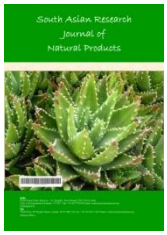

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Assessment of Antibacterial and Gas Chromatography/Mass Spectrophotometric Analysis (GC-MS) Profile of Purified Volatile Compounds from Multi-phases Solvent Extraction Method from Stem Extract of *Aframomum melegueta* [Roscoe] K. Schum

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This research work aims to ascertain the composition and potency of the bioactive component of Volatile compounds derived from *Aframomum melegueta* stem extract through multi-phase solvent extraction Gas Chromatography /Mass spectrophotometric analysis and antimicrobial assay against selected clinical isolates. *Aframomum melegueta* stem extract is used as a remedy against stomachache, diarrhea, and snakebite. The fresh stem of *Aframomum melegueta* plant was collected from Owo forest reserve, Ondo State, Nigeria, 500g of each dried and powdered plant sample was weighed separately into corked containers containing 1500ml each of acetone and ethanol, the mixture was shaken vigorously and left for 9 days, Solvent of extraction are n-Hexane (StAMH)(153 g), Dichloromethane,(StAMD (103 g), Ethyl acetate, (StAME) (45.5 g), Methanol (StAMM)(50 g). For the StAMD Elution, StAMD was absorbed in silica gel of 200-400 mesh from ZICO-TEK laboratory, GC-MS analysis of this extract was performed using a Perkin Elmer GC Claurus 500 system and gas chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with Elite-1 fused silica capillary column (30mx1µl was Mdf. Composed of 100%

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Dimethylpolysiloxane). 17 and 48 compounds were analyzed in the stem extract of purified Volatile compounds from Multi-phases solvent extraction of stem extract of *Aframomum melegueta*. The composition, chemical structure, molecular weight, and molecular formulation of different Volatile compounds of StD+ and StD++ from purified Volatile compounds. The antimicrobial assessment were performed using Kirby-Bauer disc diffusion method (Agar well diffusion method).*E. coli* and *Staphylococcus aureus* has the highest zones of inhibition of 19,0 mm at 100mg/ml. In contrast, *Klebsiella planticola and Citrobacter diversus* have the lowest zones of inhibition of 3.0 mm at 12.5 mg/ml for StD+ and *Staphylococcus aureus* (12%) and *E. coli* (12%), has the highest. In contrast, *Staphylococcus aureus* (6%), has the lowest percentage frequency distribution of purified Volatile compounds of *Aframomum melegueta (*StD+) against selected clinical isolates. *E. coli* has the highest zones of inhibition of 20 mm at 100 mg/ml, while *Klebsiella planticola* has the lowest inhibition of 1.0 mm at 12.5 mg/ml for *(*StD++). In conclusion, the uses and importance of Volatile compounds fractions from *Aframomum melegueta have* limitless potential.

Keywords: Aframomum melegueta; bacterial isolates; volatile compounds; extraction solvent.

1. INTRODUCTION

This plant is a member of the ginger family (Zingiberaceae) and is cultivated in tropical areas of West Africa. The plant seeds are used to flavor foods and as components of traditional African folk medicine. In medieval Europe, they were a highly prized spice that was eventually replaced by black pepper and other spices. Ethnobotanically, the stem extract is used as a remedy against stomachache, diarrhea, and snakebite [1,2].

In Addition, there are reported studies on the different medicinal use of *Aframomum melegueta,* they are*;* Antiulcer, Cytoprotective, and Antimicrobial activities as well as the sexual performance-enhancing effects of grains of paradise [3-6]. The aqueous seed extract has been shown to reduce the frequency of abdominal constrictions induced by acetic acid in mice and has significant anti-inflammatory activity [7]. It was later reported that the same extract has peripheral analgesic activity. Additionally, it was suggested that the extract has membrane-stabilizing activity along with antioxidant effects [8,9] as well as hypotensive and antihypertensive activity in humans [10],[11]. It has been also found that the extract has an effect on the whole-body energy expenditure and visceral fat in humans [12], However, in some of the literature studies, it should be mentioned that the seed of *Aframomum melegueta* derived Volatile compound contains humulene and caryophyllene occurred in higher proportions in the volatile oil [13].

The major components of the leaf oil were found to be myrtenyl acetate, isolimonene while caryo phyllene oxide, myrtenyl acetate, β-eudesmene, and β-caryophyllene make up the composition of the stem oil [14] whereas the root essential oil comprised of myrtenyl acetate and pinocarvyl acetate. However, the seed is comprised mainly of α-humulene, β-caryophyllene.

In literature, the major constituents of the leaf essential oil [15] was identified as sabinene,α-pinene and β-caryophyllene. The seed essential oil of *Aframomum melegueta* presents a characteristic composition with β-caryophyllene, α-humulene, and their epoxides as main constituents [16]. Volatile compound are extracted from plants to produce essential oils, and are some of the most important medicinally active plant constituents. An essential oil is a concentrated hydrophobic liquid containing volatile chemical compounds from plants. Essential oils are also known as volatile oils, ethereal oils, aetheroleum, or simply as the oil of the plant from which they were extracted, such as oil of clove [16,17].

Eugenol occurred in abundance in the oil [17], β-Pinene predominates in the essential oils of the leaves and seeds of *Aframomum melegueta* [18]. GC/MS analysis of the hexane and methanol extracts of *Aframomum melegueta* seed yielded gingerol, zingiberone, paradol, *trans*-6-shogaol, *cis*-isoelemicin, β-bisabolene, α-guaiene, aroma dendrene, *trans*-β-farnesene and geraniol. The essential oil of *Aframomum melegueta* displayed insect repellency against *Rhyzopertha dominica* [19] antimicrobial activity [20], antifungal effect [21], moderate inhibition of acetyl-cholinesterase [22], antioxidant[23], meta bolites exhibit poly pharmacology against SARS-CoV-2 drug targets [24].

2. MATERIALS AND METHODS

Collection of fresh stem *Aframomum melegueta* [Roscoe] K. Schum

The fresh stem of *Aframomum melegueta* [Roscoe] K. Schum plant were collected from Owo forest reserve, Ondo State, Nigeria The experimental site is located between coordinates 6.96879 and 5.5626 and an altitude of 415 m on the month June 20th, 2020 and the plant samples were authenticated at the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria.

Preparation of fresh stem *Aframomum melegueta* [Roscoe] K. Schum

For the extraction of each plant part, 500g of each dried and powdered plant sample was weighed separately into corked containers containing 1500ml each of acetone and ethanol, the mixture was shaken vigorously and left for 9 days. The mixture was in a ratio of 1:1. All mixtures were filtered using sterile what man No. 1 filter papers, and the filtrates were collected directly into sterile crucibles. The filtrate was extracted using a soxhlet extractor, and the residues obtained were kept at room temperature [23].

Extraction of fresh stem *Aframomum melegueta* [Roscoe] K. Schum. **Multi-Phase Solvent Extraction of fresh** stem of *Aframomum melegueta* [Roscoe] K. Schum **Bioactive Compound (**Volatile compounds **Fraction).**

Aframomum melegueta was serially extracted using the multiphase extraction method at room temperature for 72 hours varying solvents based on their polarity using organic solvents. Solvent of extraction are: n-Hexane, (StAMH) (153 g), Dichloromethane, (StAMD (103 g), Ethyl acetate, (StAME) (45.5 g), Methanol, (StAMM) (50 g). For the StAMD Elution, StAMD was absorbed in silica gel of 200-400 mesh from ZICO-TEK laboratory. The following table shows detailed multiphase extraction of the essential fraction of *Aframomum melegueta.* A schematic extraction tree follows the table below.

At 100% EtOAc elution, all phyto-constituents in the column have been eluted as revealed on the TLC plate developed. The elutes were spotted on the TLC plate and developed by HEX: DCM as the mobile phase at a ratio of 50:50. Bulking was done as follows; 1 -9 a, 11-13 b, 15 -19 c, 21 – 31 d, 33 – 35 e, 37 – 51 f. a – f were all chlorophyll and were discarded and tagged A.52 – 55(B),56 – 63(C),64 – 69 (D), 70 – 74(E), 75 – 84(F), 85 – 92(G), 93 – 97(H). Further bulking as guided by TLC developed by mobile phase DCM: EtOAc 2: 8; EtOAc 100%. B, C, and D bulked and labeled D+ pure compound I. E and F bulked as D++, G and H bulked to H+ - discarded due to insufficient quantity.

Gas chromatograph and mass spectroscope (GC-MS) Analysis of fresh stem extract of *Aframomum melegueta* [Roscoe] K. Schum.

GC-MS technique was used in this study to identify the components present in the extract of entire parts of *Aframomum melegueta* [Ros coe] K. Schum. GC-MS technique was carried out at the School of Chemistry and Physics, Westville Campus, University of KwaZulu-natal Durban, South Africa. GC-MS analysis of this extract was performed using a Perkin Elmer GC Claurus 500 system and gas chromatograph interfaced to a Mass spectrophotometer (GC-MS) equipped with Elite-1 fused silica capillary column (30mx1µl was Mdf. Composed of 100% Dimethylpolysiloxane). For GC-MS detection, an electron ionization energy system with ionization energy of 70eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1ml/min. and an injection volume of 2µl was employed (Split ratio of 10:1). The injector temperature was 250ºC. The oven temperature was programmed from 110ºC (isothermal for 2min.), with an increase of 10ºC/min to 200ºC, then 5ºC /min. to 280ºC, ending with a 9min. isothermal at 280ºC. Mass spectra were taken at 70eV; a scan-interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total area. Software adapted to handle mass spectra and chromatograms was a Turbo mass Ver 5.2.0. Compounds identification was obtained by comparing the retention times with those of authentic compounds and the spectral data obtained from the library data of the corresponding compounds. The given sample was extracted with ethyl acetate and analyzed in GC-MS for different components [23, 24, 25].

Identification of components:

The identity of the components in the extract was assigned by the comparison of their retention time and mass spectra fragmentation patterns with those stored in the computer library and also with published literature. NIST library sources were also used for matching the identified components from the plant material.

Table 1. Details of Multi-phase solvent extraction process; (Volatile compounds) Phase/Fraction)

n-Hex	DCM	EtOAc	Volume(mL)	Test-tube Numbering
100	-	-	300	500 Conical flask
90	10	-	200	1-5
50	50	-	200	6-14
45	55	-	100	15-20
30	70	-	100	21-27
20	80	-	400	28-57
-	100	-	100	58-67
-	90	10	300	68-79
-	50	50	100	80-86
	-	100	200	87-97

Key- n-Hex-Normal hexane, EtOAc-Ethyl acetate, DCM- Dichloromethane, Met- Methanol, Hexane-Hexane

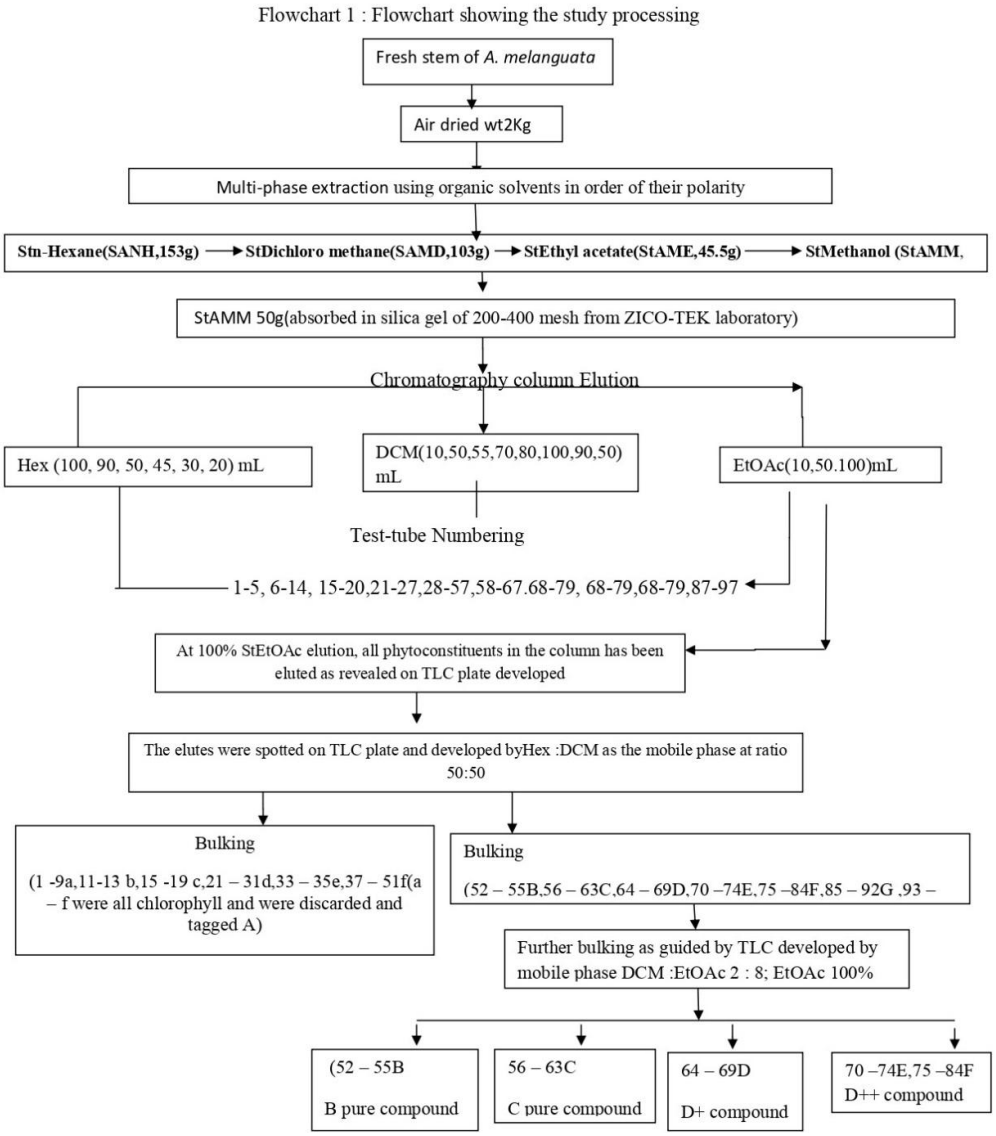


Chart 1. Schematic diagram of multi-phase extraction of bioactive compounds (Volatile compound), SD^{+} and SD^{++})

Standardization of plant extracts for antimicrobial assay:

At aseptic conditions, the extracts were reconstituted by adding 1g of each extract to 2.5ml of DMSO and 7.5ml of sterile distilled water to make 100mg/ml. The serial concentration was prepared to get concentration of 50 mg/ml, 25 mg/ml and 12.5 mg/ml respectively [25,26]

Test organisms:

The selected clinical test isolates used were: *E. coli, Staphylococcus aureus, Klebsiella. pneumoniae, Proteus mirabilis, P. aeruginosa, Staphylococcus aureus, Corynebacterium cystitidis, Klebsiella planticola, Salmonella choleraesuis, Citrobacter freundii, Citrobacter diversus.*

Standardization of test organisms:

The test organisms used were obtained from the stock culture of the laboratory of the Department of Microbiology, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. The test organisms were sub-cultured to obtain pure cultures of the organisms. The broth cultures of the test organism were prepared according to 0.5 McFarland's standard [26].

Antimicrobial screening of purified Volatile Compounds of *Aframomum melegueta***. (StD+ and StD++) extracts against selected clinical isolates using** Kirby-Bauer method (Agar well diffusion method).

The agar well diffusion method according to Osuntokun et al was used. The overnight broth culture of the respective bacteria strains was adjusted to 0.5 McFarland standard. Mueller-Hinton agar plates were swabbed using sterile cotton swabs with the adjusted broth culture of the respective bacteria strains. Wells (6 mm in diameter) were made equidistance in each of the plates using a sterile cork borer. 100 µl (0.1 ml) of each concentration of the extract were respectively introduced into the wells using sterile automatic pipettes, with the stock solution in the center well with different concentrations of the extracts (50, 25, and 12.5 mg/ml). The plates were allowed to diffuse at room temperature for 2 hours and were incubated at 37°C for 24 hours for the bacterial isolates and 24°C for 48 hours for the fungal isolates. The zones of inhibition were measured to the nearest millimeter (mm) using a standard transparent meter rule. The antibacterial activity was expressed as the mean zone of inhibition diameters (mm) produced by the plant extract [23,25].

3. RESULTS

The following represented the result obtained during the course of this research work. two Tables (2 and 3,), state the spectral analysis of *Aframomum melegueta*, Figs. 1-11) which demonstrate the antibacterial activity zones of inhibition and percentage frequency distribution of purified essential oil against the clinical organisms/isolates.

Table 2; Gas chromatography and mass **spectrophotometric**(GC-MS) analysis of *Aframomum melegueta* stem extract of purified Volatile Compounds(*St*D^{+}),table 3; Gas chromategraphy and mass spectroscope(GC-MS) analysis of *Afra momum melegueta* stem extract of purified Volatile compounds(*St*D++), Fig 1; Antibacterial activity of purified Volatile compounds of *Aframomum melegueta (*StD+) stem extracts against selected clinical isolates, Fig 2; Percentage frequency distribution of antibacterial activity of *Aframomum melegueta (St*D^{+}) stem against clinical isolates at *(St*D^{+})100mg/ml, Fig 3; Percentage frequency distribution of anti bacterial activity of *Aframomum melegueta* stem against clinical isolates at *(St*D^{+}) 50mg/ml, Fig 4; Percentage frequency distribution of anti bacterial activity of *Aframomum melegueta* stem against clinical isolates at *(St*D^{+}) 25mg/ml, Fig 5; Percentage frequency distribution of anti bacterial activity of *Aframomum melegueta* stem against clinical isolates at *(St*D^{+}) 12.5mg/ml, Fig 6; Antibacterial activity of purified Volatile compound of *Aframomum meleg ueta.(St*D^{++})stem extracts against selected clinical isolates, Fig 7; Percentage frequency distribution of antibacterial activity of *Aframomum melegueta* stem against clinical isolates at *(St*D^{++}) 100mg/ml, Fig 8; Percentage frequency distribution of antibacterial activity of *Aframomum melegueta* stem clinical isolates at *(St*D^{++}) 50mg/ml, Fig 9; Percentage frequency distribution of antibacterial activity of *Aframo mum melegueta* stem against clinical isolates at *(St*D^{++}) 25mg/ml, Fig 10; Percentage freq uency distribution of antibacterial activity of *Aframomum melegueta* stem against clinical isolates at *(St*D^{++}) 12.5mg/ml. Spectral 1; Gas chromatography and mass spectroscope (GC-MS)spectra analysis of *Aframomum melegueta* purified Volatile compounds stem extract (*St*D^{++}).

Spectral 2; Gas chromatography and mass spectroscope (GC-MS) spectra analysis of *Aframomum melegueta* stem extract of purified Volatile compounds(*St*D^{++}).Table 2 and 3 represents the Gas chromatograph and mass spectroscope (GC-MS) Spectra Analysis of *Aframomum melegueta* purified Volatile Comp ounds (*St*D+ and StD^{++}).

Fig. 1 represents percentage frequency distribution of antibacterial activity against clinical isolates at *(*StD^{+}) and SD^{++}) at 100, 50,25, and 12.5mg/ml.

Table 2; Gas chromatography and mass **spectrophotometric** (GC-MS) analysis of *Aframomum melegueta* stem extract purified Volatile Compound (*St*D+). It was observed that 17 compounds were analyzed. It revealed the composition, chemical structure, molecular weight, and molecular formulation of different Volatile compounds. This include 1,1-Dimethyl-chloropropanol($C_5H_{11}ClO$,MolWgt,122Da),Alpha.-Hydroxyisocaproic acid ($C_6H_{12}O_3$, Mol W, Mol Wgt1,32Da),Dimethylsulfoxonium formylmethy lide ($C_4H_8O_2S$, Mol Wgt 120),Cyclohexasiloxane, dodecamethyl-($C_{12}H_{36}O_6Si_6$,MolWgt444Da),Cycl oheptasiloxane,tetradecamethyl-($C_{14}H_{42}O_7Si_7$,MolWgt518Da),Heptasiloxane,hexadecam ethyl-($C_{16}H_{48}O_6Si_7$,MolWgt518Da),Cyclooctasiloxane, hexadecamethyl-($C_{16}H_{48}O_8Si_8$,MolWgt592Da), Cyclononasiloxane, octadecaethyl-($C_{18}H_{54}O_9Si_9$, Mol Wgt 666Da), Heptasiloxane,hexadeca meth yl-($C_{16}H_{48}O_6Si_7$,Mol Wgt 532 Da),Cyclonona siloxane, octadecamethyl-($C_{18}H_{54}O_9Si_9$,Mol Wgt 666Da), Heptasiloxane, hexadecamethyl-($C_{16}H_{48}O_6Si_7$, Mol Wgt 532Da), Cyclodecasiloxane, ei cosa methyl ($C_{20}H_{60}O_{10}Si_{10}$, Mol Wgt 7740Da), Dibutyl phthalate ($C_{16}H_{22}O_4$,,Mol Wgt 278 Da), l-(+)-Ascorbic acid 2,6 dihexade canoate ($C_{38}H_{68}O_8$,MolWgt652Da),Heptasiloxane,hexadecameth yl-($C_{16}H_{48}O_6Si_7$,Mol Wgt532Da) and Cycloocta siloxane,hexadecamethyl-($C_{18}H_{54}O_9Si_9$, Mol Wgt 592Da).

Table 3: Gas chromatography and mass **spectrophotometric** (GC-MS) analysis of *Aframomum melegueta* stem extract of purified Volatile compounds**(*St*D++)**, this table denote the chemical nature of *Aframomum melegueta* stem extract of Volatile compounds. It was observed that 48 compound were analyzed this include Dimethyl sulfoxonium formylmethylide ($C_4H_8O_2S$, Mol Wgt 120Da), 2-Hexanol, 2-methyl-($C_7H_{16}O$,Mol Wgt 116 Da), Ethanol,2-butoxy-($C_6H_{14}O_2$,, Mol Wgt,118 Da), Cyclopentasiloxane, deca methyl ($C_{10}H_{30}O_5Si_5$, Mol Wgt 370Da), Cyclohexa siloxane, dodecamethyl ($C_{12}H_{36}O_6Si_6$,Mol Wgt 444Da), Hexasiloxane, tetradecamethyl-($C_{14}H_{42}O_5Si_6$,MolWgt458Da),Cycloheptasiloxane, tetrad eca methyl-($C_{14}H_{42}O_7Si_7$ Mol Wgt 518Da), 1,4,7,-Cycloundecatriene,1,5,9,9-tetramethyl-,Z, ($C_{15}H_{24}$ Mol Wgt 204 Da), Phenol, 2,4-bis(1,1-dimethylethyl)-($C_{14}H_{22}O$ Mol Wgt 206Da),Hepta siloxane, hexadecamethyl-($C_{16}H_{48}O_6Si_7$ Mol Wgt 532 Da),Caryophylleneoxide($C_{15}H_{24}O$ Mol Wgt 220Da), 2-Oxatricyclo [4.3.1.0 (3,8) decane ($C_9H_{14}O$ Mol Wgt138Da), Methanol, [6, 8,9-tri methyl-4-(1-propenyl)-3-oxabicyclo($C_{15}H_{24}O_2$ Mol Wgt 236 Da),Cyclooctasiloxane,hexadecamethyl ($C_{16}H_{48}O_8Si_8$Mol Wgt 592 Da), Isoaromadendren eepoxide($C_{15}H_{24}O$ mol Wgt Tricyclo [3.2.1.02, 7]oct-3-ene,2,3,4,5-tetramethyl ($C_{12}H_{18}$ Mol Wgt 162 Da),1-Oxaspiro[2.5] octane,5,5-dimethyl-4-(3-methyl($C_{14}H_{22}O$ Mol Wgt 206Da), alpha.-Cadinol($C_{15}H_{26}O$ Mol Wgt 222 Da), Heptasilo xane,hexadecamethyl($C_{16}H_{48}O_6Si_7$ Mol Wgt 532 Da),Longifolenaldehyde ($C_{15}H_{24}O$ mol Wgt 220 Da),Alloaromadendrene oxide-(1) ($C_{15}H_{24}O$ Mol Wgt 220 Da), Cyclononasiloxane, octadeca methyl-($C_{18}H_{54}O_9Si_9$Mol Wgt 666 Da), 6-epi-shyobunol ($C_{15}H_{26}O$ Mol Wgt 22 Da), Tetra decanoic acid, ethylester ($C_{16}H_{32}O_2$ Mol Wgt 256Da), 4-Hexen-1-ol,6-(2,6,6-trimethyl-1-cyclo hexene($C_{16}H_{28}O$ Mol Wgt 236 Da), Hepta siloxane, hexadecamethyl($C_{16}H_{48}O_6Si_7$ Mol Wgt 532Da),2-Pentadecanone,6,10,14-trimethyl-($C_{18}H_{36}O$ Mol Wgt 268 Da),1,2-Benzene dicarboxylIc acid,bis(2-methylpro ($C_{16}H_{22}O_4$ Mol Wgt 278 Da) ,Cyclodecasiloxane, eicosamethyl-($C_{20}H_{60}O_{10}S I_{10}$ Mol Wgt 740 Da), Hexadecanoic acid, methyl ester($C_{17}H_{34}O_2$ Mol Wgt 270Da), 1,4-Methano azulene-9-methanol,decahydr4($C_{15}H_{26}O$ Mol Wgt 222 Da), Di butyl phthalate ($C_{16}H_{22}O_4$ Mol Wgt 278Da),Naphtho(2,3-b)furan-2(3H)-one,decahy dro-8($C_{15}H_{20}O_2$ Mol Wgt 232Da), l-(+)-Ascorbic acid 2,6-dihexadecanoate($C_{38}H_{68}O_8$) mol Wgt 652 Da),Menthol, 1'-(butyn-3-one-1-yl)-,(1R, 2S ,5R)- ($C_{14}H_{22}O_2$ Mol Wgt 222Da), 4-Hexen-1-ol, 6-(2,6,6-trimethyl-cyclohexen($C_{16}H_{28}O$,MolWgt 238Da),Heptasiloxane,hexadecamethyl($C_{16}H_{48}O_6Si_7$Mol Wgt 532Da),Hexadecanoic acid, ethyl ester ($C_{18}H_{36}O_2$ Mol Wgt 284Da),(3H) –Benzo furanone,6-ethenylhexahydro-6-($C_{15}H_{20}O_2$)Mol Wgt 232Da),3,7,11,15-Tetramethyl hexadeca-1,6,10,14-tetr($C_{20}H_{34}O$)Mol Wgt 290Da),Cycloo ctasiloxane,hexadecamethyl-($C_{16}H_{48}O_8Si_8$ Mol wgt 592 Da),7a-Isopropenyl-4,5-dimethylocta hydroinden-($C_{15}H_{26}O$ MolWgt 22Da),Linoleic acid ethylester $C_{20}H_{36}O_2$ Mol Wgt 308Da), Hepta siloxane, hexadecamethyl-($C_{16}H_{48}O_6Si_7$ Mol Wgt 532Da), Allo aromadendrene oxide-(1)($C_{15}H_{24}O$ Mol Wgt 220 Da). Fig.1.denotes the Antibact erial activity of purified Volatile compounds of

*Aframomum melegueta (*StD++) stem extracts against selected clinical isolates (StD^{+}). It was observed that all the selected test organisms were susceptible to the purified Volatile compounds of *Aframomum melegueta* at varying degrees of concentration of 100, 50,25, and 12,5mg/ml. respectively. *E.coli* and *Sta phylococcus aureus* has the highest zones of inhibition of 19,0mm at 100mg/ml while *Klebsiellae planticola and Citrobacter diversus* has the lowest zones of inhibition of 3.0mm at 12.5mg/ml. other relatively high zones of inhibition were *Corynebacterium cystitidis* (18.0mm), *Klebsiella .pneumoniae* (17.0 mm*), Proteus vulgaris* (17.0mm), *Citrobacter freundii* (15.0mm)*and Citrobacter diversus (*13.0mm) *and Salmonella choleraesuis* (12.0 mm). The lowest zones of inhibition were observed in *Staphylococcus aureus and E. coli* (7.0mm), *Corynebacterium cystitidis* (6.0mm), and *Klebsiella .pneumoniae* and *Citrobacter freundii* (5.0mm), *Proteus vulgaris, P. aeruginosa and Salmonella choleraesuis* (4.0mm) respectively.

Fig. 2,3,4,5 represents percentage frequency distribution of antibacterial activity against selected clinical isolates at *(*StD+), Fig. 2 depicts the percentage frequency distribution of antibacterial activity against clinical isolates at *(*StD+) at 100mg/ml. At 100mg /ml, *Staphylo coccus aureus* (12%) and *E.coli* (12%), have the highest while *Staphylococcus aureus* (6%), has the lowest percentage frequency distribution of purified Volatile compounds of *Aframomum melegueta* (StD+) against selected clinical isolates. Other isolates were *Citrobacter diversus* (8%), *Proteus vulgaris* (10%), *Klebsiella .pneu moniae* (11%), *P. aeruginosa* (7%), *Staphylo coccus aureus*((6%), *Coryne bacterium cystitidis* (11%), *Klebsiella planticola* (7%), *Salmonella choleraesuis*(7%),*Citrobacter freundii* (9%) respectively.

Fig. 3 depicts the percentage frequency distribution of antibacterial activity against clinical isolates at *(*StD+) at 50mg/ml. At 50mg/ml, *E.coli* (13%) and *Staphylococcus aureus* (13%), have the highest while *Staphylococcus aureus* (6%), has the lowest percentage frequency distribution of purified Volatile compounds of *Aframomum melegueta (*StD+) against selected clinical isolates. Other isolates were *Klebsiella .pneumoniae* (12%), *Proteus vulgaris* (10%), *P. aeruginosa* (7%), *Klebsiella planticola* (7%), *Corynebacterium cystitidis* (10%),*Salmonella choleraesuis* (7%), *Citrobacter freundii* (8%), *Citrobacter diversus* (7%) respectively.

Fig. 4 depicts the percentage frequency distribution of antibacterial activity against clinical isolates at *(*StD+) at 25mg/ml. At 25mg/ml, *E. coli* (12%) and *Staphylococcus aureus* (12%), has the highest while *Proteus vulgaris* (7%), has the lowest percentage frequency distribution of purified Volatile compounds of *Aframomum melegueta (*StD+) against selected clinical isolates. Other isolates were *Citrobacter freundii* (9%), *Citrobacter diversus* (9%), *Klebsiella pneumonia (*10%), *Proteus vulgaris* (7%), *P. aeruginosa* (9%), *Klebsiella planticola* (9%), *Staphylococcus aureus* (6%), *Corynebacterium cystitidis* (9%), *Salmonella choleraesuis* (8%), *Citrobacter freundii*(9%), Fig. 5 depicts the percentage frequency distribution of antibacterial l activity against clinical isolates at *(*StD+) at 12.5mg/ml. At 12,5mg/ml, *E. coli* (13%), and *Staphylococcus aureus* (13%) have the highest while *Citrobacter diversus* (6%), has the lowest percentage frequency distribution of purified Volatile compounds of *Aframomum melegueta (*StD++) against selected clinical isolates. Other isolates were *Klebsiella.pneumoniae* (9%), *Proteus vulgaris* (8%), *P. aeruginosa* (8%), *Staphylococcus aureus* (9%), *Corynebacterium cystitidis* (11%), *Klebsiella planticola* (6%), *Citrobacter freundii* (9%), *Salmonella choleraesuis* (8%).

Fig. 6 depicts the antibacterial activity of purified Volatile compounds of *Aframomum melegueta (*StD++) stem extracts against selected clinical isolates. *E. coli* has the highest zones of inhibition of 20mm at 100mg/ml, while *Klebsiella planticola* has the lowest inhibition of 1.0mm at 12.5mg/ml. the order of decreasing zones of inhibition as follows *Klebsiella.pneumoniae, P.aeruginosa P.aeruginosa,* and *Staphylococcus aureus* (17.0mm), *Corynebacterium cystitidis,* and *Citrobacter diversus* (14.0mm), *Citrobacter freundii* (13.0mm), *Salmonella choleraesuis* (12 .0mm) and *Proteus. mirabilis* (11.0mm) respectively. The decreasing order of zones of inhibition was as follows *Salmonella choleraesuis* (2.0mm), *Klebsiella pneumoniae (3.0mm), Klebsiella pneumoniae, and Corynebacterium cystitidis* (4.0mm), *Staphylococcus aureus, P. aeruginosa*,(5.0mm) *and E.coli* (6.0mm*)* respectively.

Fig. 7 depicts the percentage frequency distribution of antibacterial activity of purified Volatile compounds of *Aframomum melegueta* (StD++) stem extracts against selected clinical isolates. At 100mg/ml, *E. coli* (12%) and *Staphylococcus aureus* (12%) has the highest

while *Klebsiella planticola* (6%), has the lowest percentage frequency distribution of purified Volatile compounds of *Aframomum melegueta (*StD++) against selected clinical isolates. Other isolates were *Citrobacter freundii* (8%), *Citro bacter diversus* (9%), and *Klebsiella. Pneumonia* (10%), *Proteus. mirabilis* (7%), *P. aeruginosa* (10%),*Staphylococcus aureus* (10%), *Coryne bacterium cystitidis* (9%), *Salmonella cholerae suis*(7%) and *Citrobacter freundii* (8%) respec tively.

Fig. 8 shows percentage frequency distribution of antibacterial activity of purified Volatile compounds of *Aframomum melegueta* (StD++) stem extracts against selected clinical isolates at 50mg/ml. *E.coli* (15%) has the highest while *Citrobacter freundii* (5%) has lowest percentage frequency distribution of purified Volatile compounds of *Aframomum melegueta (*StD++)against selected clinical isolates, other isolates were *Citrobacter diversus* (7%), *Staphylococcus aureus* (12%), *Klebsiella pneumonia (*11%),*Proteus. mirabilis* (9%), *P. aeruginosa* (9%), *Staphylococcus aureus* (10%), *Corynebacterium cystitidis* (8%), *Klebsiella planticola* (6%), *Salmonella choleraesuis* (8%).

Fig. 9 shows the percentage frequency distribution of antibacterial activity of purified Volatile compounds of *Aframomum melegueta (*StD++) stem extracts against selected clinical isolates at 25mg/ml. *Staphylococcus aureus* (13%) has the highest while *Citrobacter freundii* (4%) has the lowest percentage frequency distribution of purified Volatile compounds of *Aframomum melegueta (*StD++) against selected clinical isolates. Other isolates were *Klebsiella planticola* (6%), *Salmonella choleraesuis* (7%), *Citrobacter freundii* (4%), *Citrobacter diversus* (7%), *E. coli* (18%), *Staphylococcus aureus* (7%), *Klebsiella pneumoniae* (9%), *Proteus. mirabilis* (8%) , *P. aeruginosa* (10%) and Corynebacterium cystitidis (11%).

Fig. 10 shows the percentage frequency distribution of antibacterial activity of purified Volatile compounds of *Aframomum melegueta (*StD++) stem extracts against selected clinical isolates at 12.5mg/ml. *Staphylococcus aureus* (14%) has the highest while *Klebsiella planticola* (2%) has the lowest percentage frequency distribution of purified Volatile compounds of *Aframomum melegueta (*StD++) against sele cted clinical isolates. Other isolates were *Citro bacter diversus* (5%), *E. coli* (12%), *Staphylo coccus aureus* (12%), *Klebsiella. Pneumonia* (7%), *Proteus. mirabilis* (9%), *P.aeruginosa* (12%),*Corynebacterium cystitidis* (10%), *Kleb siella planticola* (2%) *Salmonella choleraesuis* (5%) and *Citrobacter freundii* (10%).

4. DISCUSSION

This research work aims to ascertain the composition and potency of the bioactive component of Volatile compounds derived from *Aframomum melegueta* stem extract through multi-phase solvent extraction Gas Chromatography /Mass Spectroscopic analysis and antimicrobial assay against selected clinical isolates. In this present study, it was observed that *Aframomum melegueta* stem extract has overwhelming potential uses in our-to-day human activity especially the antibacterial potency on clinical isolate. It can be observed the *Aframomum melegueta* stem extract has various degrees of antibacterial potency on clinical isolates which is corroborated with different results gathered in the table and figures.

In StD+ Fraction,(Antibacterial activity of purified Volatile compounds of *Aframomum melegueta (*StD+) stem extracts against selected clinical isolates), *E.coli* and *Staphylococcus aureus* has the highest zones of inhibition of 19,0 mm at 100mg/ml while *Klebsiella eplanticola* and *Citrobacter diversus* has the lowest zones of inhibition of 3.0mm at 12.5mg/ml. other relatively high zones of inhibition were *Corynebacterium cystitidis* (18.0mm), *Klebsiella .pneumoniae* (17. 0mm*), Proteus vulgaris* (17.0mm), *Citrobacter freundii* (15.0mm) *and Citrobacter diversus (*13. 0mm) *and Salmonella choleraesuis* (12. 0mm). The lowest zones of inhibition were observed in *Staphylococcus aureus and E.coli* (7.0mm), *Corynebacterium cystitidis* (6.0mm), *Klebsiella .pneumoniae* and *Citrobacter freundii* (5.0mm), *Proteus vulgaris, P. aeruginosa* and *Salmonella choleraesuis* (4.0mm) *while In StD++,* fraction,(Antibacterial activity of purified Volatile compounds of *Aframomum melegueta (*StD++) stem extracts against selected clinical isolates)*, E. coli* has the highest zones of inhibition of 20mm at 100mg/ml, while *Klebsiella planticola* has the lowest of inhibition of 1.0mm at 12.5 mg/ml. the order of decreasing zones of inhibition as follows *Klebsiella.pneumoniae, P.aeruginosa P.aeruginosa* and *Staphylococcus aureus* (17. omm),*Corynebacterium cystitidis* and *Citrobacter diversus*(14.0mm),*Citrobacter freund Ii*(13.0mm), *Salmonella choleraesuis(*12.0mm) and *Proteus. mirabilis* (11.0mm) respectively.The decreasing order of zones of inhibition was as follows *Salmonella choleraesuis* (2.0mm),

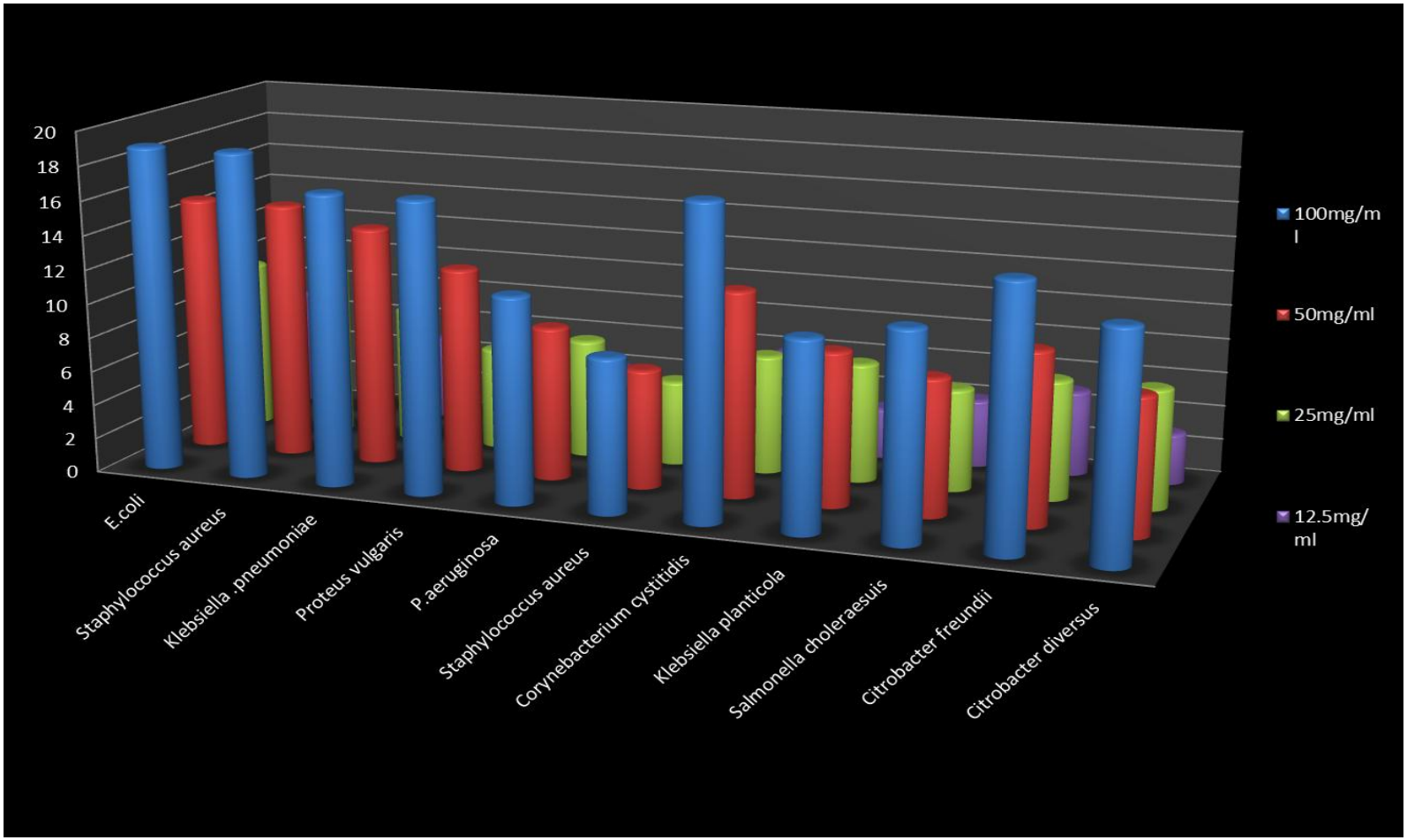


Fig. 1. Antibacterial activity of purified Volatile Compounds of *Aframomum melegueta* (StD+) stem extracts against selected clinical isolates

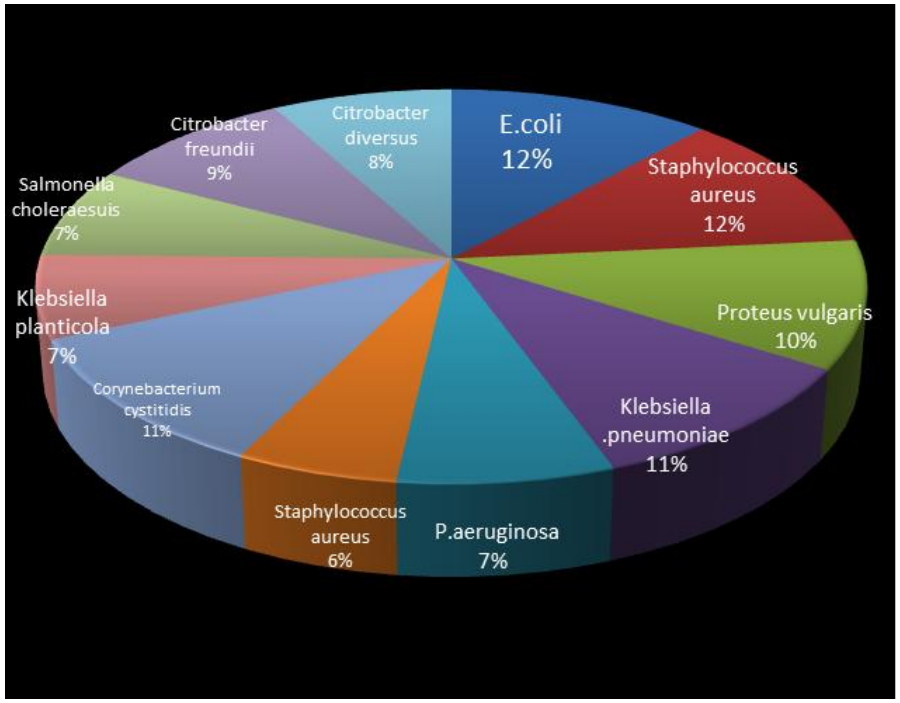


Fig. 2. Percentage Frequency Distribution of Antibacterial Activity of Volatile Compounds of *Aframomum melegueta* Stem extracts against selected clinical isolates at (StD+) 100mg/ml

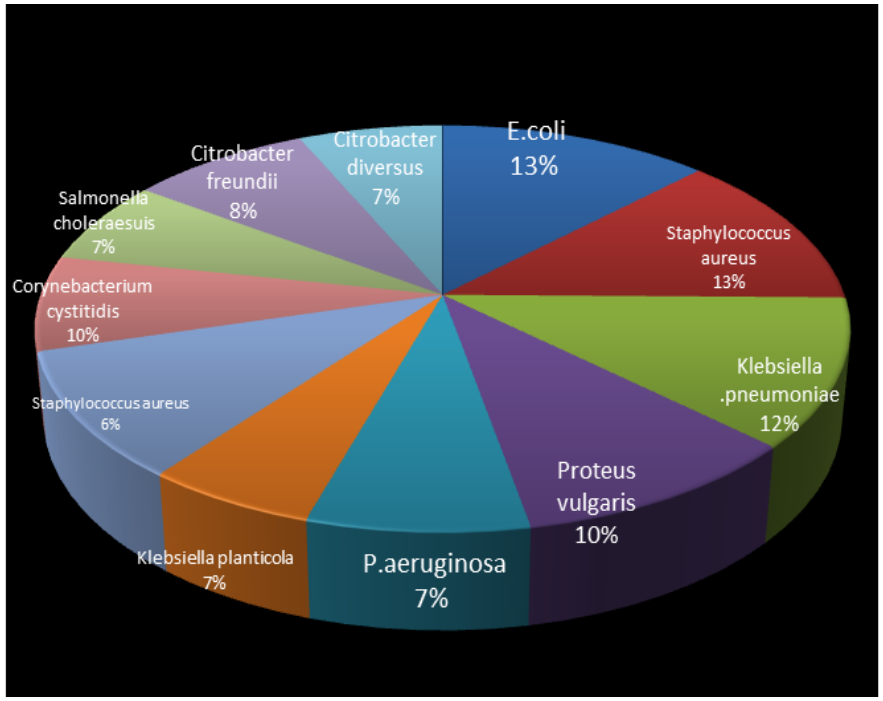


Fig. 3. Percentage Frequency Distribution of Antibacterial Activity of Volatile Compounds of *Aframomum melegueta* Stem extracts against selected clinical isolates at (StD+) 50mg/ml

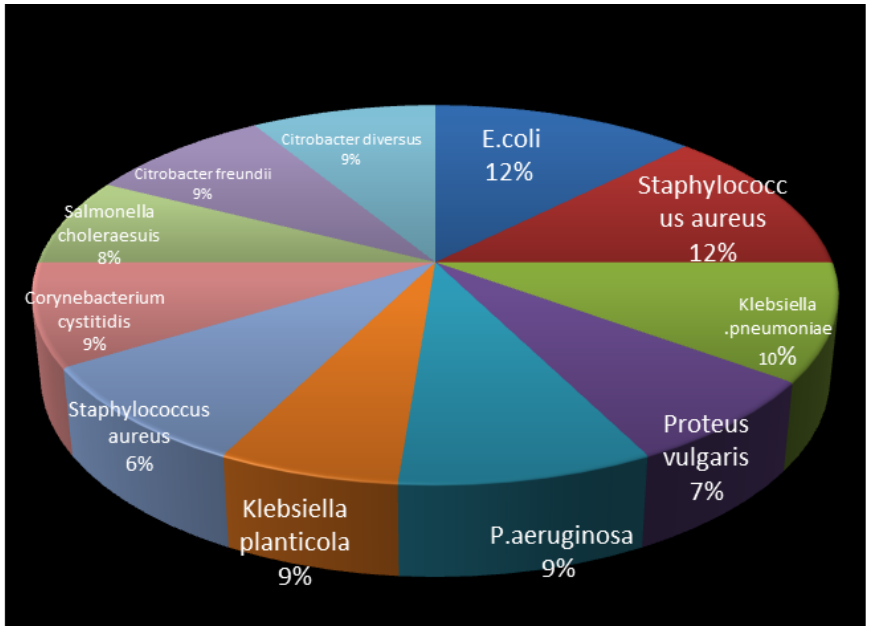


Fig. 4. Percentage Frequency Distribution of Antibacterial Activity of Volatile Compounds of *Aframomum melegueta* Stem extracts against selected clinical isolates at (StD+) 25mg/ml

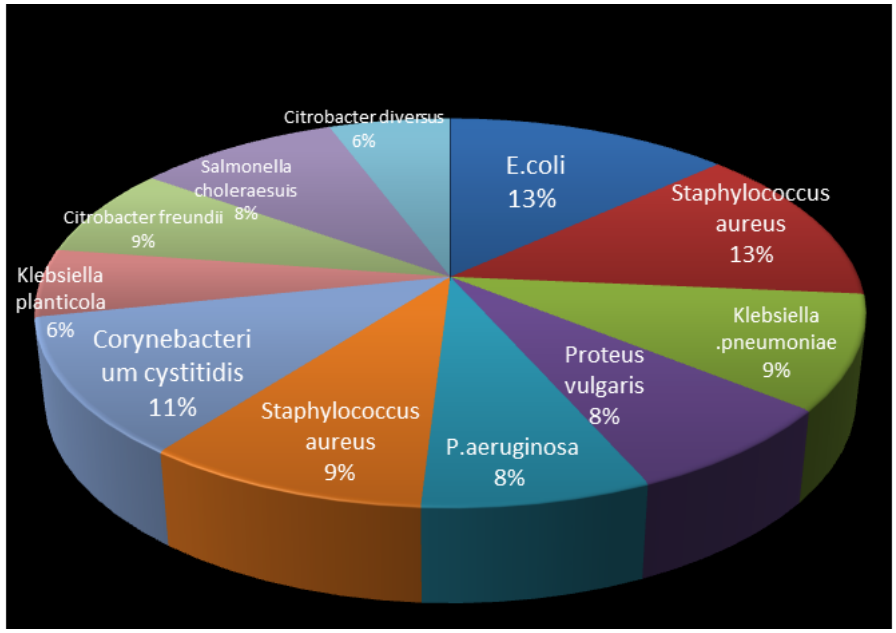


Fig. 5. Percentage Frequency Distribution of Antibacterial Activity of Volatile Compounds of *Aframomum melegueta* Stem extracts against selected clinical isolates at (StD+) 12.5mg/ml

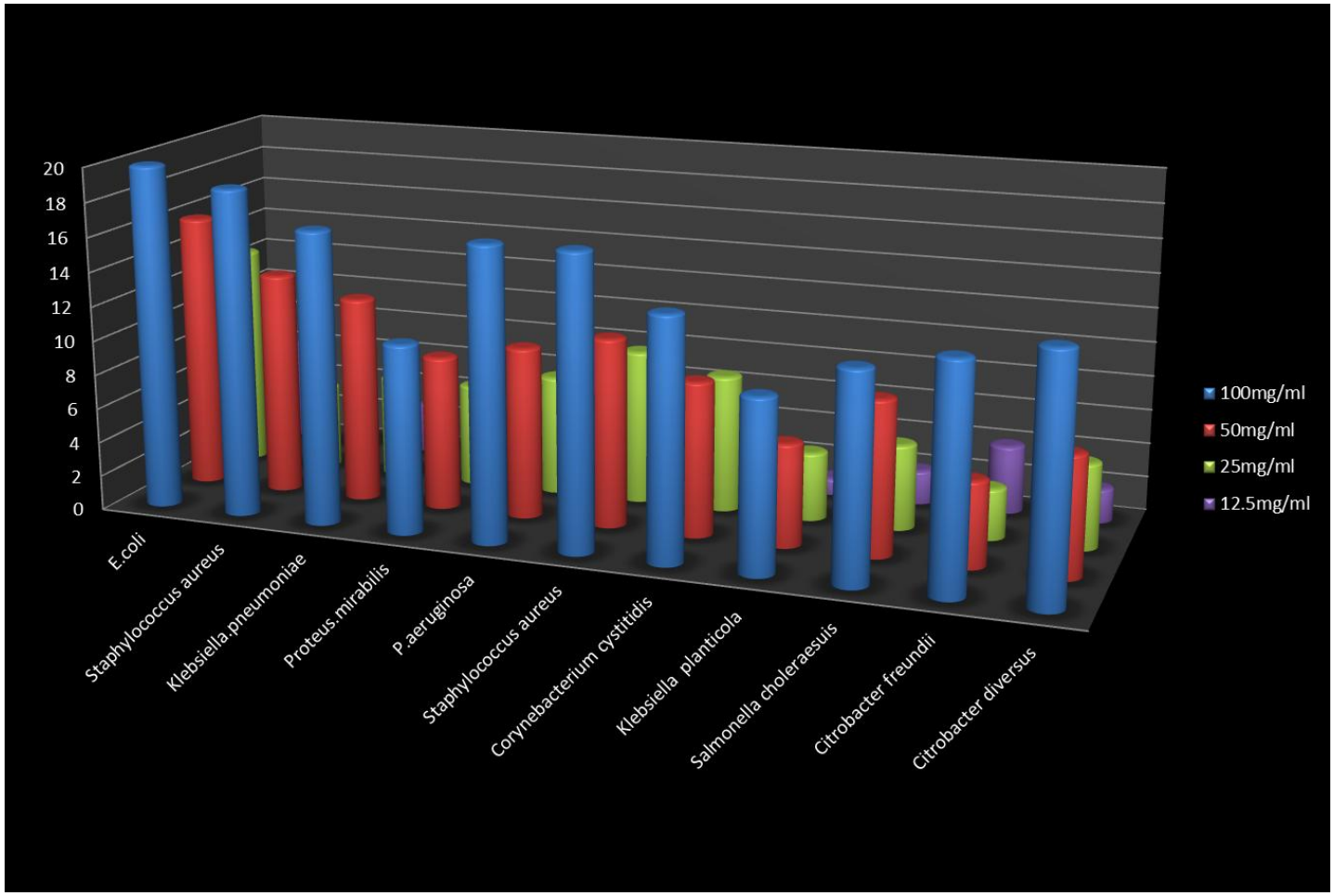


Fig. 6. Antibacterial Activity of Purified Volatile Compounds of *Aframomum melegueta*; (SD++)Stem Extracts Against Selected Clinical Isolates

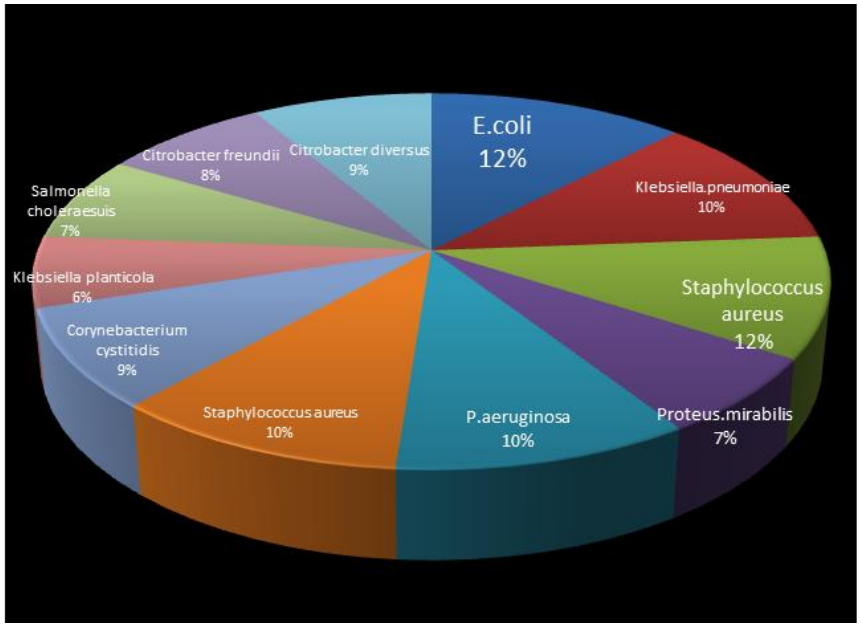


Fig. 7. Percentage Frequency Distribution of Antibacterial Activity of Volatile Compounds of *Aframomum melegueta* Stem extracts against selected clinical isolates at (StD++) 100 mg/ml

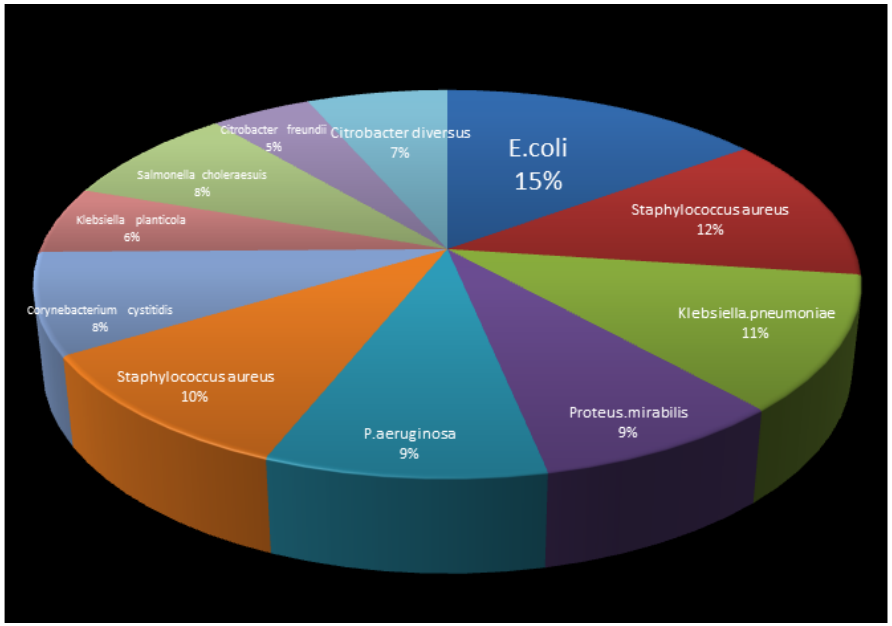


Fig. 8. Percentage Frequency Distribution of Antibacterial Activity against of Volatile Compounds of *Aframomum melegueta* Stem extracts against selected clinical isolates at (StD++) 50 mg/ml

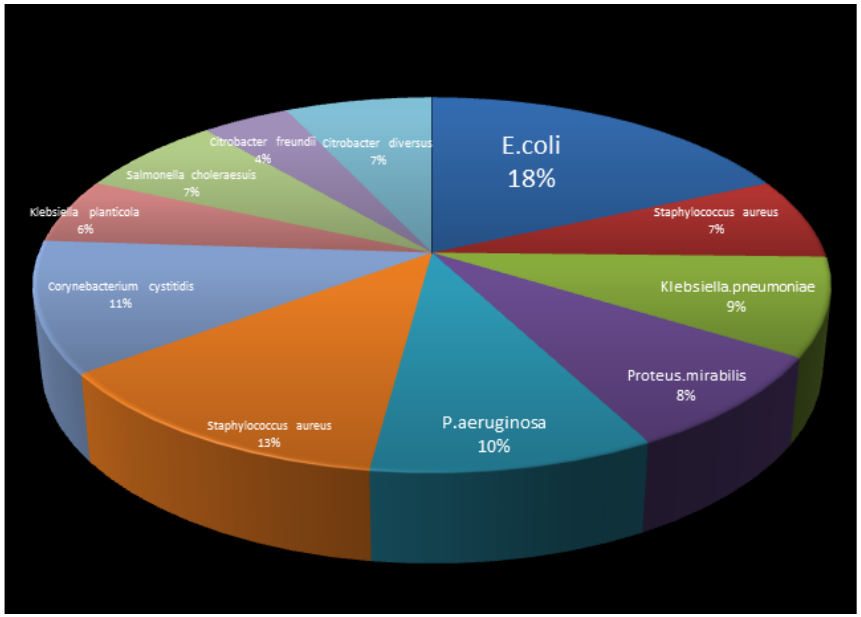


Fig. 9. Percentage Frequency Distribution of Antibacterial Activity of Volatile Compounds of *Aframomum melegueta* Stem extracts against selected clinical isolates at (StD++) 25mg/ml

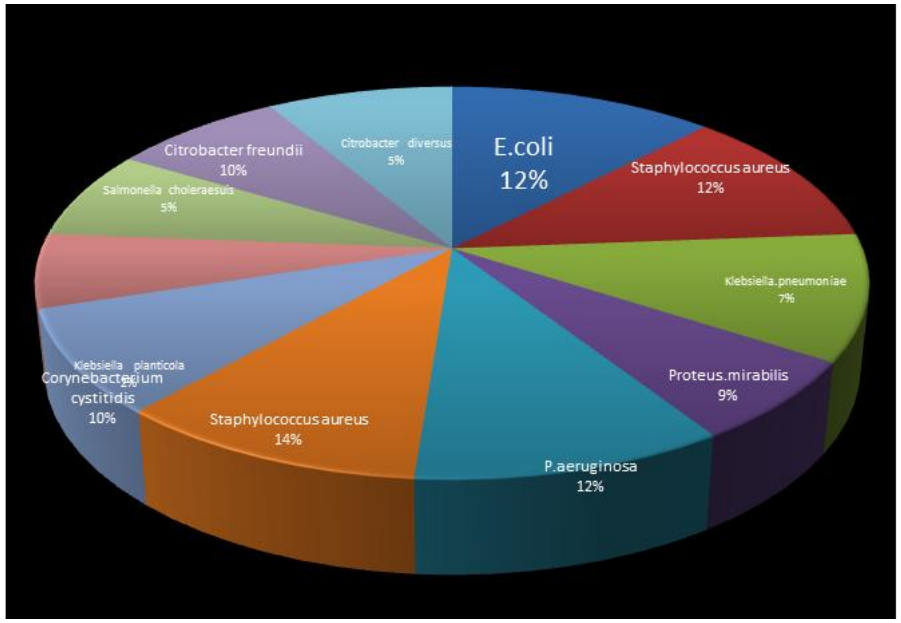


Fig. 10. Percentage Frequency Distribution of Antibacterial Activity of Volatile Compounds of *Aframomum melegueta* Stem extracts against selected clinical isolates at (StD++) 12.5mg/ml

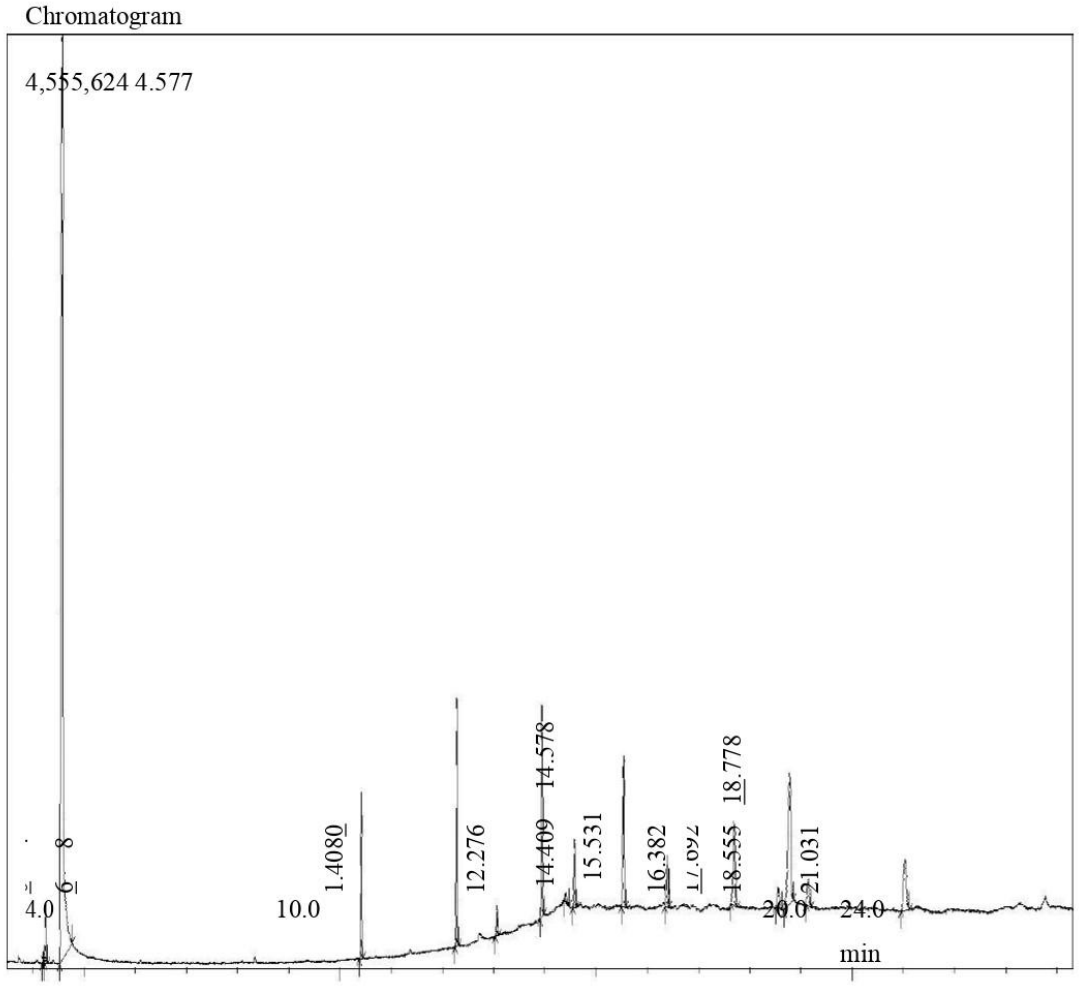
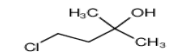
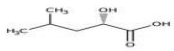
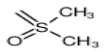
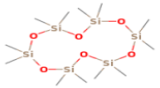
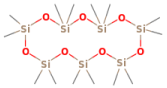
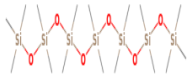
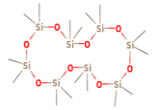
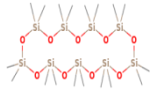
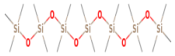
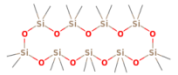
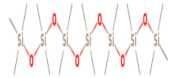
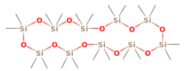
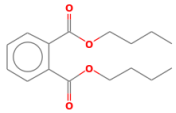
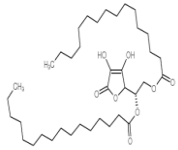
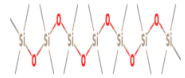
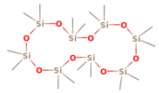


Fig. 11. Spectral 1;Gas chromatography/mass spectrophotometric (GC-MS) Spectra Analysis of *Aframomum melegueta* Purified Volatile Compounds stem extract (*St*D+)

Table 2. Gas chromatography/mass spectrophotometric (GC-MS) Analysis of *Aframomum melegueta* stem extract of Purified Volatile Compounds **stem extract (*St*D+)**

Retention time	Area	Area %	Height	Height%	A/H	Mol weight	Name	Structure formula	Structure
206	64426	0.25	53502	0.49	1.20	122	1,1-Dimethyl- chloropropanol	$C_5H_{11}ClO$	
4.248	356893	1.41	285635	2.61	1.25	132	alpha.-Hydroxyisocaproic acid	$C_6H_{12}O_3$	
4.577	13651447	53.84	4511552	41.18	3.03	120	Dimethylsulfoxonium formyl me thylide	:$C_4H_8O_2S$	
10.408	990350	3.91	812257	7.41	1.22	444	Cyclohexasiloxane, dodecame thyl-	$C_{12}H_{36}O_6Si_6$	
12.276	1517873	5.99	1209032	11.04	1.26	518	Cycloheptasiloxane,tetradecamethyl-	$C_{14}H_{42}O_7Si_7$	
13.060	173527	0.68	149045	1.36	1.16	518	Heptasiloxane, hexadecamethyl-	$C_{16}H_{48}O_6Si_7$	
13.939	1254825	4.95	1036069	9.46	1.21	592	Cyclooctasiloxane, hexadecame thyl-	$C_{16}H_{48}O_8Si_8$	
14.409	97955	0.39	43036	0.39	2.28	666	Cyclononasiloxane, octadeca ethyl-	$C_{18}H_{54}O_9Si_9$	
14.578	437659	1.73	331899	3.03	1.32	532	Heptasiloxane, hexadecamethyl-	$C_{16}H_{48}O_6Si_7$	

Retention time	Area	Area %	Height	Height%	A/H	Mol weight	Name	Structure formula	Structure
15.531	1230441	4.85	740050	6.76	1.66	666	Cyclononasiloxane, octadecame thyl-	$C_{18}H_{54}O_9Si_9$	
16.382	493275	1.95	251641	2.30	1.96	532	Heptasiloxane, hexadecamethyl-	$C_{16}H_{48}O_6Si_7$	
17.692	1070365	4.22	414010	3.78	2.59	740	Cyclodecasiloxane, eicosameth yl-	$C_{20}H_{60}O_{10}Si_{10}$	
18.555	273288	1.08	98037	0.89	2.79	278	Dibutyl phthalate	$C_{16}H_{22}O_4$	
18.778	2352943	9.28	639712	5.84	3.68	652	l-(+)-Ascorbic acid 2,6 dihexad ecanoate	$C_{38}H_{68}O_8$	
19.139	371477	1.47	131485	1.20	2.83	532	Heptasiloxane, hexadecamethyl-	$C_{16}H_{48}O_6Si_7$	
21.031	1019984	4.02	247666	2.26	4.12	592	Cyclooctasiloxane,hexadecamethyl-	$C_{18}H_{54}O_9Si_9$	

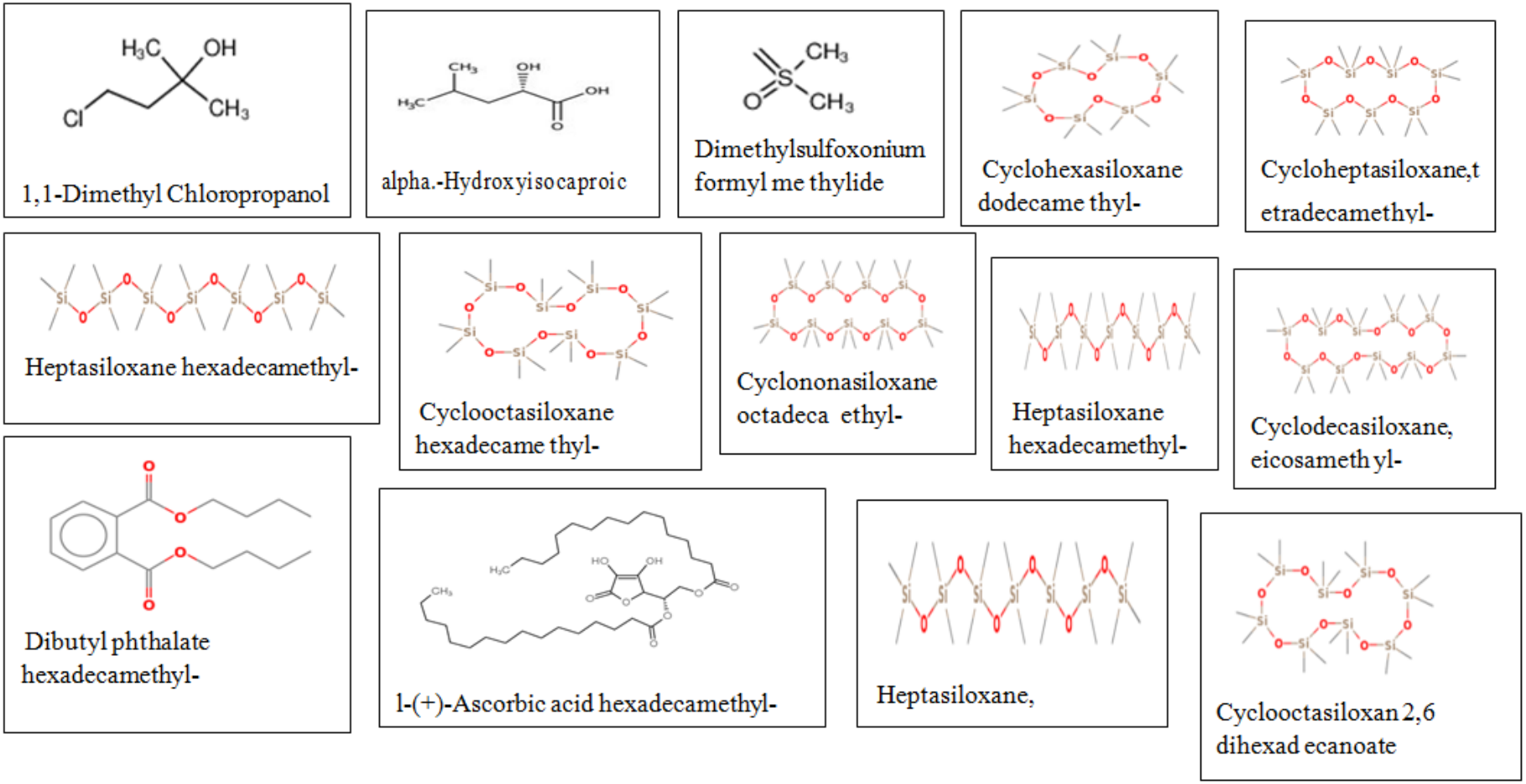


Fig. 12. Summary of Gas chromatography/mass spectrophotometric (GC-MS) Analysis of *Aframomum melegueta* stem extract of Purified Volatile Compounds stem extract (*St*D$^+$)

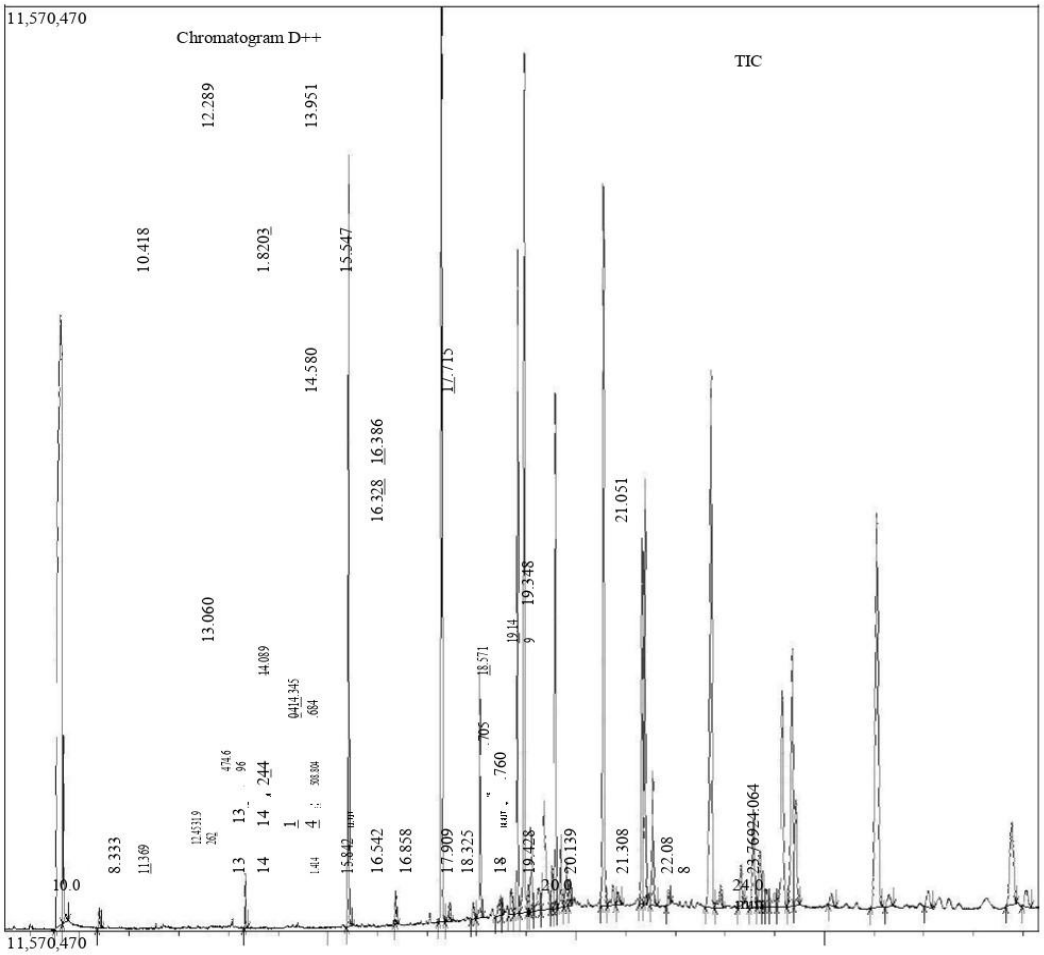
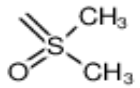
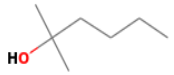
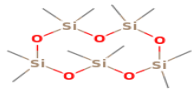
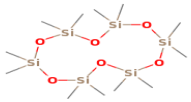
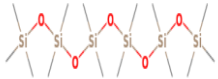
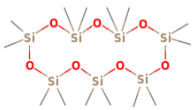
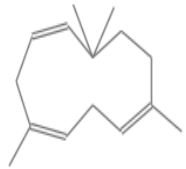
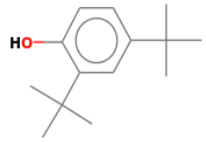
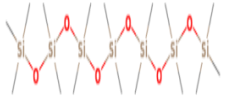
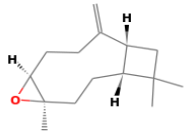
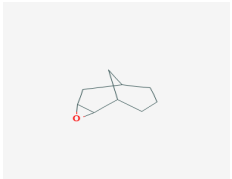
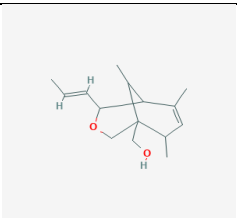
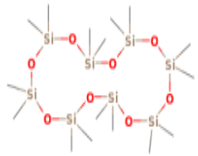
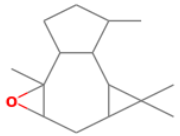
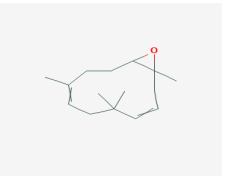
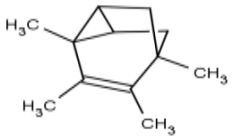


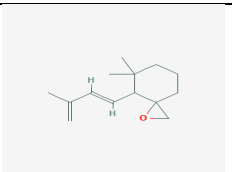
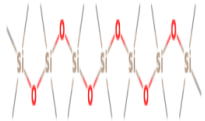
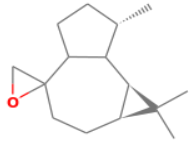
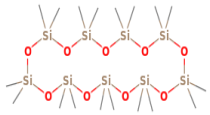
Fig. 13. Spectral 2; Gas chromatograph/ mass spectrophotometric (GC-MS) Spectra Analysis of *Aframomum melegueta* stem extract of Purified Volatile Compounds stem extract (*St*D++)

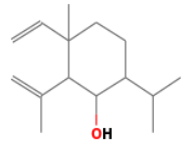
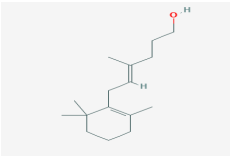
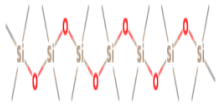
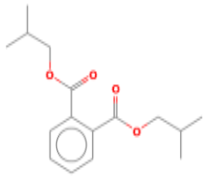
Table 3. Gas chromatography/mass spectrophotometric(GC-MS) Analysis of *Aframomum melegueta* stem extract of Purified Volatile Compounds **stem extract (*St*D++)**

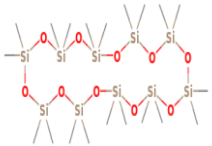
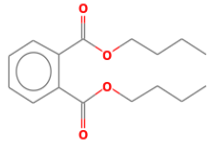
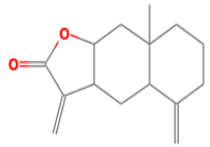
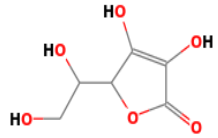
Peak	Retention time	Area	Area %	Height	Height%	A/H	Mol weight	Name	Structure formula	Structure
1	4.619	43145466	15.72	7641497	6.76	5.65	120	Dimethylsulfoxonium formylmethylide	$C_4H_8O_2S$	
2	4.674	2493029	0.91	2352888	2.08	1.06	116	2-Hexanol, 2-methyl-	$C_7H_{16}O$	
3	5.399	353165	0.13	255303	0.23	1.38	118	Ethanol, 2-butoxy-	$C_6H_{14}O_2$	
4	8.333	837959	0.31	681373	0.60	1.23	370	Cyclopentasiloxane, decamethyl	$C_{10}H_{30}O_5Si_5$	
5	10.418	15200809	5.54	9650386	8.54	1.58	444	Cyclohexasiloxane, dodecamethyl	$C_{12}H_{36}O_6Si_6$	
6	11.369	434180	0.16	396118	0.35	1.10	458	Hexasiloxane, tetradecamethyl-	$C_{14}H_{42}O_5Si_6$	
7	12.289	19927221	7.26	11446136	10.13	1.74	518	Cycloheptasiloxane, tetradecamethyl-	$C_{14}H_{42}O_7Si_7$	

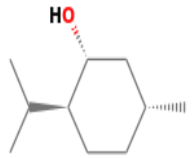
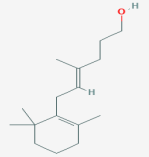
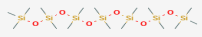
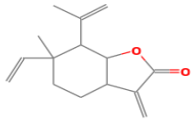
Peak	Retention time	Area	Area %	Height	Height%	A/H	Mol weight	Name	Structure formula	Structure
8	12.453	388191	0.14	234747	0.21	1.65	204	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,	$C_{15}H_{24}$	
9	12.926	477280	0.17	199124	0.18	2.40	206	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	
10	13.060	3804513	1.39	3075160	2.72	1.24	532	Heptasiloxane, hexadecamethyl-	$C_{16}H_{48}O_6Si_7$	
11	13.474	836300	0.30	253446	0.22	3.30	220	Caryophyllene oxide	$C_{15}H_{24}O$	
12	13.520	245557	0.09	163184	0.14	1.50	138	2-Oxatricyclo [4.3.1.0 (3,8)]decane	$C_9H_{14}O$	

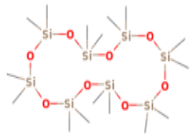
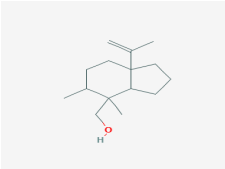
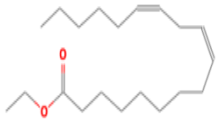
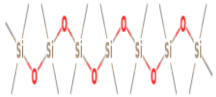
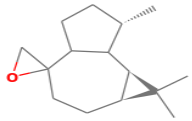
Peak	Retention time	Area	Area %	Height	Height%	A/H	Mol weight	Name	Structure formula	Structure
13	13.696	962949	0.35	317368	0.28	3.03	236	Methanol, [6,8,9-trimethyl-4-(1-propenyl)-3-oxabicyclo	$C_{15}H_{24}O_2$	
14	13.951	18269598	6.66	10747963	9.51	1.70	592	Cyclooctasiloxane, hexadecamethyl	$C_{16}H_{48}O_8Si_8$	
15	14.040	540950	0.20	342696	0.30	1.58	220	Isoaromadendrene epoxide	$C_{15}H_{24}O$	
16	14.089	2073681	0.76	1061617	0.94	1.95	:220	12-Oxabicyclo [9.1.0] dodeca-3,7-diene, 1,5, 5,8-	$C_{15}H_{24}O$	
17	14.244	1046953	0.38	286100	0.25	3.66	162	Tricyclo[3.2.1.02,7]oct-3-ene, 2,3,4,5-tetramethyl	$C_{12}H_{18}$	

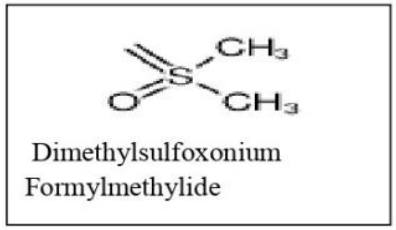
Peak	Retention time	Area	Area %	Height	Height%	A/H	Mol weight	Name	Structure formula	Structure
18	14.345	5991489	2.18	1361670	1.20	4.40	206	1-Oxaspiro [2.5] octan e , 5,5-dimethyl-4-(3-methyl	$C_{14}H_{22}O$	
19	14.508	1342836	0.49	547372	0.48	2.45	222	.alpha.-Cadinol	$C_{15}H_{26}O$	
20	14.580	8855880	3.23	6434881	5.69	1.38	:532	Heptasiloxane, hexadecamethyl	$C_{16}H_{48}O_6Si_7$	
21	14.804	1309248	0.48	592766	0.52	2.21	220	Longifolenaldehyde	$C_{15}H_{24}O$	
22	14.864	574571	0.21	334118	0.30	1.72	220	Alloaromadendrene oxide-(1)	$C_{15}H_{24}O$	
23	15.547	20440142	7.45	9029655	7.99	2.26	666	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	

Peak	Retention time	Area	Area %	Height	Height%	A/H	Mol weight	Name	Structure formula	Structure
24	15.737	887291	0.32	256295	0.23	3.46	222	6-epi-shyobunol	$C_{15}H_{26}O$	
25	15.842	522807	0.19	165137	0.15	3.17	256	Tetradecanoic acid, ethyl ester	$C_{16}H_{32}O_2$	
26	16.328	10165724	3.70	4566247	4.04	2.23	236	4-Hexen-1-ol, 6-(2,6,6-trimethyl-1-cyclohexen	$C_{16}H_{28}O$	
27	16.386	10377796	3.78	5313878	4.70	1.95	532	Heptasiloxane, hexadecamethyl-	$C_{16}H_{48}O_6Si_7$	
28	16.542	3556482	1.30	1672036	1.48	2.13	268	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	
29	16.858	424896	0.15	189288	0.17	2.24	278	1,2-Benzene dicarboxylic acid, bis(2-methyl pro	$C_{16}H_{22}O_4$	

Peak	Retention time	Area	Area %	Height	Height%	A/H	Mol weight	Name	Structure formula	Structure
30	17.715	20188562	7.36	6714905	5.94	3.01	740	Cyclodecasiloxane, eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$	
31	17.909	917069	0.33	285819	0.25	3.21	270	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	
32	18.325	1483764	0.54	498584	0.44	2.98	222	1,4-Methanoazulene-9-methanol, decahydro-4	$C_{15}H_{26}O$	
33	18.571	3674794	1.34	1229358	1.09	2.99	278	Dibutyl phthalate	$C_{16}H_{22}O_4$	
34	18.705	2343459	0.85	706972	0.63	3.31	232	Naphtho(2,3-b)furan-2(3H)-one, decahydro-8	$C_{15}H_{20}O_2$	
35	18.760	1236194	0.45	452806	0.40	2.73	652	l-(+)-Ascorbic acid 2,6-dihexadecanoate	:$C_{38}H_{68}O_8$	

Peak	Retention time	Area	Area %	Height	Height%	A/H	Mol weight	Name	Structure formula	Structure
36	18.837	797999	0.29	227887	0.20	3.50	222	Menthol, 1'-(butyn-3-one-1-yl)-, (1R,2S,5R)-	$C_{14}H_{22}O_2$	
37	18.998	971942	0.35	174673	0.15	5.56	236	4-Hexen-1-ol, 6-(2,6,6-trimethyl-1-cyclohexen	$C_{16}H_{28}O$	
38	19.149	8785281	3.20	2696162	2.39	3.26	532	Heptasiloxane, hexadecamethyl-	$C_{16}H_{48}O_6Si_7$	
39	19.348	11148953	4.06	3221931	2.85	3.46	284	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	
40	19.428	4118606	1.50	1328485	1.18	3.10	232	2(3H)-Benzofuranone, 6-ethenylhexahydro-6-	$C_{15}H_{20}O_2$	
41	20.139	528888	0.19	154535	0.14	3.42	290	3,7,11,15-Tetramethylhexadeca-1,6,10,14-tetr	$C_{20}H_{34}O$	

Peak	Retention time	Area	Area %	Height	Height%	A/H	Mol weight	Name	Structure formula	Structure
42	21.051	20063075	7.31	4929341	4.36	4.07	592	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$	
43	21.308	652829	0.24	149423	0.13	4.37	222	(7a-Isopropenyl-4,5-dimethyloctahydroinden-	$C_{15}H_{26}O$	
44	22.088	925406	0.34	202243	0.18	4.58	308	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	
45	23.769	5274359	1.92	1025615	0.91	5.14	532	Heptasiloxane, hexadecamethyl-	$C_{16}H_{48}O_6Si_7$	
46	24.064	1051570	0.38	215439	0.19	4.88	220	Alloaromadendrene oxide-(1)	$C_{15}H_{24}O$	

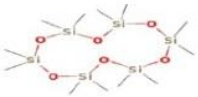


Dimethylsulfoxonium Formylmethylide

2-Hexanol, 2-methyl-

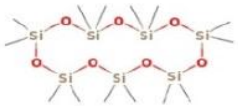
Ethanol, 2-butoxy-

Cyclopentasiloxane decamethyl

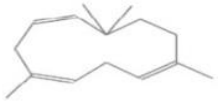


Cyclohexasiloxane, dodecamethyl

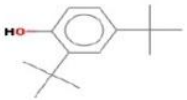
Hexasiloxane tetradecamethyl-



Cycloheptasiloxane, tetradecamethyl-

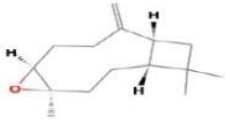


1,4,7, Cycloundecatriene 1,5,9,9-tetramethyl-, Z



Phenol, 2,4-bis(1,1-dimethylethyl)-

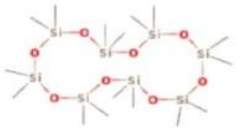
Heptasiloxane, hexadecamethyl-



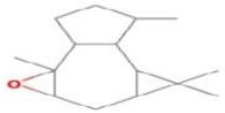
Caryophyllene Oxide epoxide

2-Oxatricyclo [4.3.1.0 (3,8)]decane

Methanol, [6,8,9-trimethyl

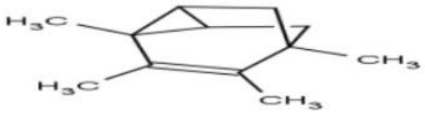


Cyclooctasiloxane 4-(1-propenyl)-3- oxabicyclo

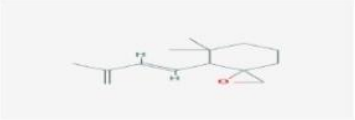


Isoaromadendrene hexadecamethyl

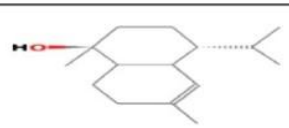
12-Oxabicyclo [9.1.0]



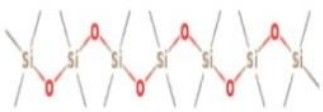
Tricyclo[3.2.1.02,7]oc dodeca-3,7-diene, 1,5, 5,8- hexadecamethyl



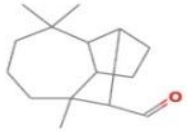
1-Oxaspiro [2.5] octane t-3-ene, 2,3,4,5-



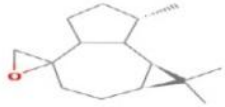
alpha.-Cadinol



Heptasiloxane, 5,5-dimethyl-4-(3-methyl

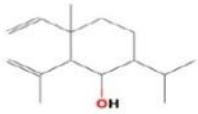


Longifolenaldehyde

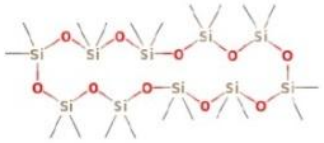


Alloaromadendrene oxide-(1)

Cyclononasiloxane octadecamethyl-

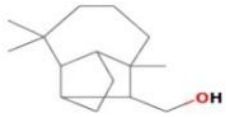


6-epi-shyobunol

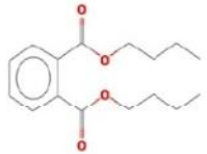


Cyclodecasiloxane eicosamethyl-

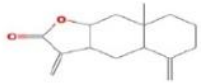
Hexadecanoic acid, methyl ester dihexadecanoate



1,4-Methanoazulene-9-methanol, decahydro-4

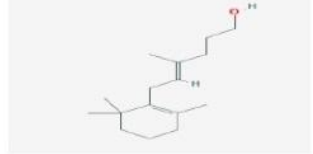


Dibutyl phthalate



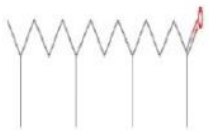
Naphtho(2,3-b)furan-2(3H)-one, decahydro-8

Tetradecanoic acid ethyl ester trimethyl-1-cyclohexen

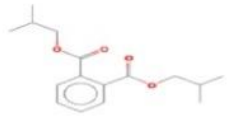


4-Hexen-1-ol, 6-(2,6,6-trimethyl-1-cyclohexen

Heptasiloxane, hexadecamethyl-



2-Pentadecanone 6.10.14-trimethyl



1,2-Benzene dicarboxylic acid, bis(2-methyl

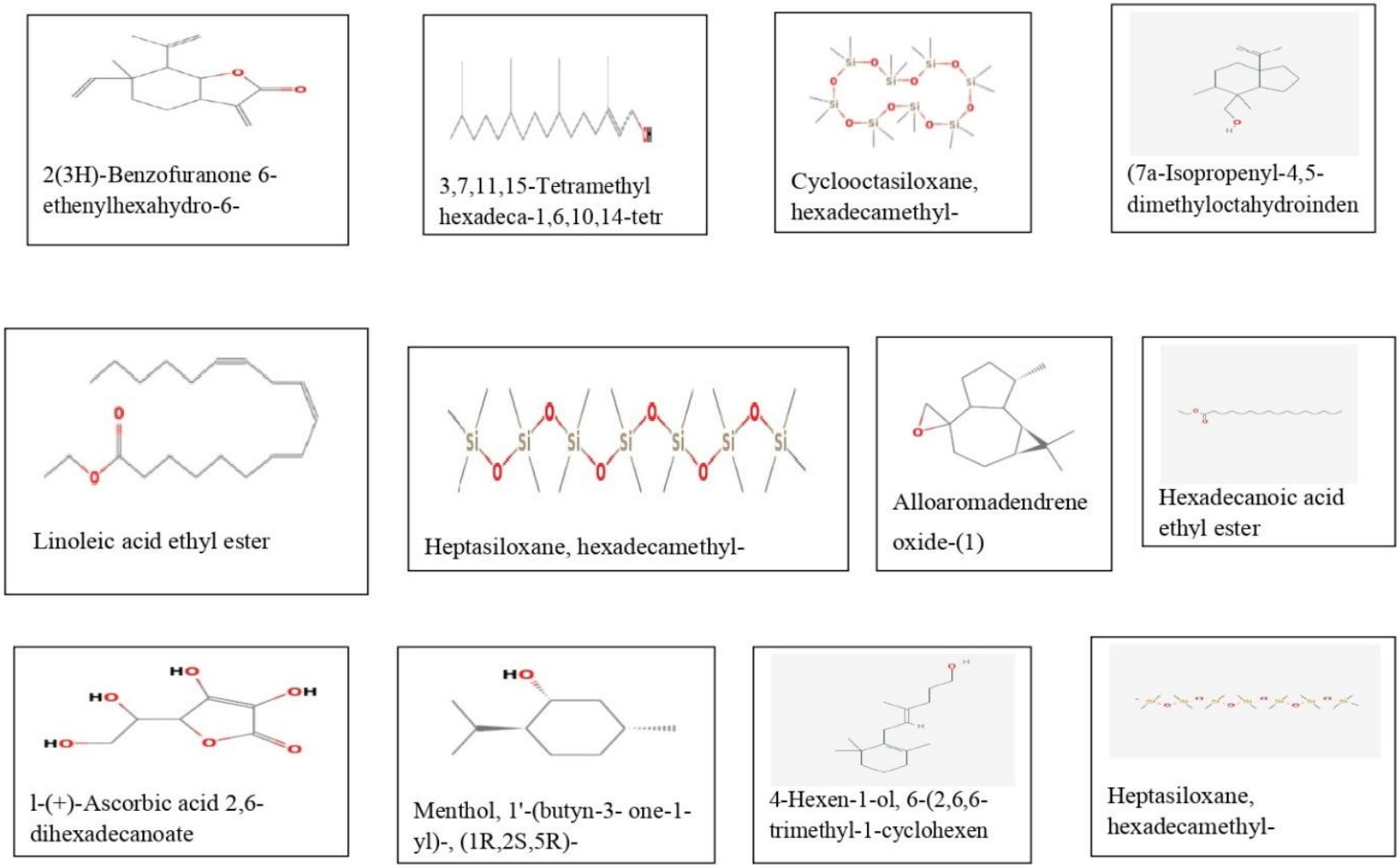


Fig. 14. Summary of Gas chromatography/mass spectrophotometric (GC-MS) Analysis of *Aframomum melegueta* stem extract of Purified Volatile Compounds stem extract (StD++)

Klebsiella. Pneumonia (3.0mm), Klebsiella. Pneum oniae, and *Coryne bacterium cystitidis* (4.0 mm), *Staphylococcus aureus, P. aeruginosa*, (5.0mm) and *E. coli*, (6.0 mm). The result shows that *Aframomum melegueta* stem extract possesses some levels of antibacterial potential and is in agreement with previous findings on oils of *V. danielli and Aframomum melegueta* [26,27] [28,29,20].

Some researchers propound that the presence of secondary metabolite i.e Phyto-constituents is the defector to the mechanism of action of essential oil from *Aframomum melegueta* stem extract, like Flavonoids are ketone-containing secondary metabolites found in plants. Flavonoids such as myricetin, quercetin, and morin have been extensively investigated and proven to possess anti-inflammatory, antimicro bial, and antiproliferative activities [30].

Alkaloids are bioactive molecules with nitrogen, constituting a key component of their molecular architecture, with distinct antimicrobial properties example are furoquinolones, indole alkaloids and acridones which is recent, with a plethora of studies reporting the efficacy of alkaloids against protozoan parasites [31]. The aglycone and hyd rophilic sugar moieties are active bio surfactants that have several therapeutic applications including immunostimulatory, molluscicidal, hypo cholesterolemic, antimicrobial, and antio xidant activities [32]. Cardiac glycoside is a cardiotonic steroid, that generally contains a steroid-like structure, and induces a cardiotonic effect via selective inhibition of Na+/K+-ATPase [33]. Tannins are moieties linked to a carbohydrate core (hydrolyzable tannins), and have wide therapeutic properties including antiproliferative and antimicrobial activities [34].

However, apart from the secondary metabolite which is responsible for the metabolic activity of the Volatile compounds of *Aframomum mele gueta* stem extract, the Gas chromatographic chemical constituent also reveals the presence of arrays of chemical architecture ranging from alkyl group to the alkanoates. The prominent ones are from Std+ fraction are Cycloheptasiloxane, tetra decamethyl($C_{14}H_{42}O_7Si_7$), l-(+)-Ascorbic acid 2,6-dihexadecanoate($C_{38}H_{68}O_8$) and Dodecanoic acid ($C_{12}H_{36}O_6Si_6$) [34].

The first highest peak from (16) sixteen components in GCMS analysis of Volatile compounds fraction (SD+) of *Aframomum melegueta* stem extract was Cycloheptasiloxane, tetradecamethyl.Cycloheptasiloxane, tetradeca methyl- ($C_{14}H_{42}O_7Si_7$) compound which belongs to the class of organic compounds known as organo-heterosilanes. These are organosilicon compounds where the tetravalent silicon atom is linked to one or more heteroatoms. Cyclo trisiloxane (CAS No. 541-05-9) is the cyclic dimethyl polysiloxane that conforms to the generic structure of cyclic dimethyl polysiloxane compounds, where n = 3, and the other components of Cyclomethicone (where n = 4, 5, 6, or 7) are present at the levels of less than 1%. Other names for cyclo-trisiloxane include cyclo-trisiloxane, hexamethyl- and hexamethyl cyclotri siloxane [35].

The presence of Cyclohexasiloxane, dode-camethyl in *Aframomum melegueta* stem extract is used in personal care products, antiperspirants and antifungals alluded to the report of [36] that the plant extract of *Aframomum melegueta* is an effective antifungal agent in the treatment of fungi infection and a promising alternative /adjunct/ supplement to the azole and allylamine group. Cyclohexasiloxane, dodecamethyl in *Aframomum melegueta* stem extract may be used as personal care compositions and cosmetic compositions can be in the form of a solution, emulsion, foam, mousse, cream, gel, lotion, ointment, solid, powder, paste, semi-solid, stick, spray or a combination thereof. Exemplary personal care compositions or cosmetic com positions include deodorants, antiperspi rants, insect repellants, anesthetics, skin conditioners, skin lotions, skin moisturizers, skin toners, skin sanitizers, skin cleansing compositions,skin soothing and lubricating compositions, sun screen, anti-aging products, concealer products, soaps, foaming bath products, shower gels, cleansing products, shampoos, hair conditioners, hair styling gels, hair anti-dandruff compo sitions,hair growth promoter compositions, hair colorant compositions, hair bleaching agent com positions, hair anti-frizzing agent composition s, hair shining compositions, hair relaxer compo sitions, mousses, styling gels, hair sprays, hair dyes, hair waving products, hair straightening products, shaving product com positions, personal lubricant compositions, spermicidal gel compositions, manicure products, nail polish, nail polish remover, nail creams and lotions, cuticle softeners, color cosmetics, lipsticks, lip balms, foundations, face powders, eye liners, eye shadows, blushes, makeup, mascaras and color cosmetic removers [37], this shows the various ways in which Cyclohexasiloxane, dodecamethyl in *Aframomum melegueta* stem extract can be put to biological use.

The second highest peak from (16) sixteen components in GC/MS analysis of Volatile compounds fraction (SD+) of *Aframomum melegueta* stem extract was l-(+)-Ascorbic acid 2,6-dihexa decanoate ($C_{38}H_{68}O_8$) It is a vitamin C compound. Ascorbic acid is often used for preventing and treating the common cold, gum disease, acne, and other skin infections, bronch itis, stomach ulcers, tuberculosis, dysentery, boils, and wounds [38], "Ascorbic acid is also used to prevent glaucoma, cataracts, gallbladder disease, dental cavities, constipation, hay fever, asthma, arthritis, back pain, diabetes, chronic fatigue syndrome, osteoporosis and boosting the immune system" (38), "Ascorbic acid acts as an antioxidant in the skin by scavenging and quenching free radicals generated by ultraviolet radiation. The use of *Aframomum melegueta* stem in the treatment of diarrhoea, dysentery, stomach problems, ulcers, wound, fever and other form of health challenges in herbal medicine could be as a result of the presence of ascorbic acid 2,6- dihexadecanoate in the stem of the plant" [39,40]

"The combination of Cyclohexasiloxane and Dodecanoic acid also known as lauric acid has been a driving factor in the antimicrobial properties of *Aframomum melegueta* stem extract"[41]. "Tetradecanoic acid known as myristic acid is commonly added co-tran slationally to the penultimate, nitrogen-terminus, glycine in receptor-associated kinases to confer the membrane localization of the enzyme"[42].

"The first and second highest peak from [48] sixteen components in GCMS analysis of Volatile compounds fraction (SD++) of *Afr. melegueta* stem extract were Phenol, 2,4-bis(1,1-dimethyl ethyl ($C_{14}H_{22}O$) and Cyclooctasiloxane,hexadec amethyl-($C_{16}H_{48}O_8Si_8$), Cyclooctasiloxane, hexa decamethyl-($C_{16}H_{48}O_8Si_8$) has been discussed previously. Moreover, 2,4-Di-tert-butyl-phenol or 2,4-bis(1,1-dimethylethyl)-phenol (2,4-DTBP) is a common natural product that exhibits potent toxicity against almost all testing organisms, including the producing species. 2,4-DTBP can modulate the secreted EPS of *Serratia marcescens,* which in turn could facilitate the disruption of biofilms, as well as favor the diffusion of antimicrobials into the cell aggregates, resulting in the eradication of persistent and biofilms" [44].

"This compound can be used to enhance the efficacy of conventional antibiotics. Intercellular communication in bacteria (quorum sensing (QS)) is an important phenomenon in disease dissemination and pathogenesis that controls biofilm formation. 2,4-DTBP controls QS-mediated biofilm formation and simultaneously increases the hydration of the cell wall, which results in reduced biofilm formation" [43] and "3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) and plaque reduction assays showed that 2,4-DTBP exhibited significant anti-coxsack ievirus B-3 (CVB-3) and anti-herpes virus type 2 (HSV-2) activities" [45].

"The compound was found to be effective against an agriculturally important root-rot fungus *Fusarium oxysporum* by inhibiting spore germination and hyphal growth" [46,47,48]. During the fungal spore germination, 2,4-DTBP completely inhibited the germination by preventing the emergence of a normal germ tube and led to the abnormal branching and swelling of hyphae.

5. CONCLUSION

The study shows the richness of naturally occurring Volatile compounds of *Aframomum melegueta* which is deposited abundantly in the fresh stem extract and suggests its antimicrobial capability and potentials as a natural antimicrobial agent against selected multiple resistant microorganisms. *Aframomum melegueta* Volatile compounds has limitless potentials and its various uses, example of such uses was elucidate during the course of this research work. Our observation is mind blowing. fresh stem extract *of Aframomum melegueta* also contain many useful naturally occurring chemical compound which are useful in nature. *Aframomum melegueta* extracts is indeed a very rich medicinal plant

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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