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Antibacterial Potentials of Three Common Spices against Selected Pathogens

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Abstract
Humans are generally most interested in the species of pathogenic bacteria which can cause disease in humans. The antibacterial activities of *n*-hexane, dichloromethane and methanol extracts of *Curcuma longa* Linn. (Turmeric) rhizome, *Myristica fragrans* Houtt. (nutmeg) and *Zingiber officinale* Rosc. (Ginger) rhizome were evaluated against *Staphylococcus aureus* ATCC 26923, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25522, *Enterococcus faecalis* ATCC 29212, *S. aureus* ATCC 25923, Methicillin-Resistance-*Staphylococcus aureus, Klebsiella pneumoniae* ATCC 35657 and *Serratia marcescens.* Inhibition test was carried out using the agar dilution techniques while the minimum inhibitory concentration (MIC) was determined by the agar well diffusion method. Susceptibility testing at 20 mg/mL and 100mg/mL revealed the highest inhibitory activity by methanol extracts of *Curcuma longa* and *Myristica fragrans* with diameter of zone of inhibition between 16±0.0 mm and 27±0.0 mm, the most active been *Myristica fragrans* on *E. faecalis* ATCC 29212. The MIC and MBC were lowest (12.5 mg/mL) in the methanol extract of *Myristica fragrans.* Methanol extract of *Myristica fragrans* had better bactericidal activity on *Staphylococcus aureus* ATCC 26923 affording 88 %, 95 % and 97 % reduction in population at concentrations equivalent to MIC, 2 x MIC and 4 x MIC respectively at 4 hours exposure time followed by a total (100 %) kill of the population after 8 hours of exposure. The results of the study emphasize these spices as having antibacterial activity on some pathogenic bacteria isolated from human samples and their usefulness in the treatment of infections associated with the test pathogens.
Keywords: C*urcuma longa* (Linn.), *Myristica fragrans* (Houtt.), *Zingiber officinale* (Rosc), pathogenic bacteria, phytochemical screening, antibacterial activities, *in-vitro* study

1. Introduction

Medicinal plants represent a rich source of antimicrobial agents (Mahesh & Satish, 2008). Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava *et al*., 1996). A wide range of medicinal plant parts are used for extract as raw drugs and they possess varied medicinal properties. Medicinal plants have been found useful in the cure of a number of diseases including bacterial diseases. Due to a rapid increase in the rate of infections, antibiotic resistance in microorganisms and due to side effects of synthetic antibiotics, medicinal plants are gaining popularity over these drugs (Babu & Subhasree, 2009). Although medicinal plants produce slow recovery, the therapeutic use of medicinal plant is becoming popular because of their lesser side effects and low resistance in microorganisms (Seyyednejad & Motamedi, 2010). Some spices have also found use locally in the treatment of various diseases including infectious diseases. These spices include *Curcuma longa* Linn. (Turmeric), *Myristica fragrans* Houtt. (nutmeg) and *Zingiber officinale* Rosc. (Ginger). *Curcuma longa* Linn. (Turmeric) is a rhizomatous herbaceous perennial plant of the ginger family, Zingiberaceae (Chan *et al.,* 2009), distributed throughout tropical and subtropical regions of the world, being widely cultivated in Asiatic countries, mainly in India and China (Eigner & Scholz, 1999). *Curcuma longa* as a powder, called turmeric, has been in continuous use for its flavouring, as a spice in both vegetarian and non-vegetarian food preparations and it also has digestive properties (Govindarajan, 1980). Current traditional Indian medicine claims the use of its powder against biliary disorders, anorexia, coryza, coughs, diabetic wounds, hepatic disorder, rheumatism, sinusitis and urinary tract infections (Ammon *et al*., 1993). Curcumin, a hydrophobic polyphenol, is a principal active constituent of turmeric. In addition to curcumin, turmeric also contains other constituents termed curcuminoids (Ahsan et al., 1999; Sandur *et al*., 2007).

Myristica frangans Houtt. (Family- Myristicaceae) is native to the Spice Islands, located in Moluccas, Indonesia. The oils are used as medicines to treat rheumatism or as a cure for irritated skin. The oil can also be used in condiments soaps and perfumes (Wang *et al*., 2004). In traditional medicine, the seed kernel (nutmeg) is widely used as carminative, astringent, hypolipidaemic, antithrombotic, antiplatelet aggregation, antifungal, aphrodisiac (Sonavane *et al*., 2002), treating flatulence, nausea, and dyspepsia (Zaidi *et al*., 2009). In dentistry application, macelignan, an active compound from seed had strong anticariogenic activity, possessed antibacterial effect against oral microorganisms such as *Streptococcus species*, and *Lactobacillus species*, and exhibited weak activity for *Actinomyces viscosus, Porphyromonas gingivalis* and *Staphylococcus aureus* (Chung

et al., 2006). Narasimhan and Dhake (2006) reported that trimyristin, an active compound obtained from seed of *Myristica fragrans*, also exhibited good antibacterial properties against Gram-positive and Gram-negative bacteria. The main constituents of *Myristica fragrans* have been found to be alkyl benzene derivatives (myristicin, elemicin, safrole), terpenes, alpha-pinene, beta-pinene, myristic acid, trimyristin (Wang *et al*., 2004), neolignan (myrislignan), and macelignan (Chung *et al*., 2006).

Ginger (*Zingiber officinale* Rosc), belonging to a tropical and subtropical family-Zingiberaceae, originating in South East Asia and introduced to many parts of the globe, has been cultivated for thousands of years as a spice and for medicinal purposes. The underground stem or rhizome of this plant has been used as a medicine in Asian, Indian and Arabic herbal traditions since ancient times (Shukla and Singh, 2007). It has been used in Unani, Ayurvedic and Chinese herbal medicines all over the world, since antiquity, for a wide array of unrelated ailments that include arthritis, rheumatism, sprains, pains, muscular aches, sore throats, indigestion, vomiting, fever, hypertension, cramps, constipation, dementia, helminthiasis and infectious diseases (Ali *et al.,* 2008**).** Chemically ginger contains several classes of compounds. The chemical composition of dried ginger is as follows: starch 40-60%, proteins 10%, fats 10%, fibres 5%, inorganic material 6%, residual moisture 10% and essential oil (oleoresin) 1-4 per cent (Verma and Bordia, 2001). The aim of this study was to evaluate the antibacterial properties of *Curcuma longa* Linn. (Turmeric) rhizome, *Myristica fragrans* Houtt. (Nutmeg) seed and *Zingiber officinale* Rosc. (Ginger) rhizome which have been used in folklore medicine to treat various diseases including infectious diseases.

2. Materials and Methods

2.1 Plant collection, extraction and preparation of extracts

Curcuma longa Linn. (Turmeric) rhizome, *Myristica fragrans* Houtt. (Nutmeg) seed and *Zingiber officinale* Rosc. (Ginger) rhizome were collected, authenticated, extracted. Extracts were prepared for biological assays as previously reported (Ogudo *et al.,* 2014).

2.2 Phytochemical Screening

Phytochemical screening was carried out to detect the presence of secondary metabolites such as anthraquinones, tannins, saponins, alkaloids, and phenol using methods described by Harborne (1998).

2.3 Antimicrobial Agents

The chemotherapeutic agents used in the test as positive control is Gentamicin (Nicholas Laboratories Limited, England), while the negative control was 20 % Ethanol.

2.4 Strains of pathogenic bacteria and culture methods

Eight (8) isolates comprising four (4) Gram positive and four Gram negative organisms were used for this investigation. They were *Staphylococcus aureus* ATCC 26923, *Staphylococcus aureus* ATCC 25923, Methicillin-Resistant-*Staphylococcus aureus* (MRSA), *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25522, *Klebsiella pneumoniae* and *Serratia marcescens.* All the isolates were collected, characterized and identified at The National Institute of Medical Research (NIMR), Yaba, Lagos State, Nigeria. The test organisms were cultured on agar slants and stored in the refrigerator at 4 °C. Subcultures were made at two-week intervals throughout the period of the experiments.

2.5 Susceptibility Testing

Susceptibility of the test organisms to *n*-hexane, dichloromethane and methanol extracts of *Curcuma longa* (Linn.) (turmeric) rhizomes, *Myristica fragrans* Houtt. (nutmeg) seed and *Zingiber officinale* (Rosc.) (ginger) rhizomes was determined using the agar cup diffusion technique. A 0.1 mL aliquot of logarithmic phase broth culture of each bacterium (optical density equivalent to 10^7-10^8 cfu/mL) was used to seed sterile sensitivity test agar (Oxoid) medium. The seeded plates were allowed to dry in the incubator at 37 °C for 20 min. A standard cork borer (8 mm diameter) was used to cut uniform wells on the surface of the agar, into which was added 100 µL of the test extract reconstituted in 20 % ethanol to final concentrations of 20 mg/mL and 100 mg/mL. A pre-incubation diffusion of the extracts into the seeded medium was allowed for 1 hour. Plates were incubated at 37 °C for 18-24 hours after which diameters of zones of inhibition (mm) were measured. 20 % ethanol was included in each plate as a solvent control while Gentamycin (100 µg/mL) was included as the positive control. This method is similar to previous published procedures (Ogudo *et al*., 2014).

2.6 Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentrations (MIC) of active extracts were determined by a modification of standard agar dilution method procedures as previously described (Ogudo *et al.,* 2014). Extracts were tested at concentrations ranging from 6.25 mg/mL to 200 mg/mL. Gentamicin and 20 % ethanol were used as positive and negative controls respectively. The MICs were determined after 24 hours of incubation at 37 °C. The MIC was regarded as the lowest concentration that prevented visible growth from two replicates of experiment.

2.7 Determination of Minimum Bactericidal Concentration (MBC)

Minimum bactericidal concentrations (MBC) of the active extracts were determined by a modification of the method of Aibinu *et al.,* (2007). To a 0.5 mL extract at different concentration as used in the MIC assay that

showed no visible growth on the agar plates, was added 0.5 mL of test organism in a tube. These were incubated at 37 ^{o}C for about 24-48 hours. Samples were streaked out from the tubes onto sensitivity test agar to determine the minimum concentration of the extract required to kill the organisms. These concentrations were indicated by the inability of the organisms to grow on transfer to extract-free growth media. The lowest concentration that prevented bacterial growth after 24-48 hours of incubation was recorded as the minimum bactericidal concentration (MBC). The entire tests were carried out in two replicates to ensure accuracy. Agar plates without extracts and another agar plate without any inoculated organism were also incubated to serve as positive and negative control plates respectively.

2.8 Determination of bactericidal activity of the methanol extracts of Curcuma longa rhizome, Myristica fragrans seed and *Zingiber officinale* rhizome

The viable counting technique previously described (Ogudo *et al*., 2014) was used for this purpose. Bactericidal activities of active extracts with low MIC values were studied in *Staphylococcus aureus* ATCC 26923 and *S. aureus* ATCC 25923. Plates were incubated at 37 °C for 24 hours before counting the colonies. A graph of percentage viable count against time in hour was plotted to show the rate of kill of the test organisms after two replicates of experiments.

3. Results and Discussion

Medicinal plants and spices represent a rich source of antimicrobial agents used traditionally by many cultures for controlling common health complications (Mahesh & Satish, 2008; Aboaba and Efuwape 2001). The antibacterial potentials of three common spices namely *Curcuma longa* Linn. (turmeric), *Myristica fragrans* Houtt. (nutmeg) and *Zingiber officinale* Rosc. (ginger) were evaluated in *Staphylococcus aureus* ATCC 26923, *Staphylococcus aureus* ATCC 25923, Methicillin Resistant-*Staphylococcus aureus* (MRSA), *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25522, *Klebsiella pneumoniae* and *Serratia marcescens* to justify and support their local use in the treatment of diseases including infectious diseases. The extraction yield was highest in methanol for both *Curcuma longa* and *Zingiber officinale* whereas the yield was highest in dichloromethane for *Myristica fragrans*. While the former were not in agreement with the report of Cowan (1999) who ranked methanol next to dichloromethane in terms of yield in extraction of plant active components, the later agreed with Cowan's report. Results of phytochemical screening for secondary metabolites are presented in Table 1. Susceptibility testing at 20 mg/mL and 100mg/mL revealed that the highest inhibitory activity was exhibited by methanol extracts of *Curcuma longa* and *Myristica fragrans* with diameter of zone of inhibition between 16±0.0 mm and 27±0.0 mm, the most active been methanol extracts of *Myristica fragrans* on *E. faecalis* ATCC 29212 (Table 2). Methanol and *n*-hexane extracts of *Z. officinale* Rosc. (Ginger) dried rhizome had a moderate diameter zone of inhibition ranging between 14 mm and 16 mm on *S. aureus* ATCC 25923 and MRSA (Table 2). The *n*-hexane and dichloromethane extracts of *Myristica fragrans* as well as the dichloromethane extract of *Zingiber officinale* had no antibacterial activities and so the results were not presented. The antibacterial activity observed was only in the Gram positive organisms, the Gram negative organisms were resistant to the extracts at the test concentrations. This shows that the extracts have narrow spectrum of activity against the test organisms. It was also observed that the test organisms were not susceptible to low concentrations of the control drug- Gentamycin; they were only inhibited by high concentration (100 µg/mL) of Gentamycin (Table 2). The inhibitory activity of the bioactive extracts on the susceptible organisms is noteworthy even though they were not as potent as the control drug. The resistance of the Gram negative organisms to the test extracts is a function of the cell wall composition which is known to prevent the entrance of antibacterial agents into the cell of the Gram negative organisms. The MIC and MBC of active extracts against susceptible test bacteria species are presented in Table 3. The MIC and MBC were lowest (12.5 mg/mL) in the methanol extract of *Myristica fragrans* against *Staphylococcus aureus* ATCC 26923. The observed inhibitory activity on the Gram positive organisms is attributable to the presence of the phytochemical compounds detected in the samples (Table 1). These compounds are known to possess antibacterial properties. Tannins, alkaloids, anthraquinones, flavonoids and phenols except saponins were present in *Myristica fragrans* seed (Table 1) and these may be responsible for the activity demonstrated by this plant. Narasimhan and Dhake (2006) reported antibacterial properties of the seed of *Myristica fragrans* against Gram-positive and Gram-negative bacteria. The antibacterial activity exhibited by the methanol and dichloromethane extracts of *Curcuma longa* is as a result of the phytochemical compounds viz: tannins, anthraquinones, flavonoids and phenols detected in the extracts. Alkaloids and saponins were absent (Table 1). These phytochemical compounds when present in a plant sample can act additively or synergistically to elicit antibacterial action at a single or multiple target sites associated with physiological process (Tyler, 1999). Niamsa and Sittiwet in 2009 reported the antibacterial activity of aqueous extract of *C. longa* against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae* ATCC 10031 and *Staphylococcus epidermidis* ATCC 12228. *Zingiber officinale* though rich in tannins and alkaloids with a trace amount of flavonoids had weak inhibitory activity. This report correlates with the findings of Eruteya and Odunfa (2009) and Esimone et al., (2010) who

reported ginger as having weak antimicrobial effects.

The bactericidal activity was observed to be dependent on time and dose/concentration as the percentage reduction in viable count of surviving population increased with increase in exposure time and concentration of the extracts (Figures 1-3). Exposure of *Staphylococcus aureus* ATCC 26923 to dichloromethane extract of *Curcuma longa* (Turmeric) resulted in decline (> 50 %) in population after 8 hours of exposure to extract at doses equivalent to MIC, 2 x MIC, 4 x MIC and 8 x MIC followed by a total kill of the population at 24 hours. This was also observed with methanol extract of *Zingiber* officinale (Ginger) on *Staphylococcus aureus* ATCC 25923. Methanol extract of *Myristica fragrans* had better bactericidal activity on *Staphylococcus aureus* ATCC 26923 affording 88 %, 95 % and 97 % reduction in population at concentrations equivalent to MIC, 2 x MIC and 4 x MIC respectively at 4 hours of exposure time followed by a total (100 %) kill of the population after 8 hours of exposure. The control drug- Gentamycin (60 µg/mL) in contact with *Staphylococcus aureus* ATCC 26923 reduced the population by 95 % at 4 hours exposure time with a 100 % kill of the population at 8 hours of exposure. The methanol extract of *Myristica fragrans* compared fairly well with the control drug. The antibacterial activities exhibited by methanol extracts of *Curcuma longa* rhizome and *Myristica fragrans* seed suggest that these plants may be of therapeutic importance in the treatment of infections caused by *Staphylococcus aureus* and other susceptible organisms if further explored for the bioactive components.

Table 1: Results of phytochemical screening of *Curcuma longa* (Turmeric), *Myristica fragrans* (Nutmeg) and *Zingiber officinale* (Ginger)

	Curcuma longa	*Myristica fragrans*	*Zingiber officinale*
Tannins	++	++	++
Alkaloids	-	++	++
Anthraquinone	++	+	-
Saponins	-	-	-
Flavonoids	++	+	+
Phenols	+	++	-

Note: ++ = High Concentration, + = Low (Trace amount), **-** = Absent

Table 2: Antimicrobial susceptibility of test bacteria to lyophilized extracts of *Curcuma longa* (Turmeric), *Myristica fragrans* (Nutmeg) and *Zingiber officinale* (Ginger). Diameter of zone of inhibition (mm) Mean ± SEM

Pathogenic Organisms	Methanol extract of *Curcuma longa* (mg/mL)		Dichloromethane extract of *Curcuma longa* (mg/mL)		*n*-Hexane extract of *Curcuma longa* (mg/mL)		Methanol extract of *Myristica fragrans* (mg/mL)		Methanol extract of *Zingiber officinale* (mg/mL)		*n*-Hexane extract of *Zingiber officinale* (mg/mL)		Gentamicin (µg/mL)
	20	100	20	100	20	100	20	100	20	100	20	100	100
S. aureus ATCC 26923	20±0.0	24±0.2	14±0.0	19±0.2	14±0.0	22±0.2	16±0.0	21±0.1	-	-	-	-	24±0.0
P. aeruginosa ATCC 27853	-	-	-	-	-	-	-	-	-	-	-	-	21±0.0
E. coli ATCC 25522	-	-	-	-	-	-	-	-	-	-	-	-	22±0.5
E. faecalis ATCC 29212	-	-	-	-	12±0.1	16± 0.0	21±0.0	27±0.0	-	-	-	-	23±0.5
S. aureus ATCC 25923	-	-	-	-	18±0.4	24±0.0	-	-	14±0.0	16±0.0	15±0.0	16±0.0	24±0.0
MRSA	-	-	-	-	-	-	-	-	15±0.0	16±0.0	-	-	24±0.0
K. pneumoniae ATCC 35657	-	-	-	-	-	-	-	-	-	-	-	-	23±0.0
S. marcescens	-	-	-	-	-	-	-	-	-	-	-	-	23±0.0

Key: - = No activity (Resistant); Diameter of cork borer = 8 mm

Table 3: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of lyophilized extracts of *Curcuma longa* (Tumeric), *Myristica fragrans* (nutmeg) and *Zingiber officinale* (Ginger) on common pathogenic bacteria species

Pathogenic Organisms	Methanol extract of *C. longa* (mg/mL)		Dichloro-methane extract of *C. longa* (mg/mL)		*n*-Hexane extract of *C. longa* (mg/mL)		Methanol extract of *Myristica fragrans* (mg/mL)		Methanol extract of *Z. officinale* (mg/mL)		*n*-Hexane extract of *Z. officinale* (mg/mL)		Gentamicin (µg/mL)	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus ATCC 26923	100	200	12.5	75	50	200	12.5	12.5	-	-	-	-	60	100
MRSA	ND	ND	ND	ND	ND	ND	ND	ND	100	200	ND	ND	100	100
E. faecalis ATCC 29212	ND	ND	ND	ND	100	200	100	125	ND	ND	ND	ND	100	100
S. aureus ATCC 25923	ND	ND	ND	ND	12.5	50	ND	ND	75	100	200	400	80	100

Note: - = No activity (Resistant), ND = Not determined

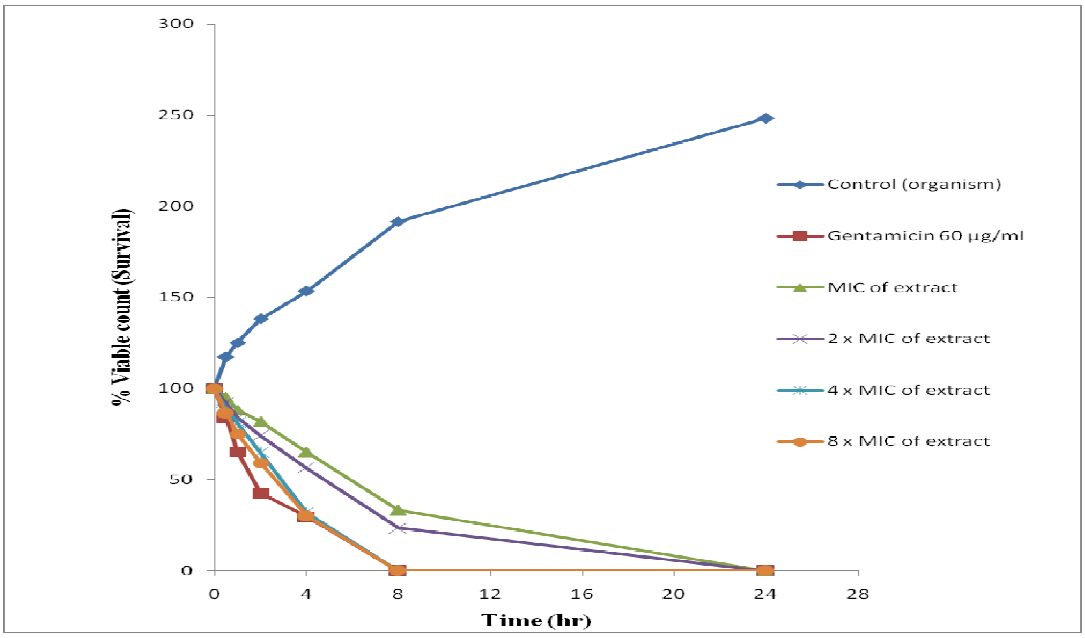

Fig. 1: Percentage viable count (Survival) vs Time (hr) of dichloromethane extract of *Curcuma longa* (Turmeric) on *Staphylococcus aureus* ATCC 26923 showing the rate of kill of the organism at the different concentrations of the extract**.**

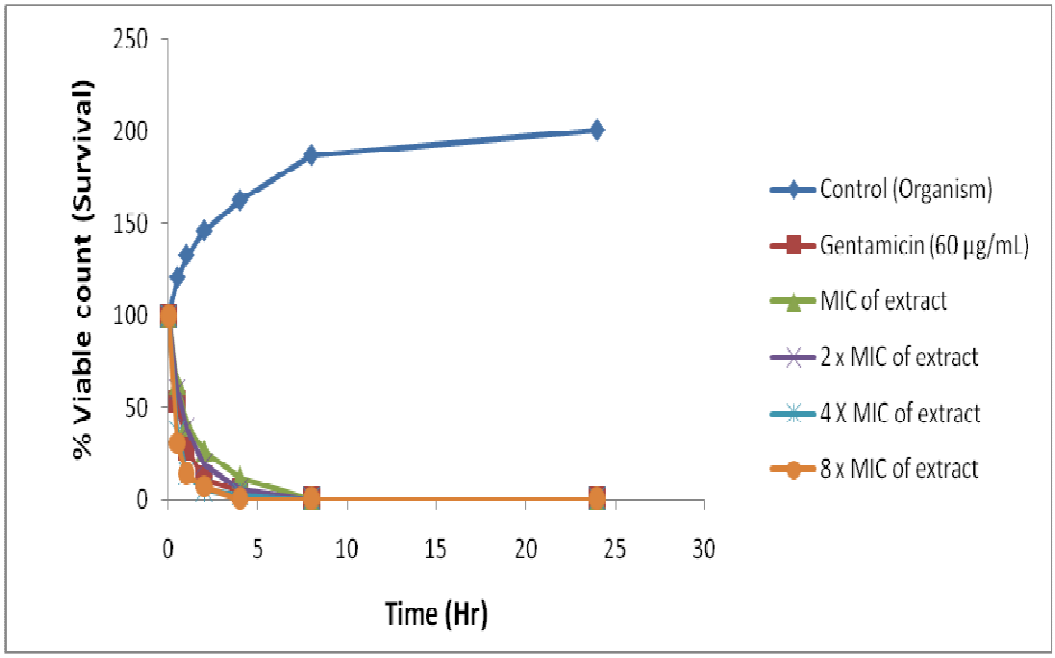

Fig. 2: Percentage viable count (Survival) vs Time (hr) of methanol extract of *Myristica fragrans* (nutmeg) on *Staphylococcus aureus* ATCC 26923 showing the rate of kill of the organism at the different concentrations of the extract.

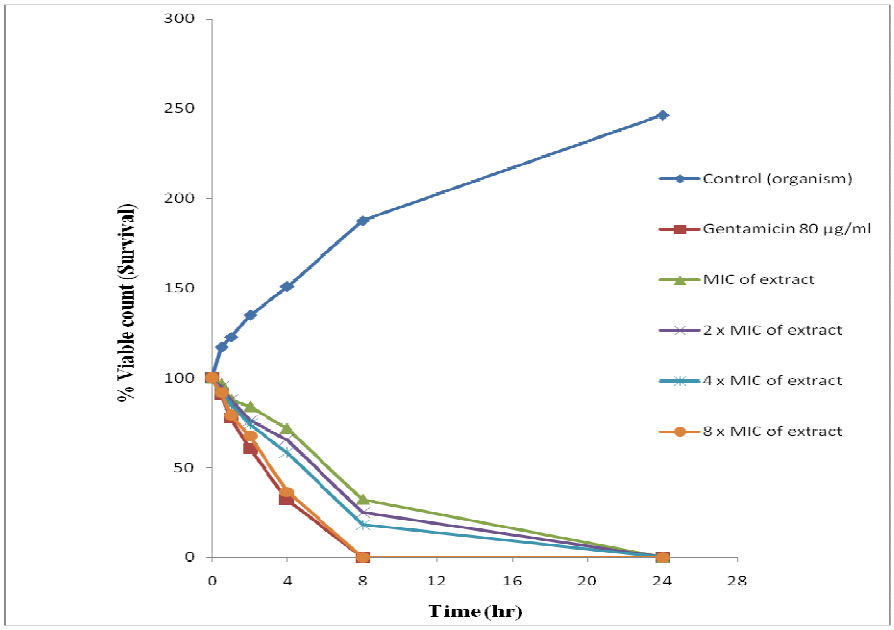

Fig. 3: Percentage viable count (Survival) vs Time (hr) of methanol extract of *Zingiber officinale* (Ginger) on *Staphylococcus aureus* ATCC 25923 showing the rate of kill of the organism at the different concentrations of the extract.

4. Conclusion

This study evaluates the antibacterial activity of *n*-hexane, dichloromethane and methanol extracts of *Curcuma longa* Linn. (Turmeric) rhizome, *Myristica fragrans* Houtt. (Nutmeg) seed and *Zingiber officinale* Rosc. (Ginger) rhizome. The results of the study emphasize these spices as having antibacterial activity on some pathogenic bacteria isolated from human samples and their usefulness in the treatment of infections associated with the test pathogens. This study also showed that turmeric, nutmeg and ginger extracts possess differences in antibacterial activities. The practice of using spices as supplementary or alternative medicine in developing countries like Nigeria will not reduce only the clinical burden of drug resistance development but also the side effects and cost of the treatment with conventional antibiotics.

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