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Antimicrobial screening, functional groups and elemental analysis of *Aframomum melegueta*

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ABSTRACT: The antimicrobial screening, functional groups and elemental analysis of *Aframomum melegueta* seeds were investigated using standard microbiological methods. The results revealed that the extracts of *Aframomum melegueta* had inhibitory effect against selected oral microorganisms such as *Staphylococcus epidermidis, Escherichia coli, Streptococcus mutans, Klebsiella pneumoniae, Aspergillus niger, Candida albicans, Rhizopus oryzae, Aspergillus flavus*. The mineral contents were high with remarkable concentration of nitrogen (22.225 %), sodium (0.24 %), calcium (0.059 %), magnesium (1431.5 mg/kg), iron (40.99 mg/mg/kg). The mineral element contents were within WHO/FAO safe limit indicating favourable nutritional balance. The trace elements of the spice were investigated to establishing its nutritional uses. Thirty two compounds in Ethanol extract were identified to be bioactive by Gas chromatography – Mass spectrometry (GC-MS). This analysis revealed the oil extracts isolated from *A. Melegueta* contain Eugenol, Caryophyllene, Octacosane, Butan-2-one,4-(3-hydroxy-2-methoxyphenyl). Comparing the nutrient and chemical constituents with recommended dietary allowance (RDA) values, the results reveal that the spices contain an appreciable amount of nutrients, minerals, trace elements and functional groups and low levels of toxicants**.**

Keywords: Antimicrobial screening, Functional groups, Elemental analysis, *Aframomum melegueta*, Ethanol extract.

Introduction

The use of traditional medicine is wide spread throughout the world. A medicinal plant is any plant which, in one or more of its organs, contain substances that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs. A number of plants have been used in traditional medicine for many years due to their antimicrobial properties (Sofowora, 1993). The medicinal values of these plants lie in some chemical substances that produce a definite physiological action in human or animal body (Edeoga *et al*., 2005).

A. melegueta is a tropical herbaceous perennial plant of the genus *Aframomum* belonging to the family *Zingiberaceae* (ginger family) of the angiosperms in the Kingdom plantae. The seeds have pungent peppery taste due to aromatic ketones (Galal, 1996). It is widely spread across tropical Africa including Nigeria, Liberia, Sierra Leone, Ghana, Cameroon, Cote D' ivoire and Togo. The phytochemicals obtained from the seed of *A. melegueta* has been used for years in the treatment of infectious diseases. The grains of *A. melegueta* possess active ingredients that may be exploited for local development of antimicrobials (Oyegade, 1999). The presence of phenolic compounds in the seed of *A. melegueta* indicates that this plant is an antimicrobial agents because phenols and phenolic compounds have been extensively used in disinfections and remain the standard with which other bactericides are compared (Okwu, 2004). Extracts from the seed of *A. melegueta* with potent antiseptic or bactericidal properties have therefore been used in treating wounds and preventions of infections (Okwu, 2004).

Materials and Methods

Collection of Samples

A. melegueta seeds were purchased from Uselu Market in Benin City, Edo Sate, Nigeria. The samples were transported in sterile polyethylene bag to the laboratory for analysis.

Extraction

The active ingredients were extracted by dissolving 10 g of the powdery *A. melegueta* seeds in 20 ml 99.99 % pure n-hexane in a well corked bottle. The mixture was allowed to stand for 72 hours to settle and the supernatant filtered into a beaker. The filtrate was rewashed with 20 ml n-hexane for two consecutive times. The combined aliquots was evaporated on a steam bath and filtered through a pasture pipette stocked with glass wool (membrane) which was packed with anhydrous sodium sulphate to remove the left over moisture. The filtrate was concentrated to 1 ml in the vial bottle and was transferred for analysis.

Source of Microorganisms

The microorganisms utilized in this study were obtained from the University of Benin Teaching Hospital (UBTH), Benin City, Edo State, Nigeria. Bacteria and fungi were sub cultured aseptically unto nutrient agar and potato dextrose agar media. Bacteria used include *Staphylococcus epidermidis, Escherichia coli, Proteus mirabilis, Klebsiella pneumonia* and *Staphylococcus aureus* while fungi include *Rhizorpus oryzae, Aspergillus flavus* and *Aspergillus niger*

Antimicrobial Susceptibility Testing

Preparation of concentration of extract:

The extract of *A. melegueta* was prepared according to the methods of Ekwenye and Elegalam (2005), concentration of 100 mg/ml of the extract was prepared by dissolving 0.1g of the extract in 1ml of sterile water. Concentrations of 50 mg/ml, 25 mg/ml 12.5 mg/ml and 6.25 mg/ml were prepared from the stock concentration (100 mg/ml) by double dilution procedure.

Microbial inoculum preparation:

The inocula were prepared by inoculating the test organisms in nutrient broth and incubated for 24 hours at 37^0C. After incubation, one millilitre (1 ml) of the cultures was inoculated onto nutrient agar at 45^0C with a Pasteur pipette.

Antimicrobial Assay:

Antimicrobial activity was evaluated by noting the zones of inhibition against the test organisms (Eloff, 1998). Twenty four hours (24 hr) old culture of each organism was transferred aseptically into 10 ml sterile normal saline in a test tube and mixed thoroughly for uniform distribution. A sterile cotton swab was then used to spread the resulting suspension uniformly on the surface of oven-dried Nutrient agar and potato dextrose agar plates for bacteria and fungi respectively.

Three (3) adequately spaced wells of diameter 4 mm per plate were made on the culture agar surface respectively using sterile metal cup-borer. Zero point two millilitre (0.2 ml) extract and control were introduced in each hole under aseptic condition with the aid of pipette pump. The plate was kept at room temperature for 1 hour to allow the agents to diffuse into the agar medium and incubated accordingly. Conventional antibiotics were used as positive controls for bacteria and fungi respectively and distilled water was used as the negative control. The plates were incubated at 37 ^{0}C for 24 hours for the bacteria strains and at 28 ^{0}C for 72 hours for fungal isolates. The zones of inhibition were measured and recorded after incubation. Zones of inhibition around the wells indicated antimicrobial activity of the extracts against the test organisms. The diameters of these zones were measured diagonally in millimetres with ruler and the mean inhibition zone diameter of each organism was recorded in triplicates.

Mineral analysis

Sodium (Na), potassium (k), calcium (Ca), Magnesium (Mg), iron (Fe) and zinc (Zn) were analysed from the indigenous spices. One gramme (1.0 g) each of oven dried spices were digested with 5 ml concentrated nitric acid (HNO_3) and 1ml each of concentrated sulphuric acid (H_2SO_4) and 60 % perchloric acid ($HClO_4$) and heated until white fumes of perchloric acid were formed. The volume of the digest was reduced by heating but not to dryness.

The flask was allowed to cool down; the content was diluted with distilled deionized water and then filtered into a 50 ml volumetric flask. The content made up to the mark with deionized water and further analysed for mineral contents using Atomic Absorption Spectrophotometer (AAS).

GC-MS Analysis

GC-MS analysis was carried out using a GC Clarus 500 Perkin Elmer system comprising an autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions. Column Elite-5MS fused Silica capillary column (30 mm X 0.25 mm X 0.25 μm df, composed of 5 % Diphenyl / 95 % Dimethyl polySiloxane), operating in electron impact mode at 7 oev; Helium (99.999 %) was used as carrier gas at a constant flow of 0.1ml min and an injection volume of 2 μl was employed (split ratio of 10:1) injector temperature 250 ^{0}C; ion-source temperature 280 ^{0}C. The oven temperature was programmed from 110 ^{0}C (isothermal for 2 min), with an increase of 10 ^{0}C/min, to 200 ^{0}C, then 5 ^{0}C/min to 280 ^{0}C, ending with a 9 min isothermal at 280 ^{0}C mass spectra were taken at 70 ev; a scan interval of 0.2 seconds and fragments from 40 to 450 Da. Total GC running time was 36 minutes.

Identification of Components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of the known compounds stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

Results

This study investigated trace minerals with varied concentration such as Mg, K, Na, Fe, Ca, Pb and Mn were analysed by atomic absorption spectroscopy in mg/kg. The mineral analysis of the spices indicated their richness in sodium, calcium, potassium, iron, phosphorous and magnesium as presented in Table 1. The Zone of inhibition of aqueous extract of *A. melegueta* on selected oral organisms are showed in Table 2. The Gas chromatographic and mass spectral data for sample – eal isolated from *A. melegueta we*re presented in Table 3. Chromatograph for the elemental analysis of *A. melegueta* is shown in Figures 1 and 2. The peak at 3451 and 2931 cm-1 are corresponded to Hydroxyl and CH stretching frequency respectively. A band at 2377 cm-1 occurs in many spectra due to inequalities in path length. The peak at 1640 and 1600 cm-1 assign to C=C and unsaturated. The strong peak at 3451.42 cm-1 assigned to the CH_3 stretching vibration and the peak at 2931.43 cm-1 assigned to C-H stretching which means that some alkane compounds existed in these rare medicinal plant. The bands between 3000 and 2000 cm-1 represent C-H stretching vibration that are mainly generated by rapids (Wolkers *et al*., 1995; Wei *et al*., 2009).The mineral element contents were within WHO/FAO safe limit indicating favourable nutritional balance as presented in Table 1.

Table 1: Mineral concentration of ethanol extracts of *A. meleguata* seeds

Mineral elements	Concentration (mg/kg)	WHO/FAO safe limit (mg/kg)
N	22.25	_
Na	0.24	_
Ca	0.059	_
Pb	ND	100
Zn	9.935	100
Mg	1431.5	_
Mn	189.35	_
Ba	ND	_
Li	ND	_
Fe	40.99	300

ND: not detected

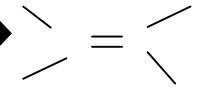
IR

3451 ➡ Strong band of O—H

2931 ➡ Saturated C—H stretch (CH_2, CH_3)

2377 ➡ O=C=O (CO_2) ➡ Occurs in many spectra due to inequalities in path length

1640

C C Unsaturation ➡  ➡

1600

1413

$^{-}$O—C ➡ CH

1313

1033 ➡ C—O stretching

Figure 1: FTIR Spectrum analysis of ethanol extract of *A. melegueta* seeds

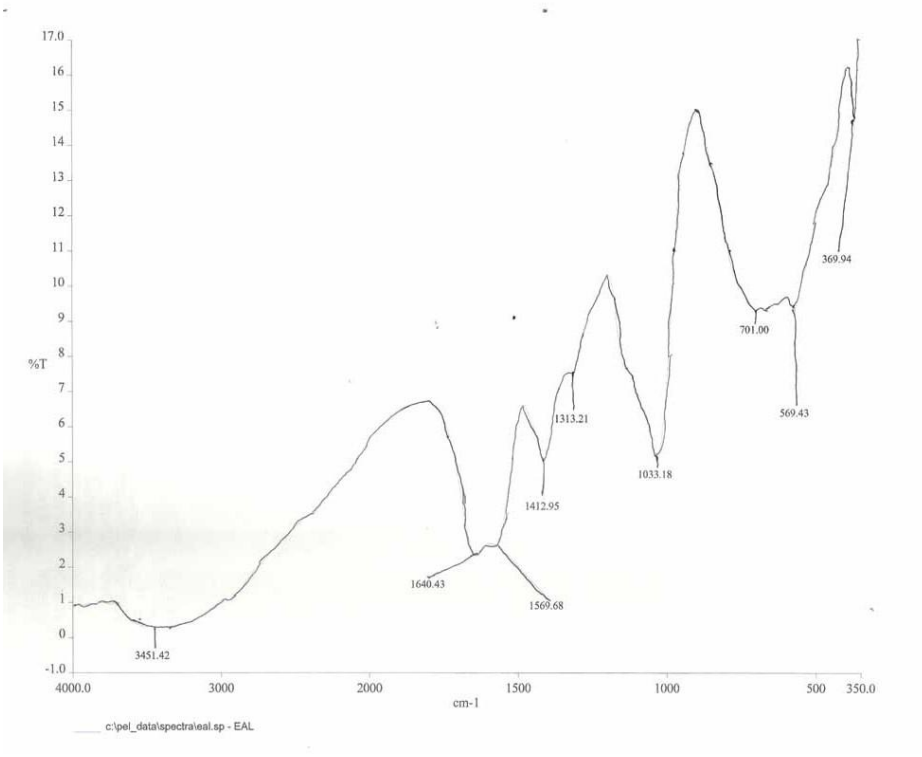


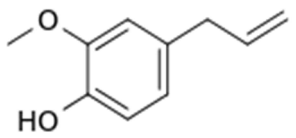
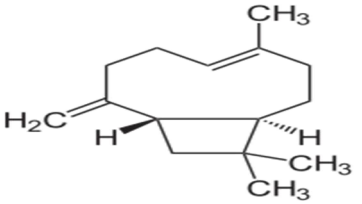
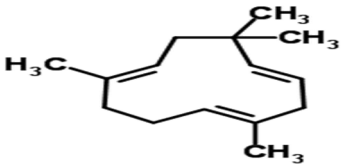
Fig. 2: GC-MS Chromatograph of *A. melegueta* seeds

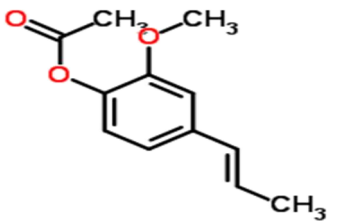
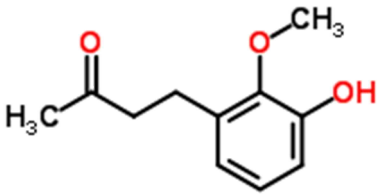
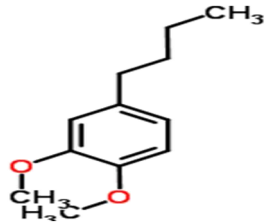
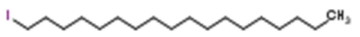
Table 2: Zone of inhibition of ethanol extract of *A. melegueta* seeds on selected oral microorganisms

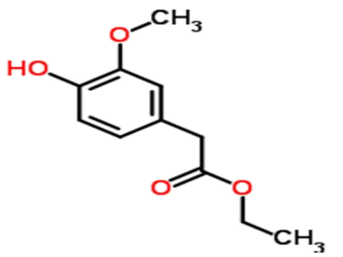
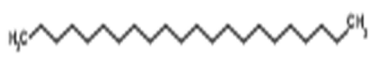
Organisms	**100mg/ml**	**50mg/ml**	**25mg/ml**	**12.5mg/ml**	**6.25mg/ml**	**S.D. H_2O**
S. epidermidis	19.47^{a}±1.52	20.67^{a}±0.58	13.37^{a}±3.04	15.00^{a}±2.00	7.47^{b}±5.76	0.00±0.00
E. coli	10.00^{a}±1.00	9.00^{a}±1.05	1.63^{b}±2.79	0.00±0.00	0.00±0.00	0.00±0.00
S. mutans	10.33^{a}±7.77	9.33^{a}±0.49	9.28^{a}±1.16	0.00±0.00	0.00±0.00	0.00±0.00
K. pneumoniae	12.53^{a}±4.97	11.67^{a}±2.89	6.57^{b}±0.58	0.00±0.00	0.00±0.00	0.00±0.00
A. niger	12.65^{a}±2.42	13.33^{a}±2.08	5.00^{b}±1.73	3.33^{b}±2.89	4.67^{b}±5.03	0.00±0.00
C. albicans	17.77±2.56	13.53±2.18	15.00±4.36	12.33±3.22	8.67±3.22	0.00±0.00
R. oryzae	18.75±0.68	14.43±7.58	18.00±4.36	15.33±2.31	10.00±1.00	0.00±0.00
A. flavus	14.00^{a}±3.61	14.33^{a}±1.89	6.00^{b}±1.00	4.27^{b}±2.08	7.33^{b}±2.31	0.00±0.00

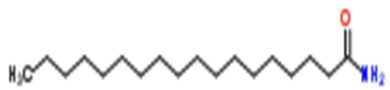
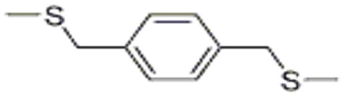
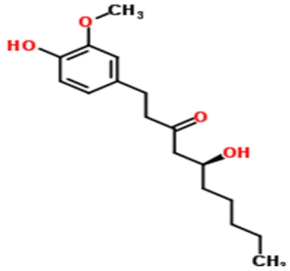
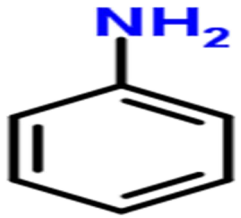
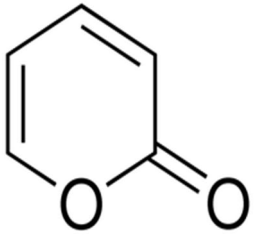
Similar letter within a row indicate that values are not significantly different from each other
Note: P<0.01 - Highly Significant; P<0.05 – Significant; *P>0.05 - Not Significant;* Mean ± SEM; S.D H_2O – Sterile distilled water

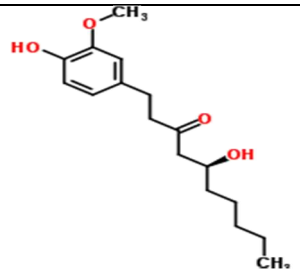
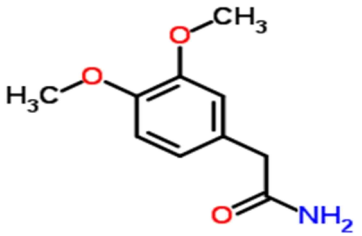
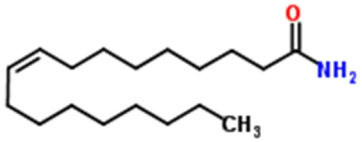
Table 3: Gas chromatographic and mass spectral data for the oil extract of eal isolated from *A. melegueta*

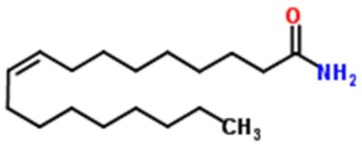
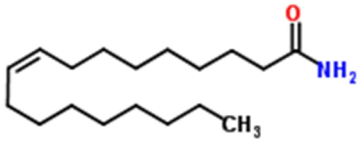
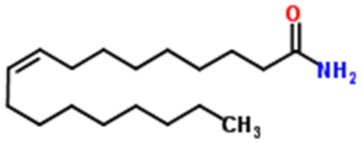
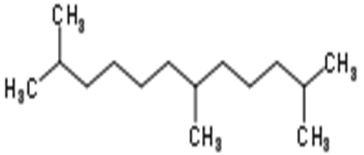
S/N	Compound	Naturl. Product Class	Rt (mins)	Molecular Mass	Molecular Formula	Peak Percentage	Compound structure
1	Eugenol	Terpenoids	15.598	164.2	$C_{10}H_{12}O_2$	3.49	
2	Caryophyllene	Terpenes	17.687	204.36	$C_{15}H_{24}$	4.30	
3	α-Caryophyllene	Terpenes	18.779	204.35	$C_{15}H_{24}$	5.15	
4	Heptacosane	Alkane	19.958	380.73	$C_{27}H_{56}$	1.21	

S/N	Compound	Naturl. Product Class	Rt (mins)	Molecular Mass	Molecular Formula	Peak Percentage	Compound structure
5	Phenol,2-methoxy-4-(2-propenyl)-acetate	Ester	20.988	206.24	$C_{12}H_{14}O_3$	1.63	
6	Octacosane	Alkane	24.192	394.76	$C_{28}H_{58}$	0.70	
7	Butan-2-one,4-(3-hydroxy-2-methoxyphenyl)	Ketone	24.793	194.23	$C_{11}H_{14}O_3$	33.54	
8	4-butyl-1,2-dimethoxy-benzene	Aromatic compound	25.955	194.27	$C_{12}H_{18}O_2$	3.40	
9	1-Iodo-octadecane	Alkane	26.361	380.39	$C_{18}H_{37}I$	2.05	
10	Heneicosane	Alkane	30.149	296.57	$C_{21}H_{44}$	0.78	

S/N	Compound	Naturl. Product Class	Rt (mins)	Molecular Mass	Molecular Formula	Peak Percentage	Compound structure
11	Octacosane	Alkane	31.488	394.76	$C_{28}H_{58}$	0.94	
12	Heneicosane	Alkane	32.140	296.57	$C_{21}H_{44}$	2.77	
13	Heneicosane	Alkane	33.199	296.57	$C_{21}H_{44}$	0.71	
14	Tetratriacontane	Alkane	35.539	478.92	$C_{34}H_{70}$	0.79	
15	Heneicosane	Alkane	36.460	296.57	$C_{21}H_{44}$	0.83	
16	Ethyl homovanillate	Ester	36.844	210.23	$C_{11}H_{14}O_4$	3.50	
17	Tetracontane	Alkane	37.113	563.08	$C_{40}H_{82}$	0.75	
18	Docosane	Alkane	37.364	310.60	$C_{22}H_{46}$	1.55	

S/N	Compound	Naturl. Product Class	Rt (mins)	Molecular Mass	Molecular Formula	Peak Percentage	Compound structure
19	Octadecanamide	Amide	38.337	283.49	$C_{18}H_{37}NO$	1.67	
20	Bis (methyl thio) methyl benzene	Aromatic compound	39.384	198.00	$C_{10}H_{14}S_2$	2.68	
21	1-(4-hydroxy-3-methoxy phenyl)-3-Decanone	Ketone	39.625	294.39	$C_{17}H_{26}O_4$	7.70	
22	Phenylamine	Aromatic amine	40.037	93.13	C_6H_7N	0.37	
23	Pyran-2-one derivative	Heterocyclic compound	40.243	96.08	$C_5H_4O_2$	0.61	

S/N	Compound	Naturl. Product Class	Rt (mins)	Molecular Mass	Molecular Formula	Peak Percentage	Compound structure
24	Gingerol	Alcohol	40.946	294.39	$C_{17}H_{26}O_4$	4.69	
25	3-4-dimethoxy benzene acetamide	Amine	41.570	195.22	$C_{10}H_{13}NO_3$	1.19	
26	Tetracontane	Alkane	41.976	563.08	$C_{40}H_{82}$	0.32	
27	Tetracontane	Alkane	42.131	563.08	$C_{40}H_{82}$	0.84	
28	9-octadecenamide	Amide	42.405	281.48	$C_{18}H_{35}NO$	4.42	

S/N	Compound	Naturl. Product Class	Rt (mins)	Molecular Mass	Molecular Formula	Peak Percentage	Compound structure
29	9-octadecenamide	Amide	42.537	281.48	$C_{18}H_{35}NO$	2.28	
30	9-octadecenamide	Amide	42.651	281.48	$C_{18}H_{35}NO$	3.19	
31	9-octadecenamide	Amide	42.949	281.48	$C_{18}H_{35}NO$	0.56	
32	2,6,11-trimethyldodecane	Alkane	47.389	212.41	$C_{15}H_{32}$	0.91	
						100%	

Discussion

The investigations on the antibacterial activity of *Aframomum melegueta* extract had inhibitory effect on the growth of selected oral microorganisms such as *S. epidermidis, E. coli, S. mutans, K. pneumoniae, A. niger, C. albicans, R. oryzae, A. flavus* (Table 2). This suggests that the plant extract is broad spectrum in activity. Higher antimicrobial activity of the extracts was observed at lowest concentration of 6.25 mg/ml on *S. epidermidis* (7.47±5.76), *C. Albicans* (8.67±3.22), *R. oryzae* (10.00±1.00) and *A. flavus* (7.33±2.31). This is in line with the earlier result obtained by Oyagade (1999), Akpulu (1994) and Oladunmoye (2007) which showed significant ($p<0.05$) activity against oral organisms. The antimicrobial effect of *A. melegueta* may be due to the phytochemical constituents present in the plant seeds. *A. melegueta* seeds recorded high phytonutrient such as flavonoids, phenolic compound, tannins, saponins, terpernoids, cardiac glycosides and alkaloids.

Medicinal plants have been reported to be used in oriental medicine for treatment of ailment ranging from common cold to cancer (Fisher, 2012). Medicinal plants are also known to contain trace elements which play vital role as structural and functional components of metalloprotein and enzymes in the living cells. Each mineral plays a number of different functions in the body. The most important pathway of metals to transport into human is from soil to plant and from plant to human. Some metals such as Calcium (Ca), Magnesium (Mg) and Zinc (Zn) have been reported to be essential for human health which does not produce any toxicity to human beings whereas others such as Lead (Pb), Cadmium (Cd) and Aluminium (Al) have been identified as toxic, while other elements are not toxic to human (Kirmani *et al*., 2011). The result showed that Magnesium (Mg), Manganese (Mn) and Iron (Fe) are higher in concentration compared to other elements analysed using the Atomic Absorption Spectrophotometer (AAS). Calcium was implicated in trace amount. Calcium is an essential element in the human body. It is one of the most abundant elements in human body and accounts for 2 to 3 pounds of our total body weight (Bachheti *et al*., 2012). The bone and teeth development need the calcium and also it regulate heart rhythm, helps in normal blood clotting, maintain proper nerve and muscle functions and lower blood pressure (Bachheti *et al*., 2012).

Gas chromatographic and mass spectral data for the oil extract isolated from *A. Melegueta* Eugenol was detected at a retention time of 15.598 minutes and peak area percent of 3.49 (Table 3). It is a phenyl propene (phenyl propanol). It's a clear to pale yellow oil liquid extracted from certain essential oils, from alligator pepper, clove oil, nut meg, cinnamon, basil and hay leaf. The Eugenolin extracted from *A. melegueta* may be used in the production of perfumeries, flavorings, essential oil and in medicine as local antiseptics and anaesthetics. Caryophyllene was detected at a retention time of 17.687 minutes and peak percent of 4.30 IUPAC – 4, 11, 11 – trimethyl -8-methylene bicyclo (7, 2, 0) undec-4-ene. It is a natural bicyclic sesquiternone that is a constituent of many essential oils, especially clove oil, the oil from the stems and flowers of *Syzygium aromaticum* (cloves), the essential oil of hemp (*Cannabis sativa*), rosemary (*Rosmarinus oficinalis*) and alligator pepper. It contributes to the spiciness of black pepper and anti-inflammatory effect in mice (Gertsch *et al.*, 2008). Tetratriacontane was detected at a retention time of (35.539 minutes) and peak area percent of 0.79. Tetratriacontane**,** neutral components in the seeds of *A. melegueta*, has medicinal importance as an anti-inflammatory, antibacterial and antiulcergenic.

Heneicosane was detected at a retention time of 30.149 minutes and peak percent of 0.78. Heneicosane is a white waxy solid combustible; incompatible with strong oxidizing agent, heneicosane is one of the vital pheromone for attracting mosquitoes of *Aedes* spp. to lay their eggs in areas of stagnant fresh water, for their subsequent destruction, thus controlling spread of dangerous disease transmission by the vectors.

Conclusion

The traditional uses of *A. melegueta* for treatment and therapeutic purposes suggest that the plant extract can be substituted for the commercial antibiotics. The presence of trace elements such as Calcium, Magnesium, Iron, Zinc, Nitrogen and the functional groups such as Ketones, Terpenoids, Terpenes are responsible for various medicinal properties of *A. melegueta*

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