit. Each experiment was repeated at least three times.

Statistical Analysis

Data generated from the study were entered into IBM SPSS version 24.0 (SPSS Inc., Chicago, IL, USA) to compute descriptive statistics. Data were also analyzed with GraphPad Prism version 6 (GraphPad Software, San Diego, CA, USA) to perform one-way analysis of variance (ANOVA) to compare between different antibacterial activities of different extract concentrations against each test bacteria. $P \leq 0.05$ was considered statistically significant in all analyses.

RESULTS

Percentage yield and Phytochemical profile of *Z. zanthoxyloides* leaves extract

The results of phytochemical screening as represented in Table 2 showed that alkaloids, flavonoids, tannins, terpenoids, saponins and phenols were detected in *Z. zanthoxyloides* leaf extract. However, phenols were not detected in the ethanol fraction.

Effect of *Z. zanthoxyloides* Leaf Extracts on Growth of Test Pathogens

All the extract fractions (PEEZZ, EEZZ, and EAEZZ) demonstrated growth inhibition against the test organisms (*L. acidophilus*, *A. actinomycetcomitans*, and *C. albicans*) compared to standards. From Figure 2, ciprofluxicillin demonstrated significant growth inhibition against L. acidophilus compared to that of the extract fractions. The order of increasing growth inhibition demonstrated by the three extractions against *L. acidophilus* was EAEZZ > EEZZ > PEEZZ (Table 3). Figure 3 shows that all the three extract fractions demonstrated growth inhibition against *A. actinomycetcomitans* even than that of metronidazole and amoxicillin. Among the three extracts fractions, PEEZZ was the most potent followed by EAEZZ (Table 4). Figure 4 shows that the three extract fractions demonstrate anti-fungal effects against *C. albicans* comparable to fluconazole. EEZZ demonstrated the most potent anti-fungal effect even more than that of fluconazole (Table 5). EAEZZ was the most potent among all the extract fractions (Table 6).

Table 2: Phytochemical profile of *Z. zanthoxyloides* leaf extracts

Compound	Test	Test results		
		Ethyl acetate	Ethanol	Petroleum ether
Alkaloids	Dragendorf	+	+	+
Flavonoids	Sodium hydroxide	+	+	-
Tanins	Ferric chloride	+	+	-
Terpenoids	Salkowski	+	+	+
Saponins	Froth	+	+	+
Phenols	Ferric chloride	+	-	+

(+) present, (-) absent

Table 3: Effect of treatments on mean absorbance of *L. acidophilus* growth

Drugs	Mean absorbances of microbial suspension at different concentrations				
	156.25 µg/mL	312.5 µg/mL	625 µg/mL	1250 µg/mL	2500 µg/mL
PEEZZ	$1.575 \pm 0.003^{+}$	$1.536 \pm 0.004^{+}$	$1.505 \pm 0.006^{+}$	$1.394 \pm 0.000^{+}$	$0.872 \pm 0.007^{+}$
EEZZ	$1.640 \pm 0.021^{+}$	$1.612 \pm 0.003^{+}$	$1.491 \pm 0.003^{+}$	$1.342 \pm 0.003^{+}$	$0.942 \pm 0.004^{+}$
EAEZZ	$1.641 \pm 0.001^{+}$	$1.600 \pm 0.002^{+}$	$1.441 \pm 0.003^{+}$	$1.352 \pm 0.001^{+}$	$1.012 \pm 0.001^{+}$
Ciprofloxacin	0.211 ± 0.003	0.091 ± 0.004	0.058 ± 0.001	0.040 ± 0.001	0.030 ± 0.000

Each value is the Mean ± SE within followed by + are significantly different Ciprofloxacin respectively at p<0.05. PEEZZ – Petroleum ether extract of *Z. zanthoxyloides*; EEZZ – EEZZ - Ethanol extract of *Z. zanthoxyloides*; EAEZZ – Ethyl Acetate extract of *Z. zanthoxyloides*; MIC – Minimum inhibitory concentration

Table 4: Effect of treatments on mean absorbance of *A. actinomycetmcomitan* growth

Drugs	Mean absorbances of microbial suspension at different concentrations				
	156.25 µg/Ml	312.5 µg/mL	625 µg/mL	1250 µg/mL	2500 µg/mL
PEEZZ	$0.464 \pm 0.002^{+}$	$0.116 \pm 0.00^{+}$	$0.055 \pm 0.00^{+}$	$0.020 \pm 0.001^{+}$	$0.019 \pm 0.000^{+}$
EEZZ	$0.993 \pm 0.011^{+}$	$0.689 \pm 0.005^{+}$	$0.063 \pm 0.001^{+}$	$0.058 \pm 0.001^{+}$	$0.056 \pm 0.00^{+}$
EAEZZ	$0.396 \pm 0.000^{+}$	$0.363 \pm 0.001^{+}$	$0.015 \pm 0.004^{+}$	$0.009 \pm 0.001^{+}$	$0.007 \pm 0.001^{+}$
Met & Amoxicillin	1.050 ± 0.000	0.099 ± 0.000	0.096 ± 0.001	0.094 ± 0.00	0.082 ± 0.001

Each value is Mean ± SE within followed by $^{+}$ are significantly different from Met & Amoxicillin respectively at p<0.05. PEEZZ – Petroleum ether extract of *Z. zanthoxyloides*; EEZZ – EEZZ - Ethanol extract of *Z. zanthoxyloides*; EAEZZ – Ethyl Acetate extract of *Z. zanthoxyloides*; MIC – Minimum inhibitory concentration

Table 5: Effect of treatments on mean absorbance of *C. albican* growth

Drugs	Mean absorbances of microbial suspension at different concentrations				
	156.25 µg/mL	**312.5 µg/mL**	**625 µg/mL**	**1250 µg/mL**	**2500 µg/mL**
PEEZZ	0.720 ± 0.000^{+}	0.698 ± 0.000^{+}	0.663 ± 0.007^{+}	0.481 ± 0.008^{+}	0.301 ± 0.001^{+}
EEZZ	0.752 ± 0.002^{+}	0.714 ± 0.000^{+}	0.703 ± 0.000^{+}	0.698 ± 0.001^{+}	0.654 ± 0.001^{+}
EAEZZ	0.764 ± 0.000^{+}	0.733 ± 0.002^{+}	0.664 ± 0.000^{+}	0.648 ± 0.001^{+}	0.603 ± 0.000^{+}
Fluconazole	0.552 ± 0.000	0.501 ± 0.000	0.450 ± 0.001	0.284 ± 0.001	0.034 ± 0.000

Each value is Mean ± SE within followed by $^{+}$ are significantly different from Fluconazole respectively at $p < 0.05$. PEEZZ – Petroleum ether extract of *Z. zanthoxyloides*; EEZZ – EEZZ - Ethanol extract of *Z. zanthoxyloides*; EAEZZ – Ethyl Acetate extract of *Z. zanthoxyloides*; MIC – Minimum inhibitory concentration

Table 6: IC_{50} values for crude extracts from the leaves of *Z. zanthozyloides* against tested microorganisms

Treatments	*L. acidophilus*		*A. actinomycetmcomitan*		*C. albicans*	
	Log (Concentration)	µg/mL	Log (Concentration)	µg/mL	Log (Concentration)	µg/mL
PEEZZ	3.031	1073.99	2.352	224.914	2.874	748.17
EEZZ	3.001	1002.31	2.615	412.09	2.537	344.35
EAEZZ	2.958	907.82	2.464	291.07	2.688	487.53
Ciprofloxacin	-	-				
Met & Amoxicillin			-	-		
Fluconazole					2.681	479.73

PEEZZ – Petroleum ether extract of *Z. zanthoxyloides*; EEZZ – EEZZ - Ethanol extract of *Z. zanthoxyloides*; EAEZZ – Ethyl Acetate extract of *Z. zanthoxyloides*; IC_{50} - Half-maximal inhibitory concentration.

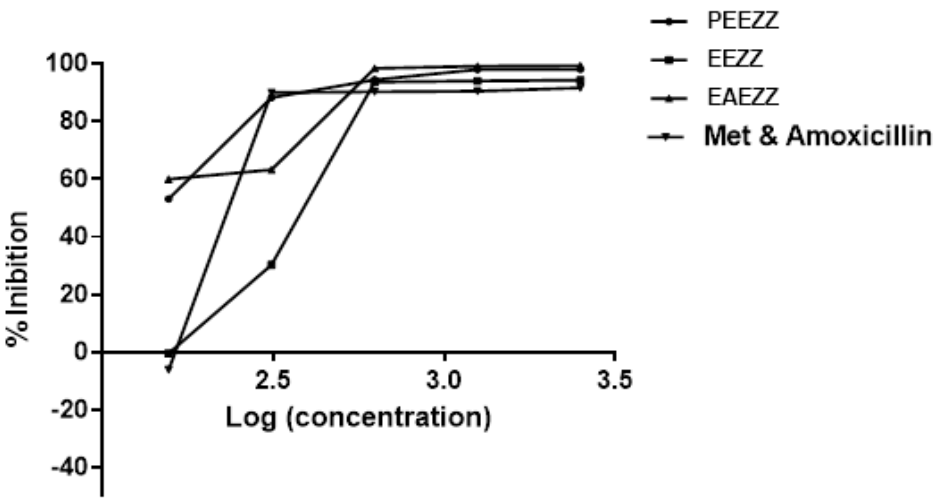

Figure 2: Effect of treatments on mean percentage inhibition of *L. acidophilus* growth

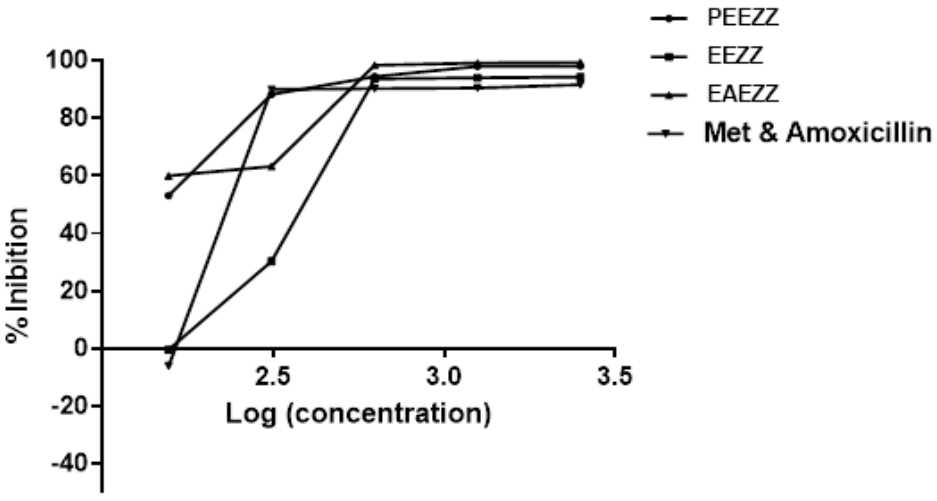

Figure 3: Effect of treatments on mean percentage inhibition of *A. actinomycetmcomitan* growth

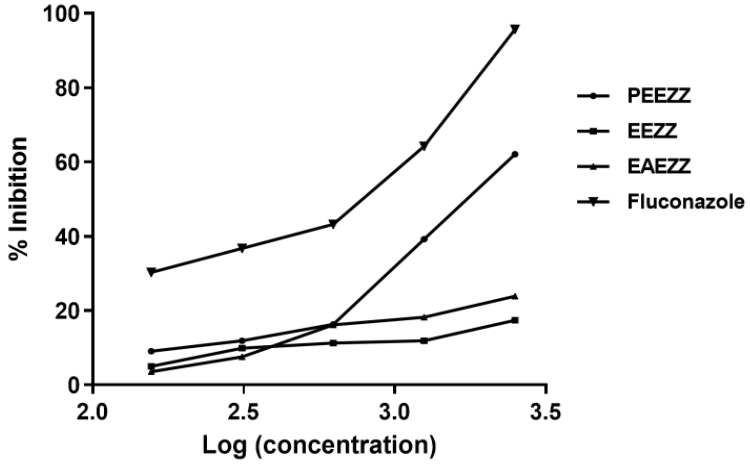

Figure 4: Effect of treatments on mean percentage inhibition of *C. albicans* growth

DISCUSSION

The results of this study demonstrate the promise of *Z. zanthoxyloides* extract for the treatment and prevention of oral infections caused by the common oral pathogens *L. acidophilus, A. actinomycetemcomitans* and *C. albicans*. The extract fractions produced concentration-dependent inhibition of growth. In the case of *L. acidophilus*, growth inhibitory effect of the extract fractions was lower compared to standard antibiotic (ciprofloxacin) indicating that the crude extract might be having insufficient concentration to give antibacterial effect, has very small amounts of the bioactive compounds to give antibacterial effect to *L. acidophilus* or *L. acidophilus* is in itself resistant towards both ethyl acetate, petroleum ether and ethanol extracts of *Z. zanthoxyloides.* No study of leaf extract of *Z. zanthoxyloides* has been done against *L. acidophilus* for comparison. However, crude aqueous methanol extract from root bark of the plant had significant antimicrobial activity against *Lactobacillus spp* [1]. In another study, *L. acidophilus* was resistant against root extracts of the plant but produced mild susceptibility which increased with an increasing concentration of the extracts [25].

For the remaining pathogens, the extract fractions produced comparable growth inhibitory effects at higher (in the case of *C. albicans*) and lower (in the case of *A. actinomycetemcomitans*) concentrations relative to standard antibiotics (Amoxacillin and Metronidazole & Fluconazole). This finding is in agreement with Bosson-Vanga's study [36], in which *C. albicans* strains were susceptible to alcoholic extract of leaves of *Z. zanthoxyloides.* Further extensive review of literature did not reveal any study done using the leaves of *Z. zanthoxyloides* against *C. albicans*, however, our results agree with the study of Adekunle and Odukoya [37] who demonstrated the finding of anti-fungal activity of ethanol and aqueous crude extracts of *Z. zanthoxyloides* stem and roots against *C. albicans*. Similarly, Adeeyo and colleagues [38] reported appreciable inhibition of *C. albicans* by extracts of *Z. zanthoxyloides* even when compared with commercial antibiotic (Nystatin). This observation may be as a result of the antifungal effect of flavonoids, saponins and tannins against *Candida albicans* due to their toxicity, astringent, molecular structure or other ways.

Reduced susceptibility to the extracts by Gram positive *Lactobacillus acidophilus* as against increased susceptibility by the Gram negative *Aggregatibacter actinomycetemcomitans* as observed in this study contradicts previous findings which suggests that Gram-negative bacteria are usually more resistant to plant-origin antimicrobials and show no effect, compared to Gram-positive bacteria [39]. It has been reported that the difference in susceptibility to antimicrobial extracts between Gram-positive and Gram-negative bacteria could be attributed to morphological differences. For instance, the outer membrane of Gram-negative bacteria has lipopolysaccharide, making the cell wall impermeable to lipophilic extracts [40]. However, the overdelicate structure of Gram-negative bacteria, which involves structural differences between the cell wall and exterior membrane, influences their susceptibility to various antimicrobial agents [41].

The observed antimicrobial activity of *Z. zanthoxyloides* can be attributed to the presence of rich phytochemicals in the extracts. The analysis of the plant extracts revealed the presence of phytochemicals such as alkaloids, flavonoids, tannins, terpenoids, saponins and phenols which are known to exhibit medical and physiological activities. These phytoconstituents, as detected in this study, have previously been identified in earlier studies involving *Z. zanthoxyloides* [26,42]. Previous studies have reported the analgesic and cytotoxic properties of alkaloids [43], as well as the anti-inflammatory and antibacterial properties of tannins and saponins, which acts by precipitating microbial protein and making nutritional proteins unavailable for bacteria use [44,45]. The antibacterial and antifungal properties of flavonoids containing plants have equally been reported [46].

With regard to the choice of solvent of extraction, ethyl acetate extraction yielded the greatest number of phyto-constituents with comparatively better antimicrobial activity against the organisms used in the study. This finding is supported by results of a study by Abha Chaudhary and colleagues [47] who have reported the existence of a high level of flavonoid and phenolic compounds in ethyl acetate extracts of *Ocimum sanctum* compared with other solvents.

The findings of this study can be considered as very promising in prospecting antimicrobials from plant sources, when considering the medical importance of tested microorganisms especially in oral infection. *L. acidophilus* has emerged as one of the most problematic gram-negative pathogens, with high antibiotic resistance rates [48] and has been implicated in the progression of dental caries, a major cause of destruction of tissues of the teeth [49]. The Gram-negative facultative anaerobe, *A. actinomycetemcomitans,* is common in periodontal diseases such as localized aggressive periodontitis and bone resorption [50]. About 8.20% of root canal infections is attributable to microscopic fungi, such as Candida species with *C. albicans* being the most frequently isolated species [51]. These findings trace the importance of discovering new drugs against these organisms. Interestingly, at least one extract tested in this study prevented the growth of these microbes. The results presented here are limited to the verification of the antimicrobial activity of *Z. zanthoxyloides* extract on oral disease-causing microbes based on individual phytochemicals present in the extract, however, phytochemicals have proven to work together to produce synergistic effect [52,53] which most likely occurred in this study.

CONCLUSION

The study showed that the *Z. zanthoxyloides* leaf extracts have comparable anti-microbial activity to standard antibiotics against *L. acidophilus*, *A. actinomycetemcomitans* and *C. albicans*. This could be attributed to the presence of phytochemicals such as alkaloids, flavonoids, tannins, terpenoids, saponins and phenols that were identified in the extracts. The study recommends further purification, identification and exploration by pharmaceuticals for active anti-microbial agents against oral infections in which the studied pathogens may be implicated.

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Conflict of interest

The authors declared no conflict of interest.

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