Antibacterial and GC-MS Profile of Purified Essential Oil From Multi-Phases Solvent Extraction Method From Stem Extract of *Aframomum melegueta* [Roscoe] K. Schum

Abstract

The purpose of this research work is to ascertain the composition and potency of the bioactive component of essential oil derived from *Aframomum melegueta* stem extract through multi-phase solvent extraction Gas Chromatography and Mass Spectroscopic 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* [Roscoe] K. Schum 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)(153g), Dichloromethane,(StAMD(103g), Ethyl acetate, (StAME)(45.5g), Methanol (StAMM)(50g). 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% Dimethylpolysiloxane). agar well diffusion method was used for the antimicrobial assay. 17 and 48 compounds were analyzed in the stem extract of purified Essential oil from Multi-phases solvent extraction of stem extract of *Aframomum melegueta* [Roscoe] K. Schum. The composition, chemical structure, molecular weight, and molecular formulation of different essential oil of StD+ and StD++ from purified Essential oil. *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 for StD+ and *Staphylococcus aureus* (12%) and *E.coli* (12%), has the highest while *Staphylococcus aureus* (6%), has the lowest percentage frequency distribution of purified essential oil of *Aframamum melegueta (*StD+) 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 for *(*StD++). In conclusion, the uses and importance of essential oil fraction from *Aframomum melegueta has* limitless potentials

.Keyword;*Aframomum melegueta,* Bacterial,Essential oil, Extraction solvent

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 (Omukoro *et al*,. 2007),(Akendengu *et al*,.1994).

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(Galal.1996),(Kamtchouing *et al*,.2002).(Alo *et al*,.2012),(Chiejina & Ukeh (2012). 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 (Mbongue *et al*,.2012). 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 (Umukoro *et al*,.2001),(Umukoro *et al*,.2008). as well as hypotensive and antihypertensive activity in humans (Lawal *et al*,.2007),(Sugita *et al*,.2013). it has been also found that the extract has an effect on the whole-body energy expenditure and visceral fat in humans(Sugita *et al*,.2014), However, in some of the literature studies, it should be mentioned that the seed of *Aframomum melegueta* derived essential oil contains humulene and caryophyllene occurred in higher proportions in the volatile oil(Aiyeoba & Ekundayo (1999).

The major components of the leaf oil were found to be myrtenyl acetate, isolimonene while caryophyllene oxide, myrtenyl acetate, β-eudesmene, and β-caryophyllene make up the composition of the stem oil (Owokotomo *et al,.(*2014) whereas the root essential oil comprised of myrtenyl acetate and pinocarvyl acetate. However, the seed comprised mainly of α-humulene, β-caryophyllene.

In literature, the major constituents of the leaf oil(Ntonifor *et al*,.2006). was identified as sabinene,α-pinene and β-caryophyllene. The seed essential oil of *A. melegueta* presents a characteristic composition with β-caryophyllene, α-humulene, and their epoxides as main

constituents (Menut *et al*,.1991). Eugenol occurred in abundance in the oil (Lamaty *et al*,.1993),β-Pinene predominates in the essential oils of the leaves and seeds of *A. melegueta* (Adefegha *et al*,.2017). GC/MS analysis of the hexane and methanol extracts of *A. melegueta* seed yielded gingerol, zingiberone, paradol, *trans*-6-shogaol, *cis*-isoelemicin, β-bisabolene, α-guaiene, aromadendrene, *trans*-β-farnesene and geraniol. The essential oil of *A. melegueta* displayed insect repellency against *Rhyzopertha dominica* (Ukeh.2008) antimicrobial activity (Uzeh & Oguntosin .2013), antifungal effect (Héritier *et al*,.2017), moderate inhibition of acetyl-cholinesterase (Owokotomo *et al*,.2015), antioxidant(Osuntokun *et al*,.2020), metabolites exhibit polypharmacology against SARS-CoV-2 drug targets Olaposi *et al*,.2020).

2.0 Material and Method

Collection of plant material

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 plant extract

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 Whatman 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

Extraction of Plant

Multi-Phase Solvent Extraction of *Aframamum melegueta* Bioactive Compound (Essential Oil Fraction)

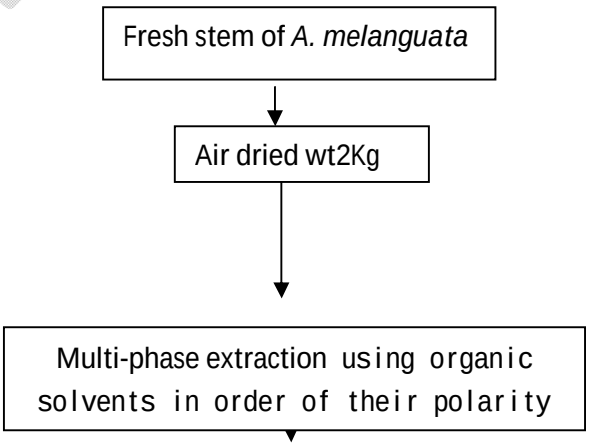
Aframamum melegueta was serially extracted using the multiphase extraction method at room temperature for 72 hours varying solvents based on their polarity using organic solvents. Solvent

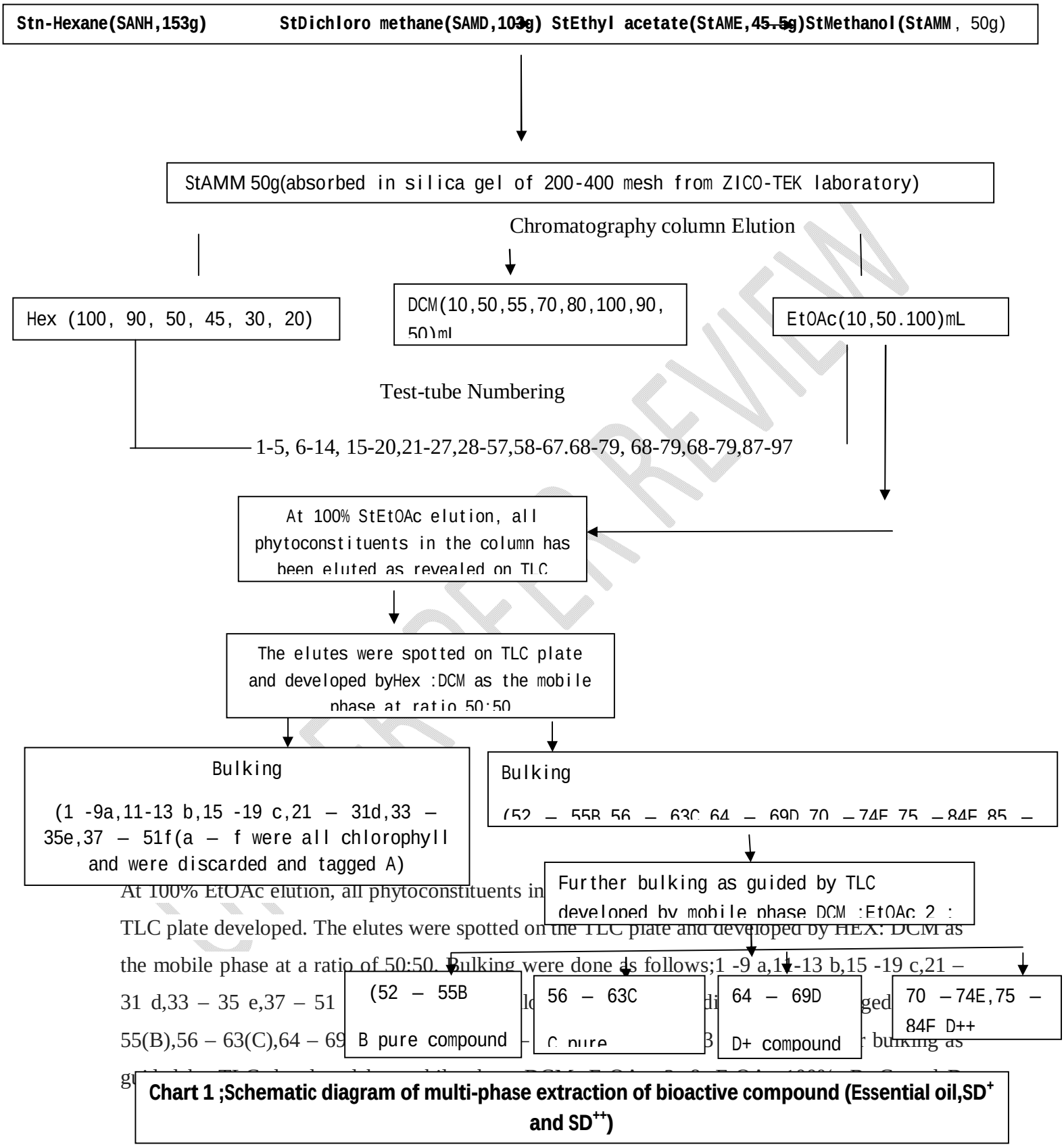
of extraction are: n-Hexane,(StAMH)(153g),Dichloromethane,(StAMD(103g),Ethyl acetate, (StAME) (45.5g), Methanol,(StAMM)(50g). For the StAMD Elution, StAMD was absorbed in silica gel of 200-400 mesh from ZICO-TEK laboratory. The following table shows detailed multiphase extraction of the essential fraction of *Aframamum melegueta*. A schematic extraction tree follows this table.**Below**

Chart 1 :Details of Multi-Phase Solvent Extraction Process. (Essential Oil 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

Flowchart 1 :Flowchart showing the study processing





At 100% EtOAc elution, all phytoconstituents in [illegible] 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 were done as follows;1 -9 a,11-13 b,15 -19 c,21 – 31 d,33 – 35 e,37 – 51 [illegible] 55(B),56 – 63(C),64 – 69 [illegible] bulking as gu[illegible]

Chart 1 ;Schematic diagram of multi-phase extraction of bioactive compound (Essential oil,SD^{+} and SD^{++})

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 *Aframomum melegueta*

GC-MS technique was used in this study to identify the components present in the extract of entire parts of *Aframomum melegueta* [Roscoe] 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 Spectrometer (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 an 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 Ver5.2.0. Compound 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 component

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.

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Standardization of Plant Extracts

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 50mg/ml, 25mg/ml and 12.5mg/ml respectively

Test Organisms

The selected clinical test isolates used were: *E.coli, Staphylococcus aureus, Klebsiella.pneumoniae,Proteus .mirabilis, P.aeruginosa, Staphylococcus aureus, Coryne bacterium 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 [9]. [9].

Antimicrobial Screening of Purified Essential oil of *Aframamum melegueta.*(StD+ and StD++) Extracts against selected clinical isolates

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 12.5mg/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

3.0 Result

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

Table 1; Gas chromatography and mass spectroscope (GC-MS) analysis of *Aframomum melegueta* stem extract of purified essential oil stem extract (*St*D +), table 2; Gas chromatography and mass spectroscope(gc-ms) analysis of *Aframomum melegueta* stem extract of purified essential oil stem extract (*St*D++), Fig 1; Antibacterial activity of purified essential oil of *Aframamum melegueta (*StD+) stem extracts against selected clinical isolates, Fig 2; Percentage frequency distribution of antibacterial activity of *Aframamum melegueta (St*D +) stem against clinical isolates at *(St*D +)100mg/ml, Fig 3; Percentage frequency distribution of antibacterial activity of *Aframamum melegueta* stem against clinical isolates at *(St*D+) 50mg/ml, Fig 4; Percentage frequency distribution of antibacterial activity of *Aframamum melegueta* stem against clinical isolates at *(St*D +) 25mg/ml, Fig 5; Percentage frequency distribution of antibacterial activity of *Aframamum melegueta* stem against clinical isolates at *(St*D +) 12.5mg/ml, Fig 6; Antibacterial activity of purified essential oil of *Aframamum melegueta.(St*D ++)stem extracts against selected clinical isolates, Fig 7; Percentage frequency distribution of antibacterial activity of *Aframamum melegueta* stem against clinical isolates at *(St*D $^{++}$) 100mg/ml, Fig 8; Percentage frequency distribution of antibacterial activity of *Aframamum melegueta* stem clinical isolates at *(St*D^{++}) 50mg/ml, Fig 9; Percentage frequency distribution of antibacterial activity of *Aframamum melegueta* stem against clinical isolates at *(St*D $^{++}$) 25mg/ml, Fig 10; Percentage frequency distribution of antibacterial activity of *Aframamum 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 essential oil stem extract (*St*D++).Spectral 2; Gas chromatography and mass spectroscope (gc-ms) spectra analysis of *Aframomum melegueta* stem extract of purified essential oil stem extract (*St*D ++).Table 1 and 2 represents the Gas chromatograph and mass spectroscope (GC-MS) Spectra Analysis of Aframomum melegueta purified essential oil (*St*D+ and StD++),

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

Table 1; Gas chromatography and mass spectroscope (GC-MS) analysis of *Aframomum melegueta* stem extract purified Essential oil (*St*D+). It was observed that 17 compound were analyzed. It reveled the composition, chemical structure, molecular weight, molecular formulation of different essential oil. This include 1,1-Dimethyl- chloro propanol ($C_5H_{11}ClO$,,MolWgt,122Da), Alpha.-Hydroxyisocaproic acid ($C_6H_{12}O_3$, Mol W, Mol Wgt1,32Da), Dimethylsulfoxonium formyl methylide($C_4H_8O_2S$, Mol Wgt 120), Cyclohexasiloxane, dodecame thyl-($C_{12}H_{36}O_6Si_6$.,Mol Wgt 444Da), Cycloheptasiloxane, tetra decamethyl-($C_{14}H_{42}O_7Si_7$.Mol Wgt 518 Da), Heptasiloxane, hexadecamethyl-($C_{16}H_{48}O_6Si_7$,Mol Wgt 518Da), Cyclo octasiloxane, hexadecame thyl-($C_{16}H_{48}O_8Si_8$,Mol Wgt 592Da), Cyclonona siloxane, octadeca ethyl-($C_{18}H_{54}O_9Si_9$,Mol Wgt 666Da), Heptasiloxane, hexadecamethyl-($C_{16}H_{48}O_6Si_7$, Mol Wgt 532Da), Cyclononasiloxane, octadecame thyl-($C_{18}H_{54}O_9Si_9$,Mol Wgt 666Da), Heptasiloxane, hexadecamethyl-($C_{16}H_{48}O_6Si_7$, MolWgt532Da), Cyclodecasiloxane, eicosamethyl ($C_{20}H_{60}O_{10}Si_{10}$, Mol Wgt 7740Da), Dibutyl phthalate ($C_{16}H_{22}O_4$,,Mol Wgt 278 Da),l-(+)-Ascorbic acid 2,6 dihexadecanoate($C_{38}H_{68}O_8$,Mol Wgt 652Da), Heptasiloxane, hexa decamethyl-($C_{16}H_{48}O_6Si_7$,MolWgt532Da) and -Cyclooctasiloxane,hexadecamethyl-($C_{18}H_{54}O_9Si_9$,Mol Wgt 592Da).

Table 2: Gas chromatography and mass spectroscope (GC-MS) analysis of *Aframomum melegueta* stem extract of purified Essential oil **(*St*D++)**, this table denotes the chemical nature of *Aframomum melegueta* stem extract of essential oil. It was observed that 48 compound were analyzed this include Dimethylsulfoxonium 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, decamethyl ($C_{10}H_{30}O_5Si_5$,Mol Wgt 370Da), Cyclohexa siloxane, dodeca methyl ($C_{12}H_{36}O_6Si_6$,Mol Wgt 444Da),Hexasiloxane, tetradecamethyl-($C_{14}H_{42}O_5Si_6$, Mol Wgt 458Da), Cyclohepta siloxane, tetradecamethyl-($C_{14}H_{42}O_7Si_7$ 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), Heptasiloxane, hexadecamethyl-($C_{16}H_{48}O_6Si_7$ Mol Wgt 532 Da),Caryophyllene oxide($C_{15}H_{24}O$ Mol Wgt 220Da), 2-Oxatricyclo [4.3.1.0 (3,8)]decane($C_9H_{14}O$MolWgt138Da),Methanol,[6,8,9-trimethyl-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), Isoaromadendrene epoxide($C_{15}H_{24}O$ mol Wgt Tricyclo[3.2.1.02,7]oct-3-ene, 2,3,4,5-tetra methyl ($C_{12}H_{18}$ 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 222Da),Heptasiloxane, hexa deca me th yl($C_{16}H_{48}O_6Si_7$ Mol Wgt 532Da), Longifolenaldehyde ($C_{15}H_{24}O$ mol Wgt220 Da), Alloaro mad endrene oxide-(1)($C_{15}H_{24}O$ Mol Wgt 220 Da),Cyclononasiloxane, octadecamethyl-($C_{18}H_{54}O_9Si_9$Mol Wgt 666 Da), 6-epi-shyobunol ($C_{15}H_{26}O$ Mol Wgt 22Da), Tetradecanoic acid, ethyl ester ($C_{16}H_{32}O_2$ Mol Wgt 256Da), 4-Hexen-1-ol, 6-(2,6,6-trimethyl-1-cyclohexen($C_{16}H_{28}O$ Mol Wgt 236 Da),Heptasiloxane, hexadecamethyl($C_{16}H_{48}O_6Si_7$ Mol Wgt 532Da), 2-Penta decanone, 6,10,14-trimethyl-($C_{18}H_{36}O$ Mol Wgt 268Da), 1,2-Benzene dicarbox ylic acid, bis(2-methylpro ($C_{16}H_{22}O_4$ Mol Wgt 278 Da), Cyclodecasiloxane, eicosamethyl-($C_{20}H_{60}O_{10}Si_{10}$ Mol Wgt 740 Da), Hexa decanoic acid, methyl ester($C_{17}H_{34}O_2$ Mol Wgt 270Da), 1,4-Methanoazulene-9-methanol, decahydro-4($C_{15}H_{26}O$ Mol Wgt 222Da), Dibutyl phthalate($C_{16}H_{22}O_4$ Mol Wgt 278Da) ,Naphtho (2,3-b)furan-2(3H)-one,decahydro-8 ($C_{15}H_{20}O_2$ Mol Wgt 232Da), l-(+)-Ascorbic acid 2,6-dihexa decanoate($C_{38}H_{68}O_8$ mol Wgt 652 Da) , Menthol, 1'-(butyn-3-one-1-yl)-, (1R,2S,5 R)-($C_{14}H_{22}O_2$ Mol Wgt 222Da), 4-Hexen-1-ol, 6-(2,6,6-trimethyl-1-cyclohexen($C_{16}H_{28}O$, Mol Wgt 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)-Benzofuranone, 6-ethenylhexahydro-6-($C_{15}H_{20}O_2$ Mol Wgt232Da),3,7,11,15-Tetramethyl hexadeca-1,6,10,14-tetr($C_{20}H_{34}O$ Mol Wgt 290Da), Cyclo octasiloxane, hexadecamethyl-($C_{16}H_{48}O_8Si_8$ Mol wgt 592 Da), 7a-Isopropenyl-4,5-dimethyl octa hydroinden-($C_{15}H_{26}O$ MolWgt 22Da),Linoleic acid ethyl ester $C_{20}H_{36}O_2$ Mol Wgt 308Da), Hepta siloxane, hexadecamethyl-($C_{16}H_{48}O_6Si_7$ Mol Wgt 532Da), Alloaromadendrene oxide-(1)($C_{15}H_{24}O$ Mol Wgt 220Da).**Figure 1** denote the Antibacterial activity of purified essential oil of *Aframamum melegueta(*StD++) stem extracts against selected clinical isolates (StD^+). It was observed that all the selected test organisms were susceptible to the purified essential oil of *Aframomum melegueta* at varying degree of concentration of 100, 50,25 and 12,5mg/ml. respectively. *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 Citro*

*bacter 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) respectively.

Figure 2,3,4,5represents percentage frequency distribution of antibacterial activity against clinical isolates at *(*StD+).

Figure 2 depicts percentage frequency distribution of antibacterial activity against clinical isolates at *(*StD+) at 100mg/ml. At 100mg /ml, *Staphylococcus aureus* (12%) and *E.coli* (12%), has the highest while *Staphylococcus aureus* (6%), has the lowest percentage frequency distribution of purified essential oil of *Aframamum melegueta* (StD+) against selected clinical isolates. Other isolates were *Citrobacter diversus* (8%), *Proteus vulgaris* (10%) ,*Klebsiella .pneumoniae* (11%), *P.aeruginosa* (7%),*Staphylococcus aureus*((6%), *Coryne bacterium cystitidis* (11%), *Klebsiella planticola* (7%), *Salmonella choleraesuis* (7%),*Citrobacter freundii* (9%) respectively.

Figure 3 depicts percentage frequency distribution of antibacterial activity against clinical isolates at *(*StD+) at 50mg/ml. At 50mg/ml, *E.coli* (13%) and *Staphylococcus aureus* (13%), has the highest while *Staphylococcus aureus* (6%), has the lowest percentage frequency distribution of purified essential oil of *Aframamum 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.

Figure 4 depicts 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 essential oil of *Aframamum melegueta (*StD+) against selected clinical isolates. Other isolates were *Citrobacter freundii* (9%),*Citrobacter diversus* (9%), *Klebsiella .pneumoniae(*10%),*Proteus vulgaris* (7%),*P.aeruginosa* (9%),*Klebsiella planticola* (9%), *Staphylococcus aureus* (6%), *Coryne bacterium cystitidis* (9%), *Salmonella choleraesuis* (8%), *Citrobacter freundii*(9%),

Figure 5 depicts 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%) has the highest while *Citrobacter diversus* (6%), has the lowest percentage frequency distribution of purified essential oil of *Aframamum 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%),

Figure 6 depicts the Antibacterial activity of purified essential oil of *Aframamum 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 were 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.

Figure 7 depicts the percentage frequency distribution of antibacterial activity of purified essential oil of *Aframamum 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 essential oil of *Aframamum melegueta (*StD++) against selected clinical isolates. Other isolates were *Citrobacter freundii* (8%),*Citrobacter diversus* (9%),and *Klebsiella. Pneumonia* (10%), *Proteus. mirabilis* (7%),*P.aeruginosa* (10%), *Staphylococcus aureus* (10%), *Corynebacterium cystitidis* (9%), *Salmonella choleraesuis* (7%) and *Citrobacter freundii* (8%) respectively .

Figure 8 shows percentage frequency distribution of antibacterial activity of purified essential oil of *Aframamum 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 essential oil of *Aframamum melegueta (*StD++)against selected clinical isolates, other isolates were *Citrobacter diversus* (7%),*Staphylococcus aureus*

(12%), *Klebsiella.pneumoniae(*11%),*Proteus.mirabilis* (9%), *P.aeruginosa* (9%), *Staphylococcus aureus* (10%), *Corynebacterium cystitidis* (8%), *Klebsiella planticola* (6%), *Salmonella chole raesuis* (8%),

Figure 9 shows the percentage frequency distribution of antibacterial activity of purified essential oil of *Aframamum melegueta (*StD++) stem extracts against selected clinical isolates at 25mg/ml. *Sta phylococcus aureus* (13%) has the highest while *Citrobacter freundii* (4%) has the lowest percentage frequency distribution of purified essential oil of *Aframamum 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 Coryneb acterium cystitidis(11%).

Figure 10 shows the percentage frequency distribution of antibacterial activity of purified essential oil of *Aframamum melegueta (*StD++) stem extracts against selected clinical isolates at 12.5mg/ml. *Staphylococcus aureus* (14%) has the highest while *Klebsiella planticola* (2%) has lowest percentage frequency distribution of purified essential oil of *Aframamum melegueta (*StD++) against selected clinical isolates. Other isolates were *Citrobacter diversus* (5%), *E.coli* (12%),*Staphylococcus aureus* (12%), *Klebsiella. Pneumonia* (7%), *Proteus. mirabilis* (9%), *P aeruginosa* (12%),*Corynebacterium cystitidis* (10%), *Klebsiella planticola* (2%) *Salmonella choleraesuis*(5%) and *Citrobacter freundii* (10%).

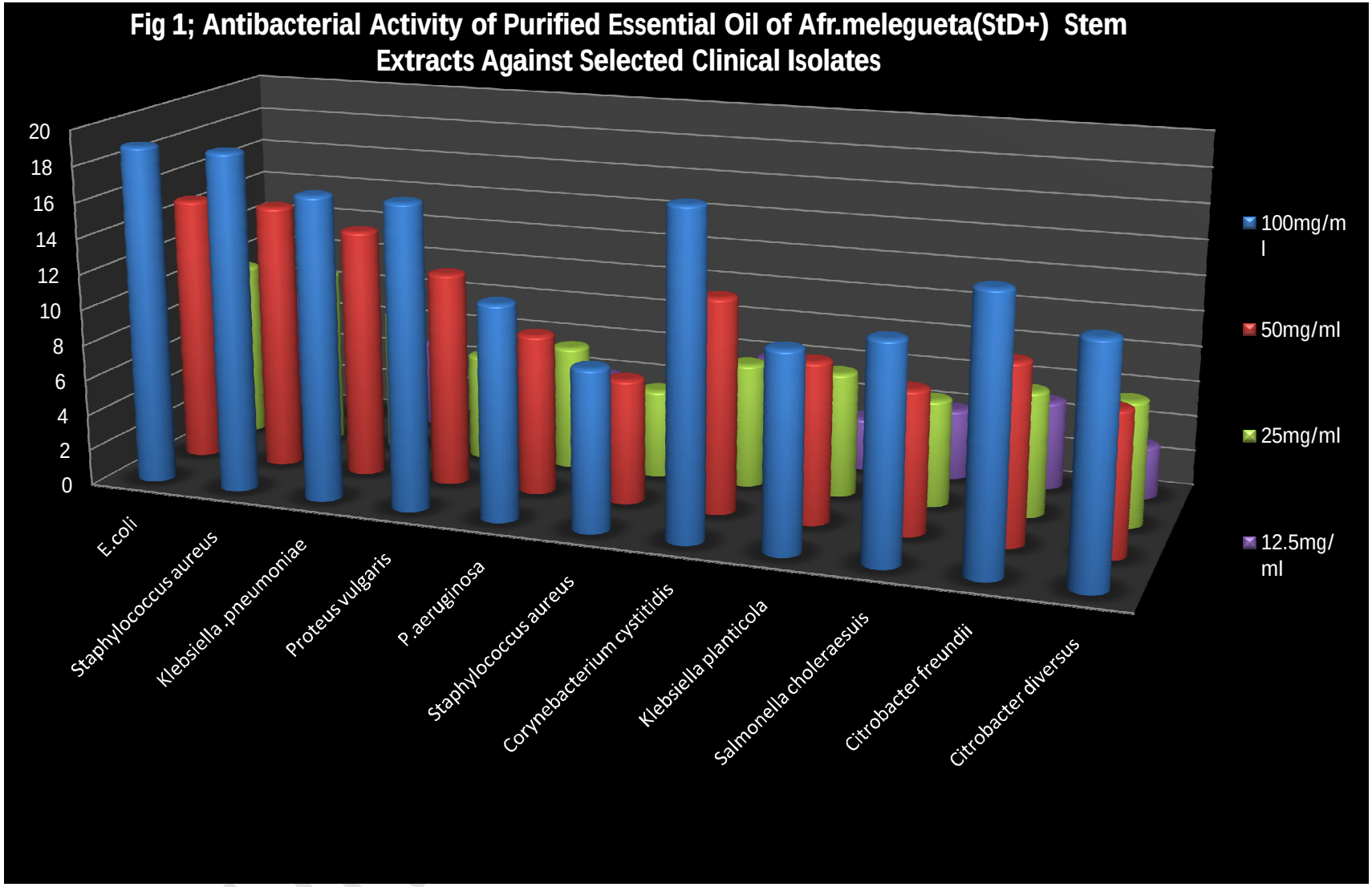


Fig 1; Antibacterial Activity of Purified Essential Oil of Afr.melegueta(StD+) Stem Extracts Against Selected Clinical Isolates
20
18
16
14
12
10
8
6
4
2
0
E.coli
Staphylococcus aureus
Klebsiella .pneumoniae
Proteus vulgaris
P.aeruginosa
Staphylococcus aureus
Corynebacterium cystitidis
Klebsiella planticola
Salmonella choleraesuis
Citrobacter freundii
Citrobacter diversus
100mg/ml
50mg/ml
25mg/ml
12.5mg/ml

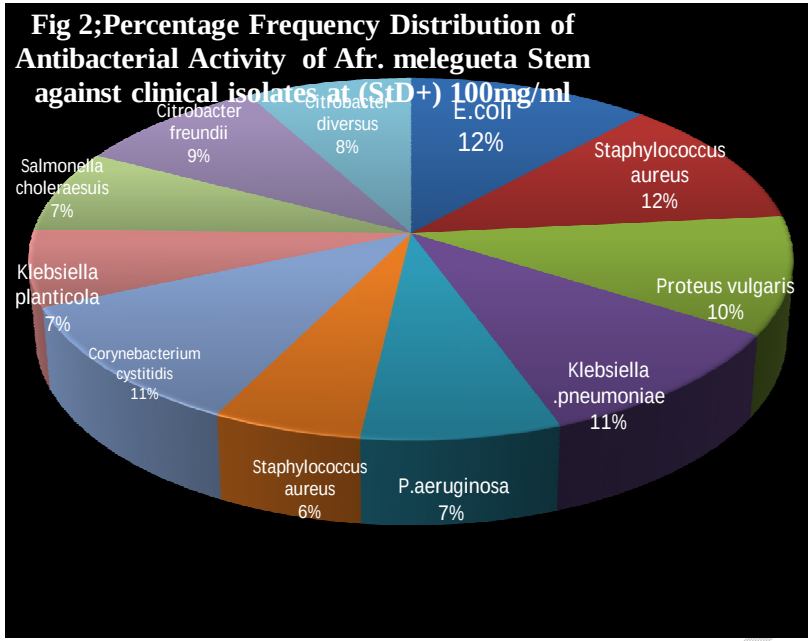


Fig 2;Percentage Frequency Distribution of Antibacterial Activity of Afr. melegueta Stem against clinical isolates at (StD+) 100mg/ml
E.coli 12%
Staphylococcus aureus 12%
Proteus vulgaris 10%
Klebsiella .pneumoniae 11%
P.aeruginosa 7%
Staphylococcus aureus 6%
Klebsiella planticola 7%
Corynebacterium cystitidis 11%
Salmonella choleraesuis 7%
Citrobacter freundii 9%
Citrobacter diversus 8%

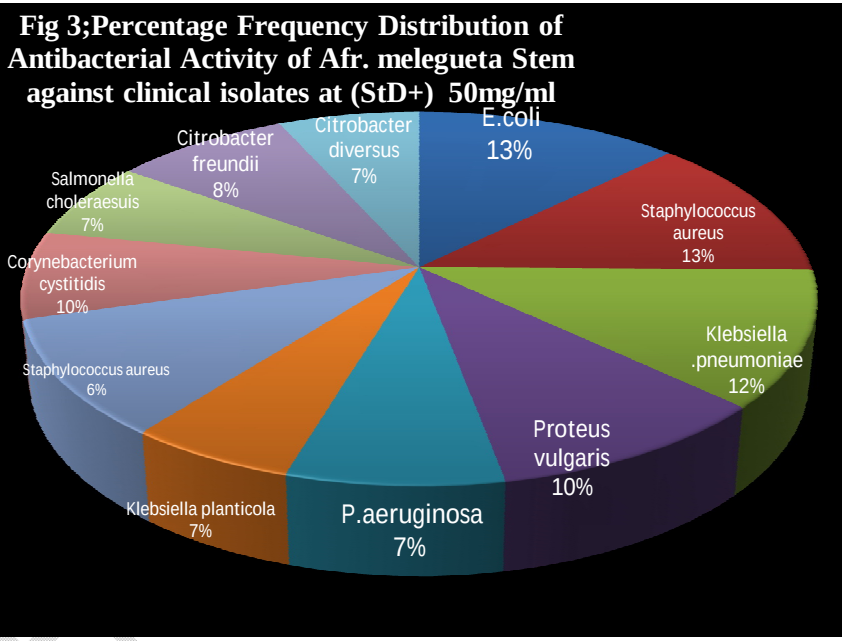


Fig 3;Percentage Frequency Distribution of Antibacterial Activity of Afr. melegueta Stem against clinical isolates at (StD+) 50mg/ml
E.coli 13%
Staphylococcus aureus 13%
Klebsiella .pneumoniae 12%
Proteus vulgaris 10%
P.aeruginosa 7%
Klebsiella planticola 7%
Staphylococcus aureus 6%
Corynebacterium cystitidis 10%
Salmonella choleraesuis 7%
Citrobacter freundii 8%
Citrobacter diversus 7%

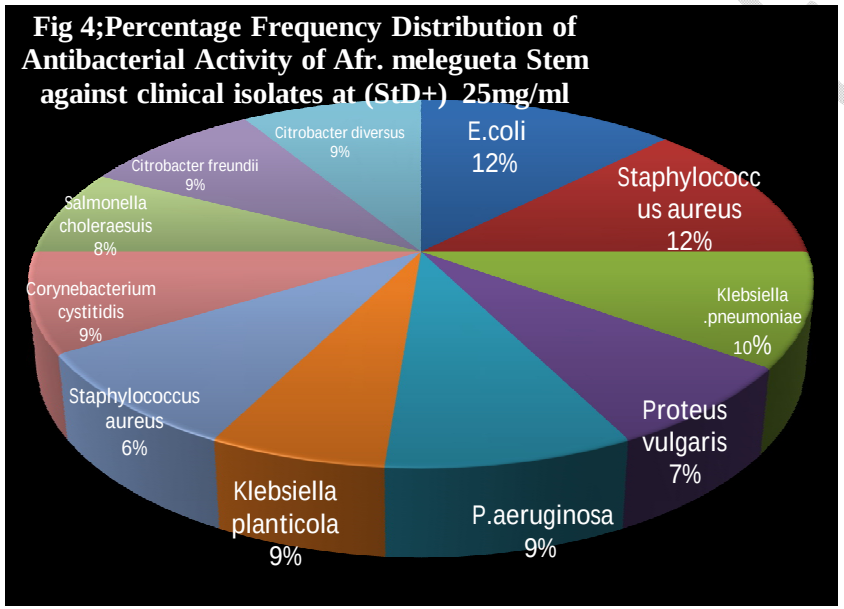


Fig 4;Percentage Frequency Distribution of Antibacterial Activity of Afr. melegueta Stem against clinical isolates at (StD+) 25mg/ml
E.coli 12%
Staphylococcus aureus 12%
Klebsiella .pneumoniae 10%
Proteus vulgaris 7%
P.aeruginosa 9%
Klebsiella planticola 9%
Staphylococcus aureus 6%
Corynebacterium cystitidis 9%
Salmonella choleraesuis 8%
Citrobacter freundii 9%
Citrobacter diversus 9%

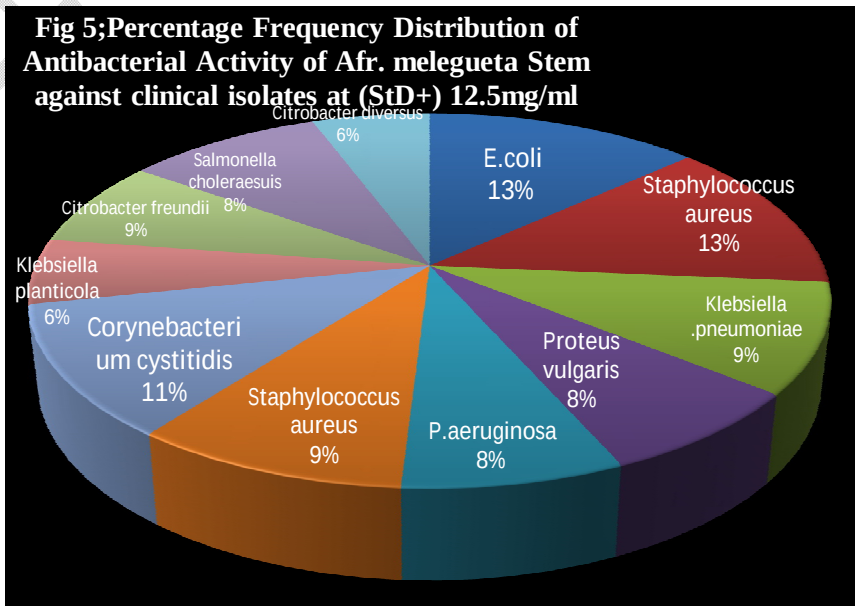
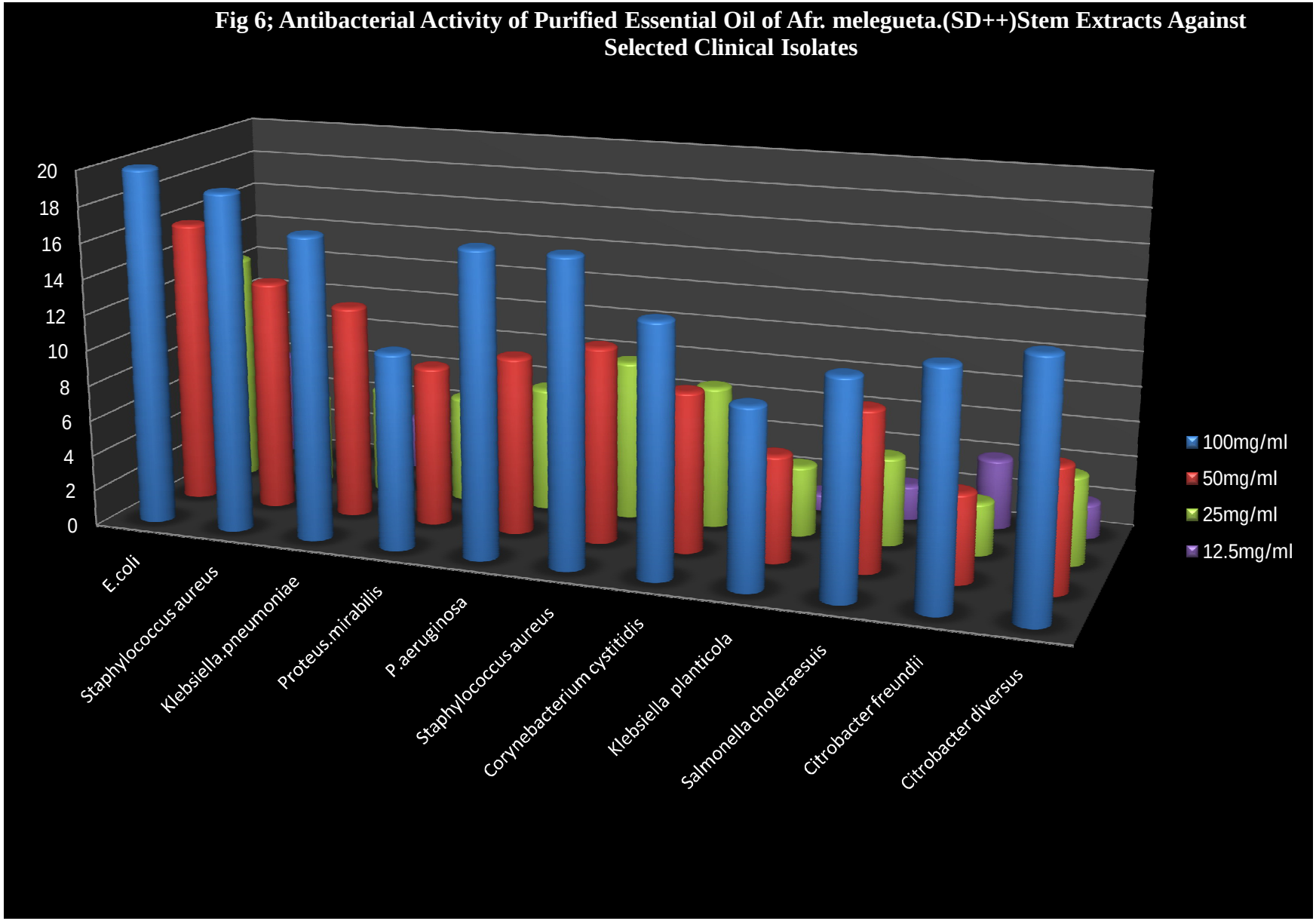


Fig 5;Percentage Frequency Distribution of Antibacterial Activity of Afr. melegueta Stem against clinical isolates at (StD+) 12.5mg/ml
E.coli 13%
Staphylococcus aureus 13%
Klebsiella .pneumoniae 9%
Proteus vulgaris 8%
P.aeruginosa 8%
Staphylococcus aureus 9%
Corynebacterium cystitidis 11%
Klebsiella planticola 6%
Citrobacter freundii 9%
Salmonella choleraesuis 8%
Citrobacter diversus 6%

Fig 6; Antibacterial Activity of Purified Essential Oil of Afr. melegueta.(SD++)Stem Extracts Against Selected Clinical Isolates



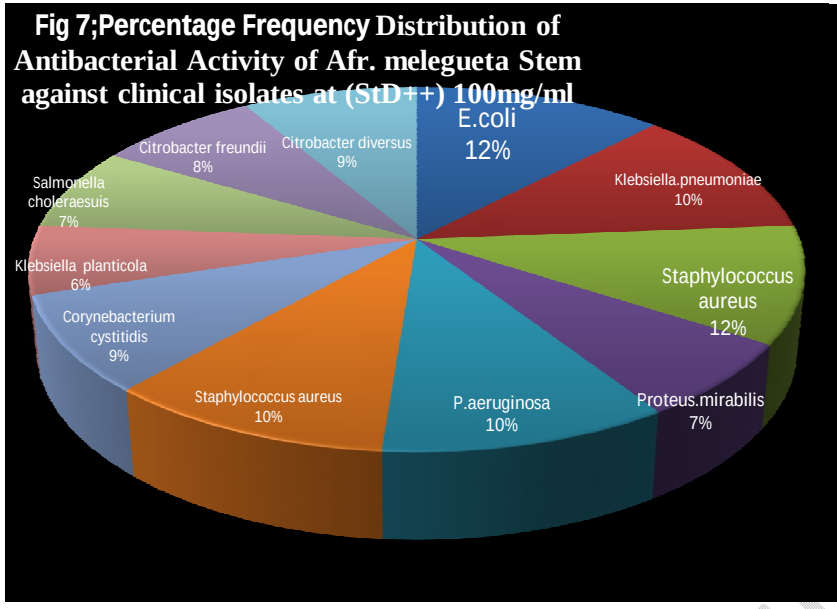


Fig 7;Percentage Frequency Distribution of Antibacterial Activity of Afr. melegueta Stem against clinical isolates at (StD++) 100mg/ml
E.coli 12%
Klebsiella.pneumoniae 10%
Staphylococcus aureus 12%
Proteus.mirabilis 7%
P.aeruginosa 10%
Staphylococcus aureus 10%
Corynebacterium cystitidis 9%
Klebsiella planticola 6%
Salmonella choleraesuis 7%
Citrobacter freundii 8%
Citrobacter diversus 9%

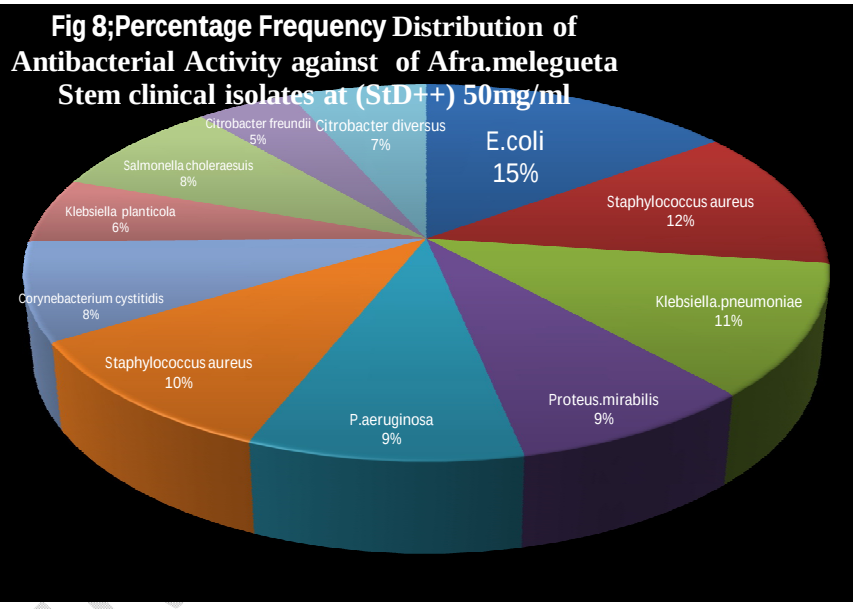


Fig 8;Percentage Frequency Distribution of Antibacterial Activity against of Afra.melegueta Stem clinical isolates at (StD++) 50mg/ml
E.coli 15%
Staphylococcus aureus 12%
Klebsiella.pneumoniae 11%
Proteus.mirabilis 9%
P.aeruginosa 9%
Staphylococcus aureus 10%
Corynebacterium cystitidis 8%
Klebsiella planticola 6%
Salmonella choleraesuis 8%
Citrobacter freundii 5%
Citrobacter diversus 7%

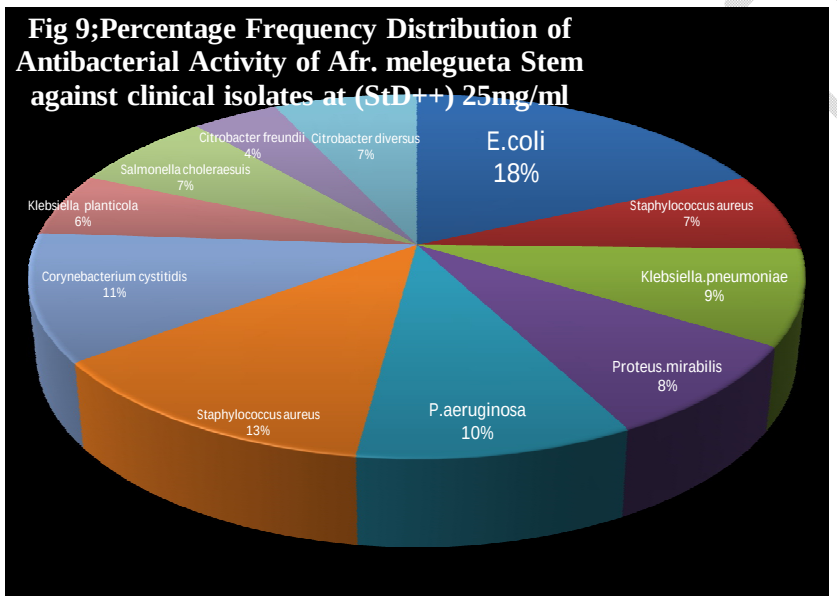


Fig 9;Percentage Frequency Distribution of Antibacterial Activity of Afr. melegueta Stem against clinical isolates at (StD++) 25mg/ml
E.coli 18%
Staphylococcus aureus 7%
Klebsiella.pneumoniae 9%
Proteus.mirabilis 8%
P.aeruginosa 10%
Staphylococcus aureus 13%
Corynebacterium cystitidis 11%
Klebsiella planticola 6%
Salmonella choleraesuis 7%
Citrobacter freundii 4%
Citrobacter diversus 7%

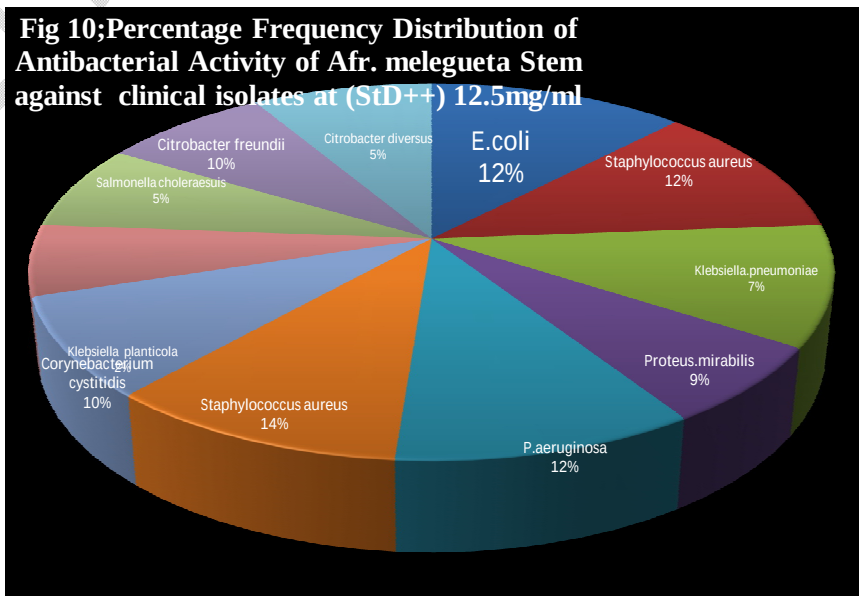


Fig 10;Percentage Frequency Distribution of Antibacterial Activity of Afr. melegueta Stem against clinical isolates at (StD++) 12.5mg/ml
E.coli 12%
Staphylococcus aureus 12%
Klebsiella.pneumoniae 7%
Proteus.mirabilis 9%
P.aeruginosa 12%
Staphylococcus aureus 14%
Corynebacterium cystitidis 10%
Klebsiella planticola 2%
Salmonella choleraesuis 5%
Citrobacter freundii 10%
Citrobacter diversus 5%

Figure 11 :Spctral 1;Gas chromatography and mass spectroscope(GC-MS) Spectra **Analysis of *Aframomum melegueta*Purified Essential oil stem extract (*St*D+)**

Chromatogram

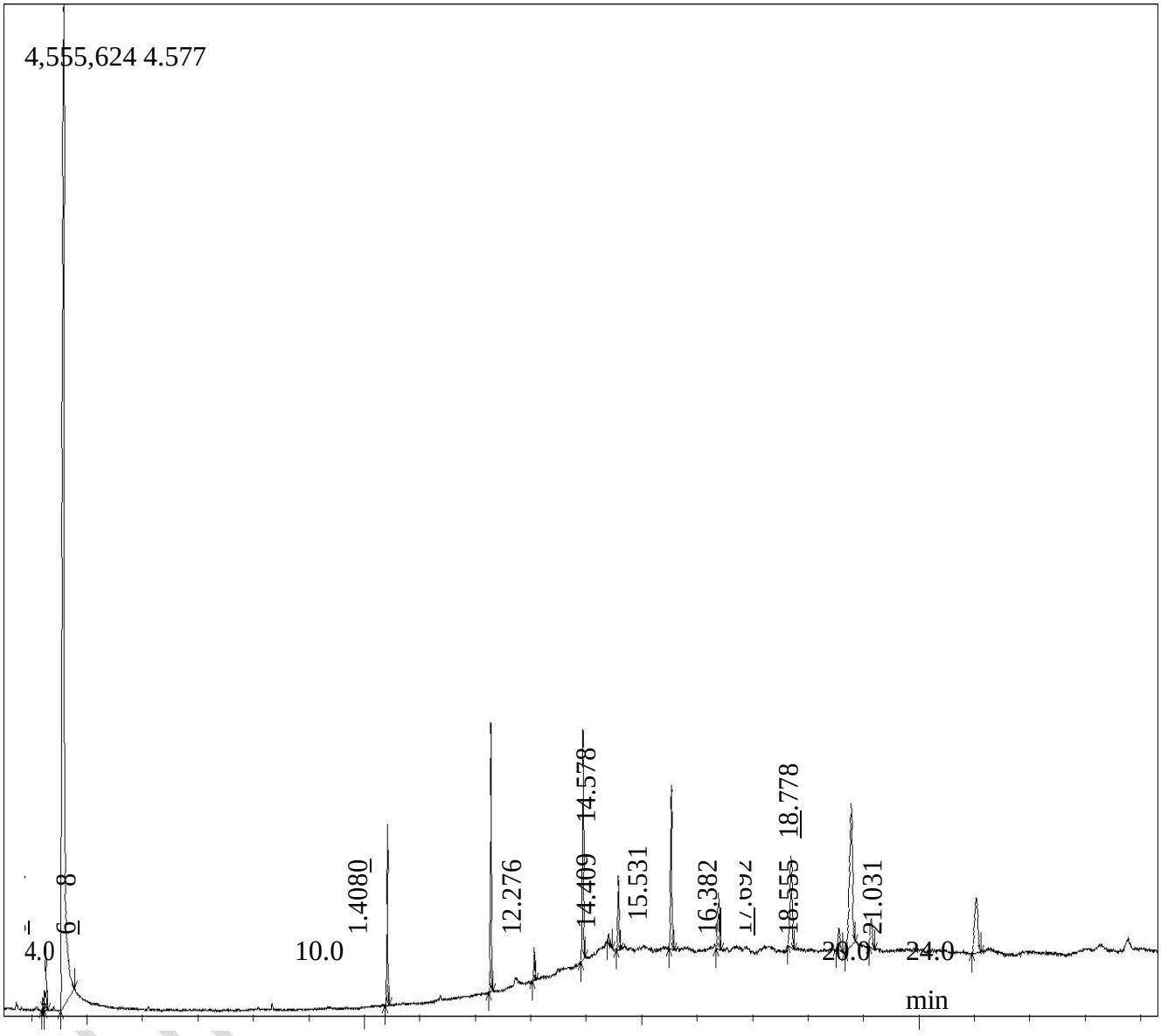
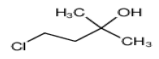
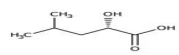
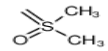
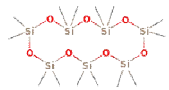
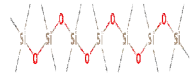
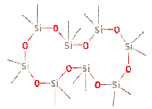


Table 1; Gas chromatography and mass spectroscope(GC-MS) Analysis of Aframomum melegueta stem extract of Purified Essential oil stem extract (*St*D+

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 methylide	:$C_4H_8O_2S$	
10.408	990350	3.91	812257	7.41	1.22	444	Cyclohexasiloxane, dodecamethyl-	$C_{12}H_{36}O_6Si_6$	
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, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$	

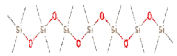
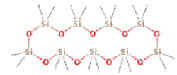
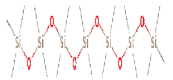
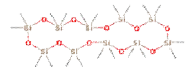
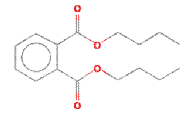
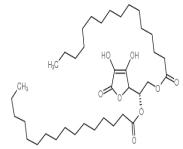
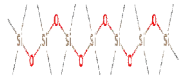
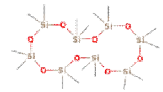
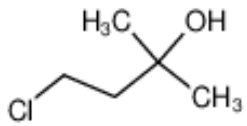
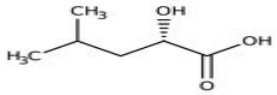
14.409	97955	0.39	43036	0.39	2.28	666	Cyclononasiloxane, octadeca ethyl-	$C_{18}H_{54}O_9Si_9$	
14.578	437659	1.73	331899	3.03	1.32	532	Heptasiloxane, hexadecamethyl-	$C_{16}H_{48}O_6Si_7$	
15.531		4.85	740050	6.76	1.66	666	Cyclononasiloxane, octadecame thyl-	$C_{18}H_{54}O_9Si_9$	
16.382	493275	1.95	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,hexadecamet hyl-	$C_{18}H_{54}O_9Si_9$	

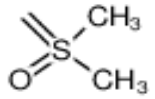
figure 12 : Summary of Gas chromatography and mass spectroscope(GC-MS) Analysis of Aframomum melegueta stem extract of Purified Essential oil stem extract (*St*D+)



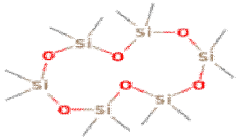
1,1-Dimethyl Chloropropanol



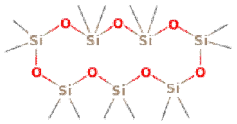
alpha.-Hydroxyisocaproic



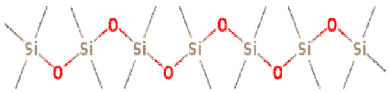
Dimethylsulfoxonium formyl me thylide



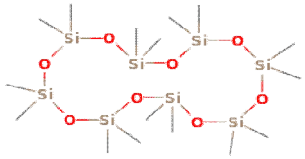
Cyclohexasiloxanedo decame thyl-



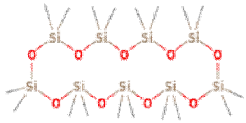
Cycloheptasiloxane,t etradecamethyl-



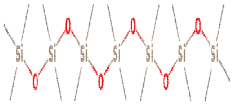
Heptasiloxanehexadecamethyl-



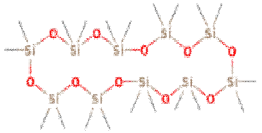
Cyclooctasiloxanehexade came thyl-



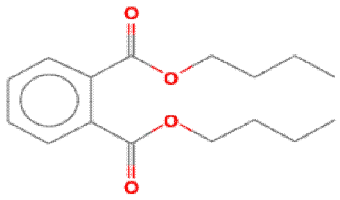
Cyclononasiloxane octadeca ethyl-



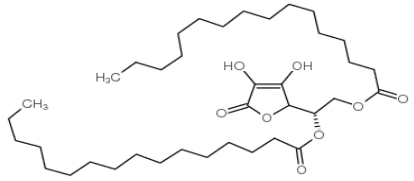
Heptasiloxanehexa decamethyl-



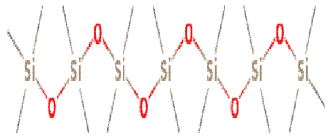
Cyclodecasiloxane,eic osameth yl-



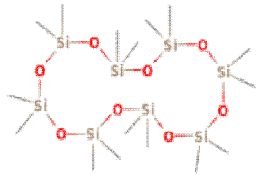
Dibutyl phthalate hexadecamethyl-



l-(+)-Ascorbic acid hexadecamethyl-



Heptasiloxane,



Cyclooctasiloxan 2,6 dihexad ecanoate

Figure 13 :Spectral 2; Gas chromatograph and mass spectroscope(GC-MS) Spectra Analysis of *Aframomum melegueta* stem extract of Purified Essential oil stem extract (*St*D++)

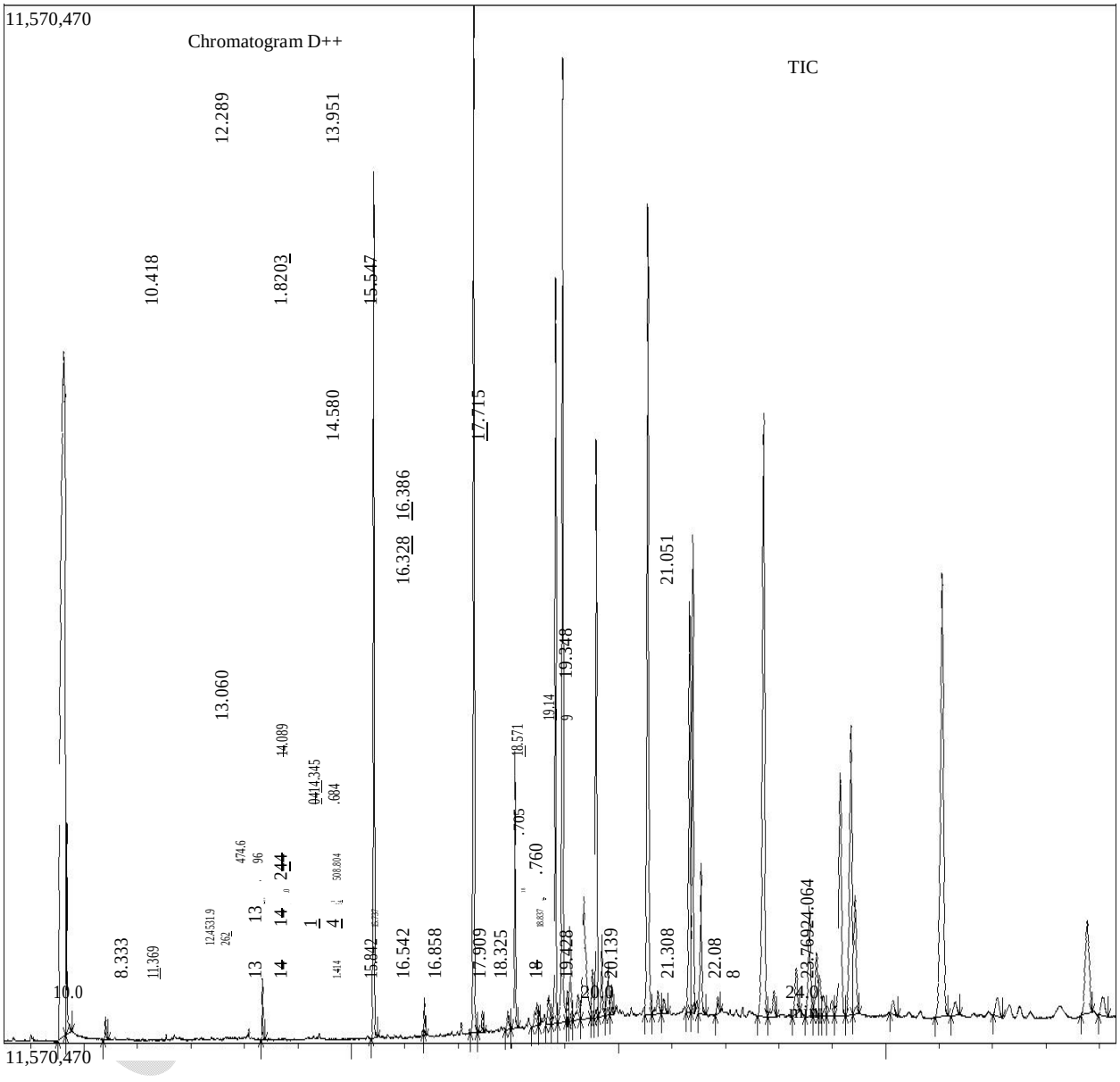
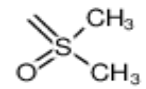
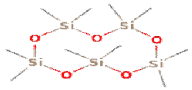
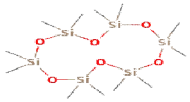
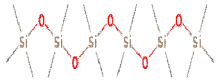
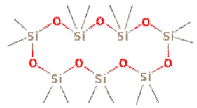
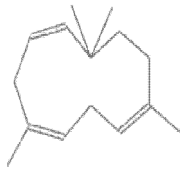
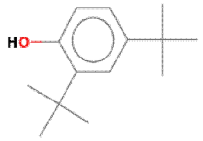
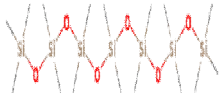
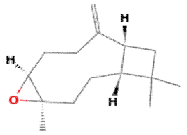
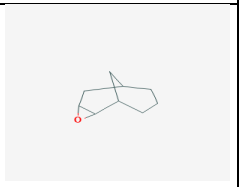
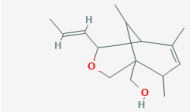
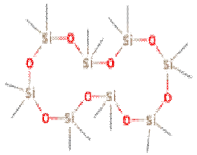
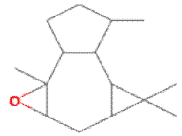
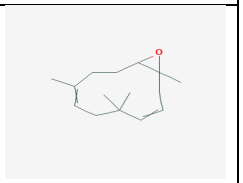
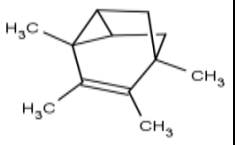
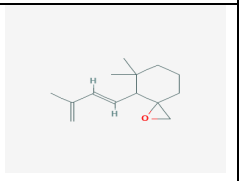
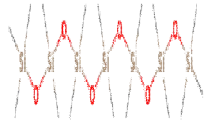
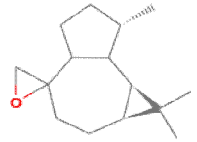
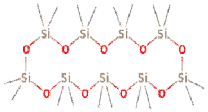
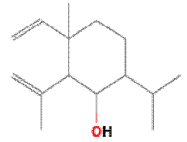


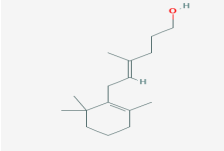
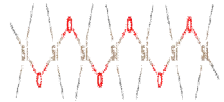
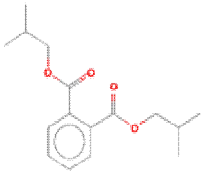
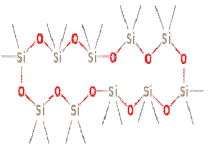
Table 2; Gas chromatography and mass spectroscope(GC-MS) Analysis of *Aframomum melegueta* stem extract of Purified Essential oil stem extract (*St*D++)

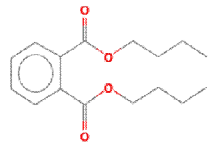
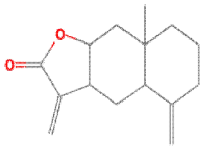
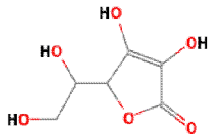
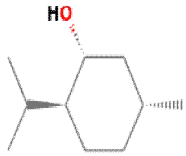
Peak	Retention time	Area	Area %	Height	Height %	A/H	Mol weight	Name	Structure formula	Structure
1	4.619	43145466	15.72	7641497	6.76	5.65	120	Dimethylsulfoxonium formylmethylide	$C_4H_8O_2S$	
2	4.674	2493029	0.91	2352888	2.08	1.06	116	2-Hexanol, 2-methyl-	$C_7H_{16}O$	
3	5.399	353165	0.13	255303	0.23	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-	$C_{14}H_{42}O_5Si_6$	

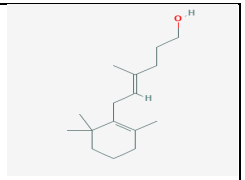
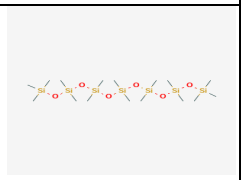
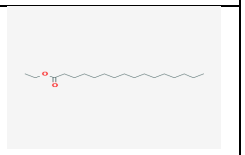
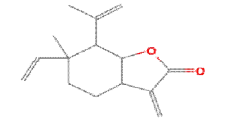
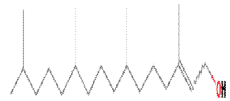
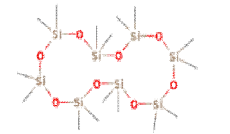
7	12.289	19927221	7.26	11446136	10.13	1.74	518	Cycloheptasiloxane, tetradecamethyl-	$C_{14}H_{42}O_7Si_7$	
8	12.453	388191	0.14	234747	0.21	1.65	204	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,	$C_{15}H_{24}$	
9	12.926	477280	0.17	199124	0.18	2.40	206	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	
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	$C_9H_{14}O$	

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	$C_{12}H_{18}$	
18	14.345	5991489	2.18	1361670	1.20	4.40	206	1-Oxaspiro [2.5] octane , 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-	$C_{18}H_{54}O_9Si_9$	
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	$C_{16}H_{22}O_4$	
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	:$C_{38}H_{68}O_8$	
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	$C_{20}H_{34}O$	
42	21.051	20063075	7.31	4929341	4.36	4.07	592	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$	

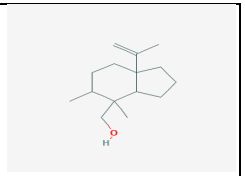
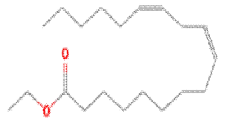
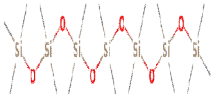
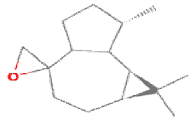
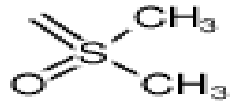
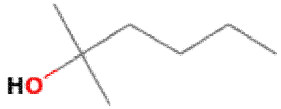
43	21.308	652829	0.24	149423	0.13	4.37	222	(7a-Isopropenyl-4,5-dimethyloctahydroinden-	$C_{15}H_{26}O$	
44	22.088	925406	0.34	202243	0.18	4.58	308	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	
45	23.769	5274359	1.92	1025615	0.91	5.14	532	Heptasiloxane, hexadecamethyl-	$C_{16}H_{48}O_6Si_7$	
46	24.064	1051570	0.38	215439	0.19	4.88	220	Alloaromadendrene oxide-(1)	$C_{15}H_{24}O$	

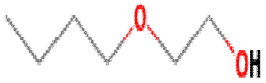
Figure 14: Summary of Gas chromatography and mass spectroscope(GC-MS) Analysis of *Aframomum melegueta* stem extract of Purified Essential oil stem extract (*St*D++)



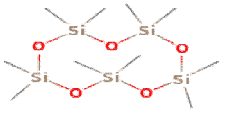
Dimethylsulfoxonium Formylmethylide



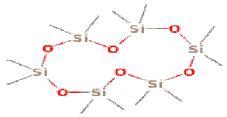
2-Hexanol, 2-methyl-



Ethanol, 2-butoxy-

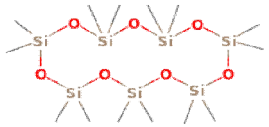


Cyclopentasiloxaned ecamethyl

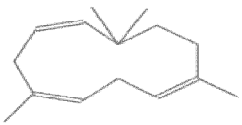


Cyclohexasiloxane,d odecamethyl

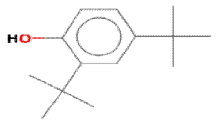
Hexasiloxane tetradecamethyl-



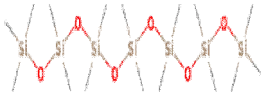
Cycloheptasiloxane, tetradecamethyl-



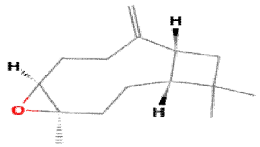
1,4,7, Cycloundecatriene1,5,9, 9-tetramethyl-, Z



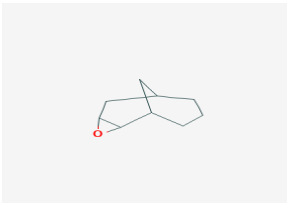
Phenol, 2,4-bis(1,1-dimethylethyl)-



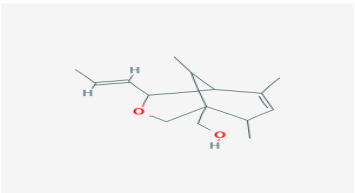
Heptasiloxane,hexade camethyl-



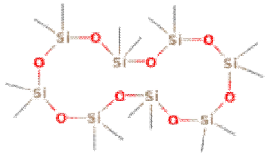
Caryophyllene Oxideepoxide



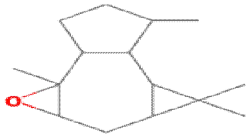
2-Oxatricyclo [4.3.1.0 (3,8)]decane



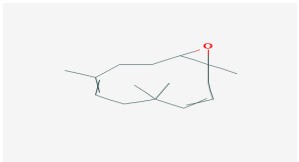
Methanol, [6,8,9-trimethyl



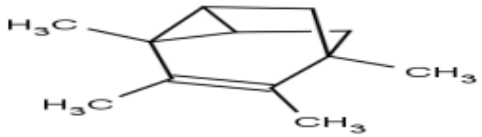
Cyclooctasiloxane 4-(1-propenyl)-3- oxabicyclo



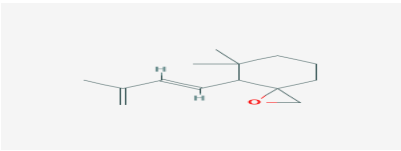
Isoaromadendrenehexad ecamethyl



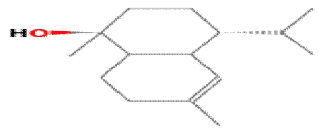
12-Oxabicyclo [9.1.0]



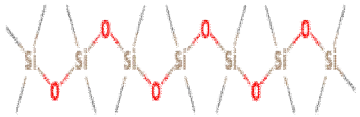
Tricyclo[3.2.1.02,7]ocdodeca-3,7-diene, 1,5, 5,8-hexadecamethyl



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

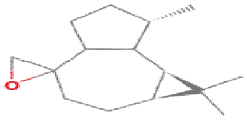


alpha.-Cadinol

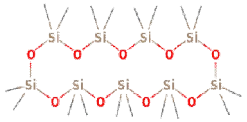


Heptasiloxane,5,5-dimethyl-4-(3-methyl

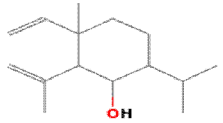
Longifolenaldehyde



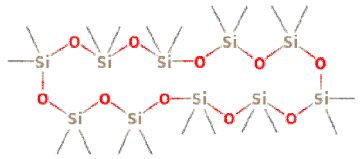
Alloaromadendreneoxide-(1)



Cyclononasiloxaneoctadecamethyl-

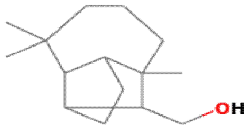


6-epi-shyobunol

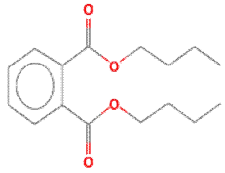


Cyclodecasiloxaneeicosamethyl-

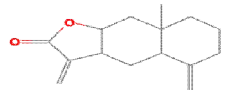
Hexadecanoic acid,methyl esterdihexadecanoate



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

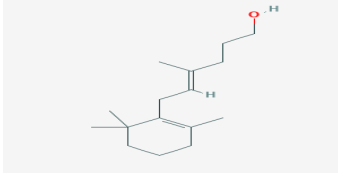


Dibutyl phthalate

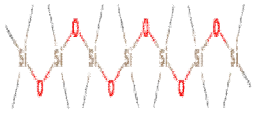


Naphtho(2,3-b)furan-2(3H)-one, decahydro-8

Tetradecanoic acidethyl estertrimethyl-1-cyclohexen

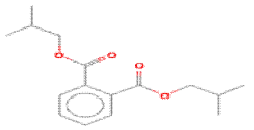


4-Hexen-1-ol, 6-(2,6,6-trimethyl-1-cyclohexen

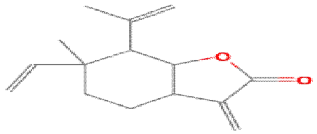


Heptasiloxane,hexadecamethyl-

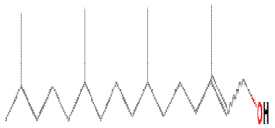
2-Pentadecanone6.1



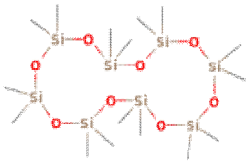
1,2-Benzene dicarboxylic acid, bis(2-methyl



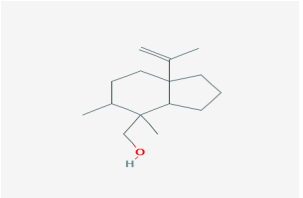
2(3H)-Benzofuranone6-ethenylhexahydro-6-



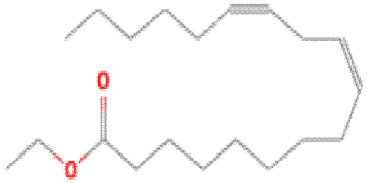
3,7,11,15-Tetramethylhexadeca-



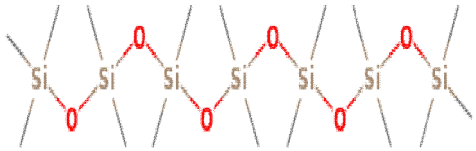
Cyclooctasiloxane,hexadecamethyl-



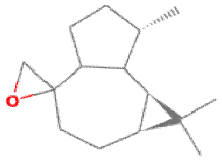
(7a-Isopropenyl-4,5-dimethyloctahydroinden



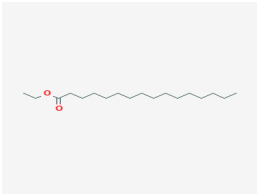
Linoleic acid ethyl ester



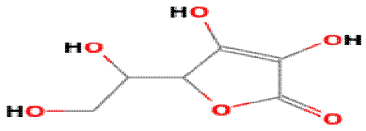
Heptasiloxane, hexadecamethyl-



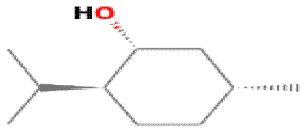
Alloaromadendrene oxide-(1)



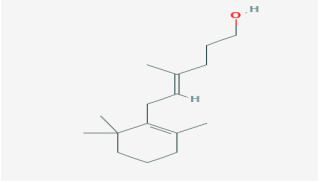
Hexadecanoic acidethyl ester



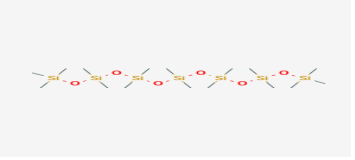
l-(+)-Ascorbic acid2,6-dihexadecanoate



Menthol, 1'-(butyn-3-one-1-yl)-, (1R,2S,5R)-



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



Heptasiloxane,hexadecamethyl-

4.0 Discussion

The purpose of this research work is to ascertain the composition and potency of the bioactive component of essential oil derived from *Aframomum melegueta* stem extract through multi-phase solvent extraction Gas Chromatography and 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 table and figures

In StD+ Fraction,(Antibacterial activity of purified essential oil of *Aframamum 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 essential oil of *Aframamum 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 12.5mg/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 freundii* (13.0mm), *Salmonella choleraesuis(*12.0mm) and *Proteus. mirabilis* (11.0mm) respectively. The decreasing order of zones of inhibition were as follows *Salmonella choleraesuis* (2.0mm), *Klebsiella.pneumoniae (3.0mm),Klebsiella. pneumoniae, and Corynebacterium cystitidis* (4.0 mm), *Staphylococcus aureus, P.aeruginosa,* (5.0mm) *and E.coli,*(6.0mm). The result shows that *Aframomum melegueta* stem extract possess some levels of antibacterial potentials and is in agreement with previous findings on oils of *A.*

danielli and Aframomum melegueta (Njimoh *et al*,.2015), Martins *et al*,.2001] and *A. melegueta* (Lawal *et al*,.2015,Uzeh and Oguntosin,2013).

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, antimicrobial, and antiproliferative activities (Semwal *et al*,.2016), 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(Amoa *et al*,.2013). the aglycone and hydrophilic sugar moieties are active biosurfactants that have several therapeutic applications including immunostimulatory, molluscicidal, hypocholesterolemic, antimicrobial, and antioxidant activities(Francis *et al*,.2002). 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 (Moses *et al*,.2014). Tannins are moieties linked to a carbohydrate core (hydrolyzable tannins), and have wide therapeutic properties including antiproliferative and antimicrobial activities(Chung *et al*,.1998),

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

The first highest peak from (16) sixteen components in GCMS analysis of essential oil fraction (SD+) of *Aframomum melegueta* stem extract was Cycloheptasiloxane, tetradecamethyl. Cycloheptasiloxane, tetradecamethyl-(($C_{14}H_{42}O_7Si_7$) 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. Cyclotrisiloxane (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 hexamethylcyclotrisiloxane (Patel *et al*,. 2011).

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 (Kumar *et al*,.2015) 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 compositions include deodorants, antiperspirants, insect repellants, anesthetics, skin conditioners, skin lotions, skin moisturizers, skin toners, skin sanitizers, skin cleansing compositions, skin soothing and lubricating compositions, sunscreen, anti-aging products, concealer products, soaps, foaming bath products, shower gels, cleansing products, shampoos, hair conditioners, hair styling gels, hair anti-dandruff compositions, hair growth promoter compositions, hair colorant compositions, hair bleaching agent compositions, hair anti-frizzing agent compositions, hair shining compositions, hair relaxer compositions, mousses, styling gels, hair sprays, hair dyes, hair waving products, hair straightening products, shaving product compositions, 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 (Thijssen *et al*,.(2005), 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 GCMS analysis of essential oil 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, bronchitis, stomach ulcers, tuberculosis, dysentery, boils, and wounds (Olumekun *et al*,.2020), 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 (Olumekun *et al*,.2020), 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(Osuntokun *et al*,.2020), (Osuntokun *et al*,.2021a)

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(Ntonifor et al,.2006). Tetradecanoic acid known as myristic acid is commonly added co-translationally to the penultimate, nitrogen-terminus, glycine in receptor-associated kinases to confer the membrane localization of the enzyme(Osuntokun *et al*,.2021b).

The first and second highest peak from (48) sixteen components in GCMS analysis of essential oil fraction (SD++) of *Aframomum melegueta* stem extract were Phenol, 2,4-bis(1,1-dimethyl ethyl ($C_{14}H_{22}O$) and Cyclooctasiloxane, hexadecamethyl-($C_{16}H_{48}O_8Si_8$), Cyclooctasiloxane, hexadecamethyl-($C_{16}H_{48}O_8Si_8$) 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 (Padmavath *et al*,.2015).

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 (Padmavathi *et al*,.2014) and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) and plaque reduction assays showed that 2,4-DTBP exhibited significant anti-coxsackievirus B-3 (CVB-3) and anti-herpes virus type 2 (HSV-2) activities (Leila *et al*,.2019).

The compound was found to be effective against an agriculturally important root-rot fungus Fusarium oxysporum by inhibiting spore germination and hyphal growth (Dharni *et al*,.2014). 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.

Conclusionthe uses and importance of essential oil fraction from *Aframomum melegueta* is limitless clinically and industrial

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