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Herbal Bio-Disinfectants: *Z. zanthoxyloides and G. latifolium* as Effective Antimicrobial Agents against Inherent Microbial Pathogens in Water.

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Abstract

Water and sanitation facilities in sub-Saharan Africa and Africa in general are appalling and for the most part absent. Progress continues with respect to the development of plant materials as potent adsorbents, disinfectants, coagulants, flocculants, wetland species and lots more as substitutes for the dangerous chemical disinfectants.

This research presents the potential of phyto-active components of *Zanthoxylum zanthoxyloides and Gongronema latifolium* as effective biocides against water microbial contaminants. Dry powder of *Zanthoxylum zanthoxyloides and Gongronema latifolium* were extracted and prepared into different concentrations with ethyl acetate and chloroform, ranging from 25 to 500 mg/ml. These fractions were then examined for antimicrobial activities against inherent bacterial and fungal water contaminants using disc diffusion assay. Fractions were afterwards screened for phytochemical active compounds using standard methods. Crude extracts of the different plant examined selectively comprise saponins, tannins, reducing sugars, anthraquinones, flavonoids, terpenoids, phlobatanins and alkaloids. All plant extracts showed broad spectrum antibiosis against selected gram positive and gram negative bacteria including *E. coli*, *P. aeruginosa*, *Klebsiella* sp, *S. pneumoniae* and *B. cereus*, as well as tested fungi, including *A. niger, A. flavus, Trichoderma sp and Candida sp.* While all extracts exhibited maximum antibiosis at 500 mg/ml, the chloroform extracts compared well than ethyl acetate extracts. The overall results revealed that antimicrobial activities of the plant extracts are dose dependent with comparative activity greater than that of commercial antibiotics at the concentration of extracts tested. *E.coli* was the most susceptible microbial isolate tested and represents the potential of the extract against a group of coliform which are important indicators of microbial pollution in water. Other microbial isolates also recorded sensitivity to extracts tested at varying degrees. The findings indicate that microbes tested were mostly susceptible to chloroform extract of *Z. zanthoxyloides* and *G. latifolium* except for the activity of ethyl acetate extract of *Z. zanthoxyloide* against *E.coli*. Results of phytochemical screening of the extract also showed the varied presence of alkaloids, saponins, tannins, flavonoids, terpenoids and anthraquinones The results indicated that plant materials investigated can be developed as effective biocides against water microbial contaminants.

Key words: Bio-disinfection; *G. latifolium;* Wate;*, Z. zanthoxyloides.*

1. Introduction

Drinking water is a basic human need [1]. Access to safe water is considered a fundamental human right and individuals are entitlement to sufficient, safe, accessible and affordable water without discrimination [2,3]. Human settlement, civilisation and religion have all evolved round water [4]. The importance of water as source of life and purification is well inscribed in history; Hindus, sprinkle water on new born child and believed that "The iota of life is created in water" (Hinduism: Atharvaveda, Asthagarideyam); Muslims feed on a drop of "zam zam" holy water from Mecca and hold the view that "By means of water, God gives life to every living thing" (Islam: Quran 21:30); Christians perform baptism and water is usually a fundamental symbol of faith, "Whoever believes in me, stream of living water will pour from within him" (Christianity: John 7:38). Excavations from the Neolithic time have found a striking correspondence between settlements and wells [3]. The importance of ample water and quality is thus apparent in human history and beliefs [4]. The Biblical book of Exodus (15:23-27) is the earliest historical reference to phyto-purification of water; "And the people murmured against Moses, saying, "What shall we drink?" And he cried unto the Lord; and the Lord showed him a tree, which when he had cast into the waters, the waters were made sweet" [4].

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Water treatment involves the processing of raw water to improve its quality to meet prescribed standards for domestic, industrial and commercial uses. It is strategically employed to prevent the ingestion of harmful contaminants [5]. However, water processing in disinfection units is not utilised fully in developing countries because of cost issues associated with the procurement and import of disinfecting chemicals. This has resulted to increased vulnerability of many rural communities in developing African countries to waterborne diseases and death [1,6-8]. Children and the elderly are mainly affected [9]. It has been recommended that household water treatment techniques [10], is a way forward to combatting drinking water problems [11]. The quest to meet the enormous water challenges of the developing countries at the household level have led to growing interest in plant-based water treatment solutions **(Table 1).** However, there is little information regarding the use of natural extracts as disinfectants in water treatment. With Africa having good percentage of medicinal plants [1] , it is important to understand the potential of African indigenous plant extracts in water treatment, especially their antimicrobial properties. Two important locally available plants found in many African countries worthy of further investigation are *Zanthoxylum zanthoxyloides* and *Gongronema latifolium* **[**8**].**

Zanthoxylum are deciduous shrubs and trees of the family *Rutaceae* which comprise 250 species used as sources of pharmaceutical and cosmetics raw materials. Traditionally, leaves and fruits are used for mouth freshing and tooth care [12,13]. *Zanthoxylum zanthoxyloides* is considered antiseptic, analgesic and diaphoretic, used in treatment of malaria, fever, sickle cell anaemia, tuberculosis, paralysis, oedema and general body weakness. It is taken to treat intestinal problems, including colic, dysentery, intestinal worms, gonorrhoea and urethritis, but also to treat pain during childbirth, migraine and neuralgia. The roots are externally applied to ulcers, swellings, haemorrhoids, abscesses, snake bites, yaws, wounds leprosy and syphilitic sores as well as rheumatic and arthritic pain and hernia [14]. The roots and stem are widely used in the treatment of sore gums, toothache and dental caries. A decoction of the roots is used as a mouthwash and against a sore throat. Sap from the pulped bark is applied as eye drops to treat eye infections, notably conjunctivitis with pus. Root and stem bark powder is taken to treat whooping cough [15]. In southern Nigeria a decoction of the stem bark and roots is taken to treat cancer. *Z. zanthoxyloides* also has numerous magico-religious uses, including protection against spirits. It also serves as fetish plant [15]. Diverse phytochemicals have been reported as constituents of the plants [16-19].

Gongronema latifolium (Asclapiadaceae) is a perennial edible plant with soft and pliable stem [20]. Reports by various authors showed that it contains essential oils, saponins, alkaloids and tannins among others [21-24]. Aqueous and ethanolic *G. latifolium* extracts had hypoglycemic, hypolipidemic, antioxidative and anti-inflammatory properties [23,25-27]. Infusion of the aerial parts is taken to treat cough, intestinal worms, dysentery, and malaria. It is also taken as a tonic to treat loss of appetite. In Sierra Leone an infusion or decoction of the stems with lime juice is taken as a purge to treat colic and stomach-ache. In Senegal and Ghana the leaves are rubbed on the joints of small children to help them walk. The boiled fruits in soup are eaten as laxative [28]. Decoction of leaves or leafy stems is commonly taken to treat diabetes and high blood pressure. The latex is applied to teeth affected by caries. It is also taken for controlling weight gain in lactating women and overall health management. Asthma patients chew fresh leaves to relieve wheezing. A cold maceration of the roots is also taken as remedy for asthma. A decoction of the roots, combined with other plant species, is taken to treat sickle cell anaemia. A maceration of the leaves in alcohol is taken to treat bilharziosis, viralhepatitis and as a general antimicrobial agent [29]. Several 17β-marsdenin derivatives (pregnane glycosides), as well as β-sitosterol, lupenylcinnamate, lupenyl acetate, lupeol, essential oils and saponins have been found in the plant. The essential oil from the leaves contains as main components linalool (19.5 %), (E)-phytol (15.3 %) and aroma dendrene hydrate (9.8 %) [30,31].

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Table 1. List of plant species used as coagulants and disinfectants.

Scientific name	Family	Genera	Plant part	Habit	Reference	Uses
Moringa oleifera Lam.	Moringaceae	Moringa	Seed	Tree	[32]	Coagulation and
Opuntia ficus indica (L.) Mill.	Cactaceae	Opuntia	Leaves	Shrub	[34]	Coagulation and disinfection
Cicer arietinum L.	Fabaceae	Cicer	Seeds	Herb	[36]	Coagulation and disinfection
Aloe barbadensis Mill.	Alloaceae	Aloe	Seeds	Herb		
Hibiscus sabdarifa L.	Malvaceae	Hibiscus	Calyx	Herb	[37,38]	
Jatropha curcas L.	Euphorbiaceae	Jatropha	Seed	Tree		
Parkinsonia aculeata L;	Fabaceae	Parkinsonia	Seed	Tree	[39]	Coagulation and disinfection
Vigna unguiculata (L.)	Fabaceae	Vigna	Seed	Herb		
Cyamopsis tetragono	Fabaceae	Cyamopsis	Seed	Herb	[40]	Coagulation and disinfection
Manihot esculenta crantz	Euphorbiaceae	Manihot	Root	Shrub	[41]	Coagulation and disinfection
Solanum incunum L.	Solanaceae	Solanum	Leaf	Shrub	[42]	Coagulation and disinfection
Phaseolus vulgaris L.	Fabaceae	Phaseolus	Seed	Herb	[43]	Coagulation

The aim of the work reported in this paper was to assess the antimicrobial effects of stem extracts from the two selected plants on inherent bacterial and fungal water contaminants as a means of investigating their use as disinfectants in developing countries where access to clean water is a big challenge.

2. Materials and Methods

2.1 Sampling

Zanthoxylum zanthoxyloides and *Gongronema latifolium* samples were collected from Oja Igbo market at Ogbomoso North local government, Oyo State. The samples were washed, air dried and grounded to powder, then sieved to get a fine powder.

2.2 Preparation of samples

50 g of dried and grounded samples of *Zanthoxylum zanthoxyloides* and *Gongronema latifolium* were extracted separately by maceration using 150 ml of ethyl acetate and chloroform as extraction solvent for 48 hours. The solvents fractions were separated using vacuum filtration aided by a sterile Whatmann No 1 filter paper and filtering crucible. The filtered extract were then dried with the help of a freeze drier and stored in sterile polypropylene airtight container at 4 ºC or dispensed in appropriate sterile dissolving solution when required.

2.3 Antimicrobial Activity

2.3.1 Preparation of antimicrobial disc and sensitivity test

Different concentrations of crude extracts of *G. latifolium* and *Z. Zanthoxyloides* ranging between 25 and 500 mg/ml were prepared by dissolving appropriate amount of crystalised extracts into ethyl acetate or chloroform. Solutions were then embedded in 6.0 mm diameter sterile disc prior to antimicrobial sensitivity testing by disc diffusion method as described by [44]. Briefly, the test organism was swabbed evenly on the surface of the agar plate using surface spread method. Impregnated paper discs with different concentrations of crude extracts were then arranged serially and radially, and pressed slightly but firmly to the surface of inoculated agar. The plates were afterward incubated at 37 ºC for 24 hours. The fungi plates were incubated at room temperature (27 ± 2 ºC) for 48 hours. The degree of sensitivity was determined by measuring the diameter in millimeter of the visible zone of inhibition of microbial growth produced by the diffusion of the extract. Test concentrations of crude extract were 500 mg/ml, 250 mg/ml, 100 mg/ml, 50 mg/ml and 25 mg/ml. Standard reference commercial

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antibiotics disc for gram negative (streptomycin), gram positive (gentamycin) and the fungi (nystatin) were used for a comparative study.

2.4 Phytochemical analyses

The phytochemical screening of the extracts was investigated according to standard methods and as modified by Harborne [45] and Adeeyo et al. [46]. Screening for tannins, saponnin, reducing sugars, alkaloids, terpenoids, flavonoids, steroids, anthraquinones and phlobatannins were carried out on extracted crude products.

2.5 Data Analysis

The Statistical Package for Social Scientists (SPSS, version 19.0) was used for the analysis of the data obtained. Two way ANOVA test was used to determine the level of significance of the crude extracts at different concentration. Also, comparison of the different solvent extraction (ethyl acetate and chloroform) was made statistically, the general antimicrobial effects of the extracts were compared with the standard antibiotics. Zones of inhibition was compared with reference standard to assign the level of activities of extracts.

3. Result and discussion

The antimicrobial activities of extracted phytobiotics against bacterial and fungal isolates tested were investigated with extract doses ranging from 25-500 mg/ml. Figure 1 shows the zone of inhibition of *G. latifolium* and *Z. zanthzyloides* against selected gram negative (*P. aeruginosa, Klebsiella* sp., *E. coli*), gram positive (*S. pneumonia, B. cereus*) and Table 2 shows the inhibition of the plant materials against fungal isolates (*A. flavus*, *A. niger, Trichoderma* sp and *Candida* sp.) using ethyl acetate and chloroform as extraction solvents.

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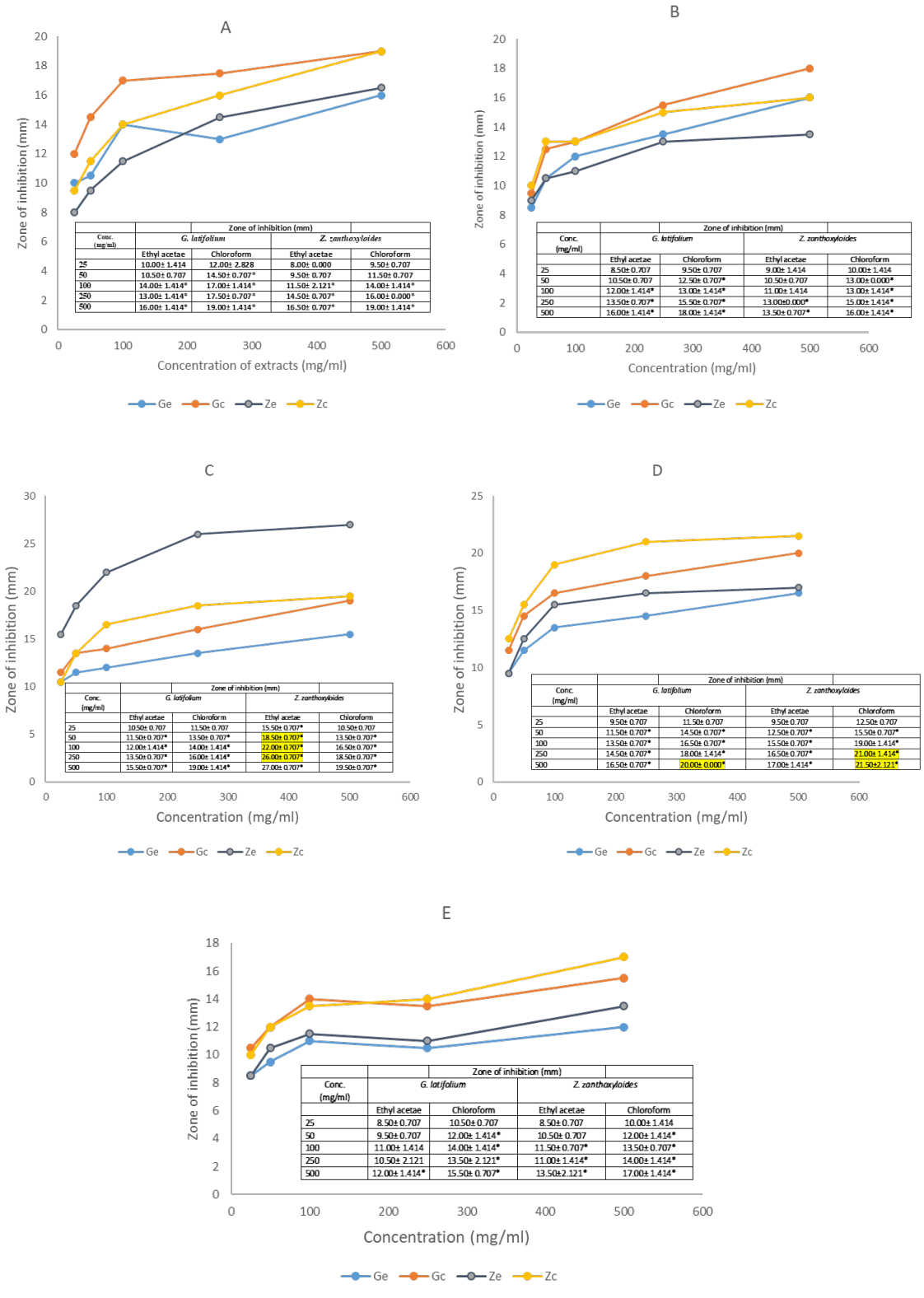

A

Conc. (mg/ml)	Zone of inhibition (mm)			
	G. latifolium		*Z. xanthoxyloides*	
	Ethyl acetae	Chloroform	Ethyl acetae	Chloroform
25	10.00± 1.414	12.00± 2.828	8.00± 0.000	9.50± 0.707
50	10.50± 0.707	14.50± 0.707*	9.50± 0.707	11.50± 0.707
100	14.00± 1.414*	17.00± 1.414*	11.50± 2.121*	14.00± 1.414*
250	13.00± 1.414*	17.50± 0.707*	14.50± 0.707*	16.00± 0.000*
500	16.00± 1.414*	19.00± 1.414*	16.50± 0.707*	19.00± 1.414*

B

Conc. (mg/ml)	Zone of inhibition (mm)			
	G. latifolium		*Z. xanthoxyloides*	
	Ethyl acetae	Chloroform	Ethyl acetae	Chloroform
25	8.50± 0.707	9.50± 0.707	9.00± 1.414	10.00± 1.414
50	10.50± 0.707	12.50± 0.707*	10.50± 0.707	13.00± 0.000*
100	12.00± 1.414*	13.00± 1.414*	11.00± 1.414	13.00± 1.414*
250	13.50± 0.707*	15.50± 0.707*	13.00±0.000*	15.00± 1.414*
500	16.00± 1.414*	18.00± 1.414*	13.50± 0.707*	16.00± 1.414*

C

Conc. (mg/ml)	Zone of inhibition (mm)			
	G. latifolium		*Z. xanthoxyloides*	
	Ethyl acetae	Chloroform	Ethyl acetae	Chloroform
25	10.50± 0.707	11.50± 0.707	15.50± 0.707*	10.50± 0.707
50	11.50± 0.707*	13.50± 0.707*	18.50± 0.707*	13.50± 0.707*
100	12.00± 1.414*	14.00± 1.414*	22.00± 0.707*	16.50± 0.707*
250	13.50± 0.707*	16.00± 1.414*	26.00± 0.707*	18.50± 0.707*
500	15.50± 0.707*	19.00± 1.414*	27.00± 0.707*	19.50± 0.707*

D

Conc. (mg/ml)	Zone of inhibition (mm)			
	G. latifolium		*Z. xanthoxyloides*	
	Ethyl acetae	Chloroform	Ethyl acetae	Chloroform
25	9.50± 0.707	11.50± 0.707	9.50± 0.707	12.50± 0.707
50	11.50± 0.707*	14.50± 0.707*	12.50± 0.707*	15.50± 0.707*
100	13.50± 0.707*	16.50± 0.707*	15.50± 0.707*	19.00± 1.414*
250	14.50± 0.707*	18.00± 1.414*	16.50± 0.707*	21.00± 1.414*
500	16.50± 0.707*	20.00± 0.000*	17.00± 1.414*	21.50±2.121*

E

Conc. (mg/ml)	Zone of inhibition (mm)			
	G. latifolium		*Z. xanthoxyloides*	
	Ethyl acetae	Chloroform	Ethyl acetae	Chloroform
25	8.50± 0.707	10.50± 0.707	8.50± 0.707	10.00± 1.414
50	9.50± 0.707	12.00± 1.414*	10.50± 0.707	12.00± 1.414*
100	11.00± 1.414	14.00± 1.414*	11.50± 0.707*	13.50± 0.707*
250	10.50± 2.121	13.50± 2.121*	11.00± 1.414*	14.00± 1.414*
500	12.00± 1.414*	15.50± 0.707*	13.50±2.121*	17.00± 1.414*

Figure 1: Antibacterial activities of *G. latifolium and Z. xanthoxyloides* Chloroform and Ethyl acetate extract on (A) *P. aeruginosa*, (B) *Klebsiella* sp, (C) *E. coli*, (D) *S. pneumonia* and (E) *B. cereus*. Inset values of inhibitions with significant difference. Extracts: Ge, *Gongronema* ethyl acetate; Gc, *Gongronema* chloroform; Ze, *Zanthoxylum* ethyl acetate; Zc, *Zanthoxylum* chloroform.

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Table 2: Antifungal activities of *G. latifolium and Z. zanthoxyloides*

Conc. mg/ml	Ethyl acetate	Chloroform
	A. flavus	
25	7.50± 0.707	9.50± 0.707
50	9.50± 0.707	11.50± 0.707*
100	10.50± 0.707	13.50± 0.707*
250	12.50± 0.707*	15.00± 0.000*
500	11.00± 1.414*	12.50± 2.121*
	A. niger	
25	9.50± 0.707	11.00± 1.414
50	9.00± 1.414	11.50± 2.121
100	11.00± 1.414	13.50± 0.707*
250	11.50±2.121	14.00± 1.414*
500	14.50± 0.707*	17.00± 1.414*
	Trichoderma sp	
25	8.50± 0.707	11.00± 1.414
50	10.50± 0.707	14.00± 1.414*
100	12.00± 1.414*	15.50± 0.707*
250	13.50± 0.707*	16.50± 0.707*
500	13.50± 0.707*	18.50± 0.707*
	Candida sp	
25	9.00± 0.000	10.50± 0.707
50	11.50± 0.707	14.50± 0.707*
100	11.50± 2.121	13.50± 2.121*
250	11.50± 0.707	13.50± 0.707*
500	11.50± 0.707	14.00± 1.414*
	G. latifolium	

Conc. mg/ml	Ethyl acetate	Chloroform
	A. flavus	
25	9.50±2.121	11.00± 1.414
50	9.50± 0.707	12.50± 0.707
100	10.50± 2.121	13.00± 2.828*
250	13.00± 1.414*	16.00± 1.414*
500	14.50± 0.707*	17.50± 2.121*
	A. niger	
25	8.50± 0.707	11.00± 1.414
50	10.00± 1.414	12.50± 0.707
100	11.50± 2.121*	14.00± 0.000*
250	12.50± 2.121*	15.00± 1.414*
500	13.00± 1.414*	16.00± 1.414*
	Trichoderma sp	
25	9.00± 1.414	11.00± 1.414
50	9.50± 0.707	13.00± 1.414*
100	11.50± 0.707	15.50± 0.707*
250	15.50± 0.707*	18.50± 0.707*
500	16.50± 0.707*	20.50± 0.707*
	Candida sp	
25	7.50± 0.707	9.50± 0.707
50	9.50± 0.707	12.00± 1.414
100	10.50± 0.707	13.00± 1.414*
250	11.50± 2.121	14.00± 1.414*
500	13.00± 1.414*	16.00± 1.414*
	Z. zanthoxyloides	

3.1 Dose dependent activities

The extent of antimicrobial activity of extract based on the zone of inhibition has been described as low (12-18 mm), moderate (19-22 mm) and strong (23-38 mm) [47]. The dose response to plant extracts can be observed in Figure 1 and Table 2. At the lower doses (25-50 mg/ml), the extracts show low to moderate antimicrobial activities across the various plant extracts irrespective of the extraction solvent. The range of inhibition zones is between 7.5- 18.50 mm. Noteworthy is the activity of ethyl acetate extract of *Z. zythoxyliodes* at this dose (18.50 mm; Fig. 1C) against *E.coli*. At the higher dosage, extracts showed moderate to high antibiosis with a range of 10.5 to 27.0 mm. Noteworthy is the activities of *Z. zythoxyloide*'s ethyl acetate extract against *E.coli* (Fig. 1C; 27.0 mm), as well as its chloroform extract against *S. pneumoniae* (21.50 mm) (Fig. 1D) and *Trichoderma* sp (Table 2; 20.50). *G. latifolium* chloroform extract also exhibited appreciable activity against all tested microbes and its activity against *S. pneumonia* (Fig. 1D; 20.00 mm) is remarkable. These results are qualitatively similar to those of Shaheed et al. [6] and Jones and Bridgeman [8] who observed increase in performances of herbal extracts at higher dosages against microbial pollutants of water.

3.2 Comparative phytobiotic activities (with commercial antibiotics)

The abilities of phytoactive extracts of plants materials investigated as compared with commercial antibiotics were investigated. Plant extracts investigated performed beyond the activities of commercial streptomycin, gentamycin and nystatin within selected concentration range investigated. Statistical analysis revealed notable significant differences from 25 mg/ml and above, but mostly at higher concentrations. The comparative antibiosis of commercial antibiotics, as well as minimum and maximum extract concentrations against tested microbes were presented in **Tables 3 and 4**. The results reveal that potent phytoactive formulations of the plant materials tested can be developed within the range researched in this work, and with better performance than available commercial antibiotics. The advent of such formulations as envisaged in this work may be the dawn of novel technology in achievement of the World Health Organisation (WHO) recommendation of zero faecal count/100 ml water sample [48] especially when developed for water disinfection. Potent antimicrobial

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activities of plant materials have been reported for different formulations such as antipsoriatic cream using different combinations of neem, sarsaparilla, bakuchi and daruhaldi which showed good antimicrobial and anti-inflammatory results [49]; herbal ointment containing *Aloe-vera*, *Azadirachta indica* and *Curcuma- longa* showed good antimicrobial activity against the selected organisms microbes when compared to standard drugs [50]. In a study where polyherbal ointment was prepared using methanolic extracts of *Azadirachta indica*, *Chromolaena odorata*, *Mimosa pudica*, *Samadera indica* and evaluated for its antibacterial and antioxidant properties, the results showed better antibacterial properties [51]. These reported research findings support phytoactivities of plant materials as reported in this work. Herbal extracts of *Ocimum samctum* and *Eucalyptus globules* as anti-microbial agents were found to exhibit significant activity against *E. coli, Pseudomonas aeuroginosa, Staphylococcus aureus, Bacillus subtilis, Sacchromyces cerevisiae* as well as *Candida albicans* and were safer for human usage when compared to reference standards Ampicillin and Amphoterecin respectively [52].

Table 3: Comparative efficiency of plant extracts with commercial antibiotics (Streptomycin and Gentamycin) against tested bacteria

		G. latifolium		*Z. zanthoxyloides*	
		Ethyl acetate	Chloroform	Ethyl acetate	Chloroform
P. aeruginosa	STR	7.50± 0.707	8.00± 1.414	7.00± 0.000	8.50± 0.707
	Min	10.00± 1.414	12.00± 2.828	8.00± 0.000	9.50± 0.707
	Max	16.00± 1.414^{+}	19.00± 1.414^{+}	16.50± 0.707^{+}	19.00± 1.414^{+}
Klebsiella sp	STR	7.50± 0.707	8.00±0.000	7.00± 0.000	7.50± 0.707
	Min	8.50± 0.707	9.50± 0.707	9.00± 1.414	10.00± 1.414
	Max	16.00± 1.414^{+}	18.00± 1.414^{+}	13.50± 0.707^{+}	16.00± 1.414^{+}
E.coli	STR	7.50± 0.707	9.00± 0.000	8.50± 0.000	7.00± 0.000
	Mın	10.50± 0.707	11.50± 0.707	15.50± 0.707^{+}	10.50± 0.707
	Max	15.50± 0.707^{+}	19.00± 1.414^{+}	27.00± 0.707^{+}	19.50± 0.707^{+}
S. pneumonia	GEN	7.50± 0.707	9.00± 0.000	7.50± 0.707	8.50± 0.707
	Min	9.50± 0.707	11.50± 0.707	9.50± 0.707	12.50± 0.707
	Max	16.50± 0.707^{+}	20.00± 0.000^{+}	17.00± 1.414^{+}	21.50±2.121^{+}
B. cereus	GEN	7.50± 0.707	7.00± 0.000	7.00± 0.000	7.50± 0.707
	Mın	8.50± 0.707	10.50± 0.707	8.50± 0.707	10.00± 1.414
	Max	12.00± 1.414^{+}	15.50± 0.707^{+}	13.502.121^{+}	17.00± 1.414^{+}

+ values with significant difference from the standard; A, Streptomycin; B, Gentamycin; Min, minimum; Max, maximum

Table 4:Comparative efficiency of plant extracts with commercial antibiotic (nystatin) against tested fungi

		G. latifolium		*Z. zanthoxyloides*	
		Ethyl acetate	Chloroform	Ethyl acetate	Chloroform
A. flavus	NIS	7.00±0.000	7.00± 0.000	7.50± 0.707	8.50± 0.707
	Min	7.50± 0.707	9.50± 0.707	9.50±2.121	11.00± 1.414
	Max	12.50± 0.707^{+}	15.00± 0.00^{+}	14.50± 0.707^{+}	17.50± 2.121^{+}
A. niger	NIS	7.50± 0.707	7.00± 0.000	7.00± 0.000	8.50± 0.707
	Min	9.50± 0.707	11.00± 1.414	8.50± 0.707	11.00± 1.414
	Max	14.50± 0.707^{+}	17.00± 1.414^{+}	13.00± 1.414^{+}	16.00± 1.414^{+}
Trichoderma sp	NIS	7.00± 0.000	8.50± 0.707	7.50± 0.707	8.50± 0.707
	Min	8.50± 0.707	11.00± 1.414	9.00± 1.414	11.00± 1.414
	Max	13.50± 0.707^{+}	18.50± 0.707^{+}	16.50± 0.707^{+}	20.50± 0.707^{+}
Candida sp	NIS	7.50± 0.707	8.50± 0.707	7.50± 0.707	8.50± 0.707
	Min	9.00± 0.000	10.50± 0.707	7.50± 0.707	9.50± 0.707
	Max	11.50± 0.707	14.00± 1.414^{+}	13.00± 1.414^{+}	16.00± 1.414^{+}

+, values with significant difference; NIS, Nystatin, Min, minimum; Max, maximum

3.3 Antibacterial activities

The antibacterial activity of the extracts varied with respect to extraction solvents and susceptibility of microbial isolates increases with concentration of extract in solution (**Figure 1**). Noteworthy is the activities of the extracts against *E.coli* which is a widely accepted indicator of water pollution. The activities of extracts tested show effectiveness of all extracts against *E.coli*. Activity of the extracts on *E.coli* showed moderate to high antibiosis at concentrations of 50 to 500 mg/ml. It is, however, notable that significant susceptibility was recorded at a concentration of 25 mg/ml of ethyl acetate extract of *Z. zanthoxyloides* which was the least concentration tested and may confirm possible potent antimicrobial activities of the investigated plants against groups of coliforms

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and microbial pathogens in water. The activities of the plants extracts against *E.coli, Kelsiella sp* and *P. aeruginosa* indicate the ability of these plant materials against a range of gram negative bacteria. For *P. aeruginosa*, susceptibility to plant extracts increases with concentration of the extracts in solution from 25 to 500 mg/ml. At dosages of 25 and 50 mg/ml, maximum values of 12.0 and 14.50 mm zones of inhibition respectively were observed when compared across all plant materials and extraction solvent use, which are low levels of susceptibility. The susceptibility of *P. aeruginosa* at this dose is, however, comparable to that of streptomycin. At doses of 100, 250 and 500 mg/ml maximum zones of inhibition recorded with respect to plant material and extraction solvents were 17.00, 17.50 and 19.00 mm respectively. Chloroform extracts of both plants seem to have effective antimicrobial action against *P. aeruginosa*. Considering *Klebsiella* sp., a low level susceptibility to concentrations of extracts at 25 and 50 mg/ml was observed. The minimum inhibition zones recorded at 25 and 50 mg/ml were 8.50 mm and 10.50 mm while the maximum zones were 10 and 13 mm respectively. These were low level antibiosis. However, the activity of chloroform extract of the two plants were significant at 50 mg/ml against *Klebsiella* sp. All other concentrations of extracts irrespective of solvent material possessed significant antimicrobial activity against *Klebsiela* sp. Maximum zone was observed at 18.00 mm with chloroform extract of *G. latifolium* at 500 mg/ml.

S. pneumonia and *B. cereus* are gram positive bacteria, the least antibiosis recorded for *S. pneumoniae* was 9.50 mm at concentration of 25 mg/ml of *G. latifolium* ethyl acetate extract while the optimum recorded was 20.00 mm (*G. latifolium*) and 21.50 mm (*Z. zanthozyloides*) from chloroform extract. Highest activity of plant extract against *B. cereus* was found to be 17.00 mm from chloroform extract of *Z. zanthoxyloide* at concentration of 500 mg/ml. These results also indicate the ability of extract to inhibit the growth of gram positive bacteria. The susceptibility of all tested microbial isolates to the plant extracts is therefore a clear indication that plants studied would be good candidates for disinfection product development.

3.4 Antifungal activities

Susceptibility of the fungal isolates to tested plant extracts were observed. The maximum antifungal activity of the extract recorded were in the order of 20.20 mm (*Zanthoxylum* chloroform extract against *Trichoderma* at 500 mg/ml)> 17.50 mm (*Zanthoxylum* chloroform extract against *A. flavus* at 500 mg/ml)> 17.00 mm (*Gongronema* chloroform extract against *A. niger* at 500 mg/ml)> 16.00 mm (*Zanthoxylum* chloroform extract against *Candida* sp at 500 mg/ml). Activities of extracts were significant from a concentration of 50 mg/ml to 500 mg/ml and show low to moderate antimicrobial activities (**Table 2**).

The overall results revealed that antimicrobial activities of the plant extracts are dose dependent with comparative activity greater than that of commercial antibiotics at the concentration of extracts tested. *E.coli* was the most susceptible microbial isolate tested and represents the potential of the extract against a group of coliform which are important indicators of microbial pollution in water. Other microbial isolates also recorded sensitivity to extracts tested at varying degrees. It was observed in these findings that microbes tested were most susceptible to chloroform extract of *Z. zanthoxyloides* and *G. latifolium* except for the activity of ethyl acetate extract of *Z. zanthoxyloide* against *E.coli*. Results of phytochemical screening of the extract also showed the varied presence of alkaloids, saponins, tannins, flavonoids, terpenoids and anthraquinones (**Table 5**). The observed trends of phytochemicals found in *Z. zanthoxyloides* for this work is in agreement with previous works carried out on same plant [53-55]. Plant constituents have been reported to possess antimicrobial properties [56,57]. Antimicrobial potency as reported in this work with a broad range of activity against gram-positive and gram-negative organisms is similar to that reported in the work of Adegbolagun and Olukemi [58] when reporting the activity of *Z. zanthxyloides* against selected microbial isolates. Optimum antimicrobial inhibition reported in this work is, however, greater than that reported by Adegbolagun and Olukemi [58] for *Bacillus*, *E. coli*, and *P. aeruginosa,* which may be attributed to the concentrations of extract used. Adesina [54] has reported the demonstration of antibacterial activity by many species of *Zanthoxylum* against both “gram-positive and gram-negative” microorganisms, although aqueous extracts of *Zanthoxylum* have been shown to be less effective against microbial sample of *P. aeruginosa* and *E. coli* [59,60]. Stem and root bark extracts from *Z. zanthoxyloides* have also been reported to have a higher activity against bacteria implicated in periodontal diseases [61]. In the work of Olila et al. [62], there was no antimicrobial activity reported from *Zanthoxylum chalybeum* against *E. coli* and *Candida albicans* tested. This was attributed to low dosage and solubility of extract in extraction solvent used [63].

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Table 5: Phytochemical compositions of *G. latifolium* and *Z. zanthoxyloides*

Analysis	*G. latifolium*		*Z. zanthoxyloides*	
	Ethyl acetate	Chloroform	Ethyl acetate	Chloroform
Saponins	Absent	**Present**	**Present**	Absent
Tannins	**Present**	**Present**	Absent	Absent
Reducing Sugars	Absent	Absent	Absent	Absent
Alkaloids	**Present**	**Present**	**Present**	**Present**
Flavonoids	Absent	**Present**	**Present**	**Present**
Terpenoids	Absent	Absent	**Present**	**Present**
Phlobatannins	Absent	Absent	Absent	Absent
Steroids	Absent	Absent	Absent	Absent
Anthraquinones	Absent	Absent	**Present**	Absent

The doses of antimicrobial samples used in this work were found to be effective in the treatment of the microbial isolates investigated with effectiveness against all bacterial and fungal isolates. Phytochemical products that produce minimum inhibitory concentrations (MIC) in the range 100–1000 mg mL^{-1} in *in vitro* susceptibility tests have been classified as antimicrobials [64].

The activity of *G. latifolium* as reported in this work is also similar to other reports on the activity of *G. latifolium* extracts to inhibit different microbes [23,65,66]. The presence of saponins and flavonoids in *G. latifolium* have been attributed to its antimicrobial efficacy [22,67]. In a similar manner to the findings of this study, *Staphylococcus* sp., *Shigella* sp., *Salmonella* sp., *Klebsiella Pneumonia, Pseudomonas* sp., *Escherichia coli* and *Onchrobactrum anthropi* were all inhibited at 100 mg/ml in Adeleye and Omadime [68]. The presence of essential oil was attributed to high antimicrobial and fungicidal effects against all the tested organisms including *Candida albicans*. Zones of inhibition ranged between 7.5 mm *for Pseudomonas aeruginosa* to 11.25 mm for *Shigella flexneri,* respectively. However, activity of extracts were comparatively lesser than that of commercial antibiotics at the concentration tested suggesting the inefficiency of active ingredient present at the concentration of extract tested which was not the case in this work. The extracts tested over the range of concentrations considered showed improved antimicrobial activities over all antibiotics used, mostly, with significant differences

4. Conclusion

A major conclusion is that plant samples tested revealed potent antimicrobial activities which significantly compared with commercial antibiotics at the concentration of extracts tested. Very strong potency of the plant extract was observed against *E. coli*; a water pollution indicator. High amount of crude extract needed in this work to attain effective inhibition of microbial isolates can be reduced by further identification of active ingredients and subsequent purification. Formulation and development of active component of *G. latifolium* and *Z. zanthoxyloides* into biotechnological products for water purification is recommended.

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