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Original article

Antimicrobial potencies of selected native African herbs against water microbes

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ABSTRACT

Purpose: Phyto-active components of *Zanthoxylum zanthoxyloides and Gongronema latifolium* was investigated against microbial contaminants in water for possible novel antimicrobial usage in water treatment.
Method: Crude ethyl acetate and chloroform fractions of *Zanthoxylum zanthoxyloides and Gongronema latifolium* were prepared and screened for antimicrobial and phytochemical profiles using standard methods.
Results: Crude extracts of the different plant examined selectively comprised saponins, tannins, reducing sugars, anthraquinones, flavonoids, terpenoids, phlobatanins and alkaloids. All plant extracts showed broad spectrum antibiosis against *E. coli*, *P. aeruginosa*, *Klebsiella* sp, *S. pneumoniae* and *B. cereus*, as well as *A. niger, A. flavus, Trichoderma sp and Candida* sp. Chloroform extracts compared well than ethyl acetate extracts.
Conclusion: This work represents the first report to direct the possible application of *Zanthoxylum zanthoxyloides and Gongronema latifolium* to potential application in water. Overall results revealed that antimicrobial activities are dose and plant dependent. Noteworthy is the comparatively greater antimicrobial activity of crude extracts over commercial antibiotics used at the concentration of extracts tested. These plants can therefore serve as a potent source of natural water disinfectant.

1. Introduction

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: Asthagarideyam, Atharvaveda); Muslims take drop of holy water from Mecca and hold the view that God gives life to every living thing by means of water (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 (Skandhan et al., 2011). The importance of ample water and quality is thus apparent in human history and beliefs (Jadhav, 2014). The Biblical ancient book of Exodus (15:23–27) is the oldest historical reference to phyto-purification of water; "So the people grumbled against Moses, saying, "What are we to drink?" "Then Moses cried out to the Lord; and the Lord showed him a piece of wood. He threw it into the water, and the water became fit to drink" (Jadhav, 2014). Human settlements, civilisation and religion have all evolved round water (Jadhav, 2014).

Potable water remains a fundamental human need and right (Megersa et al., 2014) with Individuals entitled to sufficient, safe, accessible and affordable water (WHO, 2003; Skandhan et al., 2011). Adequate treatment is important to prevent the occurrence of harmful contaminants in water (Hyung and Kim, 2009). However, in most developing nations, processing of water in disinfection units is rarely adequate resulting to increased exposure to waterborne diseases and fatal consequences in many rural societies in developing African nations (Shaheed et al., 2009; Dubreuil, 2013; Megersa et al., 2014; Jones and Bridgeman, 2017)

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with children and the elderly mainly affected (WHO, 2006). It has been recommended that household water treatment (WHO, 2007) is a way forward to combatting drinking water problems (WHO, 2011). Plant-based water treatment solutions is evolving as a feasibly cheap technology (Table 1). Since Africa produces good percentage of medicinal plants (Megersa et al., 2014), it is vital to understand the application of African indigenous plant extracts in water treatment, antimicrobials and as disinfectants. Two essentially important native African plants worthy of further investigations are *Zanthoxylum zanthoxyloides* and *Gongronema latifolium* (Jones and Bridgeman, 2017).

Zanthoxylum species are deciduous shrubs and trees of the family *Rutaceae* which comprise 250 species used as sources of pharmaceutical and cosmetics raw materials. It is externally applied to abscesses, ulcers, swellings, haemorrhoids and wounds. Rheumatic pain, fever, malaria, tuberculosis, oedema, sickle cell anaemia and paralysis have all been maintained using the plant material (Nacoulma, 1996). Other traditional uses include mouth freshening and tooth care (Gaur, 1999; Negi et al., 2011). Bark sap, root and stem bark powder are applied in eye infection and whooping cough treatment (Arbonnier, 2004). Water preparation of the stem, root and bark is also applied for cancer treatment and probably in psycho-active intervention for numerous magico-religious uses (Arbonnier, 2004). Diverse phytochemicals have been reported as constituents of the plants (Ngassoum et al., 2003; Ouattara et al., 2004; Fogang et al., 2012; Wouatsa et al., 2013).

G. latifolium (Asclapiadaceae) is a soft-stemmed palatable perennial plant (Okafor, 2005). It contains saponins, essential oils, alkaloids, tannins as well as other phytochemicals (Schneider et al., 1993; Morebise and Fafunso, 1998; Morebise et al., 2002; Essien et al., 2007). Water and alcoholic extracts from this plant exhibit hypoglycemic, hypolipidemic, antioxidative and anti-inflammatofry properties (Morebise et al., 2002; Ogundipe et al., 2003; Ugochukwu and Babady, 2003; Ugochukwu et al., 2003). Hot water treated fruits of *Gongronema latifolium* are eaten as laxative in soup preparation (Akuodor et al., 2010). Extract of the leaf in ethanol assists in treatment of viral hepatitis, bilharziosis, and as an all-purpose antimicrobial agent (Eja et al., 2011). Numerous 17β-marsdenin derivatives, and several other phytochemicals have been found in the plant. Essential oil of the leaves is mainly composed of linalool, (E)-phytol and aroma dendrene hydrate (Oshodi et al., 2004; Nenaah and Ahmed, 2011). No water treatment application of the above plants have been reported till date.

The aim of this report is to assess the disinfection potential of the two selected native African plant species on inherent bacterial and fungal water contaminants in order to have insight into their properties, particularly, in water purification in African countries where clean water accessibility is of major concern.

2. Materials and methods

2.1. Plant sampling and microbial culture collections

Zanthoxylum zanthoxyloides and *Gongronema latifolium* samples were collected from plant material market (8.1294°N, 4.2005°E) Ogbomoso, Oyo State, Nigeria. Plants were washed, air dried and grinded to powder, then sieved to get a fine powder. Dry running through published literatures was used in identification of emerging microbial contaminants in water and identified bacteria species (*P. aeruginosa*, *Klebsiella* sp., *E. coli, S. pneumonia*, B. cereus) and fungal isolates (*A. flavus*, *A. niger*, *Trichoderma* sp and *Candida* sp.) were sourced from local strains collection from Culture Bank, Microbiology Unit, Pure and Applied Biology Department, LAUTECH, Ogbomoso, Nigeria.

2.2. Preparation of samples

50 g of dried and grinded 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 hrs. The solvent fractions were separated using vacuum filtration aided by a sterile Whatmann No 1 filter paper and filtering crucible. The filtered extracts were then dried with the help of a freeze drier and stored in sterile polypropylene air-tight container at 4 °C or dispensed in appropriate sterile dissolving solution when required.

2.3. Antimicrobial activity, preparation of antimicrobial disc and sensitivity test

Crude extracts of *G. latifolium* and *Z. zanthoxyloides* eluted by ethyl acetate and chloroform and freeze dried were prepared into solutions of different concentrations ranging between 25 and 500 mg/ml by dissolving appropriate amount of crystallised extracts in sterile deionised water. About 20 μl of extract solution corresponding to a range of (0.5 mg – 10 mg) was then transferred

Table 1
Plant with disinfection and coagulating application.

Plant	Part	Description	Reference	Application
M. oleifera.	Seed	Tree	Koehn and Carter (2005) Gebremichael et al. (2005)	Coagulation and disinfection
Opuntia ficus indica. *D. Lablab.*	LeavesFruit	Shrub Herb	Shilpa et al. (2012)Zhang et al. (2006)	Coagulation and disinfection
Cicer arietinum L.	Seeds	Herb	Choubey et al. (2012)	Coagulation and disinfection
Aloe barbadensis *Hibiscus sabdarifa L.* *Jatropha curcas L.Citrus aurantifoliaGarcinia kola Heckel* *Carica papaya L.*	SeedsCalyxSeedFruitSeedSeed	HerbHerbTreeTreeHerbTree	Yongabi et al. (2011a) Yongabi et al. (2011b)	Coagulation and disinfection
Parkinsonia aculeate *Vigna unguiculata*	Seed Seed	Tree Herb	Marobhe and Gunaratna (2012)	Coagulation and disinfection
Cyamopsis tetragono	Seed	Herb	Pritchard et al. (2009)	Coagulation and disinfection
Manihot esculenta	Root	Shrub	Vara (2012)	Coagulation and disinfection
Solanum incunum L.	Leaf	Shrub	Kihampa et al. (2011)	Disinfection and Coagulation
P. vulgaris L.	Seed	Herb	Sciban et al. (2006)	Coagulation

on sterile 6 mm diameter sterile paper discs prior to antimicrobial sensitivity testing by disc diffusion method as described by (Adebayo et al., 2014; Lateef and Adeeyo, 2015). Briefly, 1 to 2 colonies of each bacteria culture were introduced into nutrient broth, and incubated at 37 °C for 24 hrs. A 1 to 50 dilution of the stock culture was then adjusted to tune of 0.5 McFarland standard (1.0×10^6 cfu/ml). About 100 μl of the test microbes were then swabbed over the agar plate surface using surface spread method. Treated paper discs were afterward arranged serially and radially but firmly unto seeded agar plates. The plates were afterward incubated at 37 °C for 24 hrs. Fungal plates were incubated at room temperature (27 ± 2 °C) for 48 hrs. The degree of microbial susceptibility was determined by measuring the visible diameter of inhibition on microbial growth after incubation. Test concentrations of crude extract ranged from 500 and 25 mg/ml. Standard reference commercial antibiotic disc for Gram-negative (streptomycin), Gram-positive (gentamycin) and fungi (nystatin) were used for a comparative study.

2.4. Phytochemical analyses

The screening for plant extract composition was assayed according to standard methods and as modified by Adeeyo et al., 2018. Screening for tannins, saponnin, reducing sugars, alkaloids, terpenoids, flavonoids, steroids, anthraquinones and phlobatannins were carried out on extracted crude products.

2.5. Data analysis

For Data analysis, SPSS, version 19.0 was used in analysing the different means of data obtained. ANOVA test was used to determine the level of significance of the result at various concentrations. Also, comparison of the different solvent extracts (chloroform and ethyl acetate) were made statistically. Antimicrobial efficacy of the extracts were examined against that of standard antibiotics. Zones of inhibition was compared with reference standard to assign the level of activities of extracts.

3. Result and discussion

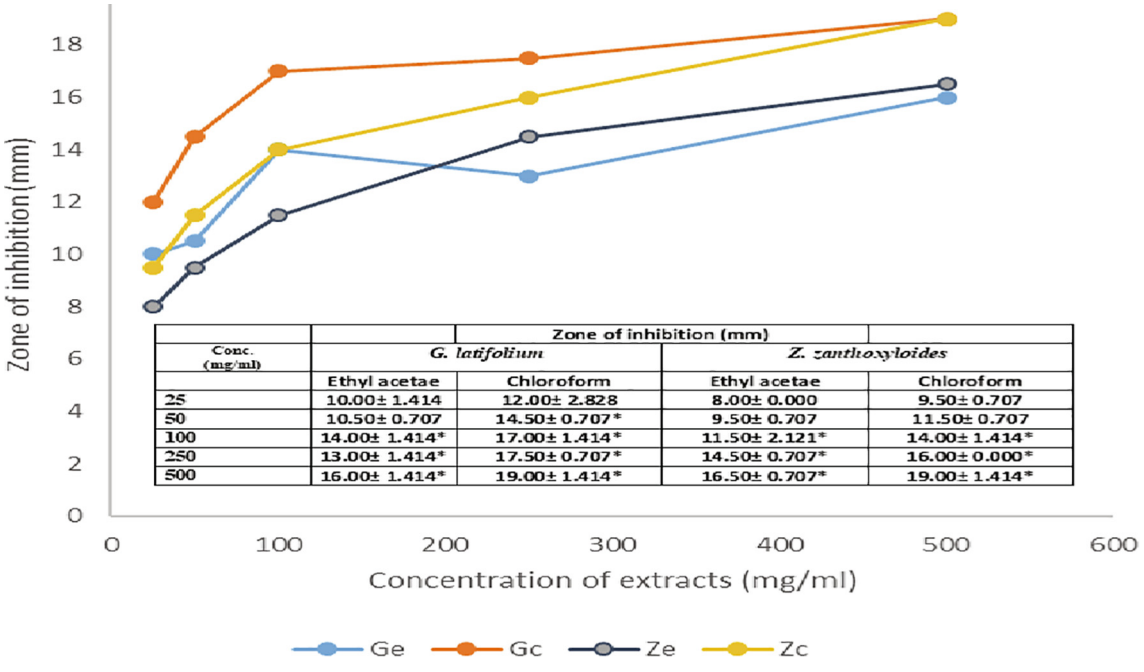
The antimicrobial activities of extracted phytobiotics against bacterial and fungal isolates tested were investigated with extract doses ranging from 25 to 500 mg/ml. Figs. 1–5 shows the zone of inhibition of *G. latifolium* and *Z. zanthzyloides* against selected Gram-negative (*P. aeruginosa, Klebsiella* 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 extracts.

3.1. Dose dependent activities

The degree of antimicrobial activity by measuring the visible diameter of inhibition on microbial growth has been described as strong (23–38 mm), moderate (19–22 mm) and low (12–18 mm) (Ahmad et al., 1999). The dose response to plant extracts can be observed in Figs. 1–5 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 zone is between 7.5 and 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 effects are qualitatively alike to that of Shaheed et al. (2009) and Jones and Bridgeman (2017) who reported increase in performances of herbal extracts at higher dosages against microbial pollutants of water.

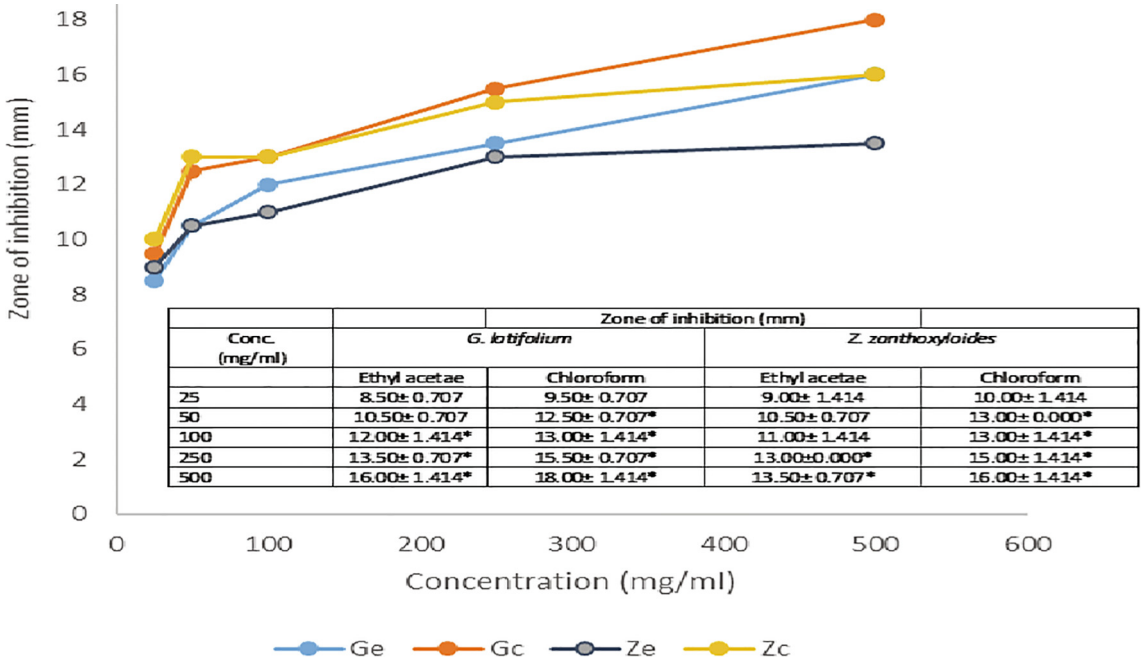
3.2. Comparative phytobiotic activities (with commercial antibiotics)

The abilities of phytoactive extracts of plants materials investigated as compared with commercial antibiotics were investigated.



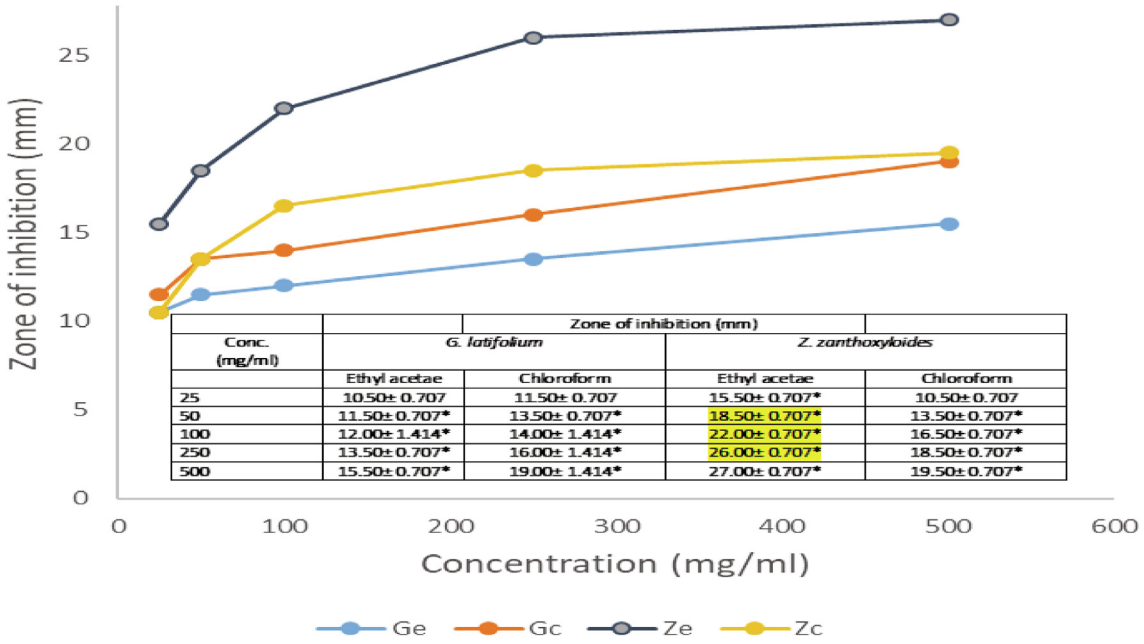
Conc. (mg/ml)	Zone of inhibition (mm)			
	G. latifolium		*Z. zanthoxyloides*	
	Ethyl acetae	Chloroform	Ethyl acetae	Chloroform
25	10.00± 1.414	12.00± 2.828	8.00± 0.000	9.50± 0.707
50	10.50± 0.707	14.50± 0.707*	9.50± 0.707	11.50± 0.707
100	14.00± 1.414*	17.00± 1.414*	11.50± 2.121*	14.00± 1.414*
250	13.00±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

Fig. 1. Antibacterial activities of *G. latifolium and Z. zanthoxyloides* Chloroform and Ethyl acetate extract on *P. aeruginosa*.



Conc. (mg/ml)	Zone of inhibition (mm)			
	G. latifolium		*Z. zanthoxyloides*	
	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*

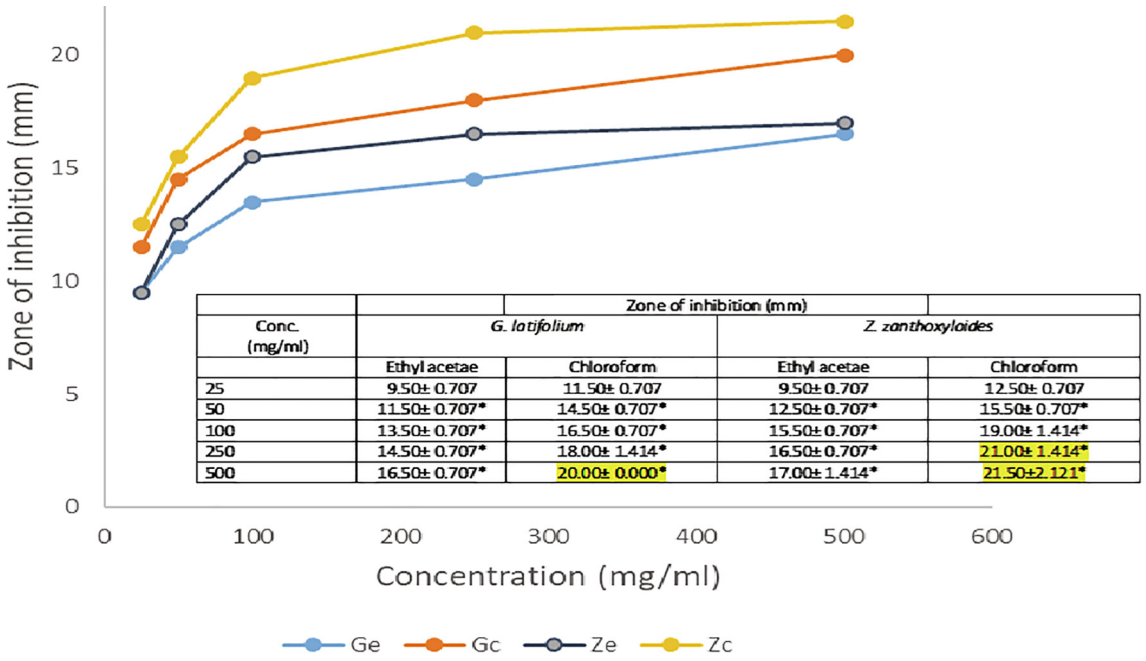
Fig. 2. Antibacterial activities of *G. latifolium and Z. zanthoxyloides* Chloroform and Ethyl acetate extract on *Klebsiella* sp,



Conc. (mg/ml)	Zone of inhibition (mm)			
	G. latifolium		*Z. zanthoxyloides*	
	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*

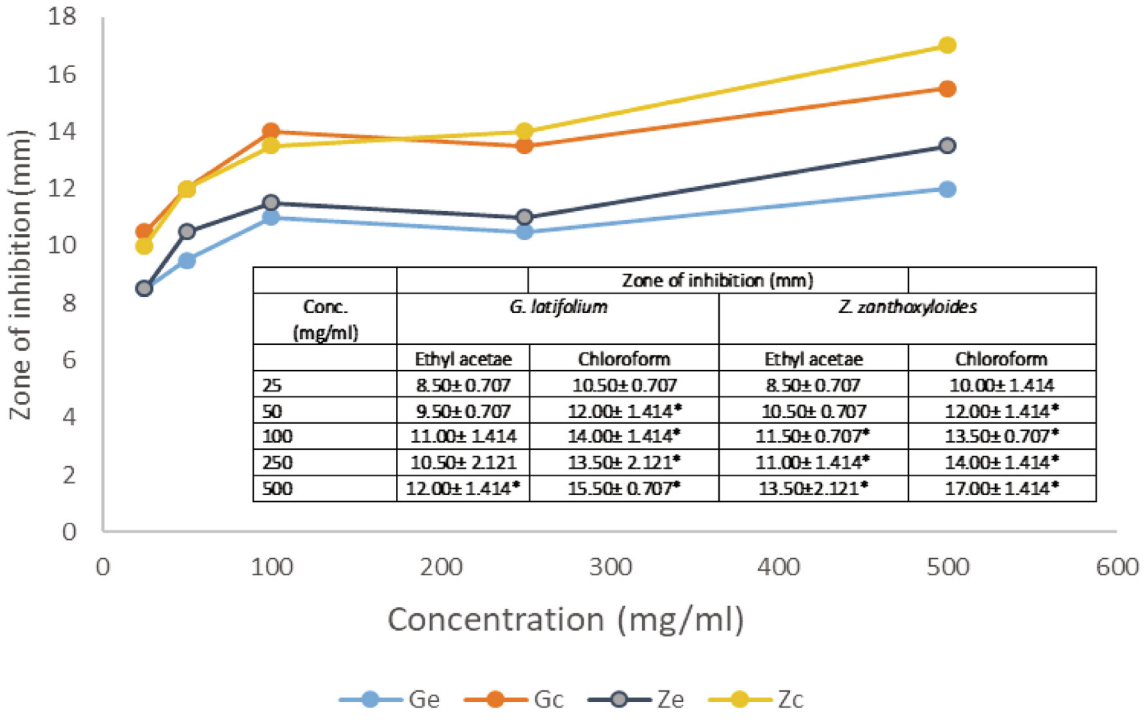
Fig. 3. Antibacterial activities of *G. latifolium and Z. zanthoxyloides* Chloroform and Ethyl acetate extract on *E. coli*.

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 revealed 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 (WHO, 1998) especially when developed for water disinfection. Potent antimicrobial activities of plant materials have been reported for different formulations such as antipsoriatic cream with varied combinations of neem, bakuchi, sarsaparilla and daruhaldi which resulted in good anti-inflammatory and antimicrobial results (Mundada et al., 2009); herbal gel comprising *Curcuma- longa, Aloe-vera* and *A. indica* resulted in a good antimicrobial activity against the tested microbes in comparison to commercially available antimicrobials (Pandey et al., 2010). In a study where polyherbal ointment was made using methanolic extracts of *M. pudica*, *S. indica A. indica*,



Conc. (mg/ml)	Zone of inhibition (mm)			
	G. latifolium		*Z. zanthoxyloides*	
	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*

Fig. 4. Antibacterial activities of *G. latifolium and Z. zanthoxyloides* Chloroform and Ethyl acetate extract on *S. pneumonia*.



Conc. (mg/ml)	Zone of inhibition (mm)			
	G. latifolium		*Z. zanthoxyloides*	
	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*

Fig. 5. Antibacterial activities of *G. latifolium and Z. zanthoxyloides* Chloroform and Ethyl acetate extract on *B. cereus*.

C. odorata, and assessed for bioactivities, the results showed better antibacterial properties (Rajasree et al., 2012). These reported research findings supported phytoactivities of plant materials as indicated in this work. Herbal extracts of *Ocimum samctum* and *Eucalyptus globules* as anti-microbial agents were found to exhibit major activity against *P. aeuroginosa, B. subtilis, Escherichia. coli, S. aureus, S. cerevisiae* as well as *C. albicans* and were safer for human usage when compared to reference standards Amphoterecin and Ampicillin respectively (Wani et al., 2013).

3.3. Antibacterial activities

The antibacterial activity of the extracts differs with respect to extraction solvents and susceptibility of microbial isolates increases with concentration of extract in solution (Figs. 1–5). Noteworthy is the activities of the extracts against *E. coli* which is a conventional indicator of water pollution. The activities of extracts tested were all notable against *E. coli*. Antibiosis of extracts on *E. coli* showed moderate to high performance at concentrations

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	

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
	Min	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
	Min	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.

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 and microbial pathogens in water. The effects of the plant extracts against *E. coli, Kelsiella sp* and *P. aeruginosa* indicated the activities 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 seemed 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 indicates 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 extracts 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 comparatively greater efficacy than commercial antibiotics at the concentration of extracts tested. *E. coli* was the most susceptible microbial isolate tested representing the potentials of the extracts against a group of coliform which are important indicators of microbial pollution in water. Other microbial isolates also recorded susceptibility to extracts tested to varying degrees. It was observed in this finding 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 revealed the varied presence of tannins, flavonoids, terpenoids, anthraquinones, alkaloids and saponins, (Table 5). The observed trends of phytochemicals of *Z. zanthoxyloides* in this work is similar with the earlier reports on same plant (Adesina, 1986; Chaaib, 2004; Adesina, 2005). Plant phytochemicals have been established to exhibit antimicrobial properties (Iwu et al., 1999; Banso and Adeyemo, 2006). 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 (2010) 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 (2010) for *Bacillus*, *P. aeruginosa and E. coli* which may be attributed to the concentrations of extract used. Adesina (2005) has described the broad spectrum antibacterial activity of many species of *Zanthoxylum* against different microbes, although aqueous extracts was reported to be less effective against microbial sample of *P. aeruginosa* and *E. coli* (Ndukwe et al., 2005; Adebiyi et al., 2009). Extracts from *Z. zanthoxyloides* have previously also been documented for notable activity against bacteria associated with periodontal diseases (Taiwo et al., 1999). In the work of Olila et al. (2001), 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 (Nair and Chanda, 2006).

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 with minimum inhibitory concentrations (MIC) ranging from 100 to 1000 mg mL^{-1} are referred to as antimicrobials (Abreu et al., 2012).

The activity of *G. latifolium* as reported in this work is also similar to other reports on the activity of *G. latifolium's* extracts to inhibit different microbes (Morebise et al., 2002; Ugochukwu and Babady, 2002; Farombi, 2003). The presence of saponins and

Table 5
Phytochemical constituents of *G. latifolium* and *Z. zanthoxyloides*.

Analysis	*G. latifolium*		*Z. zanthoxyloides*	
	Ethyl acetate	Chloroform	Ethyl acetate	Chloroform
Saponins	(−)	**(+)**	**(+)**	(−)
Tannins	**(+)**	**(+)**	(−)	(−)
Reducing Sugars	(−)	(−)	(−)	(−)
Alkaloids	**(+)**	**(+)**	**(+)**	**(+)**
Flavonoids	(−)	**(+)**	**(+)**	**(+)**
Terpenoids	(−)	(−)	**(+)**	**(+)**
Phlobatannins	(−)	(−)	(−)	(−)
Steroids	(−)	(−)	(−)	(−)
Anthraquinones	(−)	(−)	**(+)**	(−)

(+), present; (−), absent

flavonoids in *G. latifolium* have been attributed to its antimicrobial efficacy (Morebise and Fafunso, 1998). In a similar manner to the findings of this study, *Staphylococcus* sp., *Pseudomonas* sp., *Shigella* sp., *K. Pneumonia, Salmonella* sp., *E. coli* and *O. anthropi* were all inhibited at 100 mg/ml in Adeleye et al. (2011). The presence of essential oil was attributed to high antimicrobial efficacy against tested microbes including *C. albicans.* Inhibition ranged from 7.5 mm *for P. 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. This suggests 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

Inhibition activities observed against bacterial and fungal cell as exerted by phytochemicals in this study may be due to the collapsing of cell walls and membranes, resulting in leakage of cell component, interruption of proton motive force, dysfunctioning of efflux pump and enzyme, leading to cytosis (Burt, 2004). Phytochemical activities of plant extract against microbes from disruption of nucleic acid synthesis, interfering with intermediate metabolism, initiation of coagulation of cytoplasmic components and disruption of normal cell communication in quorum sensing have also been reported (Sanchez et al., 2010; Chitemerere and Mukanganyama, 2014; Anandhi et al., 2014; Nazzaro et al., 2013; Radulovic et al., 2013; Mogosanu et al., 2015). A common phenomenon is that several compounds in crude plant extract act at different target sites in pathogens and contribute to optimum efficacy of plant extracts (Gupta et al., 2014). Phytochemicals may exhibit antimicrobial effect in microbes not only through direct lethal activity but also by altering key events in pathogenesis (Brijesh et al., 2009). The activity of inhibition as observed in this study can therefore be bactericidal in which the zone of inhibition experienced results from complete lethal action against the microbes or bacteriostatic in which the inhibition zones results in a state of viable but dormant situation referred to as viable but non culturable (VBNC) state. Conventional chemically synthesised antibiotics and phytobiotics significantly differ with respect to frequency in radicals and spatial structure (Koehn and Carter, 2005). The latter is with a fewer nitrogen, sulphur, halogens, phosphorus and exhibit enhanced scaffold variety, stereo chemical abundance, diversity in ring system, molecular complexity and carbohydrate contents (Schmidt et al., 2008). Also, plant products can alter or prevent protein-protein interactions, thus acting as effective modulators of immune response, apoptosis, mitosis and signal transduction (Koehn and Carter, 2005) making them suitable alternatives to synthetic antibiotics.

4. Conclusion

A major conclusion is that plant samples tested revealed potent antimicrobial activities which notably 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. The mount 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.

Author contributions

A.O Adeeyo and K.A Odelade conceived this work, J.O Odiyo and T.A.M Msagati assisted in interpretation of data.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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