Chemical Compositions, FTIR and Larvicidal Activity of Essential Oils Extracted from Aromatic Plants

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Abstract

Essential oils are substances obtained from plant materials and are complex mixtures whose composition varies. In this study, the chemical composition, and larvicidal activity of extracted essential oils from some aromatic plants were evaluated. Modified steam distillation apparatus was used to extract essential oils from the *Citrus limon linn* leaf, *Vitex trifolia* seed and *Cananga odorata*. Furthermore, the plant materials were tested using FTIR, GC-MS, and larvicidal activity test. *Citrus limon linn* produced more amount of essential oil (1.4ml), *Vitex trifola* (0.7ml) and *Cananga odorata* (0.5ml) after 3hours of steam distillation. The result obtained from the infrared spectra indicates the functional groups of the compounds present in *Citrus limon linn*, *Vitex trifolia* and *Cananga odorata*. GC-MS analysis identified, 13, 12 and 24 chemical constituents present in *Citrus limon linn* , *Vitex trifolia* and *Cananga odorata* essential oil respectively. The three main essential oil and their percentage compositions are D-Limonene (22.82%), Citral (25.20 %), and Cis-Verbenol (20.43%) for *Citrus limon linn*. Leaf essential oil, Sabinene (39.14%), Caryophyllene (26.45%) and 1 R- α Pinene (15.20%) for *Vitex trifolia* seed essential oil, and Linalool (35.30%), Ascabiol (23.54%), Caryophyllene (20.30%), Copaene (12.76%) and Germacrene D (11.32%). However, the results from the larvicidal activity reveals that essential oil extracted from *Citrus limon linn, Vitex trifolia* and *Cananaga odorata* may serve as insecticide and can be used as potent insecticide for the control of mosquitoes.

Keyword: Larvicidal, Chemical, Aromatic, Essential oils, Fragmentation

Introduction

Medicinal and aromatic plants are important pillars of healthcare and traditional medicinal systems of the world (Ali-Shtayeh and Abu Ghdeib, 1999). A number of aromatics plants provide essential oils of unique aroma which is mainly used in flavours and fragrances, perfumery, cosmetics and pharmaceuticals (Moosavy and Shavisi, 2013).

However, an estimated 1,200 compounds, including terpenes and their corresponding aldehydes, ketones, alcohols, phenylpropanoids, hydrocarbons, esters, oxides and sulfur, have been identified in essential oils. In general, the constituents in essential oils are terpenes (monoterpenes and sesqutterpenes), aromatic compounds (aldehyde, alcohol, phenol, methoxy derivative, and so on), and terpenoids (isoprenoids) (Yahaya *et al.*, 2018). They contain volatile compounds with unique properties that have been prized worldwide for long period of time (Yahaya *et al.*, 2018).

Essential oils are used in a wide variety of consumer goods such as detergents, soaps, toilet products, cosmetics, pharmaceuticals, perfumes, confectionery food products, soft drinks, distilled alcoholic beverages (hard drinks) and insecticides. The world production and consumption of essential oils and perfumes are increasing very fast. Production technology is an essential element to improve the overall yield and quality of essential oil. The traditional technologies pertaining to essential oil processing are of great significance and are still being used in many parts of the globe. Water distillation, water and steam distillation, steam distillation, cohobation, maceration and enfleurage are the most traditional and commonly used methods. Maceration is adaptable when oil yield from distillation is poor. Distillation methods are good for powdered almonds, rose petals and rose blossoms, whereas solvent extraction is suitable for expensive, delicate and thermally unstable materials like jasmine, tuberose, and hyacinth. Water distillation is the most favored method of production of citronella oil from plant material.

2.0 Materials and Methods

2.1 Materials

2.1.1 *Plants Materials*

The leaves of *Citrus limon* leaves, Seeds of *Vitex trifolia* were collected from Girei Local government of Adamawa State, while the flower of *Cananga odorata* was obtained from Kaduna State in October 2016.

2.2 Methods

2.2.1 *Collection and preparation of plant materials*

Fresh leaves of *Citrus limon Linn*, seeds of *Vitex trifolia* and *Cananga odorata* flower were collected from Moddibo Adama University of Technology Yola, located in the North-Eastern part of the country and Unguwan Rimi, Doka district in Kaduna state respectively. The specimens were shade dried at room temperature to obtain a constant weight and then the samples were crushed to powder using an electric blender so as to enhance effective contact of solvent with sites on the plant materials.

2.2.2 *Preparation of plant extracts*

A portion (100g) of each powdered plant material that was shade dried was soaked in 300cm^3 of methanol for 24 hours. At the end of the extraction each extract was filtered using whatman filter paper under vacuum. The filtrates were further concentrated under vacuum at 40°C with rotary evaporator and stored at 4°C for further use (Runde *et al.*, 2015).

2.3 Extraction of Essential oils

The extraction of essential oils was carried out using steam distillation, adapting the method reported by (Runde *et al.*, 2015). The leaf of *Citrus limo linn, Cananga odorata* was chopped and the seed of *Vitex trifolia* was blend in other to have a good surface area. The samples were not air dried because of the fear of loss of some component(s) of the essential oil since they are highly volatile. A portion (800g) of the plant materials were placed in two ways round flasks fitted to round bottom flask containing 500cm^3 distilled water on a heating mantle. The volatile oils volatilized with the steam were condensed and made to pass through a collecting column. The oils were separated from steam in the receiver and the distillation process was carried out for a period of 3 hours. The collected oil was allowed to settled on top of deionized water in the receiver, which was later obtained with the aid of a sterilized syringe and the oil obtained was stored in 1.5ml centrifugal bottles in a refrigerator until they are required for use (Runde *et al.*, 2015). The percentage yield of oil was calculated by the following equation:

$$\text{Yield (\%)} = (\text{Oil (mL)}) / (\text{Plant (g)}) \times 100$$

2.3.1 Analysis of the essential oil

2.3.1.1 *Gas chromatography Mass spectrometry (GC/MS) analysis*

The composition analysis of essential oils obtained were carried out using an Agilent technologist gas chromatography mass spectrometry GC-MS (7890A GC system) unit, coupled with Agilent mass spectrometer (5975C inert MSD). J and W capillary column was used of 30m length and diameter of 0.250 mm with temperature limit ranging from -65°C to 325°C. 0.2µl quantity of sample was injected using splitless injection mode into the inlet at 250°C, and flow rate of 1ml/min. The oven temperature was programmed to start from 50°C and hold for 1min, and then increasing by 20°C to 300°C and hold for 8 min. The Ionization energy was 70ev in the electron ionization (EI) mode with a scan range of 50-500 amu and compositions of the essential oils were generated from the NBS75K library data base installed in the instrument. The retention indices (RI) are in relation to homologous series of n-alkanes on the GC column under the same chromatographic condition, and the component relative concentration was obtained by the peak area normalization (Ramzi *et al.*, 2013).

2.3.1.2 *Fourier Transform Infrared (FT-IR) Spectrometer*

Infrared spectrometry of the Essential Oils of *Vitex trifolia*, *Citrus limon* and *Cananga odorata* were carried out with a Buck scientific Infrared Spectrophotometer. The Essential Oils sample were placed directly on the surface of pair of rectangular sodium chloride plate at room temperature and the measurement were performed in the IR region at 4000-600 cm-^{1}. Two scans were performed at a speed of 3 cm/s for each essential oil and an air spectrum was used as reference (Wany *et al.*, 2014).

2.4 Determination of larvicidal activity of essential oil

2.4.1 *Test organisms*

The collection of mosquito larvae was performed in a breeding site located in the metropolis of Yola town. The larvae were collected using an improvised rectangular plastic semi permeable tray that inclined 45° to the water surface. Larvae harvested were maintained breeding in a 2000cm^3 conical flask with an average temperature of 26 - 28°C in the laboratory before the experiment was conducted.

2.4.2 *Preparation of the oil solution*

A sufficient amount of target oil was dissolved in normal saline using 2 ml of DMSO to produce a stock solution at 1000 ppm. This solution was used to prepared different concentration of target oil with stock solution and distilled water. Three replicates of each concentration were made. In addition to three replicates, a control containing 2ml of DMSO in normal saline was made (WHO. 1981).

2.4.3 *Bioassay Larvicidal*

Each replicate containing 100 ml of the described oil solution was placed in a 250 ml pyrex beaker. Five late 3rd instars larvae of target mosquitoes were transferred into each beaker. Three replicates were set up for each concentration, and an equal number of controls were also set up simultaneously using distilled water. After that, the beakers were left on the laboratory table for 5 hours where their motility was checked at different time interval. The total number of dead larvae in each beaker was counted after 5 hours (Mohtar *et al*., 1999).

3.0 Results and Discussions

3.1 Percentage yield of essential oils

Percentage yields of 0.175, 0.088 and 0.075 % (V/W) of essential oils of *Citrus limom* leaves, *Vitex trifolia* seeds and *Cananga odorata* flowers were obtained by the steam distillation method of essential oil extracted from 800 g of the plant materials respectively. The results obtained showed that *Citrus limon* leaves has the highest percentage yield of 0.175 %, followed by *Vitex trifolia* seeds of 0.088 % and *Cananga odorata* flowers with percentage yield of 0.075 %. Similar report shows that hydrodistillation of *Boweillia dalzielii* was reported to have yielded 1.25 % essential oils (Kubmarawa *et al.*, 2011). Another work was reported on the percentage yield of essential oils which varies with factors like site of collection, time of collection, part and form of plant used and the extraction method employed among other factors (Baser *et al*, 2010). Different percentage yield have been reported for leaves of *Ocimum americanus*, *Vosia cuspidata*, *Eucalyptus camaldulensis* and the stem bark of *Bosweilia dalzielii* to have 0.16 %, 0.05 %, 0.12 % and 0.12 % respectively (Runde *et al*., 2015). On the other hand, the percentage yield of the essential oils of leaves, stem barks and flowers of *Eucalyptus camaldulensis* obtained from Malaysia were 1.4, 0.5 and 0.46 % respectively (Elanaiem *et al*, 2015). Conversely, another research also identified the yields of essential oils of Citrus species were significantly ($p<0.05$) affected by drying treatments. The highest amount of the essential oil was obtained from oven-dried sample of *C. sinensis* peel (1.07 %) while minimum from fresh sample of *C. paradisii* peel (0.20 %). Major effect of drying on essential oil percentage was noted in *C. sinensis* (0.24-1.07 %) followed by *C. reticulate* (0.30-0.50 %) and *C. paradisii* (0.20-0.40 %) (Kamal *et al*., 2011). It was reported that more essential oils (20 ml) at 180 minutes of heating was extracted from 370 g of the orange peels than from equal mass of lemon and lime peel at the same time of heating, while lime had the least quantity of essential oils extracted (5 ml)

(Njoku *et al*., 2014). These observations are in agreement with the findings of Kamal *et al*., (2011), and the variation of essential oil yield from other related work to our work could be as a result of the environment condition before, during and after the extraction, because it was observed that the lesser the temperature of the environment during extraction the more quantity of essential oils would be obtained.

***Table 1**: Percentage Yield of Essential oils of various plants*

Plants	Plant parts and form	Volume (ml)	Appearance	% yield (V/W)
C. limon linn	Fresh leaves	1.4	Greenish yellow	0.175
V. trifolia	Fresh seeds	0.7	Yellow viscous	0.875
C. odorata	Fresh flowers	0.6	Colourless	0.075

3.2 Infrared Spectrum

3.2.1 Infrared spectrum interpretation of Citrus limon

The infrared spectra of *citrus limon linn* (figure 1) shows strong methylene/methyl band at (1442cm^{-1}) and a weak methyl band (1380cm^{-1}), plus a band at 705cm^{-1} (methylene rocking vibration) is indicative of a long-chain linear aliphatic structure. The saturated hydrocarbon C- H stretching absorptions occur at 2968cm^{-1}. The band structures observed between 3150 and 3000cm^{-1} are almost exclusively indicative of unsaturation (C=C- H) or aromatic rings and their absence in the infrared spectra obtained is thus indicative of the absence of aromatic compounds. Carbonyl compounds are often the strongest band in the spectrum and will lie between 1727 and 1452cm^{-1}.

3.2.2 *Infrared spectrum interpretation of Vitex trifolia*

In the infrared spectrum (Figure 2) of essential oil of *Vitex trifolia*, the functional groups of all of the components were observed. A broad band observed around 3400cm^{-1} is due to O-H stretch from alcoholic compounds (Vindiflorol, Terpineol). The peak at 2939cm^{-1} is a predominant asymmetric stretching of -CH3 which is observed corresponding to an alkyl saturated aliphatic group present in the compound. Also peaks at 1727 cm^{-1} and 1452 cm^{-1} indicated stretching of C=O of the aldehyde group and a bending of the -CH$_2$ group respectively. However, bending vibrations of -CH$_3$ group was observed around 1375cm^{-1}. Stretching of -C-O and vibrations of the -CH skeleton were observed between 1267 to 1023cm^{-1}.

3.2.3 *Infrared spectrum interpretation of Cananga odorata*

The infrared spectra of *Cananga odorata* (Figure 3) shows broad spectrum at 3464 cm^{-1} indicates O-H stretch of alcohol and the vibrations at 3000 -2869 cm-1 is a predominant asymmetric stretching of -CH_3 which is observed corresponding to an alkyl saturated aliphatic group present in the compound. However, the peak at 3076 indicates the presence of aromatic streach. The peak at 1726 cm^{-1} indicates stretching of C=O of the aldehyde group, while at 1451 cm^{-1} indicates bending of the -CH2 group and at 1379 cm^{-1} bending of -CH_3 group is observed. From 1267 to 1025 cm^{-1} indicates the stretching of -C-O, and also the peak around 1634 cm^{-1} confirms the presence of aromatics functional group.

Similar studies also pointed out the major component of lemon and orange essential oils are limonene. Limonene constitutes more than 90 % of the essential oil extracted from orange peel, but this percentage decreases for lemon to values slightly below 70 % (Wei *et al.*, 2010). By considering these high contents of limonene in the essential oils of lemon and orange, it is easy to deduce that the Raman spectra of these essential oils are dominated by Raman bands of limonene. The main features in the Raman spectrum of limonene appear at 1678, 1645, 1435 and 760 cm^{-1}, similar bands are clearly identifiable in the spectrum of lemon and orange essential oils, The two strong bands in the region 1680–1640 cm^{-1} are attributed to C=C stretching modes. Two bands are expected for the C=C stretching, because limonene has two double bonds in its chemical structure. The strong and broad band centered at ~1433 cm^{-1} is assigned to the CH_3 / CH_2 bending mode, while the intense signal at 763 and 757 cm^{-1} (for bitter/sweet orange and lemon essential oils, respectively) can be attributed to a ring deformation mode of limonene (Schulz *et al.*, 2007). A similar work was conducted on basil essential oils were most of the important bands in the Raman spectrum of basil essential oils, linalool type, appear at 1672, 1640, 1452, 1378, 1293, 803 and 651 cm^{-1}, these bands are similar to those observed in an earlier reported Raman spectrum of linalool (Daferera *et al*., 2002). The strong bands at 1672 and 1640 cm^{-1} are attributed to C=C stretching modes and the band at 1452 cm^{-1} appears in the typical region for CH_3/CH_2 bending modes. There are two CH3 groups directly attached to a C=C in the chemical structure of linalool; therefore, the band at 1378 cm^{-1} can be assigned to a CH_3 bending mode. The band at 1293 cm^{-1} is assigned to the =CH rocking mode, and the band at 803 cm^{-1} seems to be related to the OH group of linalool. The band 651 cm^{-1} seems to indicate the presence of camphor in this essential oil, since a very strong band in this region is expected for the ring deformation of this compound (Daferera *et al.*, 2002).

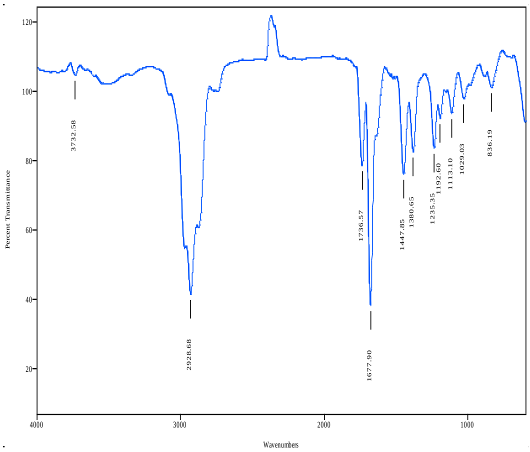


***Figure 1**: Shows the Infrared spectrum of Citrus lemon leaves*

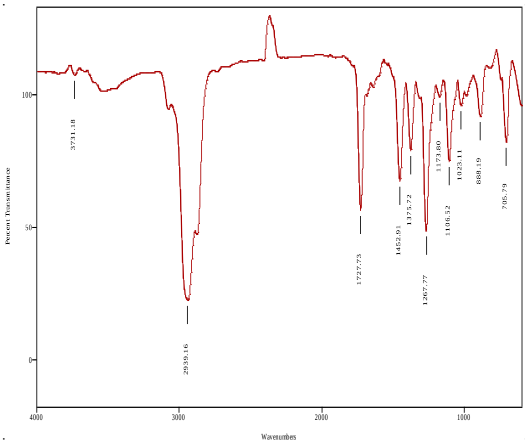


***Figure 2**: Shows the Infrared spectrum of Vitex trifolia seeds*

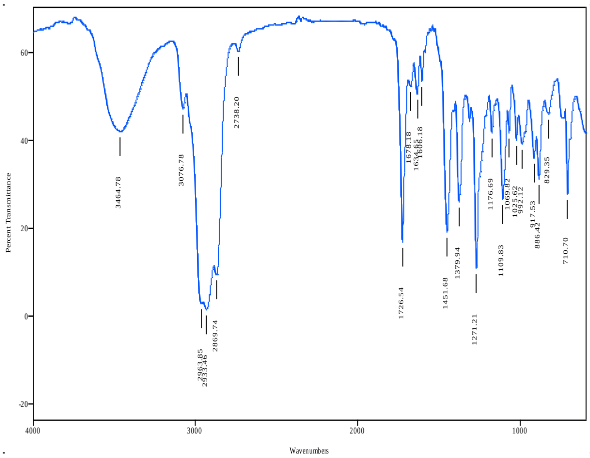


***Figure 3**: Shows the Infrared spectrum of Cananga odorata flowers*

3.4 GC-MS analysis of essential oils from *Citrus limon linn* leave

The *Citrus limon linn* leaves essential oils contained a total of 10 components. Table 2 showed the total components of the essential oils from *Citrus limon* leaves, their retention times, percent area and molecular weights. The three highest occurring components in *Citrus limon linn leaves* essential oils are α- Citral (25.20 %), D-limonene (22.82 %), Cis-Verbenol (20.43 %) and Nerol acetate (10.73 %). The amount of *Citrus limon* leaves essential oils for D-limonene and β-pinene is in agreement with the work conducted on lemon peel by Soumaya, *et al*. (2012). Findings shows that D-limonene (27.63 - 69.71 %), β- pinene (0.63 - 31.49 %), γ-terpene (0.04 - 9.96 %), and p-cymene (0.23 - 9.84 %) were the highest ones in lemon (Soumaya, *et al*. 2012). Similar work was also carried out in which three highest occurring components in orange peel essential oil where obtained, which are D-limonene (54.15 %), pulegone (11.65 %) and L-carvone (2-cyclohexen-1-one) (5.46 %) (Njoku *et al.*, 2014). Vekiari *et al*. (2002) also reported that the main components of Citrus essential oils were limonene, β-pinene, myrcene, neral, geranial, neryl acetate and β-caryophyllene. Lota *et al*., (2001) found limonene and α-pinene as the main compounds in the peel oils of sour orange. Similar work also was carried out on essential oils from the peels of Malta (*C. sinensis*), Mousami (*C. sinensis*), Grapefruit (*C. paradisi*) and *Eureka lemon* (*C. limon*) through cold pressing method. According to them the main constituents detected in Malta peel oil were limonene (61.08 %), citronellol (4.18 %), citral (7.74 %), borneol (7.63 %), α-terpinolene (2.06 %) and linalool (1.28 %). The principal compounds in Mousami essential oils were limonene (76.28 %), α-pinene (1.26 %), β-pinene (5.45 %), citral (1.74 %), and linalool (2.32 %) while limonene (86.27 %), myrcene (6.28 %), γ-terpinene (2.11 %) and α-pinene (1.26 %) in Grapefruit essential oils (Ahmad *et al*., 2006). However, when compared to what we obtained in table 3 it can be deduce what we obtained is in line with previous work conducted by others findings.

3.4.1 *Fragmentation pattern of Limonene*

The fragmentation of Limonene begins with the formation of molecular ion of the compound resulting from the loss of electron due to the bombarding effect of high energy electrons presence in the ionization chamber. The molecular ion peak appears at m/z 136. The removal of methyl group lead to the appearance of a peak at m/z 121; the subsequent loss of an ethyl group (CH_3CH_2) resulted to the appearance of another peak at m/z 107. The appearance of a peak at m/z 93 is a clear indication that a propyl ($CH_3CH_2CH_2$) group is removed. Other smaller peaks could possibly have resulted from some unidentified groups or atoms such as the peak at m/z 89 and 81. However, the peak at m/z 79 corresponded to the loss of butanyl group ($CH_2CH_2CH_2CH_3$). The next peak is the most intense peak called the base peak which appeared at m/z 68. Below are equations showing the fragmentation processes of Limonene and mass spec. (Figure 4).

$$C_{10}H_{16} \xrightarrow{-e^-} \underset{m/z\ 136}{C_{10}H_{16}^{+}}$$

$$C_{10}H_{16}^{+} \longrightarrow \underset{m/z\ 121}{C_9H_{13}^{+}} + {}^{\cdot}CH_3$$

$$C_{10}H_{16}^{+} \longrightarrow \underset{m/z\ 107}{C_8H_{11}^{+}} + {}^{\cdot}C_2H_5$$

$$C_{10}H_{16}^{+} \longrightarrow \underset{m/z\ 93}{C_7H_9^{+}} + {}^{\cdot}C_3H_7$$

$$C_{10}H_{16}^{+} \longrightarrow \underset{m/z\ 79}{C_6H_7^{+}} + {}^{\cdot}C_4H_9$$

$$C_{10}H_{16}^{+} \longrightarrow \underset{m/z\ 68}{C_5H_8^{+}} + {}^{\cdot}C_5H_8$$

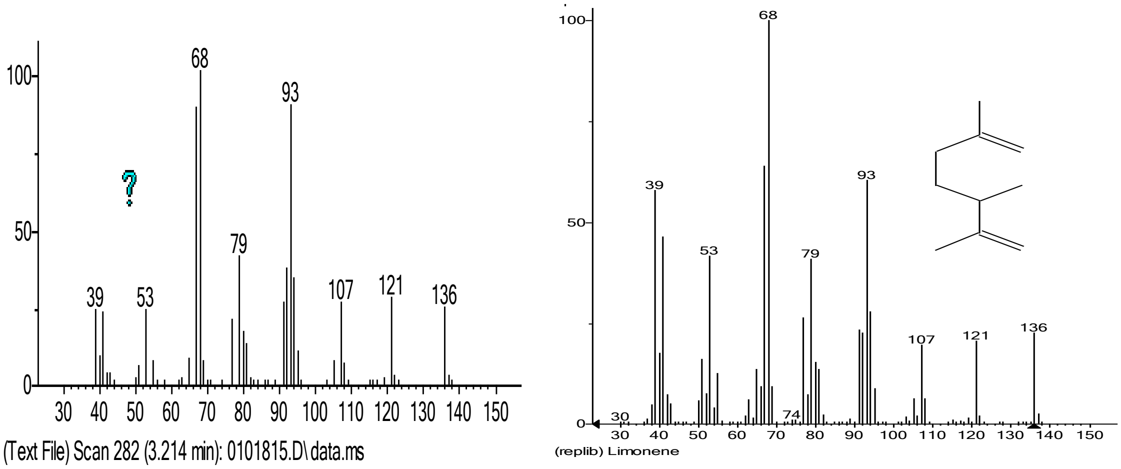


Figure 4: *Mass spectra of limonene from the analysis of Citrus limon linn*

3.*4.2 Fragmentation pattern of β- Pinene*

The molecular ion of β- pinene is formed following the knocking off of electron from the molecule of the compound by high energy electrons in the ionization chamber of the mass spectrometer instrument.

The peak that appeared at m/z 136 is the molecular ion peak of the β- pinene molecule (Figure 5).

$$C_{10}H_{16} \xrightarrow{-e^-} \underset{m/z\ 136}{C_{10}H_{16}^{+}}$$

The fragmentation continued with the loss of a methyl (CH_3) group which resulted to the appearance of peak at m/z 121.

$$C_{10}H_{16}^{+} \longrightarrow \underset{m/z\ 121}{C_9H_{13}^{+}} + {}^{\cdot}CH_3$$

Consequently another cleavage of an ethyl group (C_2H_5) resulted into the appearance of peak at m/z 107

$$C_{10}H_{16}^{+} \longrightarrow C_8H_{11}^{+} + {}^{\cdot}C_2H_5$$

m/z 107

Similar process occurred in which the next homologous group is loss which led to the appearance of peak at m/z 93. This peak has been arbitrarily assign the abundance of 100 and is called the base peak.

$$C_{10}H_{16}^{+} \longrightarrow C_7H_9^{+} + {}^{\cdot}C_3H_7$$

m/z 93

Proceeding the formation of the base peak is the formation of the peak at m/z 65 which resulted from the loss of pentanyl group (C_5H_{11}).

$$C_{10}H_{16}^{+} \longrightarrow C_5H_5^{+} + {}^{\cdot}C_5H_{11}$$

m/z 65

This fragmentation continued until another group of alkyl is loss which resulted to the appearance of less intense peak at m/z 51.

$$C_{10}H_{16}^{+} \longrightarrow C_4H_3^{+} + {}^{\cdot}C_6H_{13}$$

m/z 51

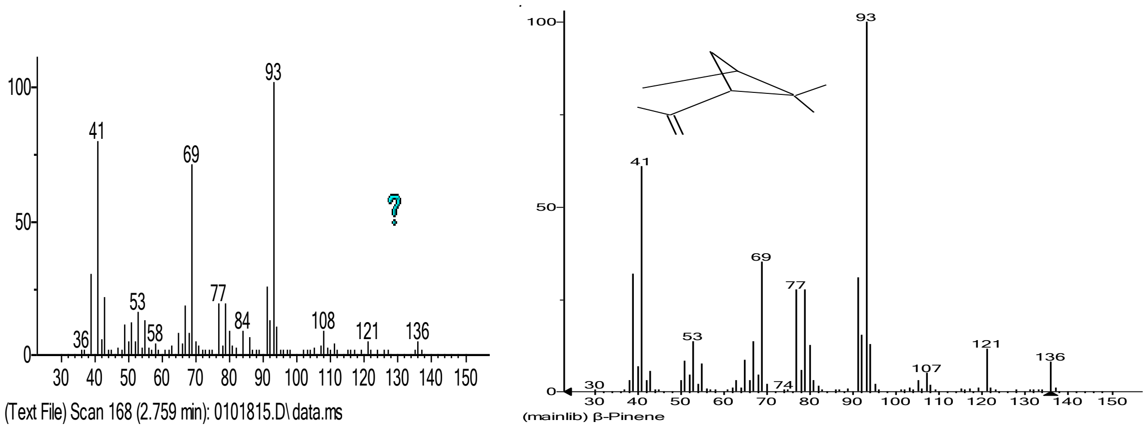


***Figure 5**: Mass spectra of β pinene from the analysis of Citrus limon linn*

***Table 2**: Components, Retention time, Area percentage, Molecular weight of essential oil from Citrus limon linn leaves.*

Component	Retention time (min)	Area %	Molecular weight
β-Pinene	2.764	4.68	136
D-Limonene	3.166	22.83	136
Linalool	3.897	7.82	154
(R)-(+)- Citronellal	4.235	2.38	154
Cis-Verbenol	4.624	20.43	152
β-Citral	5.260	25.20	152
α-Citral	5.563	2.19	152
Nerol acetate	6.235	10.73	196
Geraniol acetate	6.430		196
Caryophyllene	6.798	3.75	204

3.5 GC-MS analysis of essential oils from *Vitex trifolia* seed

The *Vitex trifolia* seed essential oil contained a total of 13 components. Table 3 showed the total components, their retention time, percentage composition and molecular weight of essential oils from *Vitex trifolia* seeds. The three highest occurring components in *Vitex trifolia* seeds essential oils are Sabinene (39.14 %), Caryophyllene (26.45 %) and 1 R- α Pinene (15.20 %). Related work also identified chemical composition of leaves and twigs of an essential oil of the *V. trifolia* were l-d-pinene casticin, luteolin, isoorientin, α-pinene, linalool, terpinyl acetate, β caryophylline, caryophylline oxide, 5-methyl artemitin, β-sitosterol, vitetrifolins,n dihydrosolidgenone abietatriene, vitetrifolin A, limonene, humulene oxide, α-humulemne, 20 hydroxycdysone, ecdysteroids, flavonoids, lignans, triterpenoids, iridoids, vitexin, β-sitosterols (Kulkani *et al*., 2011). Whereas the acetone extract of the fruits of *Vitex trifolia* Linn isolated vitetrifolin A, B and C. Pulverized leaf of V. agnus-castus oil revealed the abundance β-pinene (20.0 %),viridiflorol (9.8 %), α-pinene (9.1 %), cisocimene (8.4 %), 1,8-cineole (6.7 %), β-farnesene (5.4 %), terpinen-4-ol (4.2 %), α-terpineol (4.1 %) and β-phellandrene (4.1 %) (Hamid *et al*., 2010). Howerver, from the result of the coumpounds we obtained in our work in table 3, has a relationship with the work reported by Kukalni (2012).

3.5.1 Fragmentation pattern of caryophyllene

Formation of molecular ion of caryophyllene and the resulted appearance of peak at m/z 204 (Figure 6) is due to the bombarding effect of the high energy electrons in the ionization chamber. The loss of a methyl group resulted to the formation of peak at m/z 189. Peaks appearing at m/z 175, 161, 147, 133 and 105 correspond to the loss of ethyl, propyl, butyl, pentyl and hexyl groups respectively. The fragmentation of caryophyllen proceeded to the loss of different groups after the base peak up to the loss of least group which resulted to the appearance of peak at m/z 15 (Figure 6).

$$C_{15}H_{24} \xrightarrow{-e^-} \underset{m/z\ 204}{C_{15}H_{24}^{+}}$$

$$C_{15}H_{24}^{+} \longrightarrow \underset{m/z\ 189}{C_{14}H_{21}^{+}} + {}^{\cdot}CH_3$$

$$C_{15}H_{24}^{+} \longrightarrow \underset{m/z\ 175}{C_{13}H_{19}^{+}} + {}^{\cdot}C_2H_5$$

$$C_{15}H_{24}^{+} \longrightarrow \underset{m/z\ 161}{C_{12}H_{17}^{+}} + {}^{\cdot}C_3H_7$$

$$C_{15}H_{24}^{+} \longrightarrow \underset{m/z\ 147}{C_{11}H_{15}^{+}} + {}^{\cdot}C_4H_9$$

$$C_{15}H_{24}^{+} \longrightarrow \underset{m/z\ 133}{C_{10}H_{13}^{+}} + {}^{\cdot}C_5H_{11}$$

$$C_{15}H_{24}^{+} \longrightarrow \underset{m/z\ 105}{C_8H_9^{+}} + {}^{\cdot}C_7H_{15}$$

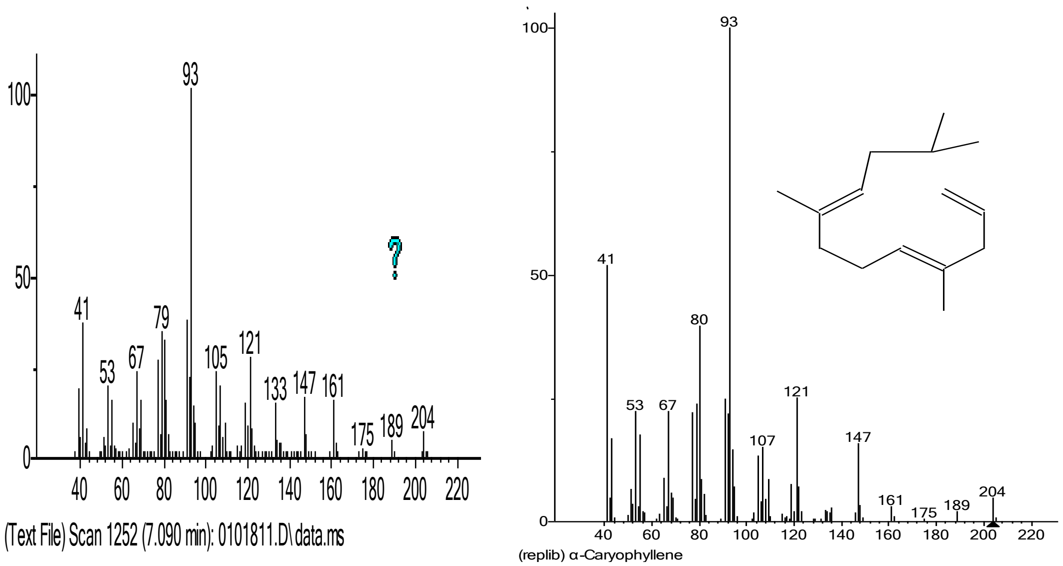


Figure 6: *Mass Spectra of caryophyllene obtained from analysis of Essential oil of Vitex trifolia seed.*

3.5.2 Fragmentation pattern of α-pinene

The mass spectrum of α-pinene was obtained from the various spectra of compounds identified in the essential oil of *Vitex trifolia*. The molecule of α-pinene exit GC-column of the GC-MS instrument and entered the ionization chamber where it is hit with high energy electron and create the molecular ion called pinene radical cation which appeared at m/z 136 in the mass spectrum (Figure 7).

$$C_{10}H_{16} \xrightarrow{-e} \underset{m/z\ 136}{C_{10}H_{16}^{+}}$$

The peaks seen in this mass spectrum are due to cations of various sizes that arrived at the detector. Peaks smaller than the molecular ion have lost a neutral fragment such as a radical due to a fragmentation reactions that occur within the ionization chamber shortly after creation of the molecule ion.

The base peak which has been arbitrarily assign 100% abundance appears at m/z 93. This is due to loss of m/z 43 neutral fragment. Other peaks which have resulted from the loss of groups are expressed in the series of equations below. The peak at m/z 121 resulted from the loss of methyl group.

$$C_{10}H_{16}^{+} \longrightarrow \underset{m/z\ 121}{C_9H_{13}^{+}} + {}^{\cdot}CH_3$$

The peak at m/z 93 is the base peak which resulted from the possible loss of isopropyl group.

$$C_{10}H_{16}^{+} \longrightarrow \underset{m/z\ 93}{C_7H_9^{+}} + {}^{\cdot}C_3H_7$$

The peak at m/z 51 resulted from the loss of a tertiary alkyl group.

$$C_{10}H_{16}^{+} \longrightarrow \underset{m/z\ 51}{C_4H_3^{+}} + {}^{\cdot}C_6H_{13}$$

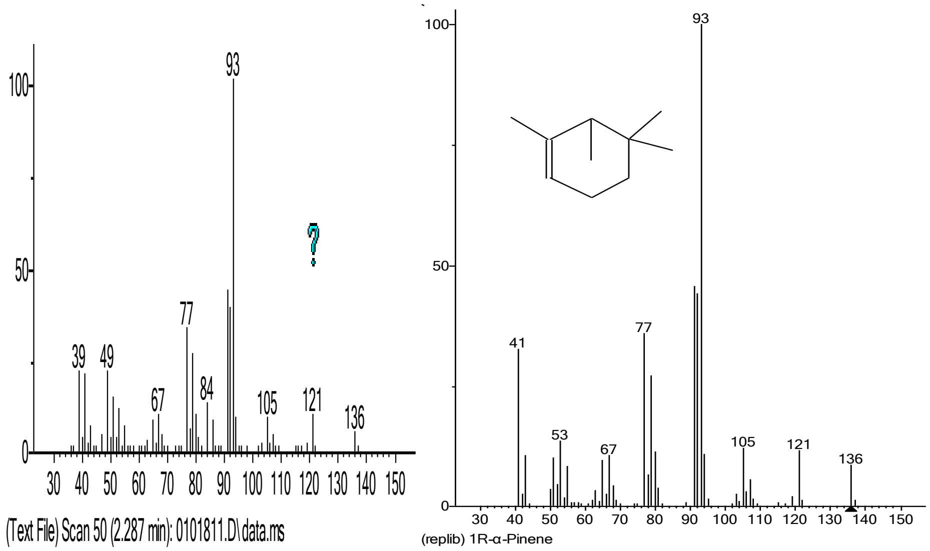


***Figure 7**: Mass Spectra of α Pinene obtained from analysis of Essential oil of Vitex trifolia seed.*

***Table 3:** Components, Retention time, Area percentage, and Molecular weight of essential oil from Vitex trifolia seed.*

Composition	**Retention time (Min)**	**Area %**	**Molecular weight**
1 R- α Pinene	2.287	15.20	136
Sabinene	2.603	29.14	136
Bicyclo[3,10]hexan-2-ol,methyl-5-[methylethyl]-,[1α,2α,5α]-	3.138	3.15	154
Terpinene	3.434	2.31	136
4,4- Terpineol	4.724	2.08	154
Copaene	6.430	1.05	204
Caryophyllene	6.798	26.45	204
α-Caryophyllene	7.114	2.59	204
Germacrene D	7.317	3.58	204
γ-Gujunene	7.485	2.94	204
Caryophyllene oxide	8.224	2.40	220
Vindiflorol	8.896	6.71	222
1-Heptatriacotanol	18161	2.05	536

3.6 GC-MS analysis of essential oils *Cananga odorata* flowers

The *Cananga odorata* flower essential oils contained a total of 9 components. Table 4 shows the total components retention time, percentage composition and molecular weight of essential oils from *Cananga odorata* flower. The highest occurring components in *Cananga odorata* flower essential oils are linalool (35.30 %), Ascabiol (23.54 %), Caryophyllene (20.30 %) Copaene (12.76 %) and Germacrene (11.32 %). Study revealed that the main components identified from the oxygenated fraction of essential oils from *Cananga odorata* leaves were methylanisole methyl benzoate, and benzyl benzoate, benzyl acetate, geranyl acetate, cinnamyl acetate, and (E, E) - farnesyl acetate, linalool, geraniol, and benzyl salicylate. Linalool was shown to be main component present in oxygenated fraction (28 %) that is responsible for the floral smell of ylang-ylang. Meanwhile, the hydrocarbon fraction of ylang-ylang oils consisted of mainly sesquiterpenes and monoterpenes whereby both germacrene D, and -caryophyllene represented 63 % of the total hydrocarbon fraction of *Cananga odorata* oils (Brokl *et al*., 2013) More recently, a study focused on the variation in the chemical profiles of essential oils from *C. odorata* among the Western Indian Ocean islands such as Union of Comoros, Madagascar, and Mayotte as they are known to be the current main producers of *Cananga odorata* essential oils (Benini *et al*., 2012). Study also revealed the chemical constituent in *Cananga odorata* leaves which includes geranyl acetate, linalol, geraniol, farnesol, benzyl acetate, geranial, methyl chavicol, beta-caryophyllene, eugenol, pinene and Farnesene (Evans, 2002). The results from previous work above showed clear evidence that most of the chemical constituent present in their work are similar to the compounds obtained in table 4.

3.6.1 Fragmentation pattern of copaene

The molecular ion of copaene is formed following the knocking off of electron from the molecule by high energy electrons in the ionization chamber of the mass spectrometer instrument (figure 8). The result shows the appearance of peak at m/z 204 called the molecular ion peak. This peak appeared as a result of loss of electron from the copaene molecule which is detected by detector.

$$C_{15}H_{24} \xrightarrow{-e^{-}} \underset{m/z\ 204}{C_{15}H_{24}^{+}}$$

The peak at m/z 189 is due to the loss of methyl group (CH_3)

$$C_{15}H_{24}^{+} \longrightarrow \underset{m/z\ 189}{C_{14}H_{21}^{+}} + {}^{\cdot}CH_3$$

The cleavage of the methyl group occurs on one of the two side methyl groups present on the copaene molecule. Other smaller peaks could possibly be due to loss of hydrogen ion.

The peak at m/z 175 resulted from the loss of CH_3CH_2 group.

$$C_{15}H_{24}^{+} \longrightarrow \underset{m/z\ 175}{C_{13}H_{19}^{+}} + {}^{\cdot}C_2H_5$$

The next peak appeared at m/z 161 which is also called the base peak. The cleavage that occurs on this peak resulted from the loss of isopropyl group (C_3H_7).

$$C_{15}H_{24}^{+} \longrightarrow \underset{m/z\ 161}{C_{12}H_{17}^{+}} + {}^{\cdot}C_3H_7$$

Next peak after the base peak appeared at m/z 147 and this indicate that there was a loss of butyl group

$$C_{15}H_{24}^{+} \longrightarrow \underset{m/z\ 147}{C_{11}H_{15}^{+}} + {}^{\cdot}C_4H_9$$

Similarly the cleavage at m/z 119 and 105 resulted to the loss to pentanyl and hexanyl group respectively as shown below.

$$C_{15}H_{24}^{+} \longrightarrow \underset{m/z\ 119}{C_{10}H_{13}^{+}} + {}^{\cdot}C_5H_{11}$$

$$C_{15}H_{24}^{+} \longrightarrow \underset{m/z\ 105}{C_9H_{11}^{+}} + {}^{\cdot}C_6H_{13}$$

Cleavage that led to the appearance of a peak at m/z 77 resulted from the loss of octanyl group.

$$C_{15}H_{24}^{+} \longrightarrow \underset{m/z\ 77}{C_7H_7^{+}} + {}^{\cdot}C_8H_{17}$$

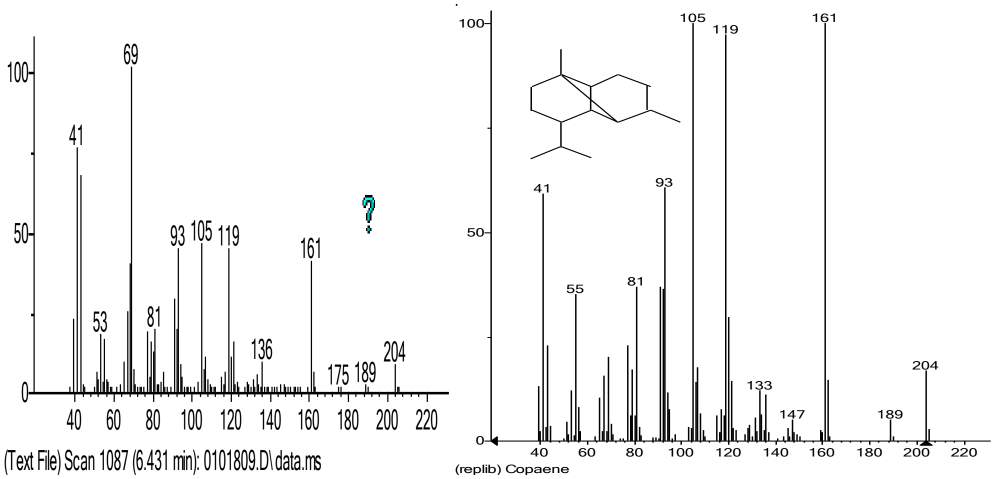


Figure 8: *Mass Spectra of Copaene obtained from the analysis of essential oil of Cananga odorata*

3.6.2 Fragmentation pattern of linalool

The molecular ion of Linalool is formed from following the knocking off of electron from the molecule by high energy electrons in the ionization chamber of the mass spectrometer instrument (figure 9, see also appendices 29-38 for Gas Chromatography-Mass Spectra). The result shows the appearance of peak at m/z 154 called the molecular ion peak. This peak appeared as a result of loss of electron from the Linalool molecule which is detected by detector.

O M/Z 154

The peak at m/z 126 is due to the loss of (CH_2O) group.

O + CH_2O M/Z 126

The peak at m/z 83 resulted from the loss of C_5H_{11} group.

+ C_5H_{11} M/Z 83

The cleavage that occurs on this peak at m/z 55 resulted from the loss of (C_7H_{15}) group.

+ C_7H_{15} M/Z 55

Similarly the cleavage at m/z 43 resulted to the loss of (C_8H_{15}) as shown below.

+ C_7H_{15} M/Z 43.

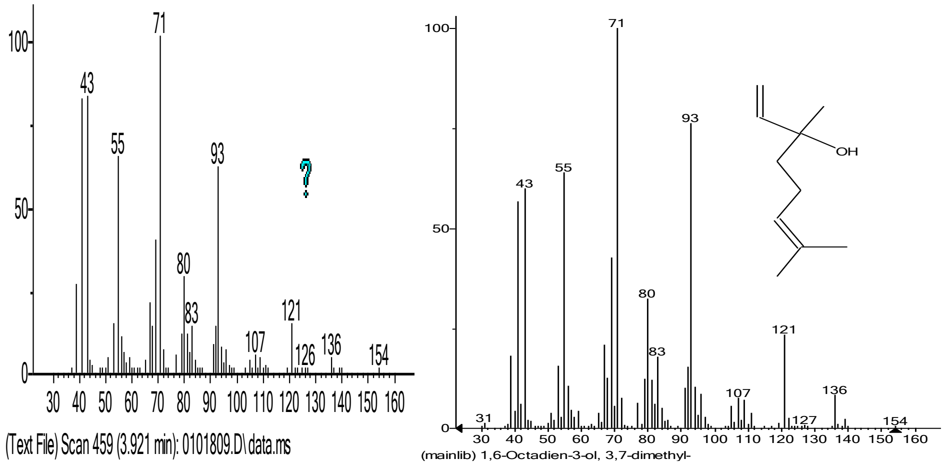


Figure 9: *Mass Spectra of Linalool obtained from the analysis of essential oil of Cananga odorata*

Table 4: *Components, Retention time, Area percentage, Molecular weight and retention indices of essential oils from Cananga odorata flowers.*

Composition	Retention time (min)	Area %	Molecular weight
Linalool	3.897	35.30	154
Copaene	6.383	12.76	204
Caryophyllene	6.846	20.30	204
α-Carophyllene	7.138	5.07	204
Germacrene D	7.429	11.32	204
α-Famesene	7.553	4.52	204
Caryophyllene oxide	8.189	9.48	220
Ascabiol	11.043	23.54	212
1-Heptatriacotanol	14.549	0.13	536

3.7 Determination of larvicidal activity of essential oils

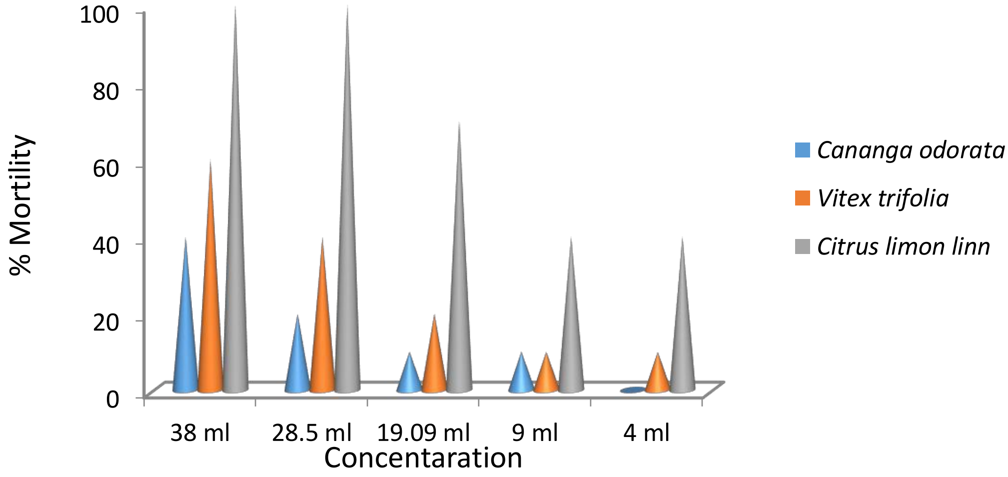
The larvicidal activities of the essential oils of Cananga odorata flowers, Vitex trifolia seeds and Citrus limon leaves were obtained using a sufficient amount of target oil in normal saline using 2ml of DMSO to produce a stock solution at 1000 ppm. This solution was used to prepare serial dilutions of target oil at different concentration through dilution of the stock solution with distilled water; three replicate of each concentration were made. In addition to three replicates, the control contains 2ml of DMSO and normal saline only. The percentage mortility of the Anopheles mosquito were monitored at the interval between 30 minutes to 5 hours. From the result all the samples have exhibited larvicidal activity as shown in table 5 and figure 10. However, the lowest larvicida activity was observed in the lowest concentrations while the highest mortility was obtained in the higher concentrations of the essential oils. Studies have indicated that the essential oils of Cananga odorata prepared in soybean oils possessed certain degree of repellent activity against the adult mosquito of A. aegypti, A. dirus, and C. quinquefasciatus with the ED 50 of 0.045, 2.149, and < 0.003 mg / cm2. The essential oil of Cananga odorata also demonstrated a moderate time of protection against A. aegypti, A. dirus, and C. quinquefasciatus at a duration of 8.4, 24.0, and 60.0 minutes, respectively (Phasomkusolsil et al., 2011).

Other studies showed that insect borne disease was targeted with *Vitex trifolia* Methyl-p-hydroxybenzoate, a crystalline compound was isolated from the leaves methanolic extract which exhibited 100 % mosquito larvae mortality against *C. quinquesfasciatus* and *A. aegypti* (Kannathasan, 2011). Similar studies showed the potential toxicity Citrus essential oils may owe to their high content in limonene. Many studies have demonstrated the insecticidal activity of pure forms of this molecule in all stages of certain mosquito species (Mansour *et al.*, 2004). However, the results show that the essential oil of *C. limon* peel is more effective despite its somewhat lower content in limonene (91.43 %) compared with that of *C. sinensis* (94.92 %). This suggests that the effectiveness of essential oils from pericarp of *C. limon*, *C. reticulata* and *C. sinensis* is not related to limonene only (Odalo *et al.*, 2005). It can be observed from the cited work that the percentage larval mortality increased with increasing concentrations of the oil, which is similar to our work in table 5 below.

***Table 5**: Larvicidal activities of essential oils from Cananga odorata flowers, Vitex trifolia seeds and Citrus limon leaves against Anopheles mosquito after 5 hours of treatment*

Concentration (ml)	38	28.5	19.09	9	4
Cananga odorata					
% Mortility	40	20	10	10	0
Vitex trifolia					
% Mortility	60	40	20	10	10
Citrus limon linn					
% Mortility	100	100	70	40	10

***Figure: 10**: Percentage mortality of larvae in cananga odorata, Vitex trifolia and Citrus limon essential oil*



Conclusion

The result obtained in the infrared region shows various spectrum which indicate the functional groups of the entire compounds present in the essential oils extracted from *Citrus limon, Vitex trifolia* and *Cananga odorata.* Analysis using Gas Chromatography-Mass Spectrometer confirmed that the essential oils contained the major components which are expected to be biologically and pharmacologically active. However, the major compounds identified in *Citrus limon*, *Vitex trifolia* and *Cananga odorata* were Pinene, linalool, Ascabiol, Caryophyllene, Copaene, Germacrene, Citral and limonene. Nevertheless, the results of the larvicidal activities revealed that essential oils extracted from *Citrus limon, Vitex trifolia* and *Cananaga odorata* may serve as insecticide and can be used as potent substance for the prevension of mosquitoes. The order of larvicidal activity is thus; *Citrus limon linn* > *Vitex trifolia* > *Cananga odorata.*

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