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Assessment of Bacterial Inhibitory Properties of *Zingiber officinale* (ginger) Ethanol Extract on Some Clinical Isolates and Evaluation of Its Bioactive Compounds

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ABSTRACT

The increased usage of antibiotics has induced microorganisms to acquire resistance factors which have become a burning predicament. As a result, there is an urgent need to find an alternative to chemotherapeutic drugs in disease treatment particularly those of plant origin which are easily available and have considerably fewer side effects. In this study, *in-vitro* antibacterial activity of ethanol extract of *Zingiber officinale* (ginger) was investigated using the agar diffusion method on some clinical isolates (*Klebsiella pneumoniae, Streptococcus mutans, Escherichia coli, Staphylococcus aureus,* and *Shigella dysenteriae*). Phytochemical analysis of the extract was carried out using gas chromatography-mass spectrometry (GC-MS). Data obtained were subjected to one-way analysis of variance (ANOVA) procedure using SPSS (version 21) computer software. The extract of *Zingiber officinale* was active against all bacterial isolates with varying zones of inhibition that ranged from 12mm-24mm. *Zingiber officinale* extract had the highest zone of inhibition (24mm) at the concentration of 100mg/ml on *E. coli* followed by *S. aureus* (21mm) and *K. pneumonia* (18mm). Results of the phytochemical analysis of the *Z. officinale* extract showed the presence of some identified bioactive compounds (Geranyl acetate, α-Pirene, Camphene, Eucalyptol, Camphore, Terpin-4-ol, Citronellol, Geraniol, 2-Heptanol, Terpinolene, hexamethyl- etc.) that have antimicrobial properties. Therefore, results obtained from this study showed that the ethanol extract of *Zingiber officinale* possesses antibacterial properties against the clinical isolates tested which invariably make it a potential candidate for the treatment of some bacterial infections.

INTRODUCTION

In recent times, there is worldwide renewed interest in traditional medicine due to the realization that modern or orthodox medicine is not widespread in poor and many developing countries of the world (Mostafa, 2018; Gull *et al.*, 2012). Hence, the great need to look into the use of herbs as an alternative to these conventional means of treating bacteria and other infections caused by other organisms.

Similarly, bacterial resistance to conventional antibiotics that occurs due to indiscriminate use and self-medication of these drugs has been a problem facing modern-day medicine. Lack of proper diagnosis, use of underdose and the absence of a rational program for antimicrobial use are many factors that contribute to the increased prevalence of drug-resistant microorganisms, rendering antibiotics ineffective (Sa-Nguanpuag *et al.,* 2011).

Zingiber Officinale is a flowering plant whose rhizome, ginger root or ginger is widely used as a spice and folk medicine. It belongs to the family *Zingiberaceae* and is a herbaceous perennial plant that grows aerial pseudostem, about one meter tall bearing narrow leaf blades (Mele, 2019; Prasad and Tyagi, 2015). Ginger (*Zingiber officinale*) is a known plant used in traditional medicine against different diseases because of its various properties such as antimicrobial, anti-inflammatory, anticoagulant, antioxidant, etc (Mostafa and Singab, 2018; Abdel-Azeem *et al*., 2013).

Several studies have been carried out to characterize and isolate its main bioactive compounds, to determine/evaluate its antimicrobial activity against pathogenic microorganisms (Choudhari and Kareppa, 2013; Adeshina *et al.,* 2011; Ansari *et al*., 2006; Sa-Nguanpuag *et al.,* 2011; Yoo *et al*., 2005). Findings by these researchers indicate that ginger contains many bioactive compounds such as monoterpenoids, sesquiterpenoids, phenolic compounds, and its derivatives, aldehydes, ketones, alcohols, esters, which provide a broad antimicrobial spectrum against different microorganisms and make it an interesting alternative to synthetic antimicrobials (Mostafa and Singab, 2018; Choudhari and Kareppa, 2013). *Zingiber officinale* has been reported to have an antibacterial effect especially against the staphylococci species and also exhibits antifungal activity against a wide variety of fungi including *candida albicans* (Ficker *et al.,* 2003). It is noteworthy that the efficacy of ginger extract on bacteria inhibition is greatly affected by many factors such as the method of extraction, antibacterial assay conditions, genetic variations among bacterial strains and its sources (Yu *et al.,* 2009; Nikolić *et al.,* 2014; Habib *et al*., 2008; Overy and Frisvad, 2005). Therefore, the main objective of this study was to investigate the bacterial inhibitory properties of the ethanol extracts of *Zinger Officinale* on some clinical isolates and to determine its phytochemical composition.

MATERIALS AND METHODS

Collection, Identification and Preparation of Plants Samples:

Fresh ginger rhizomes obtained from the local market in Lagos state Nigeria were washed with distilled water, drained, peeled, sliced and shade dried for 21days. The dried gingers were grinded in a clean blender using a clean stainless bowl to collect the powder. The powder obtained was stored in an airtight dry container.

Sample Extraction:

Extraction of the bioactive component from the ginger powder was carried out according to the method described by *Herbert et al.* (2015). Briefly, 50g of dried ginger powder was weighed into a sterile 1000 ml beaker. About 500ml (1:10ml) of ethanol was added and allowed to stand for 24hrs with intervals of shaking using a rotary shaker. Afterward, it was filtered using a sterile Watmann filter paper and the filtrate was put in a water bath at the temperature of 40°C till the ethanol vented out. The extract was then stored in dry sterile container prior to antibacterial assay.

Sources of Test Organisms and Preparation *of bacterial suspension:*

The test organisms used in this study were *Klebsiella pneumoniae*, *Streptococcus mutans*, *Escherichia coli*, *Staphylococcus aureus,* and *Shigella dysenteriae*. These organisms were clinical isolates from the Nigerian Institute of Medical Research Yaba (NIMR) and State hospital, Ogun state. The organisms were re-identified using the standard biochemical tests. They were subcultured on nutrient agar slant and stored

at 4°C until required for the study. Pure bacterial isolates grown in nutrient agar were mixed with 10 ml sterile distilled water in McCartney bottles and the turbidity of the suspension was compared with that of 0.5 McFarland standard equivalents to 1.5×10^5 cfu/ml.

Antibacterial Sensitivity Assay:

The method previously described by Kareem *et al*. (2012) was adopted for the determination of the antibacterial sensitivity of the ethanol extract of ginger on the test organisms. Briefly, Mueller-Hinton agar plates were inoculated with the various already prepared isolates from stock cultures using a sterile swab in order to ensure even distribution of the inoculums. Then, 4 mm equidistant wells were made in the inoculated agar and the wells were filled with 100 µl each concentration of the extracts. They were then kept in the refrigerator for 1 hour for adequate diffusion of the extracts and thereafter were incubated at 37 °C for 24 h. After incubation, the diameter of the zones of inhibitions around each well was measured to the nearest millimetre.

Determination of Minimum Inhibitory Concentration (MIC):

Minimum Inhibitory Concentration (MIC) was determined by standard broth dilution method procedures as described by Owuama (2017). Briefly, a loopful (10 µl) of 24 h cultures, 0.5 McFarland standard (Eucast, 2003), was inoculated into test tubes containing 1 ml of the various concentrations of ginger ethanol extract in nutrient broth. The tubes were incubated at 37 °C for 18 to 24 h and thereafter observed for growth or turbidity. Subsequently, a loopful of broth from each test tube not showing growth was inoculated into a nutrient agar plate. Thereafter, equal volumes of sterile nutrient broth were added into the test tube cultures and incubated further for 24 h at 37 °C. Then, the tubes and agar plates were examined for growth or turbidity. These experiments were repeated three times.

Ginger Ethanol Extract GC-MS Analysis:

GC-MS analysis of the *Zingiber offinale* extracts was performed using a Shimadzu gas chromatograph model QP2010 plus (Tokyo, Japan), gas chromatograph (GC) system, equipped with a mass selective detector and auto-injector (SSQ 7000; Thermo-Finnigan, Bremen, Germany). Compounds were separated on the capillary column (30 m x 0.25 mm, film thickness 0.25 um). A sample of 1.0 µl was injected using the split mode (split ratio 1:1000). For GC/MS detection, an electron ionization system, with ionization energy of 70eV, was used. Column oven temperature program was the same as previously used in GC analysis. Helium was used as a carrier gas at a flow rate of 1.5 ml min^{-1}. The mass scanning range was 40-700 *m/z* while injector and MS transfer line temperatures were set at 220 and 290 °C, respectively (Choudhari and Kareppa, 2013)

Bioactive components Identification:

The identification of the compounds was based on a comparison of their mass spectra with those of the National Institute of Standards and Technology (NIST) mass spectral library, as well as on comparison of their retention either with those of authentic compounds or with literature values.

Statistical Analysis*:*

Data obtained were subjected to one-way analysis of variance (ANOVA) procedure using SPSS (version 21) computer software. Duncan multiple range tests was used to separate the mean at a 1% level of significance.

RESULTS AND DISCUSSION

Antibacterial Activity Assay:

Results of the antibacterial susceptibility assay of the test organisms *Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Streptococcus mutans,* and *Shigella dysenteriae* to ethanol extract of ginger at 100 mg/ml,50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.12mg/ml are shown on Table 1. It was observed that all the bacteria were susceptible to ethanol extract of *Z. officinale*. *E. coli* showed the highest zones of inhibition to all the different concentrations of ginger extract that inhibited the growth of the test organisms and it ranged between 24mm-12mm followed by *S. aureus* (21mm-12mm) and *K.*

pneumoniae (18mm-10mm) in that order (Table 1). No zones of inhibition was observed in the control well (DMSO in the water at the same used concentrations). Antimicrobial activity of the ginger extract was studied previously by Pankaj *et al.* (2012) where they reported that the growth of *Klebsiella pneumoniae, Escherichia coli* and *Staphylococcus aureus* was inhibited by ethanol extract of *Z. officinale* at the concentration of 25-100mg/ml which is similar with the findings from this study. It is noteworthy that the sensitivity of these organisms to *Z. officinale* ethanol extract is concentration-dependent. All the organisms were sensitive to ethanol extract of ginger at higher concentrations compared to low concentrations of the extract. Onyeagba *et al.* (2004) found the antimicrobial activity of the ethanol extract of ginger against a broad range of bacteria including *Bacillus* spp., *Staphylococcus aureus, Escherichia coli,* and *Salmonella* spp also to be dependent on concentration. The high susceptibility of the enteric pathogens to the ethanol further supports the possibility that the active ingredients may be alkaloid or essential oils (Draughon, 2004).

Minimum inhibitory concentration (MIC) results of ginger ethanol extract against the test organisms are shown in Table 2. *Escherichia coli* and *Staphylococcus aureus* had MIC of 3.12mg/ml and of 12.5mg/ml respectively while *K. pneumonia, Streptococcus mutans,* and *Shigella dysenteriae* had an MIC of 6.25mg/ml, 25.0mg/ml respectively.

Table 1: Antibacterial activities of ethanolic extracts of *Z. officinale* (ginger) against the test organisms

Conc. (mg/ml)	Zones of inhibition (mm)				
	E. coli	***K. pneumoniae***	***S. dysenteriae***	***S. mutans***	***S. aureus***
100	16.00 ^{a}±0.00	16.00 ^{a}±0.00	16.00 ^{a}±0.00	16.00 ^{a}±0.00	16.00 ^{a}±0.00
50	16.00 ^{d}±0.00	10.00 ^{d} ±0.00	18.00 ^{b}±0.00	16.00 ^{c}±1.41	10.00 ^{a}±0.00
25	14.00 ^{d}±0.00	8.90 ^{c}±1.41	16.00 ^{c}±1.41	15.00 ^{a}±0.00	8.00 ^{c}±1.41
12.2	10.00 ^{c}±0.00	7.00 ^{e}±0.00	11.00 ^{c}±0.00	8.00 ^{e}±0.00	3.00 ^{a}±0.00
6.25	11.00^{a}±0.00	6.00 ^{b}±1.41	9.00 ^{a}±0.00	6.00 ^{b}±0.00	0.00 ^{e}±0.00
3.12	9.00 ^{b}±1.41	4.00 ^{b}±0.00	6.00 ^{a}±1.41	3.00 ^{d}±1.41	0.00 ^{e}±0.00
1.56	0.00 ^{d}±0.00	0.00 ^{f}±0.00	0.00 ^{d}±0.00	0.00 ^{e}±0.00	00.00 ^{a}±0.00

^{abcdef} Means with different superscripts along the same column are significantly ($p<0.01$) different by Duncan's Multiple range tests. Values are mean of three replicates ± Standard Deviation

Table 2: Minimum Inhibitory Concentration of the ethanol extract of ginger against the test microorganisms

Conc. (mg/ml)	Test Organisms				
	E. coli	***S. aureus***	***S. mutans***	***K. pneumoniae***	***S. dysenteraie***
100	-	-	-	-	-
50	-	-	-	-	-
25	-	+	+	-	+
12.5	+	+	+	-	+
6.25	+	+	+	+	+
3.12	+	+	+	+	+
1.56	+	+	+	+	+

(-) means Absence of growth (+) means Presence of growth

Phytochemical Analysis of Ginger Ethanol Extract:

The quantitative phytochemical analysis of the ethanolic extract of ginger (Table 3) revealed the presence of seven (7) phytochemicals which were tannin, phenol, cardiac glycoside, flavonoid, alkaloid, terpenoids and reducing sugar. Saponins, steroid, phlobatannin and anthraqunione were however absent. Tannin was more abundant (55.68mg/ml) while terpenoids were the least available constituent (21.34mg/ml). A total of 43 compounds were detected after the phytochemical analysis of the ethanol extract of *Z. officinale* (Table 4). Jolad *et al* (2004) reported that they identified 51 compounds on organically grown fresh ginger, where 31 compounds were previously reported as constituents of ginger and additional 20 are yet to be characterized. The identified compounds included gingerols, shogaols, paradols, dihydroparadols, [3]-dihydroshogaols, acetyl derivatives of gingerols, gingerdiols, mono- and di-acetyl derivatives of gingerdiols, 1-dehydrogingerdiones, diarylheptanoids, zingiberene, phellandrene and methyl ether derivatives of some of these compounds. It is noteworthy that the source of plant sample is an important factor that directly affects the type and amount of bioactive compounds the can be identified during phytochemical analysis since plant constituents can be affected by geographic variations, environmental conditions and physiological factors that influence their bioactive phytochemical compounds. These volatile compounds (eucalyptol (8.293), terpenolene (10.067), á-pinene, borneol, camphene, and linalool) detected in this study has been reported earlier by many researchers to be responsible for the antimicrobial activities of ginger (Liu *et al.*, 2020; Duarte *et al.*, 2016; Sa-Nguanpuag *et al*., 2011; Park *et al.*, 2008; Yoo *et al.*, 2006; Singh *et al.*, 2005). Liu *et al.* (2020) in their study reported that linalool had an inhibitory effect on *Pseudomonas aeruginosa* with minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of 43 μg/ml and 862 μg/mL, respectively. The results of scanning electron microscopy (SEM) revealed that linalool disrupted the normal morphology of the cell. The release of nucleic acids, as well as the decrease in the membrane potential, proved that the membrane integrity of *P. aeruginosa* was destroyed. Moreover, the respiratory chain was damaged by respiratory chain dehydrogenase determination as to the absorbance at 490 nm decreased.

Table 3: Quantitative phytochemical screening of ginger ethanol extract

Phytochemicals	Constituents in mg/100g
Tannin	55.68 ± 0.60
Phenol	24.36 ± 0.66
Cardiac glycoside	31.27 ± 0.98
Flavonoid	39.06 ± 0.13
Alkaloid	36.24 ± 0.57
Terpenoid	21.34 ± 0.28
Reducing sugar	48.82 ± 0.47

Values are means ± Standard Deviation of duplicates

Table 4: Bioactive compounds of *Zingiber officinale* roscoe rhizomes

S/N	Compound	Molecule structure	Molecule Weight (g/mol)	Retention time (min)	Area (%)
1	2-Heptanone	$C_7H_{14}O$	114	4.484	8.22
2	2-Heptanol	$C_7H_{16}O$	116.2	4.687	0.53
3	Tricyclene	$C_{10}H_{16}$	136.23	5.228	0.17
4	Camphene	$C_{10}H_{16}$	136.24	5.963	4.9
5	Methylheptenone	$C_8H_{14}O$	126.2	7.09	8.59
6	α-Phellandrene	$C_{10}H_{16}$	136.23	7.462	0.63
7	δ-3-Carene	$C_{10}H_{16}$	136.23	7.4	0.2
8	Eucalyptol	$C_{10}H_{16}O$	154.249	8.293	1.77
9	1-Octanol,	$C_8H_{18}O$	130.23	9.504	0.13
10	Menthol	$C_{10}H_{20}O$	156.27	9.763	0.28
11	Terpinolene,hexamethyl-	$C_{10}H_{16}$	136.23	10.067	0.31
12	2-Nonalene	C_9H_{18}	126.24	10.194	0.47
13	Linalool	$C_{10}H_{18}O$	154.25	10.490	1.44
14	Trans-2-Pinanol	$C_{10}H_{18}O$	154.25	11.161	0.13
15	Camphore	$C_{10}H_{16}O$	152.23	11.98	0.18
16	Camphene hydrate,	$C_{10}H_{18}O$	154.25	12.054	0.18
17	Borneol	$C_{10}H_{18}O$	154.25	12.697	1.34
18	Terpin-4-ol	$C_{19}H_{24}O_4$	316.4	13.041	0.20
19	Cryptone	$C_9H_{14}O$	138.21	13.343	0.21
20	Myrtenol	$C_{10}H_{16}O$	152.233	13.707	0.12
21	n-Decanal	$C_{10}H_{20}O$	156.26	13.984	0.13
22	Isogeranial	$C_{10}H_{18}O$	154.25	14.13	0.33
23	2,3-Epoxygeria	$C_{11}H_{20}O$	168.276	14.602	0.11
24	Citronellol	$C_{10}H_{20}O$	156.27	14.919	2.59
26	Myrtenol	$C_{10}H_{16}O$	152.233	13.702	0.12
27	Citronellol	$C_{10}H_{20}O$	156.27	14.919	2.59
28	Cyclosativene	$C_{15}H_{24}$	204.35	19.225	0.28
29	Copaene	$C_{15}H_{24}$	204.36	19.605	0.45
30	Geranyl acetate	$C_{12}H_{20}O_2$	196.29	19.881	0.82
31	α-Cubene	$C_{15}H_{24}$	204.35	20.061	0.11
32	β-Elemene	$C_{15}H_{24}$	204.35	20.126	2.98
33	α-Fenebrene	$C_{15}H_{24}$	204.35	20.547	0.27
34	(E)-Caryophyllene	$C_{15}H_{24}$	204.25	20.986	0.12
35	α-Bergamotene	$C_{15}H_{24}$	204.35	21.480	0.16
36	α-Guaiene	$C_{15}H_{24}$	204.35	21.897	0.18
37	Trans-β-bergamotene	$C_{15}H_{24}$	204.35	22.143	0.69
38	9-epi-Caryophyllene	$C_{15}H_{24}O$	220.35	22.281	0.36
39	Heptacos-1-ene	$C_{27}H_{54}$	378.7	22.939	2.34
40	Aryl-Curcumene, octa	$C_{15}H_{22}$	202.33	23.048	3.95
41	α-Funebrene	$C_{15}H_{24}$	204.35	23.496	12.46
42	α-Murrolene	$C_{15}H_{24}$	204.35	23.572	0.28
43	α-Farnesene, 3,5-bis(1	$C_{15}H_{24}$	204.35	23.739	7.27

CONCLUSION

Ginger is an important herb that exhibits many medicinal and ethnomedicinal properties as a result of a number of bioactive compounds present in its crude extract. In vitro studies have revealed that ginger exhibits a significant potential of antibacterial activity due to the presence of several bioactive compounds detected from this study.

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