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Original Research Article

ANTIBACTERIAL ACTIVITY OF *Aframomum melegueta* (ROSCOE) K. SCHUM AGAINST GASTROINTESTINAL PATHOGENS AND GC-MS PROFILING OF BIOACTIVE COMPOUNDS

IFEOMA AGATHA UGBOR[1*] CHINONYE JUDITH ENEOVO[1] CHUKWUMA EZEKIEL OGBUANYA[1] ONYIYECHI CHIDIEBUBE KENECHUKWU [2] JAMES NNABUIKE EZEMA[3] OYENIYI STEPHEN BEJIDE[4], DINEBARI PHILIP BEREBON[5]

1. Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, State University of Medical and Applied Sciences, Igbo-Eno, Enugu State, Nigeria.
2. Department of Microbiology, Faculty of Natural Sciences, Caritas University, Enugu State, Nigeria.
3. Department of Medical Microbiology, Faculty of Basic Clinical Sciences, College of Medicine, State University of Medical and Applied Sciences, Igbo-Eno, Enugu State, Nigeria.
4. Department of Biological Sciences, Augustine University, Ilara-Epe, Lagos State, Nigeria
5. Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka.

ABSTRACT

Gastrointestinal tract infections are a key cause of morbidity and mortality worldwide. The problem is further compounded by the growing rates of antibiotic resistance among gastrointestinal pathogens and the continuous appearance of new pathogens thus requiring the continuous search for new drugs. *Aframomum melegueta* is a plant that has long been utilized in ethnomedicine for the management of several illnesses. This research evaluated the antibacterial activity of *Aframomum melegueta* ethanol extracts on some bacterial isolates implicated in gastrointestinal tract infection, after phytochemically screening the extract for possible bioactive compound present. The antibacterial screening of *Aframomum melegueta* seed extracts showed both concentrations (100 and 500 mg/ml) exhibited antibacterial effects against the tested isolates with 500mg/ml being more effective. With 500mg/ml of *Aframomum melegueta* ethanolic extract, *Staphylococcus sp.* was the most sensitive isolate having an IZD of 28.33 ± 0.88 mm, followed by *Klebsiella sp.* with an IZD of 23.67.00 ± 0.88mm, then *Escherichia sp.* with 21.00 ± 1.16mm and then *Pseudomonas Sp.* with an IZD of 16.67 ± 0.88mm. It was observed that the gram negative bacteria (*Escherichia sp.*, *Klebsiella sp.* and *Pseudomonas sp.*) exhibited less sensitivity compared to the gram positive bacteria (*Staphylococcus sp.)*. Their Minimal Inhibitory Concentration was within 31.25 to 125 mg/ml while their Minimal Bactericidal Concentration was within the range of 62.5 to 250mg/ml. GC-MS profiling reveals the presence of twenty (20) compounds belonging to the Monoterpenes (Alcohols), Sesquiterpenes, Sesquiterpene Oxides, Aldehydes, Ketones, Fatty Acids and Phenylpropanoids(Gingerols) groups many of which are good antimicrobial agent. Altogether the result obtained supports it use in ethnomedicine for the treatment of stomach illnesses while necessitating further validation via toxicity and biological mechanistic studies.

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***Corresponding author:** ifeoma.ugbor@sumas.edu.ng; +234 813 525 7025
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INTRODUCTION

Gastrointestinal tract infection is an inflammation of the stomach and intestines brought on by bacteria, viruses or parasites occasioning nausea, vomiting, diarrhea and cramping. Globally, gastrointestinal tract infections—particularly diarrheal illnesses—are a key cause of morbidity and mortality [1]. According to reports by the World Health Organization (WHO), diarrheal illness is the third predominant cause of death in children under five years, accounting for around 443,832 deaths annually [2]. Older patients with weak immune systems, and the elderly are also vulnerable to more serious illness and consequences. Large epidemics of food- and waterborne outbreaks of gastrointestinal diseases are made more likely by poor sanitation practices, poor water supplies, and growing globalization of food transportation systems. The growing rates of antibiotic resistance among gastrointestinal pathogens and the continuous appearance of new pathogens pose a rising danger to treatment efficacy when clinically indicated, thus requiring the continuous search for new drugs and in this search for new drugs, there is a renewed interest in plant derived medicine because it is believed that they are safe with fewer adverse effects compared to synthetic alternative. Since ancient times, people have utilized natural remedies to improve their health, and modern medicine relies heavily on medications derived from natural sources [3]. Many antimicrobial substances for the treatment and management of infectious pathogens were previously identified from natural materials [4].

Aframomum melegueta (Roscoe) K. Schum is a plant that has long been utilized in folkloric medicine. Its seeds are employed in the management of stomach distress, heal wounds, treat leprosy and measles, as a stimulant, an aphrodisiac, a purgative, an anthelminthic and hemostatic agent, control lactation and postpartum hemorrhage, and be used in veterinary preparations [5,6,7]. It is equally used for entertainment, where it is given to visitors alongside with Kola nuts [8], diviners attempting to invoke spirits during religious ceremonies uses it [9], the leaves and seeds are used to make a variety of meals [10]. Additionally, the beer industry utilizes it to flavor and enhance alcoholic drinks including wine, ale and gin [11]. This *Aframomum melegueta* is a perennial plant belonging to the Kingdom–Plantae (plants), Division:-Magnoliophyta (Angiosperms), Class-Liliopsida (monocots), Order-Zingiberales, Family: Zingiberaceae (ginger family), Genus: *Aframomum* and Species: *Aframomum melegueta*. This plant possesses a tufted leafy stem that can reach a height of 1.5 meters with pinkish-orange top flowers that can grow into indehiscent, fleshly pods. The pods are about 5-7 cm long and contains numerous tiny, comestible, reddish brown seeds which have strong peppery taste [12], It is generally called alligator pepper or grains of paradise. In Nigeria, it is commonly called Ose orji by the Igbos, Oburo, ata, or ata-ire by the Yorubas and Chilla or citta by the Hausas. The rain forests of Nigeria, Sierra Leone, Liberia, Cameroon, Ghana, Togo, Cote d'Ivoire and other tropical African countries are home to *Aframomum melegueta* [13]. The need to continuously search for new drugs for the treatment of infections, the extensive use of plants in ethnomedicine, and the necessity to preserve medicinal plants and information about them from impending extinction overemphasizes the need to continuously study medicinal plants. This present research evaluated the antibacterial potential of ethanolic seed extract of *Aframomum melegueta* on some gastrointestinal tract pathogens and determined the bioactive components exhibiting such antibacterial property via gas chromatography-mass spectrometry.

MATERIALS AND METHODS

Collection and Preparation of the Plant Materials

Aframomum melegueta was procured from Ogige market in Nsukka, Enugu State, Nigeria and was properly identified and authenticated by Mr. Ossai I. Isaac, the Chief Taxonomist/Herbarium Curator at the International Center for Ethnomedicine and Drug Development (InterCEDD), under Bioresources Development and Conservation Program (BDCP); 110, Aku Road, Nsukka. The voucher specimen (InterCEDD/E13/004 _*Aframomum melegueta*) was prepared and deposited in the center's herbarium. The names and family of the plant were cross checked and confirmed through search on International Plant Names Index http://www.ipni.org, World Checklist of Selected Plant Families 2021 and "The Plant List". The bud was then washed with tap water at the laboratory to remove dust, sand and other impurities. It was then opened to release the seed. The plant material was dehydrated at room temperature for 1 week to preserve compositional integrity. After drying, the sample was grounded using a clean electric grinder and put into an air tight bottle and stored

Extraction

A Soxhlet extraction apparatus (Sharma Scientific Instrument, SSI-063, India) was used for the extraction. One hundred grams (100g) of the pulverized sample were extracted with 500ml of 70% ethanol, the extract was collected in a round bottom

flask and concentrated using a TOPTION RE-52AA 1L rotary evaporator.

Phytochemical Study

GC-MS analysis of phytochemical compounds present in *Aframomum melegueta*

Aframomum melegueta ethanol extract was subjected to phytochemical screening using a Perkin Elimer Turbo Mass Spectrophotometer (Norwalk, CTO6859 and USA) which includes a Perkin Elmer Auto sampler XL GC. The Perkin Elmer Elite-5 capillary column, which measured 30 m + 0.25 mm and had a 0.25 mm film thickness made of 95% dimethyl polysiloxane, was utilized. The carrier gas utilized was Helium (99.99%) at a flow rate of 0.5ml per minute. A 1 µl sample injection volume was used, the inlet temperature was kept at 250°C, and the oven temperature was designed to start at 110°C for 4 minutes, rise to 240°C, and then rise to 280°C at the rate of 20°C for 5 minutes, for a total run time of 90 minutes. The Mass Spectrometry transfer line was kept at 200°C while the source temperature was upheld at 180°C. Gas chromatography-Mass spectrometry was studied via electron impact ionization at 70 eV and data was evaluated using total ion count (TIC) for compound identification and quantification. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS library, which comprised the National Institute of Standards and Technology's (NIST) database with over 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the library. The components of the test materials were identified by their names, molecular weights, and structures. Turbo-Mass-OCPTVS-Demo SPL software was used to measure the peak areas and process the data [14].

Antimicrobial Study

Preparation of Culture Media and collection of Clinical Isolates

Following the manufacturer's instructions; Nutrient agar, Nutrient broth and Muller Hilton agar were prepared and autoclaved. Nutrient agar was used to collect pure clinical isolates of gastrointestinal pathogens isolated from patients attending the Enugu State University Teaching Hospital, Parklane, Enugu State.

Identification of the Clinical Isolates

The identities of the organisms were confirmed by Gram staining and standard biochemical tests including Catalase, Coagulase, Oxidase and Indole following the procedures outlined in Onah *et al*. [15]. The organisms were then sub-cultured on nutrient agar slants in bijou bottles and incubated at 37°C for 24h. The organism were then stored at 4°C until needed.

Reconstitution of the Extracts

One (1) g of the extract was reconstituted with 0.1ml of 100% di-methyl sulphoxide (DMSO) and 1.9 ml of sterile distilled water, then vortexed to obtain 500 mg/ml concentration. Then 200µl of the 500mg/ml was diluted with 800ul of diluent (40ul DMSO and 760ul ml distilled water) to obtain 100mg/ml concentration.

Disc Preparation

The discs were prepared using Whatman No 1 filter paper. A cork borer (6mm in diameter) was used to create an impression in the filter paper. It was then cut off using sterile surgical blade. The discs cut out were soaked in ethanol for 5 minutes and dried in an oven at 60°C for 20 minutes thus sterilizing it. The discs were afterwards impregnated with the 100mg/ml and 500mg/ml of *Aframomum melegueta* extracts separately. This was kept in the oven to dry at 37°C for 1h. After drying the disc was used for the disc diffusion test as described by Kirby-Bauer [16].

Preparation of the Test Organism (Inoculum Standardization)

The isolates collected earlier and stored, were sub cultured twice and then used to prepare a stock microbial culture in nutrient broth. These were standardized by transferring 3 to 4 loopful of the bacterial species from the stock culture into 5ml of nutrient broth and mixed properly. It was then incubated for 3h until growth reaches the turbidity of 0.5 McFarland standard which is equivalent to approximately 1.0×10^8 CFU/ml of bacteria

Disc Diffusion Test

Within 15 minutes of adjusting turbidity, 1ml of the test organism was introduced into the already prepared sterile Muller Hilton Agar plate. This was rocked on the work bench to ensure even distribution of the microorganism, after the excess was decanted into a container containing a disinfectant. The inoculated plate was allowed to stand for 15 minutes before applying the disc; this is to ensure that the organisms are well seeded into the growth medium. The disc was them applied manually using a sterile forceps, which was sterilized by dipping into 70% ethanol and flaming it. The sterilize forceps was used to pick the sterile and impregnated disc into the microorganism seeded petri dish, a gentle pressure was applied using the forceps to ensure complete contact of the disc with the seeded microorganism in the petri dish. It was ensured that just four (4) disc (including the standard antibiotic disc, Gentamicin 10µg) were placed on the

Petri dish. This was incubated for 24 h at 37°C. At the end of which the zone of inhibition created by the extract in the disc was measured with a clean transparent ruler to the nearest whole millimeter and recorded accordingly. The diameter of the zone of inhibition gives a clue to whether the organisms are susceptible, intermediate or resistant to the *Aframomum melegueta* extract.

Minimum Inhibitory Concentration (MIC)

Ajaiyeoba *et al*.'s description of the tube dilution method was used. Five hundred (500) mg/ml extract solution was prepared as above and serially diluted by two fold to obtain 250 mg/ml, 125mg/ml, 62.5mg/ml and 31.25mg/ml concentrations. One milliliters of each dilution were added to one milliliters of sterile nutrient broth in test tubes. The combination was then incubated at 37^{o} C for 24 h after being infected with 0.1 ml of the test organism that had beforehand been standardized to 1.0 × 10^{8} CFU/ml of bacteria. The minimum inhibitory concentration (MIC) was determined to be the lowest concentration of extract without turbidity [17].

Minimum Bactericidal Concentration (MBC)

The MBC of the plant extracts was obtained by subculturing 0.1ml from each of the tubes in the MIC test that did not exhibit turbidity into sterile nutrient agar plates; the lowest concentration at which no growth was seen is the MBC [17].

Statistical analysis

The collected data are presented as mean ± standard error of mean (Mean ± SEM). Two way analysis of variance (ANOVA) with a subsequent Tukey's post hoc test was employed to test for significance. $P<0.05$ was regarded as significant. This statistical analysis was conducted with Graph Pad Prism (version 6.0) software.

RESULTS

Isolate identity and Extraction Yield

The isolates collected were identified as *Escherichia sp., Staphylococcus sp., Pseudomonas sp. and, klebsiella sp.* as shown in Table 1 below

Table 1: Identity of the clinical isolates

Gram stain reaction	Shape	Catalase	Coagulase	Oxidase	Indole	Possible Microorganism
Gram Negative	Rod shaped	positive	Negative	negative	Positive	*Escherichia sp.*
Gram Negative	Rod shaped	Positive	Negative	positive	Negative	*Pseudomonas sp.*
Gram Positive	Cocci shaped	Positive	Positive	negative	Negative	*Staphylococcus sp.*
Gram Negative	Rod shaped	Positive	Negative	negative	Negative	*Klebsiella sp.*

The ethanolic extraction of *Aframomum melegueta* yielded 3.99g extract. Some other physical parameters of the extract are shown in Table 2

Table 2: Physical properties of *Aframomum melegueta* extract

Physical parameters	Extract
Weight of extract	3.99g
% yield	3.99%
Colour	Brown
Smell	Peppery
Texture	Sticky

Bioactive compounds present in ethanol extract of *Aframomum melegueta* seeds

GC-MS profiling of the *Aframomum melegueta* seeds extract revealed the presence of 20 compounds as shown in Table 3. These compounds isolated are members of the Monoterpenes/Alcohols, Sesquiterpenes, Sesquiterpene Oxides, Aldehydes, Ketones, Fatty Acids and Phenylpropanoids/Gingerols groups as shown in Table 4. The GC-MS chromatogram is shown in Figure 1 while the Structures of the different compounds are depicted in Figure 2.

Table 3: Bioactive compounds present in *Aframomum melegueta* seed ethanol extract

S/N	RT (min)	Name of the Compound	Molecular formula	MW (daltons)	Peak height / abundance	Peak area %
1	3.456	Octanal	$C_8H_{16}O$	128	1766644	1.40
2	8.629	Caryophyllene	$C_{15}H_{24}$	204	10680746	2.27
3	9.091	Humulene	$C_{15}H_{24}$	204	10670986	5.16
4	9.612	(3S,3aS,8aR)-6,8a-Dimethyl-3-(prop-1-en-2-yl)-1,2,3,3a,4,5,8,8a-octahydroazulene	$C_{15}H_{24}$	204	6239122	1.04
5	10.286	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	$C_{15}H_{26}O$	222	7196066	1.32
6	10.640	Caryophyllene oxide	$C_{15}H_{24}O$	220	7428244	1.52
7	10.960	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo [9.1.0]dodeca-3,7-diene	$C_{15}H_{24}O$	220	10409590	2.17
8	11.520	3-Buten-2-ol,4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-,(3E)	$C_{13}H_{22}O$	194	16374005	
9	11.892	4-(3,4-Dimethoxyphenyl)butan-2-one	$C_{12}H_{16}O_3$	208	9475672	2.05
10	14.624	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	6233597	3.19
11	16.390	cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282	8836157	10.13
12	17.219	3-Decanone, 1-(4-hydroxy-3-methoxyphenyl)-	$C_{17}H_{26}O_3$	278	17177903	11.73
13	17.842	Gingerol	$C_{17}H_{26}O_4$	294	14328508	10.91
14	18.219	1-(4-Hydroxy-3-methoxyphenyl)decane-3,5-dione	$C_{17}H_{24}O_4$	292	15934560	16.19
15	18.625	5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)decan-3-one	$C_{17}H_{26}O_4$	294	12887502	4.97
16	18.945	1-(4-Hydroxy-3-methoxyphenyl)tetradecane-3,5-dione	$C_{21}H_{32}O_4$	349	8414739	1.61
17	19.248	3-Decanone, 1-(4-hydroxy-3-methoxyphenyl)-	$C_{17}H_{26}O_3$	278	5325932	1.89
18	19.413	1-(4-Hydroxy-3-methoxyphenyl)dodec-4-en-3-one	$C_{19}H_{28}O_3$	308	10945479	2.70
19	19.716	1-(4-Hydroxy-3-methoxyphenyl)dodecane- 3,5-dione	$C_{19}H_{28}O_4$	320	10924472	3.11
20	20.088	(E)-1-(4-hydroxy-3-methoxyphenyl)dec-1-ene-3,5-dione	$C_{17}H_{22}O_4$	290	2466675	1.06

Keys: RT= Retention Time; MW= molecular weight

Table 4: Classes of Bioactive compounds present in *Aframomum melegueta* seed ethanol extract

Compound Class	Compounds	No. of compound
Monoterpenes/Alcohols	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	1
Sesquiterpenes	Caryophyllene, Humulene, (3S,3aS,8aR)-6,8a-Dimethyl-3-(prop-1-en-2-yl)-1,2,3,3a,4,5,8,8a-octahydroazulene	3
Sesquiterpene Oxides	Caryophyllene oxide, (1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo [9.1.0]dodeca-3,7-diene	2
Aldehydes	Octanal	1
Ketones	Butan-2-one, 4-(3-hydroxy-2-methoxyphenyl)-, 4-(3,4-Dimethoxyphenyl)butan-2-one, 3-Decanone, 1-(4-hydroxy-3-methoxyphenyl)-	5
Fatty Acids	n-Hexadecanoic acid, cis-13-Octadecenoic acid	2
Phenylpropanoids/Gingerols	Gingerol, 1-(4-Hydroxy-3-methoxyphenyl)decane-3,5-dione, 5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)decan-3-one, 1-(4-Hydroxy-3-methoxyphenyl)dodecan-3-one, 1-(4-Hydroxy-3-methoxyphenyl)dodec-4-en-3-one, 1-(4-Hydroxy-3-methoxyphenyl)dodecane-3,5-dione, (E)-1-(4-hydroxy-3-methoxyphenyl)dec-1-ene-3,5-dione	7

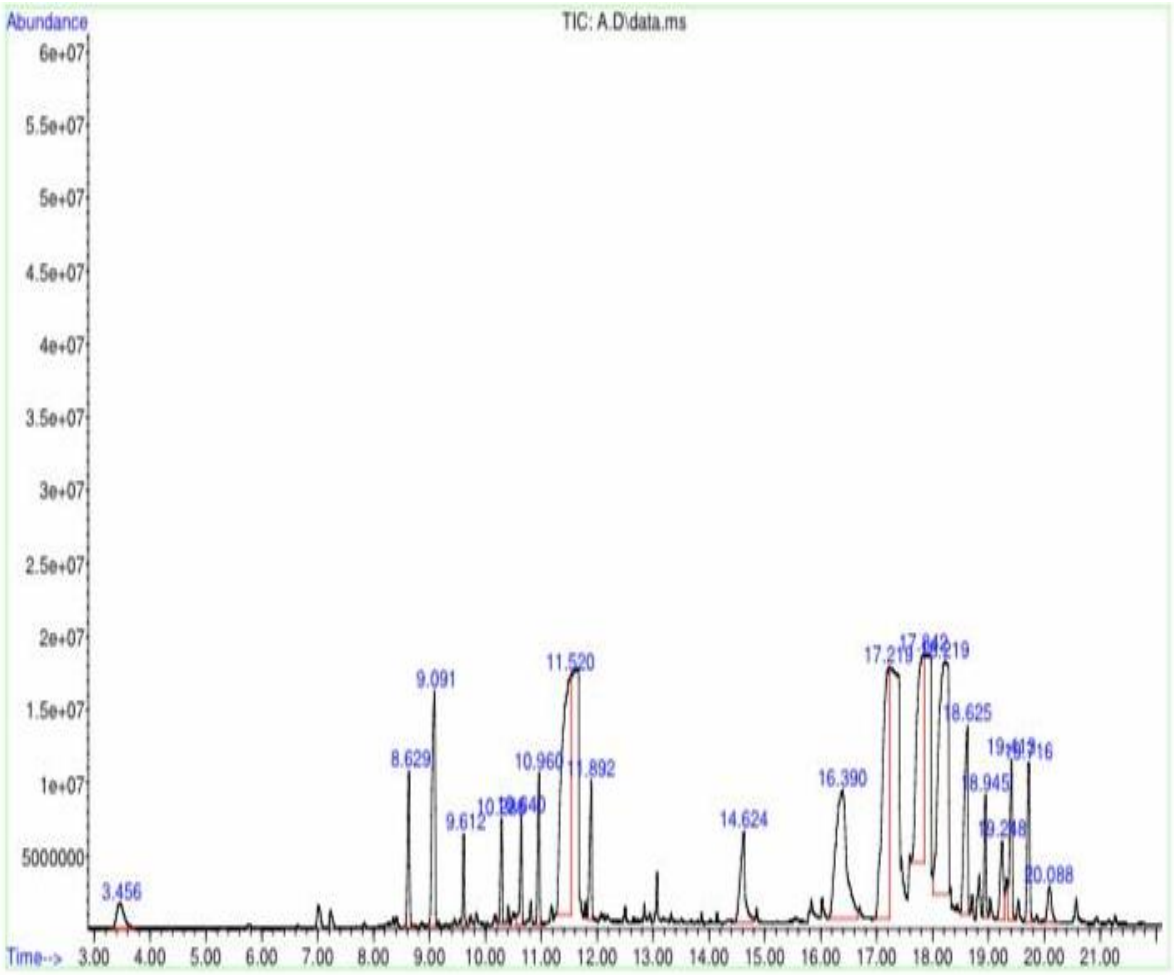
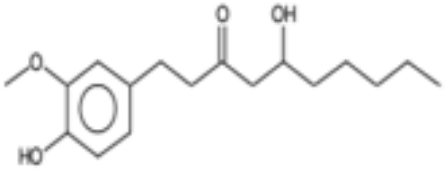


Fig 1: GC-MS Chromatogram of *Aframomum melegueta* seed ethanol extract



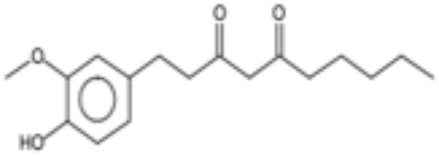
Octanal



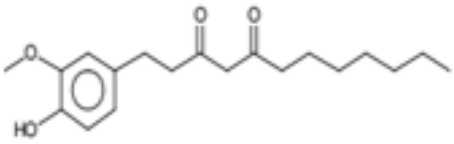
5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl) decan-3-one



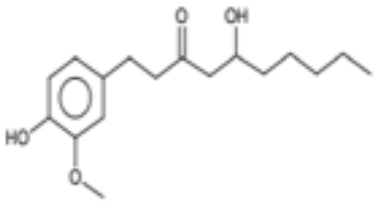
Caryophyllene



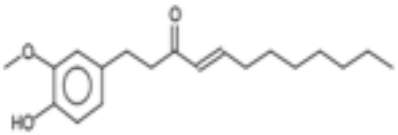
1-(4-Hydroxy-3-methoxyphenyl)decane-3,5-dione



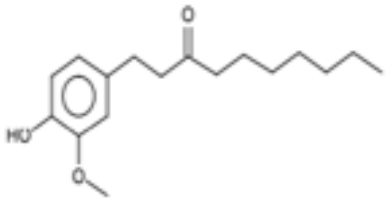
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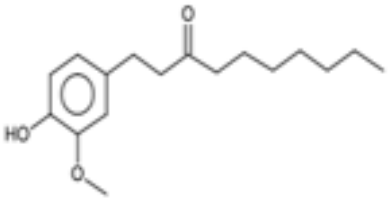
Gingerol



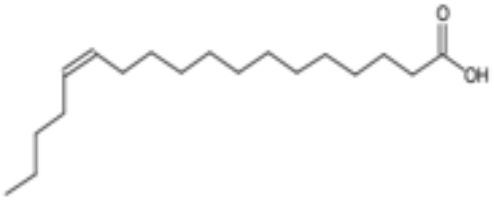
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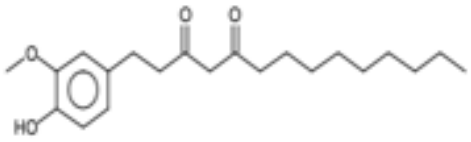
3-Decanone, 1-(4-hydroxy-3-methoxyphenyl)-



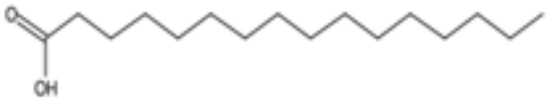
3-Decanone, 1-(4-hydroxy-3-methoxyphenyl)-



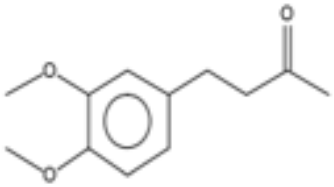
cis-13-Octadecenoic acid



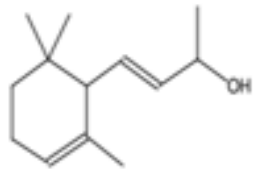
1-(4-Hydroxy-3-methoxyphenyl)tetradecane-3,5-dione



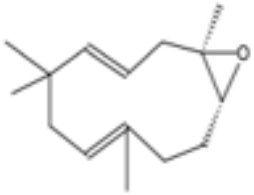
n-Hexadecanoic acid



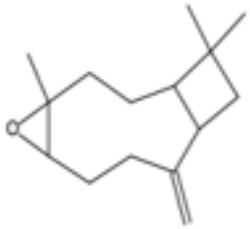
4-(3,4-Dimethoxyphenyl)butan-2-one



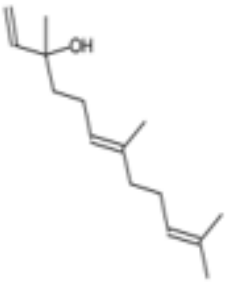
3-Buten-2-ol,4-(2,6,6-trimethyl-2-cyclohexen-1-yl),(3E)



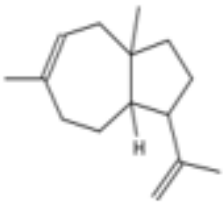
(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo [9.1.0]dodeca-3,7-diene



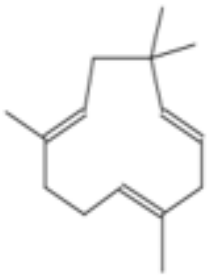
Caryophyllene oxide



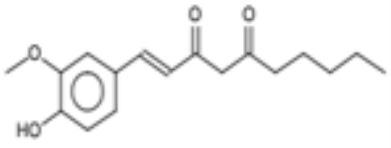
1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-



(3S,3aS,8aR)-6,8a-Dimethyl-3-(prop-1-en-2-yl)-1,2,3,3a,4,5,8,8a-octahydroazulene



Humulene



(E)-1-(4-hydroxy-3-methoxyphenyl)dec-1-ene-3,5-dione

Fig 2: Structures of compounds found in ethanol extract of *Aframomum melegueta*

Antibacterial activity of *Aframomum melegueta* seed extracts

The outcome of the antibacterial susceptibility testing of the extracts against the clinical isolates depicted by the inhibition zone diameters (IZD) in (mm) are shown in Figure 3. The extract was effective on all tested isolates with 500mg/ml been more effective. The minimum inhibitory

concentration was within the range of 31.25mg/ml to 125mg/ml as shown in Table 5 while the minimum bactericidal concentration was between 62.5mg/ml to 250mg/ml as depicted in Table 6.

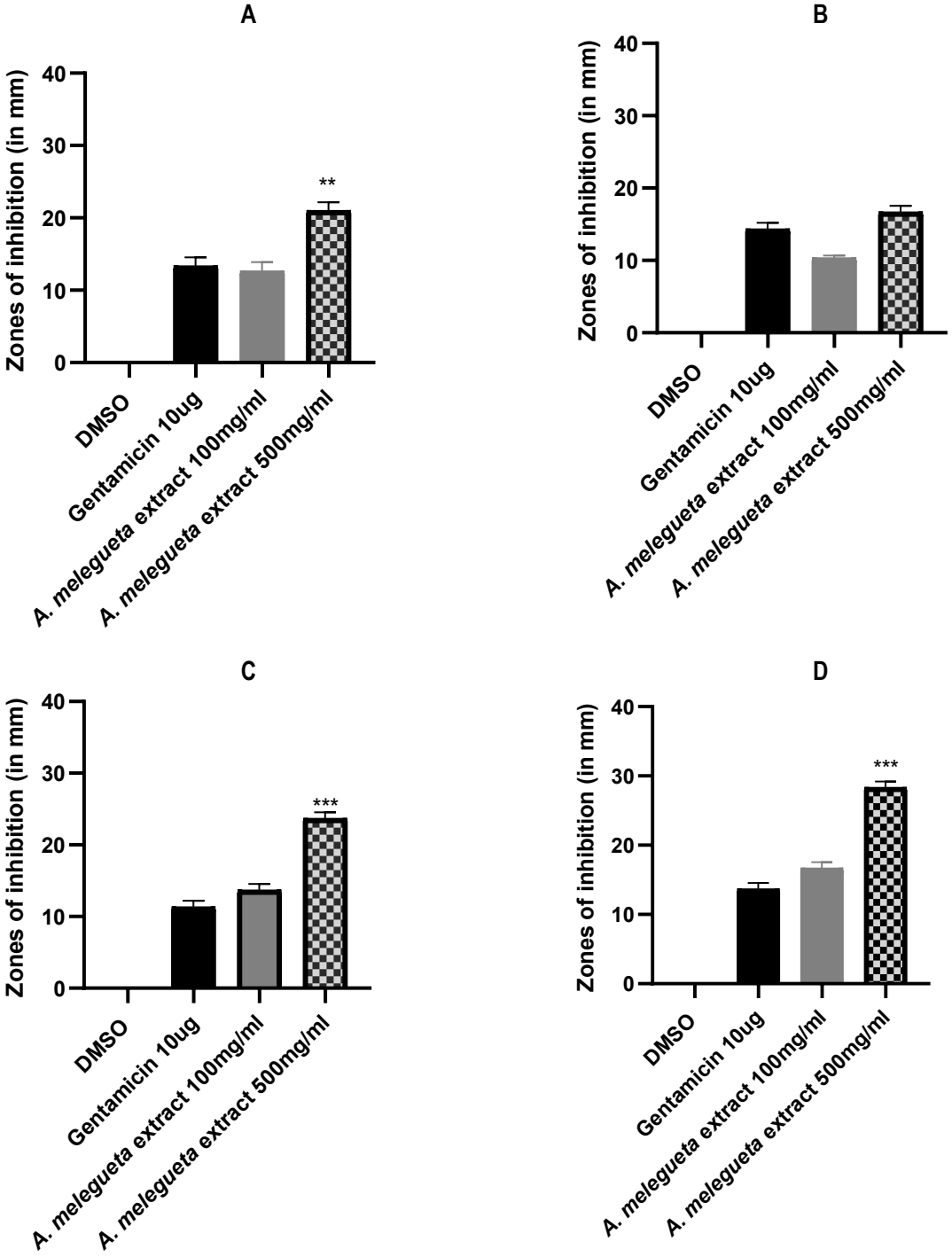


Fig. 3: Inhibition Zone Diameter (in mm) obtained with the different concentration of *Aframomum melegueta* seed extracts. (Effect on A: *Escherichia sp.*, B: *Pseudomonas sp.*; C: *Klebsiella sp.*, D: *Staphylococcus sp.*).

Table 5: Minimum Inhibitory Concentration (MIC) of the *Aframomum melegueta* ethanol Extracts

Extract conc. / Isolates	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml
Escherichia sp.	_	_	_	-	+
Pseudomonas sp.	_	_	_	+	+
Klebsiella sp.	_	_	_	+	+
Staphylococcus sp.	_	_	_	-	-

key: + = growth, - = no growth

Table 6: Minimum Bactericidal Concentration (MBC) of the *Aframomum melegueta* ethanol Extracts

Extract conc. / Isolates	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml
Escherichia sp.	_	_	_	+	+
Pseudomonas sp.	_	_	+	+	+
Klebsiella sp.	_	_	_	+	+
Staphylococcus sp.	_	_	_	-	+

DISCUSSION

The antibacterial properties of the ethanolic extract of *Aframomum melegueta* seed was measured using concentrations of 100 and 500 mg/ml. The result showed both concentrations exhibited antibacterial effects against the tested isolates with 500mg/ml being more effective depicting its effectiveness is concentration dependent. The MIC and MBC which was within 31.25 to 125 mg/ml and 62.5 to 250mg/ml respectively shows even lower concentrations of the extract can inhibit and /or kill the pathogens. With 500mg/ml of *Aframomum melegueta* ethanolic extract, *Staphylococcus sp.* was the most sensitive isolate having an IZD of 28.33 ± 0.88 mm, followed by *Klebsiella sp.* with an IZD of 23.67 ± 0.88 mm, then *Escherichia sp.* with 21.00 ± 1.15 mm and then *Pseudomonas sp.* with an IZD of 16.67 ± 0.88. It was observed that the gram negative bacteria (*Escherichia sp.*, *Klebsiella sp.* and *Pseudomonas sp.*) exhibited less sensitivity compared to the gram positive bacteria (*Staphylococcus sp.)* this could be because of the differences in their cell wall architectures. Bacteria that are gram negative have an external membrane that serves as a barrier with restricted permeability, preventing some medications and antibiotic components from entering the cell. Gram-positive bacteria typically lack this outer membrane on their cell walls. This existence of an outer membrane is one of the key factors causing Gram negative bacteria to be more resistant to antibiotics than Gram positive bacteria [18]. This is in line with the study by Abodunrin *et al.* [19] and Odo *et al*. [20]. Abodunrin and co-workers reported the antibacterial activity of this same plant extract on Urinary tract pathogens. Our study shows *Pseudomonas* sp. was the least sensitive. This could be as a result of it outer membrane as it is well recognized that *Pseudomonas sp.* outer membrane acts as a major barrier to antimicrobial agent penetration [21]. Ten (10) ug of gentamicin was used as control which equals 0.0lmg/ml and it was effective. This level of efficacy at such concentration may be due to excellent purification and concentration of active antibacterial compounds in the antibiotic control.

Abodunrin and co - workers have initially evaluated the Qualitative and Quantitative Phytochemical analysis of the methanol and pet - ether extracts of *Aframomum melegueta* seeds and shown its methanol extract contains Alkaloids, flavonoids, saponins, tannins and carbohydrates with Flavonoids being the most abundant phytocompound [19]. Based on this preliminary evidence we did the GC-MS analysis of the ethanol extract of *Aframomum melegueta* seeds and it revealed the presence of 20 different compounds which are categorized into the Monoterpenes/Alcohols, Sesquiterpenes and Sesquiterpene Oxides, Aldehydes, Ketones, Fatty Acids and Phenylpropanoids/Gingerols. The Phenylpropanoids/Gingerols have been reported by Ogwu *et al* to possess antimicrobial and anti-inflammatory effect [22]. Another research also shows they inhibit prostaglandin and leukotriene biosynthesis [23]. Sesquiterpene such as Caryophyllene have been reported to possess antimicrobial and anticancer activity against colorectal cancer cells [22, 24]. Other reports have shown caryophyllene is a selective agonist of cannabinoid receptor type-2 (CB2) and has strong cannabimimetic, anti-inflammatory, anti-nociceptive, neuroprotective, and anti-alcoholic properties in mice

[25]. It has been shown to avert osteoporosis, lower cholesterol, ease anxiety and pain, and treat seizures [26]. Fatty acids such as n-Hexadecanoic acid have been described to possess antibacterial effect [27]. Hudiadah *et al* demonstrated the antibacterial activity of n-Hexadecanoic acid and α-tocopherol against acne-causing bacteria, their finding emphasizes the potential of these compounds being used as anti-acne agent. n-Hexadecanoic acid aside being an antibacterial agent, has also been reported to possess antioxidant, anti-inflammatory, hypocholesterolemic, nematicide, pesticide, lubricant, anti-androgenic activities [25, 28]. The study by Massano *et al*., shows the Monoterpenes has potential of being used as a biofilm control agent and as an antibiotic resistance modifier [29]. Hpoo *et al*., have reported the antifungal activity of the Aldehyde Octanal [30]. Passos *et al.* reported that Humulene is a monocyclic sesquiterpene which exhibits anti-inflammatory properties and decreases skin allergy caused by allergen intake [31]. The existence of these compounds as shown by the GC-MS profiling provides some biochemical explanation for its use in ethnomedicine for the treatment and prevention of numerous illnesses. These bioactive compounds accounts for the numerous activity exhibited by *Aframomum melegueta* seed. The antibacterial activity seen in this present study could be ascribed to the presence of Gingerols, Sesquiterpene, Monoterpenes and Fatty acids as these class of compounds have previously been reported to have antibacterial effect. The ability of these extract to inhibits gastrointestinal pathogens infers it could be used in the management of gastrointestinal tract infections and is also justifies its use in ethnomedicine for the treatment of stomach related problems.

CONCLUSION

Aframomum melegueta seed extracts has been shown in this study to possess antibacterial effect against bacterial gastrointestinal pathogens. Its efficacy against this gastrointestinal pathogens provides the scientific validation for its traditional use in the treatment of stomach related problem and can be further studied for possible use in healthcare systems, especially in underdeveloped nations.

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AUTHORS' CONTRIBUTION

IAU and DPB designed the study, IAU, OCK, CEO and JNE carried out the practicals and collected data. CJE and OSB statistically analyzed the data collected, IAU, OCK and DPB drafted the manuscript. All authors read and corrected the manuscript. All authors read and approved the final manuscript. IAU, CJE, CEO, OCK, JNE, OSB and DPB meet the requirement for authorship.

CONFLICT OF INTEREST

The authors hereby declare that there is no conflict of interest.

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