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Qualitative Phytochemical Screening and GC-MS analysis in the Leaf Methanolic Extracts of *Kamettia caryophyllata* (Roxb.)Nicolson&Suresh.

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ABSTRACT

The present investigation was carried out for the qualitative detection of major phytochemical groups and for preparing the profile of specific bioactive principles present in the leaf methanolic extracts of *Kamettia caryophyllata*. *The qualitative phytochemical screening* revealed the presence of major phytochemical groups like alkaloids, phenolics, glycosides, saponins, flavonoids, tannins and carbohydrates. The GC-MS analysis revealed the presence of 39 bioactive compounds with different peak area percentages and structural details. The major chemical groups and bioactive compounds detected in the studied plant are found to have important biological activities and the study indicates *K. caryophyllata* may be a good plant source for therapeutic industry.

KEYWORDS *Kamettia caryophyllata,* qualitative phytochemical screening, GC-MS analysis, bioactivity

Phytochemicals present in plants are important for their own protection from the attack of different insects, pests and disease causing pathogenic microorganisms and understanding these facts and knowing their immense medicinal properties, people started using plants as a source of therapeutic agents from ancient times. The World Health Organization estimated that about 80% of the populations in developing countries rely on traditional herbal medicines for their primary basic needs. Extraction of bioactive compounds from medicinal plants permits the demonstration of their physiological activity and facilitates pharmacological studies leading to synthesis of a more potent drug with reduced toxicity (Pamplona, 1999). Now a day, the screening of plant extracts has been of great interest to scientists for the discovery of new drugs effective in the treatment of several diseases (Dimayuga & Garcia, 1991). The selection of a suitable plant for a pharmacological study is a very important and decisive step. Generally, the most common strategies we use to select a plant of pharmacological interests are either by collecting the traditional knowledge regarding the use of natural plant resources in folk medicine or by random phytochemical screening of plant resources to find out bioactive compounds with known medicinal properties. The use of medicinal plants as raw materials in the production of drugs is ever increasing because of their potentials in combating various diseases including the problem of drug resistance in micro-organisms. Further the plants are relatively cheap source of biological material having a vast variety of primary and secondary metabolites available in them for selecting the molecule of desired biological activity and also as they have comparatively less or no side effects on the host. Therefore the demand of plants having therapeutic value is increasing day by day both in developing as well as in developed countries and the research on herbal medicine is one of the leading areas of worldwide research. The present investigation is an attempt to assess the pharmacological interests of the plant *Kamettia caryophyllata* (Roxb.)Nicolson&Suresh, a climber belonging to the family Apocynaceae, native to India and known to have uses in treatment of leprosy, arthritics, itches and scabies in the Indian traditional system of medicine. To our knowledge, no detailed scientific chemical analysis has been previously reported on this plant. The study includes qualitative determination of major phytochemical groups and establishment of a profile of specific bioactive compounds present in the methanolic leaf extracts with the aid of GC-MS analysis.

MATERIALS AND METHODS

Collection and identification of plant material

The plant material of *Kamettia caryophyllata* (Roxb.) Nicolson&Suresh is collected in fresh condition from S.N Puram, a place belonging to the Coastal Belt of Thrissur District, Kerala. The study area lies at 10.520 N 76.210 E and has an average altitude of 2.83m. The taxonomic identity of the plant was confirmed with the Dept of Botany, University of Calicut and the voucher specimens of the plant has been deposited in the Herbarium collection, Dept. of Botany, S.N. College, Nattika, Thrissur for further reference.

Extraction of plant sample

Fresh and healthy leaf material of *K. caryophyllata* is collected and washed thoroughly under running tap water. The collected material is then air dried under shade and then powdered. Then the suitable quantity of the powdered plant material is placed in soxhlet apparatus and subjected to extraction using methanol. Subsequently, the extract is filtered and the filtrate is then evaporated using vacuum evaporator under reduced pressure at □ 40°C temperature to dryness till constant weight is obtained. The crude dried extract obtained after evaporation is stored in desiccators for further studies.

Preliminary phytochemical analysis

Different biochemical tests are performed for establishing a qualitative profile of various active phytochemical groups present in the leaf methanolic extracts of *K. caryophyllata*. Various qualitative phytochemical tests were carried out using the standard procedures described in Experimental Phytopharmacognosy (Khadabadi *et al*., 2013).

a) Detection of alkaloids

The residue is dissolved in 2 N HCl. The mixture is filtered and the filtrate is divided into 3 equal portions.

Mayer's test:
One portion is treated with few drops of Mayer's reagent. The creamish precipitate indicates the presence of alkaloids.

Wagner's test:
One portion is treated with equal amount of Wagner's reagent. The orange precipitate indicates the presence of alkaloids.

Dragendroff's test:
One portion is treated with equal amount of Dragondroff reagent. The brown precipitate indicates the presence of alkaloids.

b) Detection of tannins and phenolic compounds

Lead Acetate Test:

Add 3 ml of 10% lead acetate solution to the plant extract, a bulky white precipitate formed indicate the presence of tannins and phenolic compounds.

Ferric Chloride Test:
To the extract, few drops of neutral 5% ferric chloride solution are added. A dark green colour indicates the presence of tannins and phenolic compounds.

c) Detection of glycosides
Legal's test:
To the extract, few drops of 10% NaOH are added to make it alkaline. Then freshly prepared sodium nitroprusside is added to the solution. Presence of blue coloration indicates the presence of glycosides in the extract.

d) Detection of steroids and Triterpenoids
Libermann Burchard's Test:
Extract is treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulfuric acid is added from the sides of the test tube, shows a brown ring at the junction of two layers and the upper layer turning green shows the presence of steroids and formation of deep red colour indicate the presence of triterpenoids.

Salkowski test:
Extract is treated with few drops of conc. sulfuric acid, shaken well and allowed to stand for some time, red color at the lower layer indicate the presence of steroids and formation of yellow colored lower layer indicate the presence of Triterpenoids.

e) Detection of Saponins
Frothing test:
Add 2 mL of extract in a test tube and were shaken vigorously to obtain a stable persistent froth. Mixing of two drops of olive oil in the froth allowed for the formation of an emulsion, which indicated the presence of saponins.

f) Detection of flavonoids
Shinoda test:
To the test solution, few fragments of Magnesium ribbon are added and concentrated Hydrochloric acid is added drop wise, pink scarlet, crimson red or occasionally green to blue color appears after few minutes.

Alkaline reagent test:
To the test solution few drops of sodium hydroxide solution is added; formation of an intense yellow color, which turn colourless on addition of few drops of dilute acid indicate the presence of flavonoids.

g) Detection of carbohydrates
Molisch's test:
To 2 ml of extract, two drops of alcoholic solution of □-naphthol is added, the mixture is shaken well and 1 ml of conc. H_2SO_4 is added slowly along the sides of the test tube and allowed to stand. A violet ring indicates the presence of carbohydrates.

Fehling's test:
1ml of Fehling solution A and 1ml of Fehling solution B mixed and boiled for 1 minute and then equal volume of test solution is added to the above mixture. This is followed by heating the above preparation in boiling water bath for 5-*10 minutes*. Formation of yellow and then brick red precipitate indicates the presence of carbohydrates

h) Detection of Proteins and Amino acids
Biuret Test:
2 ml of extract is heated with 1 drop of 2 % CuSO4 solution. To this 1 ml of ethanol (95%) is added, followed by excess of KOH pellets. Pink colour in the ethanolic layers indicates the presence of proteins.

Millon's Test:
To 2 ml extract, few drops of Millon's reagent are added. A white precipitate indicates the presence of proteins.

GC-MS screening for volatile bioactive compounds
GC-MS screening of methanolic leaf extracts of *K. caryophyllata* are carried out using GC Agilent Technologies (Model – 5975C) system interfaced to a mass spectrometer (GC-MS) instrument (MS 7890A) employing the following conditions: column DB5-MS fused silica capillary column (30 X 0.25 mm ID X 0.25 mm film thickness, composed of 5% Phenyl, 95% Dimethyl Polysiloxane), operating in electron impact mode at 70 eV, helium (99.999%) is used as carrier gas at a constant flow of 1 mL/min, injector temperature 250°C; ion-source temperature 150°C. The oven is programmed with initial temperature 40°C for 5 min, with an increase of 5°C/min, to 280°C hold for 10 Min. Mass spectra is taken at 70 eV, a scan interval of 0.2 s and fragments are scanned from 50 to 550 Da. Total GC running time was 63 minutes. The constituents were identified after comparison with those available in the Computer Library (NIST ver. 2.1) attached to the GC-MS instrument and reported.

RESULTS
The details obtained from the qualitative phytochemical investigation in the methanolic leaf extract of *Kamettia caryophyllata* are depicted in table 1. The investigation showed the presence of major phytochemical groups like alkaloids, phenolics, glycosides, saponins, flavonoids, tannins and carbohydrates. This is a clear indication that the plant may have some potent therapeutic properties useful for the treatment of various human ailments. The phytochemicals like phenolics, flavonoids and tannins are gaining more attention by the researchers in the recent times due to their influence in the biological activities and their potentiality in preventing human diseases. The phenolic compounds are well known for their role in plant defense mechanisms by eliminating harmful bacteria and parasites (Sofowora, 1993). Flavanoids are usually synthesized in plants in response to microbial attack and reported to have an effective antimicrobial activity against a wide array of pathogenic microorganisms (Harborne, 1973). Flavonoids are also reported to have anti-inflammatory, anti allergic and strong anticancer activities (Ndukwe & Ikpeama, 2013). The phytoconstituent tannins are also gaining immense interest due to anti-viral, anti-tumor, anti-inflammatory and anti-ulcer effects. This ability of tannins has been attributed to their antioxidant properties (Prieto *et al*., 1999). The antimicrobial property of tannins may be due to their ability to specifically interact with vital proteins and carbohydrates, causing considerable reduction in the digestibility of these macromolecules which has inhibitory effect on *microbial* growth (Nwogu *et al*., 2008). Further tannins are known to have astringent properties, hasten the healing of wounds and inflamed mucous membranes (Ndukwe & Ikpeama, 2013). Alkaloids can be considered as one of the most efficient and therapeutically significant plant constituent having wide applications in medicine for the development of drugs. Alkaloids are known to be a good source of free radical scavengers (Jang *et al*., 2009) having analgesic, antispasmodiac, antihypertensive and bactericidal properties (Stray, 1998). Saponins are generally considered as antinutrients as they are able to decrease the uptake of certain nutrients including glucose and cholesterol. However saponins are believed to have human health promoting effects by lowering cholesterols (Gomathy *et al*., 2012). Saponins are also reported to have significant therapeutic values because of their relationship with compounds such as diuretic steroids, sex hormones, cortisones, vitamin D etc (Evans &Saunders, 2001). Besides, saponins have anti cancer, antitumor and anti-inflammatory properties (Ndukwe & Ikpeama, 2013). Glycosides is an important phytoconstituent containing a carbohydrate and non-carbohydrate residue in the same molecule and their biological effects such as antioxidant, antidiabetic, anti-viral, anticancer, antiallergic and anti-inflammatory activities have been reported (Loew & Kaszkin, 2002; Priyanga *et al*., 2014). Carbohydrates are the energy reserves of plants formed from photosynthesis, which when ingested, combine with oxygen and provide energy for vital processes. Plants containing carbohydrates, glycosides etc. are known to exert a beneficial action on our immune system by

increasing body strength and hence are valuable as dietary supplements.

The gas chromatography coupled with mass spectrometry (GC-MS) analysis performed in many ethno botanical plants revealed that they are rich sources of many secondary metabolites and these metabolites have wide range of influence on the biological activities on physiological systems (Olagunju *et al*., 2006). Neha & Patni (2013) revealed the presence of about twenty one phytocompounds in the GC-MS analysis of methanolic extract of *Woodfordia* while Santhosh et al. (2014) identified about thirty seven phytocompounds in the methanolic extract of *Adiantum capillusveneris.* The GC-MS analysis performed in the leaf methanolic extract of *K. caryophyllata* in the present study showed the presence of thirty nine bioactive compounds that might contribute to the medicinal property of the plant. The name of bioactive compounds with their peak number, retention time (RT), percentage of peak area, molecular formula and molecular weight are depicted in the table 2. The major compounds that were identified in the leaf component of *K. caryophyllata* based on peak area percentage includes p-Dioxane-2,3-diol (11.389%); Diethyl Phthalate (9.982%); cis-Vaccenic acid (9.873%); 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester (9.654%); n-Hexadecanoic acid (7.284%); Octadecanoic acid (5.983%); Phytol (5.818%); trans-13-Octadecenoic acid, methyl ester (3.957%); Hexadecanoic acid, methyl ester (3.477%); 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester (3.3.46%); Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (3.017%); 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (3.012%) and □-Sitosterol (2.036%).

The substances that inhibit the growth of pathogens and least toxic to host cells can be considered as good candidates for the development of new antimicrobial drugs. The detection and identification of specific bioactive compounds like diethyl phthalate; pentadecanoic acid methyl ester; phytol; octadecanoic acid; octadecanoic acid methyl ester; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; 9-hexadecenoic acid, methyl ester, (Z)-; cis-Vaccenic acid etc. in the GC-MS analysis of methanolic leaf extract of *K.caryophyllata* confirms its antimicrobial properties and the possibility of the use of the plant in the development of new antimicrobial drugs or in enhancing the efficiency of already existing antimicrobial drugs through synergistic effects using the antimicrobial principles present in the plant. The investigation further found that the methanolic leaf extract contains compounds like Methyl tetradecanoate; dl-□-Tocopherol; Tetradecanoic acid; 9,12-Octadecadienoic acid (Z,Z)-, methyl ester; trans-13-Octadecenoic acid, methyl ester; Squalene; Dodecanoic acid; Dodecanoic acid, methyl ester; Hexadecanoic acid, methyl ester; n-Hexadecanoic acid; Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester; Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester; p-Dioxane-2,3-diol etc. Presence of these bioactive compounds indicates that the plant also has good antioxidant and anticancer properties and therefore *K.caryophyllata* may be useful in the development of antioxidant and anticancer therapeutics in the future.

The bioactive compounds like dl-□-Tocopherol; □-Sitosterol; 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol; trans-13-Octadecenoic acid, methyl ester; Phytol; 9, 12-Octadecadienoic acid (Z,Z)-; cis-Vaccenic acid etc. are reported to have anti-inflammatory activities and their presence in the leaf component of *K.caryophyllata* indicate that the plant possess anti-inflammatory properties. According to Wang et al. (2008), the presence of tannins, phenolics and flavanoids have been associated with various degrees of anti-inflammatory and analgesic activities. The GC-MS analysis further reveals that the leaf component of *K.caryophyllata* possess hypocholesterolemic activity due to the presence of bioactive compounds like Methyl tetradecanoate; Tetradecanoic acid; Hexadecanoic acid, methyl ester; n-Hexadecanoic acid; trans-13-Octadecenoic acid, methyl ester; Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester and dl-□-Tocopherol (Lalitharani, 2009; Ravikumar *et al*., 2012; Krishnamoorthy &Subramaniam, 2014; Markkas & Madhuramozhi, 2015).

Table 1: Preliminary qualitative phytochemical screening of methanolic leaf extract of *Kamettia caryophyllata*

Sl. No	Phytochemical constituents	Chemical test(s)	Results		
			R1	R2	R3
1	Alkaloids	Dragendroff's test	+	+	+
		Mayer s test	+	+	+
		Wagner's test	+	+	+
2	Phenolics	Lead Actetate test	+	+	+
		Ferric Chloride test	+	+	+
3	Glycosides	Legal's test	+	+	+
4	Steroids	Salkowski test	-	-	-
		Libermann Burchard s test	-	-	-
5	Triterpenoids	Salkowski test	-	-	-
		Libermann Burchard s test	-	-	-
6	Saponins	Frothing test	+	+	+
7	Flavonoids	Shinoda test	+	+	+
		Alkaline reagent test	+	+	+
8	Tannins	Ferric Chloride test	+	+	+
		Lead Actetate test	+	+	+
9	Carbohydrates	Benedict's test	+	+	+
		Molisch s test	+	+	+
10	Proteins and Amino acids	Biuret test	-	-	-
		Millon s test	-	-	-

(+) indicate present (-) indicate absent

Table 2: GC-MS analysis of leaf methanolic extract of *Kamettia caryophyllata* showing the details of bioactive compounds

Peak	Retention Time (min)	Compound name	Molecular formula	Molecular weight (g/mol)	Peak area%
1	5.458	1,3,5,7-Tetroxane	$C_4H_8O_4$	120.104	1.891
2	6.122	Benzene, [(methylsulfinyl) methyl]-	$C_8H_{10}OS$	154.23	1.504
3	6.735	p-Dioxane-2,3-diol	$C_4H_8O_4$	120.104	11.389
4	7.675	Propanoic acid, 2-oxo-, methyl ester	$C_4H_6O_3$	102.09	0.746
5	8.708	2-Pentanone, 4-hydroxy-4-methyl-	$C_6H_{12}O_2$	116.16	0.420
6	13.699	2-Hydroxy-gamma-butyrolactone	$C_4H_6O_3$	102.09	0.753
7	24.110	Eugenol	$C_{10}H_{12}O_2$	164.201	1.027
8	28.310	Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	214.349	0.293
9	29.487	Dodecanoic acid	$C_{12}H_{24}O_2$	200.322	0.318
10	29.930	Diethyl Phthalate	$C_{12}H_{14}O_4$	222.24	0.312
11	31.855	Diethyl Phthalate	$C_{12}H_{14}O_4$	222.34	9.982
12	32.917	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242.397	0.500
13	33.899	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.371	0.685
14	35.053	Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	256.424	0.271
15	35.266	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.539	0.820
16	36.143	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.539	0.352
17	36.601	9-Hexadecenoic acid, methyl ester, (Z)-	$C_{17}H_{32}O_2$	268.435	0.615

18	37.093	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	3.477
19	37.989	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.539	7.284
20	40.278	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294.479	3.012
21	40.406	trans-13-Octadecenoic acid, methyl estr	$C_{19}H_{36}O_2$	296.495	3.957
22	40.606	Phytol	$C_{20}H_{40}O$	296.539	5.818
23	40.898	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298.504	0.953
24	41.145	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.445	2.807
25	41.284	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.468	9.873
26	41.690	Octadecanoic acid	$C_{18}H_{36}O_2$	284.477	5.983
27	42.798	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.445	0.832
28	43.876	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{35}H_{68}O_5$	568.924	0.813
29	45.396	Hexanedioic acid, bis(2-ethylhexyl) ester	$C_{22}H_{42}O_4$	370.574	0.900
30	46.775	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{40}O_4$	356.547	3.346
31	47.451	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330.509	3.017
32	47.646	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390.564	0.936
33	49.366	9-Octadecenoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester	$C_{27}H_{56}O_4Si_2$	500.902	0.344
34	49.436	Heptanoic acid, docosyl ester	$C_{29}H_{58}O_2$	438.789	0.730
35	50.180	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{40}O_4$	356.547	9.654
36	51.724	Squalene	$C_{30}H_{50}$	410.739	0.431
37	56.124	Tetratetracontane	$C_{44}H_{90}$	619.184	1.298
38	56.649	dl-α-Tocopherol	$C_{29}H_{50}O_2$	430.717	0.623
39	60.761	□-Sitosterol	$C_{29}H_{50}O$	414.707	2.036

Table 3: Nature and biological activity of bioactive compounds detected in the GC-MS analysis of leaf methanolic extract of *Kamettia caryophyllata*

Name of compound	Molecular formula	Nature of compound	Bioactivity
1,3,5,7-Tetroxane	$C_4H_8O_4$	Heterocyclic cholic acid derivatives	Antimalarial, non-central analgesic, antipyretic or antiinflammatory agents (http://pubchem.ncbi.nlm.nih.gov/compounds/bis)
Benzene, [(methylsulfinyl)methyl]-	$C_8H_{10}OS$	Phenolic compound	Antiallergic, antiparasitic, antibacterial, antiasthmatic. (Dr.Duke's phytochemical and Ethnobotanical Database)
p-Dioxane-2,3-diol	$C_4H_8O_4$	Dialdehyde	Anticancer, anticarcinomic, antidote pancreaprotective, antiasthmatic, (Dr. Duke's Phytochemical and Ethnobotanical Database)
Propanoic acid, 2-oxo-, methyl ester	$C_4H_6O_3$	Pyruvic acid methyl ester	Acidifier, acidulant, inhibit production of uric acid (Dr.Duke's Phytochemical and Ethnobotanical Database)
2-Pentanone, 4-hydroxy-4-methyl-	$C_6H_{12}O_2$	Beta-hydroxy ketone	Inhibitor of 17-beta-hydroxysteroid dehydrogenase, inducer of Testosterone-Hydroxylase, (Dr.Duke's Phytochemical and Ethnobotanical Database)
2-Hydroxy-gamma-butyrolactone	$C_4H_6O_3$	Carbonyl compounds	analgesic, antibacterial and anti-diabetic (Moorthy & Boominathan, 2011)
Eugenol	$C_{10}H_{12}O_2$	Phenyl propanoids	Antioxidative property, sexual attractants (Gupta et al., 2002; Silva et al., 2003).
Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	Fatty acid ester	Antioxidant activity (Lalitharani, 2009)
Dodecanoic acid	$C_{12}H_{24}O_2$	Saturated fatty acid	Antioxidant activity (Lalitharani, 2009)
Diethyl Phthalate	$C_{12}H_{14}O_4$	Diethyl ester	Antimicrobial (Premjanu &Jayanthy, 2014)
Diethyl Phthalate	$C_{12}H_{14}O_4$	Diethyl ester	Antimicrobial (Premjanu &Jayanthy, 2014)
Methyl tetradecanoate	$C_{15}H_{30}O_2$	Fatty acid ester	Antioxidant,cancer-preventive, hypercholesterolemic, lubricant, nematicide (Ravikumar et al., 2012)
Tetradecanoic acid	$C_{14}H_{28}O_2$	Saturated fatty acid	Antioxidant activity, anticancer, hypocholesterolemic, nematicide (Lalitharani, 2009; Santhosh etal., 2014)
Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	Fattyacid ester	Antimicrobial, antifungal (Belakhdar et al., 2015)
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	Acyclic diterpene	Antimicrobial, anti-inflammatory (Lalitha et al., 2014)
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	Acyclic diterpene	Antimicrobial, anti-inflammatory (Lalitha et al., 2014)
9-Hexadecenoic acid, methyl ester, (Z)-	$C_{17}H_{32}O_2$	Palmitic acid Ester	Antibacterial and antifungal (Dr. Duke's Phytochemical and Ethnobotanical Database)
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	Palmitic acid Ester	Antioxidant, hypocholesterolenic, antiandrogenic, flavor, nematicide (Lalitha et al., 2014)
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	Palmitic acid	Antioxidant activity, nematicide, , hypocholesterolemic, antiandrogenic, Hemolytic (Lalitharani, 2009; Santhosh et al., 2014)
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	Fatty acid ester	Anti-cancer (Yu et al., 2005)
trans-13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	Fatty acid ester	Anti-inflammatory, antileukotriene-D4, hypocholesterolemic, cancer preventive (Krishnamoorthy & Subramaniam, 2014)
Phytol	$C_{20}H_{40}O$	Acyclic diterpene	Antimicrobial, antiinflammatory, diuretic, anticancer (Lalitha et al., 2014; Santhosh et al., 2014)

Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	Fatty acid ester	Antimicrobial (Belakhdar et al., 2015)
9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$		Anti-inflammatory and antiarthritic (Jones, 2002)
cis-Vaccenic acid	$C_{18}H_{34}O_2$	Omega-7fatty acid	Antivirus substance and inactivation of T5 phage (Hirotani et al., 1991), Anti-inflammatory (Haider et al., 2016)
Octadecanoic acid	$C_{18}H_{36}O_2$	Saturated fatty acid	Antimicrobial activity (Rahuman, 2000)
9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	Unsaturated fatty acid	Anti-inflammatory and antiarthritic (Jones, 2002)
Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{35}H_{68}O_5$	Fatty acid ester	Antioxidant, hypocholesterolemic, antiandrogenic, hemolytic,Alpha reductase inhibitor (Markkas & Madhuramozhi, 2015)
Hexanedioic acid, bis(2-ethylhexyl) ester	$C_{22}H_{42}O_4$	Fattyacid ester	1. Acidifier, acidulant, antiuric acid production (Dr. Duke's Phytochemical and Ethnobotanical Database)
9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{40}O_4$	Fatty acid ester	Inhibition of proliferative effect in keloid fibroblast(Godswill et al.,2014)
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	Fatty acid ester	Hemolytic, pesticide, antioxidant (Gnanavel & Mary Saral, 2013).
1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	Dicarboxylic ester	plasticizer (Dr. Duke's Phytochemical and Ethnobotanical Database)
9-Octadecenoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester	$C_{27}H_{56}O_4Si_2$	Oleic acid propyl ester	Urine acidifier, urinary acidulant, antidote, anti HIV integrase, improve cerebral hypoxia (Dr. Duke's Phytochemical and Ethnobotanical Database)
Heptanoic acid, docosyl ester	$C_{29}H_{58}O_2$	Acid methyl ester	Acidifier, acidulant, arachidonic acid inhibitor, inhibit production of uric acid (Dr. Duke's Phytochemical and Ethnobotanical Database)
9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{21}H_{40}O_4$	Fatty acid ester	Inhibition of proliferative effect in keloid fibroblast (Godswill et al.,2014)
Squalene	$C_{30}H_{50}$	Triterpene alcohol	Antitumor, cancer preventive, pesticide,immunostimulant, chemo preventive (Santhosh et al., 2014)
Tetratetracontane	$C_{44}H_{90}$	Long chain alkane	Hypoglycaemic, antioxidant activities (Sivakumar & Gayathri, 2015)
dl-□-Tocopherol	$C_{29}H_{50}O_2$	Fat soluble vitamin	Analgesic, antidiabatic, antitumor, antiinflammatory, antioxidant, antidermatitic, antiageing, anticancer, antileukemic, hypocholesterolemic (Rajalakshmi & Mohan,2016)
□-Sitosterol	$C_{29}H_{50}O$	Pentacyclic triterpenoids	Anti-inflammatory effect (Dr. Duke's Phytochemical and Ethnobotanical Database)

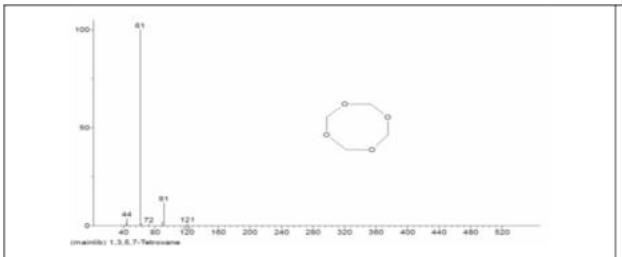

Fig. 1: Structure of 1, 3, 5, 7-Tetroxane with RT value 5.458 present in *Kamettia caryophyllata*.

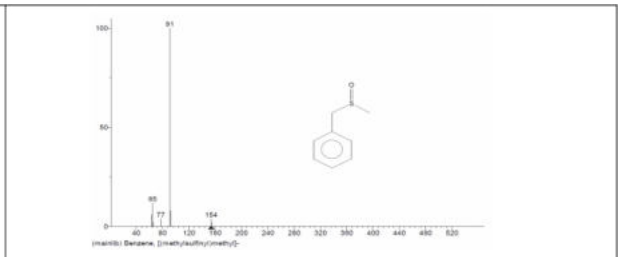

Fig. 2: Structure of Benzene, [(methylsulfinyl)methyl]- with RT value 6.122 present in *Kamettia caryophyllata*.

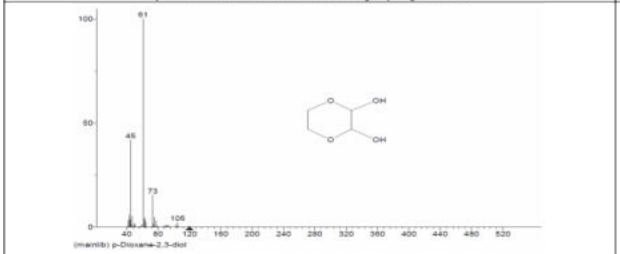

Fig. 3: Structure of p-Dioxane-2,3-diol with RT value 6.735 present in *Kamettia caryophyllata*.

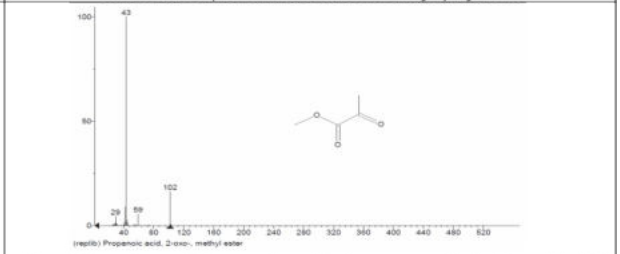

Fig. 4: Structure of Propanoic acid, 2-oxo-, methyl ester with RT value 7.675 present in *Kamettia caryophyllata*.

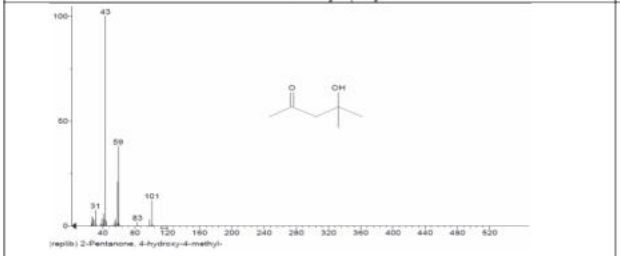

Fig. 5: Structure of 2-Pentanone, 4-hydroxy-4-methyl- with RT value 8.708 present in *Kamettia caryophyllata*.

Fig. 6: Structure of 2-Hydroxy-gamma-butyrolactone with RT value 13.699 present in *Kamettia caryophyllata*.

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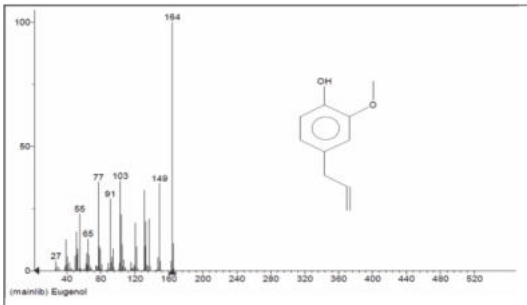

Fig. 7: Structure of Eugenol with RT value 24.110 present in *Kamettia caryophyllata*.

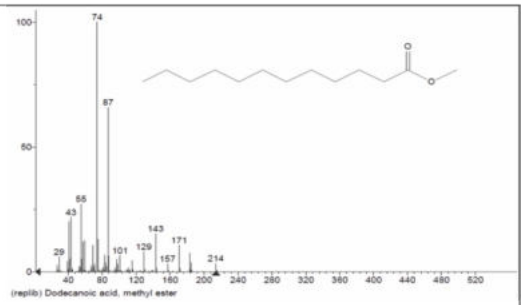

Fig. 8: Structure of Dodecanoic acid, methyl ester with RT value 28.310 present in *Kamettia caryophyllata*.

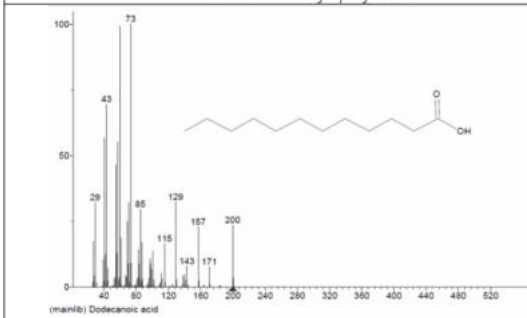

Fig. 9: Structure of Dodecanoic acid with RT value 29.487 present in *Kamettia caryophyllata*.

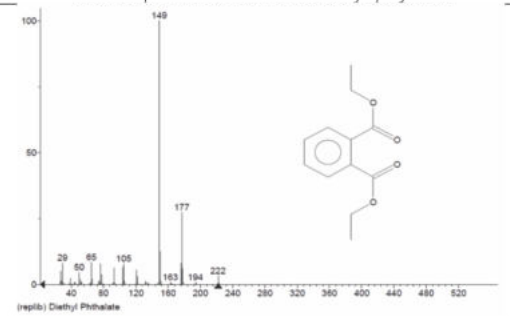

Fig. 10: Structure of Diethyl Phthalate with RT value 29.930 present in *Kamettia caryophyllata*.

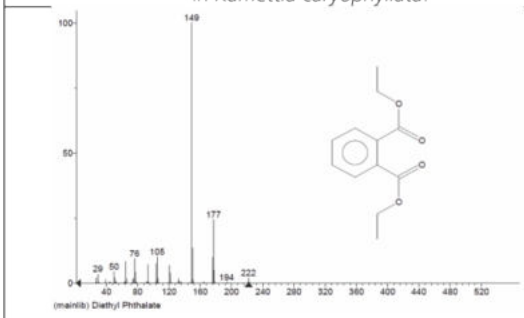

Fig. 11: Structure of Diethyl Phthalate with RT value 31.855 present in *Kamettia caryophyllata*.

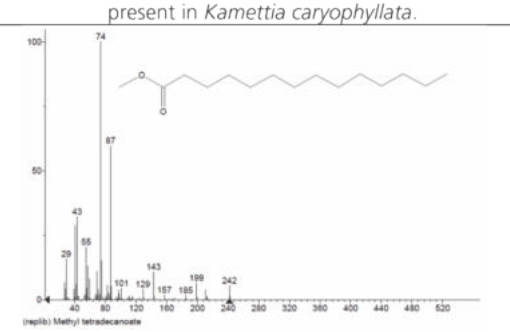

Fig. 12: Structure of Methyl tetradecanoate with RT value 32.917 present in *Kamettia caryophyllata*.

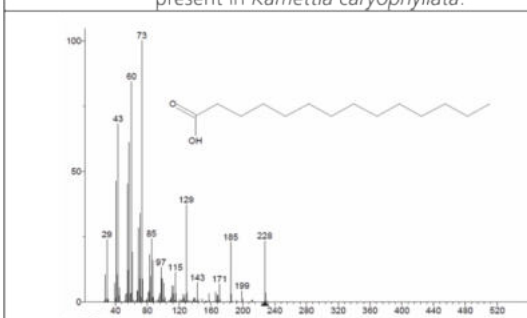

Fig. 13: Structure of Tetradecanoic acid with RT value 33.899 present in *Kamettia caryophyllata*.

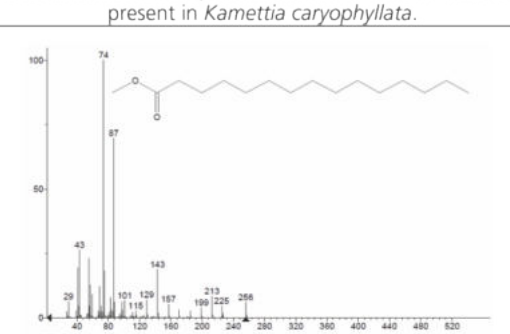

Fig. 14: Structure of Pentadecanoic acid, methyl ester with RT value 35.053 present in *Kamettia caryophyllata*.

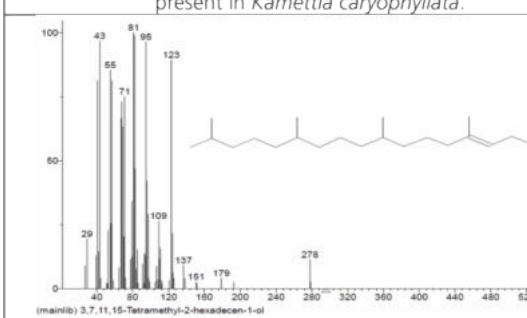

Fig. 15: Structure of 3,7,11,15-Tetramethyl-2-hexadecen-1-ol with RT value 35.266 present in *Kamettia caryophyllata*.

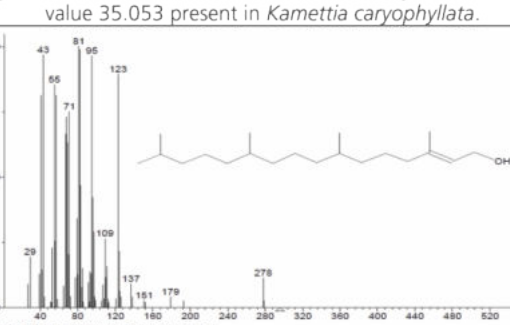

Fig. 16: Structure of 3,7,11,15-Tetramethyl-2-hexadecen-1-ol with RT value 36.143 present in *Kamettia caryophyllata*.

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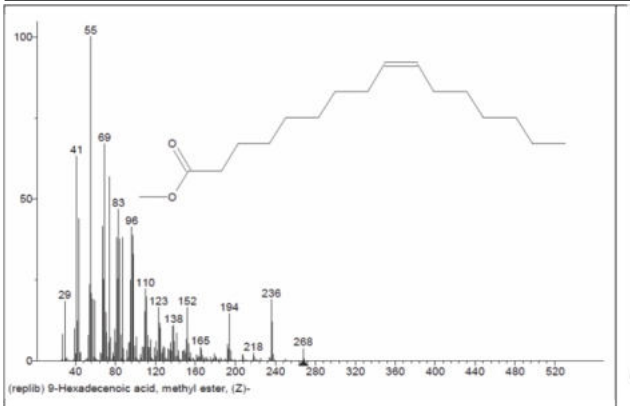

Fig. 17: Structure of 9-Hexadecenoic acid, methyl ester, (Z)- with RT value 36.601 present in *Kamettia caryophyllata*.

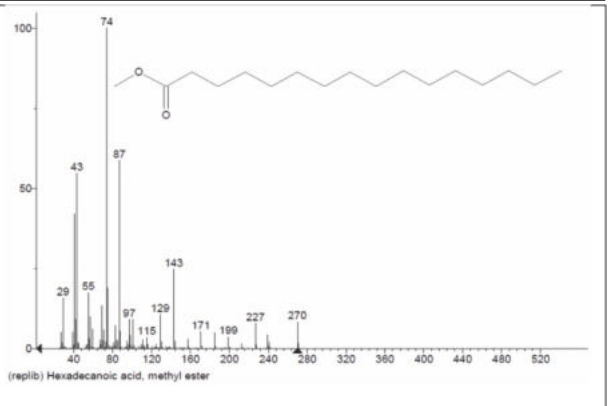

Fig. 18: Structure of Hexadecanoic acid, methyl ester with RT value 37.093 present in *Kamettia caryophyllata*.

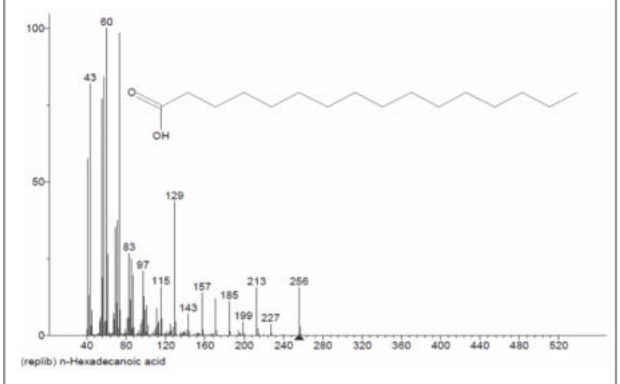

Fig. 19: Structure of n-Hexadecanoic acid with RT value 37.989 present in *Kamettia caryophyllata*.

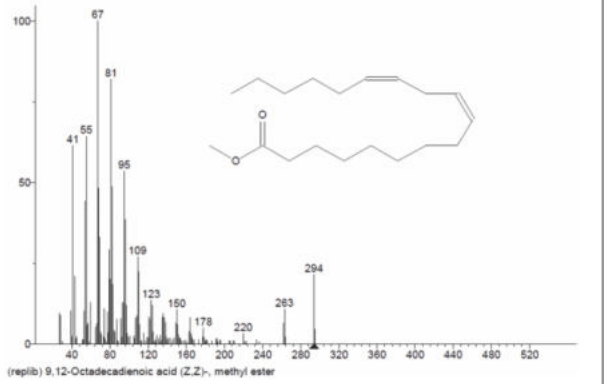

Fig. 20: Structure of 9,12-Octadecadienoic acid (Z,Z)-, methyl ester with RT value 40.278 present in *Kamettia caryophyllata*.

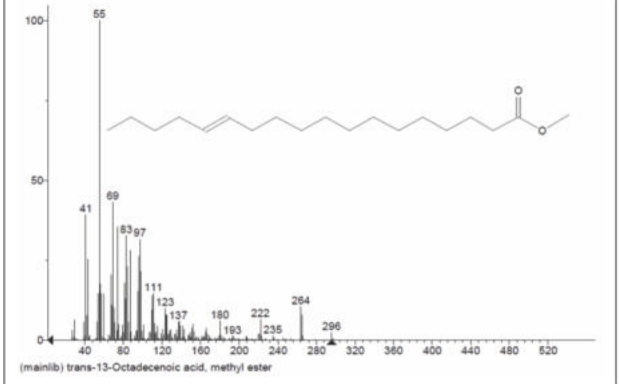

Fig. 21: Structure of trans-13-Octadecenoic acid, methyl ester with RT value 40.406 present in *Kamettia caryophyllata*.

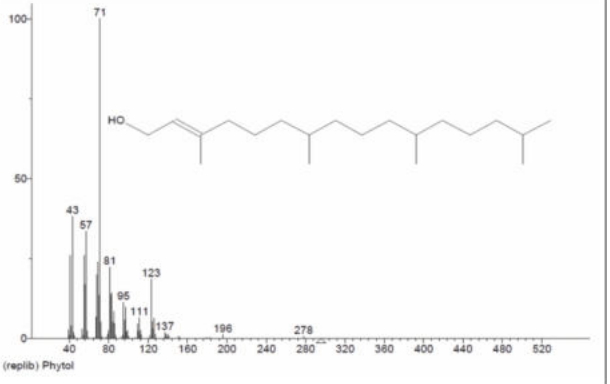

Fig. 22: Structure of Phytol with RT value 40.606 present in *Kamettia caryophyllata*.

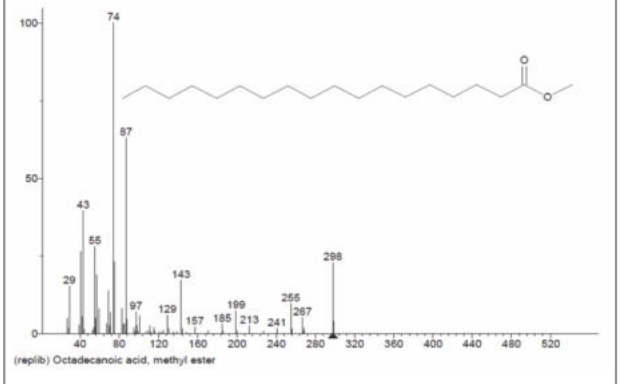

Fig. 23: Structure of Octadecanoic acid, methyl ester with RT value 40.898 present in *Kamettia caryophyllata*.

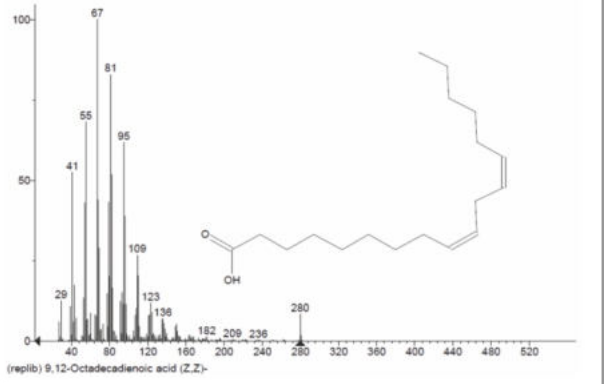

Fig. 24: Structure of 9,12-Octadecadienoic acid (Z,Z)- with RT value 41.145 present in *Kamettia caryophyllata*.

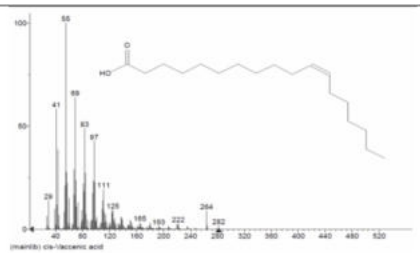

Fig. 25: Structure of cis-Vaccenic acid with RT value 41.284 present in *Kamettia caryophyllata*.

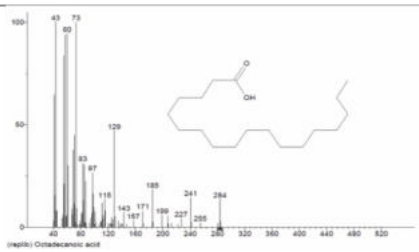

Fig. 26: Structure of Octadecanoic acid with RT value 41.690 present in *Kamettia caryophyllata*.

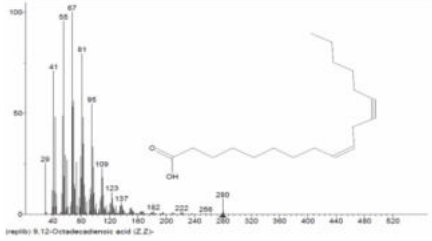

Fig. 27: Structure of 9,12-Octadecadienoic acid (Z,Z)- with RT value 42.798 present in *Kamettia caryophyllata*.37.98

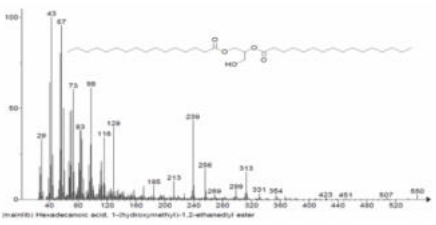

Fig. 28: Structure of Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester with RT value 43.876 present in *Kamettia caryophyllata*.

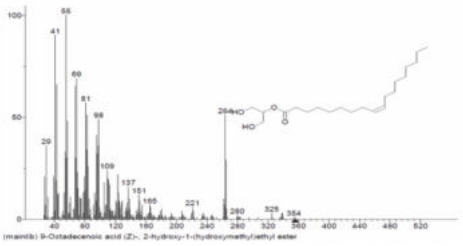

Fig. 29: Structure of Hexanedioic acid, bis(2-ethylhexyl) ester with RT value 45.396 present in *Kamettia caryophyllata*.

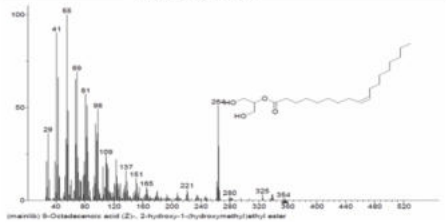

Fig. 30: Structure of 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester with RT value 46.775 present in *Kamettia caryophyllata*.

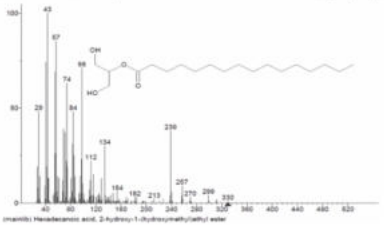

Fig. 31: Structure of Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester with RT value 47.451 present in *Kamettia caryophyllata*.

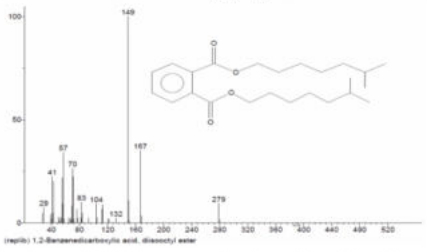

Fig. 32: Structure of 1,2-Benzenedicarboxylic acid, diisooctyl ester with RT value 47.646 present in *Kamettia caryophyllata*.

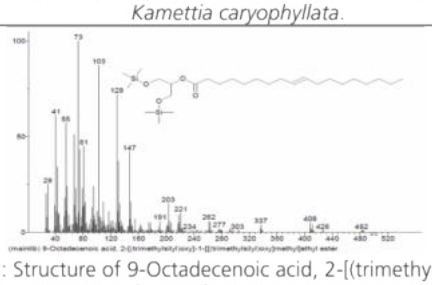

Fig. 33: Structure of 9-Octadecenoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl] ethyl ester with RT value 49.366 present in *Kamettia caryophyllata*.

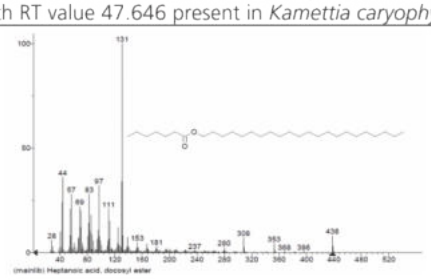

Fig. 34: Structure of Heptanoic acid, docosyl ester with RT value 49.436 present in *Kamettia caryophyllata*.

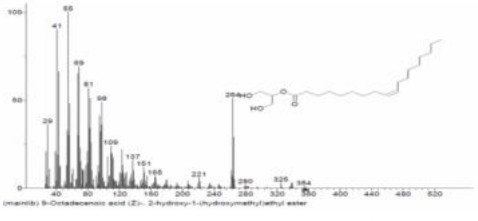

Fig. 35: Structure of 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester with RT value50.180 present in *Kamettia caryophyllata*.

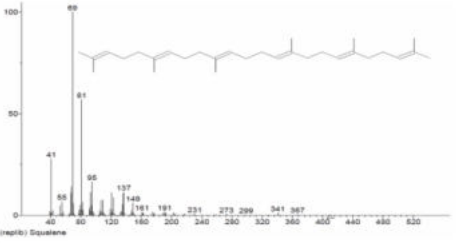

Fig. 36: Structure of Squalene with RT value 51.724 present in *Kamettia caryophyllata*.

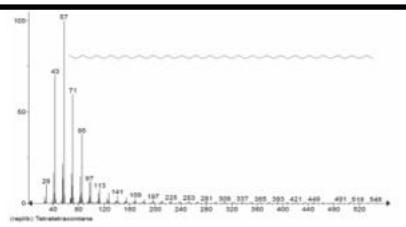

Fig. 37: Structure of Tetratetracontane with RT value 56.124 present in *Kamettia caryophyllata*.

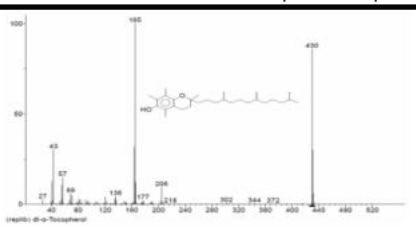

Fig. 38: Structure of dl-α-Tocopherol with RT value 56.649 present in *Kamettia caryophyllata*.

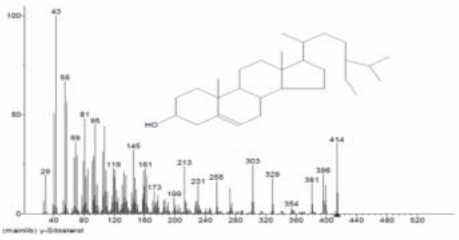

Fig. 39: Structure of γ-Sitosterol with RT value 60.761 present in *Kamettia caryophyllata*.

Figure 1-39: Spectral properties and structure of bioactive compounds detected in the GC-MS analysis of methanolic leaf extract of *Kamettia caryophyllata*

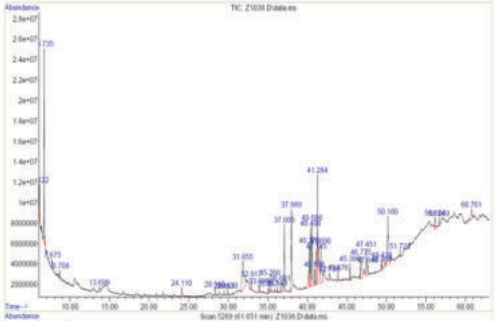

Figure 2: GC-MS chromatogram of methanolic leaf extract of *Kamettia caryophyllata*

CONCLUSION

The results of the present investigation indicate that *Kamettia caryophyllata* may be a good plant source of therapeutic values. The qualitative phytochemical screening and GC-MS analysis done on the leaf methanolic extract clearly revealed the presence of major phytoconstituents like flavonoids, tannins, alkaloids, phenolics, glycosides, saponins etc. and specific bioactive compounds like Diethyl Phthalate; Phytol; cis-Vaccenic acid; Decanoic and Dodecanoic acid compounds; dl- -Tocopherol; -Sitosterol; Squalene etc.. This kind of plant derived phytochemicals and bioactive compounds were previously confirmed as having different activities such as antioxidant, antimicrobial, anticancer, hypocholesterolemic , anti-inflammatory etc. in biological systems and are also used by pharmaceutical industries for drug formulation. Therefore the study concludes that the information regarding the biologically active principles present in the leaf component of *K. caryophyllata* will be useful for researchers and pharmaceutical industries that are involved in new active compound profiling and development of drugs against various diseases.

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