

Gc/Ms Study of Phytocompounds from The Leaves of Sweet Basil

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Abstract

Sweet basil (*Ocimum basilicum*) is referred to as the King of herb and is of the family lamiaceae. The present study was aimed at investigating the probable bioactive components of leaves of *Ocimum basilicum* using Gas chromatography - mass spectrometry (GC-MS) study. Six compounds were identified in the leaf methanol extract; they are hexadecanoic acid (8.37%), hepta - 9,10,11 - trienoic acid (17.04%), octadecenoic acid (8.37%), 5-(hydroxymethyl) heptadecane (13.75%), eicosane aldehyde (37.36%) and octadecyl vinyl ether (15.12%) as the major chemical constituents. The company of these phytocompounds in the plant extract may at least be conscientious for the acclaimed remedial and protective potentials of *Ocimum basilicum* and thus recommended as plant of phytopharmaceutical consequence.

Keywords: Medicinal plant; Sweet basil; Chemical constituents; Biological activity

Introduction

Plants are a valuable source of new natural products (Uraku, *et al.* 2015). Despite the availability of different approaches for discovery of therapeutics, natural products still remain as one of the best reservoirs of new structural types (Manju, *et al.* 2012). Researches have shown that about 25% of prescription drugs sold in the United States are of natural products, while another 25% are for structural modifications of a natural product (Cochrane, *et al.* 2008). Furthermore, 74% of the 119 most important drugs currently contain ingredients from plants used in traditional medicine (Grifo, *et al.* 1997, Omotoyinbo, *et al.* 2015). Of the several hundred thousand plant species around the globe, only a small proportion has been investigated both phytochemically and pharmacologically (Uraku, *et al.* 2015). When one considers that a single plant may contain up to thousands of constituents, the possibilities of making new discoveries become evident. The crucial factor for the ultimate success of an investigation into bioactive plant constituents is thus the selection of plant material. In view of the large number of plant species potentially available for study, it is essential to have efficient systems available for the rapid chemical and biological screening of the plant extracts selected for investigation.

Ocimum basilicum (commonly known as scent leaf) is native to Africa, Asia and Pacific Island and is of the family lamiaceae. The plant is mostly annual or perennial herbs and the genus contains between 50 and 150 species. They are found in the tropical regions of Asia, Africa, and Central and South America (Daniel, *et al.* 2011). The plant derived its name from Greek *Ozo* which means to smell and this is in reference to the strong odors of the species within the genus (Gabi, *et al.* 2012). In French, it is frequently given the name "Herbe Royale" revealing the positive light in which it is viewed (Jamal, *et al.* 2002). It is sometimes referred to "King of herb" and this name may have been derived from Greek *Basileus*, or king (Tomar, 2010). Basil's affiliation with the crown may be in part due to its use in regal medicine (Gabi, *et al.* 2012). Sweet basil (*Ocimum basilicum*) grows to a size of 1-2 feet in height and prolifically produces large green leaves, measuring around 2 inches in length, throughout the summer. Basil flowers are white, and are commonly removed to increase yield of leaves.

Basil (*Ocimum basilicum* L.), a member of the Lamiaceae family, is used both as a culinary and ornamental herb (Devendran and Balasubramanian, 2011). Traditionally basil has been used as a medicinal plant in the treatment of headaches, coughs, diarrhea, constipation, warts, worms, and kidney malfunctions (Gabi., *et al.* 2012). Externally, Basil can be used as an ointment for insect bites, and its oil is applied directly to the skin to treat acne (Tomar, 2010). Natural components from basil have long been used to flavor foods and dental and oral products (Singh., *et al.* 2007). Iranian basil plants are used to treat fevers, throat congestions, and stomach ache (Gabi., *et al.* 2012).

Despite the popular use of Basil (*O. basilicum* L.) leaves as vegetable and for treating various disorders, there is limited data available regarding Gas chromatography–mass spectrometry (GC/MS) analysis of chemical constituents of locally grown Sweet basil leaves in Nigeria. This study therefore intends to evaluate the gas chromatography/mass spectrometry (GC/MS) analysis of the chemical constituents of the methanol extract of *O. basilicum* L. leaves grown in Nigeria.

The study was aimed at investigating the phytochemical constituents of methanol extract of leaf of *O. basilicum* L by GC/MS analysis.



Figure 1: *Ocimum basilicum* plant.

Materials and Methods

Collection and Identification of Plant Material

Fresh leaves of *Cymbopogon citratus* were collected with gloved hands from the Presco Campus of Ebonyi State University, Abakaliki, Nigeria. The plant samples were identified and authenticated by Dr. Nnamani, K., a taxonomist in the Department of Applied Biology, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria.

Preparation of plant material

The leaves of *Cymbopogon citratus* were sorted, washed thoroughly with distilled water to remove dirt and debris, cut into smaller pieces before it was shade dried for 3 weeks at room temperature ($28\pm 3^{\circ}\text{C}$). The dried leaves were pulverized into fine powder using electric blender (CORONA-REF. 121, Landers and Qlink blender, Model No. OBL-15L40). The powdered materials were stored in air tight polyethylene bags protected from direct sunlight until required for use.

Plant sample extraction

Fifty (40) grams of the powdered leaves were extracted with 100 ml of 40% methanol overnight in a stopped bottle and with occasional stirring at room temperature ($28\pm 3^{\circ}\text{C}$). The sample was first sieved using muslin cloth and then filtered using Whatman No.1 filter paper. This process was repeated three times. The filtrate was concentrated under reduced pressure at 40°C for 45 min in a rotary

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vacuum evaporator; and then lyophilized to get a brown aromatic solid extract. The yield of the extract was expressed in terms of the percentage of the dry weight of initial plant material used (yield 35.37% w/w). The dry extract obtained was kept in a refrigerator at 4°C until required for use.

Column Fractionation of Ethanol extract

The dry crude extract was subjected to column chromatography according to the standard method. The sample for the column was prepared by adsorbing 20 g of the ethanol extract of *Cymbopogon citratus* with 60 g of silica gel G (60-120 mesh). The mixture was air dried and carefully layered on top of the packed silica gel in the column (14 cm length) using a glass funnel. The extract in the column was eluted with 100 ml of methanol at the rate of 1 ml/min. The eluates were concentrated and labeled. The percentage yield of the fraction was recorded. The methanol fraction of *Cymbopogon citratus* leaves was analyzed by Gas Chromatography-Mass Spectrometry (GC-MS).

Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analysis was carried out on a GC-MS (Model: QP2010 PLUS Shimadzu, Japan) comprising a AOC-20i auto-sampler and chromatograph interfaced to a mass spectrometer (GC-MS). The instrument is equipped with a VF 5 ms fused silica capillary column of 30 m length, 0.25 mm diameter and 0.25 µm film thickness. The temperatures employed were; column oven temperature 80°C, Injection Temp 250°C at a pressure of 108.0 kPa, with total flow and column flow of 6.20 ml/min and 1.58 ml/min respectively. The linear velocity was 46.3 cm/sec and a purge flow of 3.0 ml/min. The GC program ion source and interface temperature were 200.00°C and 250.00°C respectively with solvent cut time of 2.50 min. The MS program starting time was 3.00min which ended at 30.00 min. with event time of 0.50 sec, scan speed of 1666 µl/sec, scan range 40-800u and an injection volume of 1 µl of the plant extract (split ratio 10:1). The total running time of GC-MS was 30 min. The relative percentage of the extract was expressed as a percentage with peak area normalization.

Identification of phytocompounds

Interpretation on the mass spectrum was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The fragmentation pattern spectra of the unknown components were compared with those of known components stored in the NIST library (NIST Ver. 2.0 of 2005). The compound bioactivity prediction is based on Dr. Duke's Phytochemical and Ethnobotanical Databases (Dr. Duke Database, 2014). The relative percentage amount of each phyto-component was calculated by comparing its average peak area to the total area. The name, molecular weight and structure of the components of the test materials were ascertained.

Results

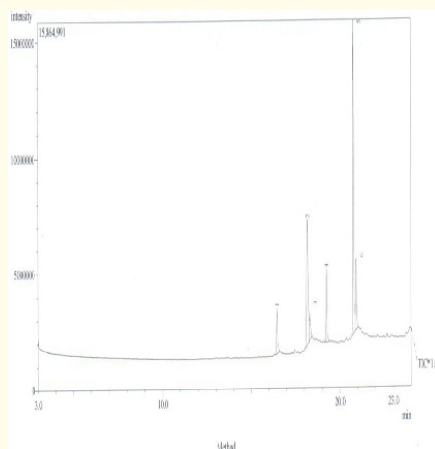


Figure 2: GC-MS chromatogram of *O. basilicum* leaves.

Peaks	Compounds	Molecular formulae	Molecular weight	Retention time	Mass peak	Base peak	Percentage content
1	Hexadecanoic acid (Palmitic acid)	[C ₁₆ H ₃₂ O ₂] ⁺	256.48	16.46	67	43	8.37
2	Hepta-9, 10, 11-trienoic acid	[C ₁₇ H ₂₈ O ₂] ⁺	264.40	18.18	94	41	17.04
3	Octadecanoic acid (Stearic acid)	[C ₁₈ H ₃₆ O ₂] ⁺	284.48	18.31	75	41	8.37
4	5-(Hydroxymethyl) heptadecane	C ₁₈ H ₃₇ O ⁺	269.49	19.26	75	43	13.75
5	Eicosane aldehyde	[C ₂₁ H ₄₁ O] ⁺	309.59	20.77	162	55	37.36
6	Octadecyl vinyl ether	[C ₂₀ H ₄₀ O] ⁺	297.53	20.92	91	43	15.12

Table 1: Phytoconstituents identified in *Ocimum basilicum* leaves by GC-MS assay.

Peak no.	Name of compound	Type of compound	Biological activity
1	Hexadecanoic acid	Fatty acid	Antioxadant, hypocholesterolemic, lubricant, nematocide, and pesticide, antiandrogenic and flavour
2	Hepta-9,10, 11-trienoic acid	Fatty acid	Anti-inflammatory, antiandrogenic, anemigenic, 5,α-reductase inhibitor, α-reductase inhibitor, lubricant, antitumor, choleric, dermatitogenic, immunostimulant, anti-leucotriene-D4, lipoxygenase inhibitor, allergenic, flavour, hypocholesterolemic, insectifuge, irritant, percutaneo-stimulant, perfumery and propepic.
3	Octadecanoic acid (Stearic acid)	Fatty acid	Antiviral, antiinflammatory, 5-α-reductase inhibitor, hypocholesterolemic, propepic, suppository, flavour and cream formulation.
4	5-(Hydroxymethyl) heptadecane	Fatty alcohol	Nf
5	Eicosane aldehyde	Aldehyde	Anti-inflammatory and anti-therogenic
6	Octadecyl vinyl ether	Aliphatic	Nf

Table 2: Biological activity of phytochemicals identified from *Ocimum basilicum* leaves.

NF: Not found. Source: Dr. Duke's phytochemical and ethnobotanical database.

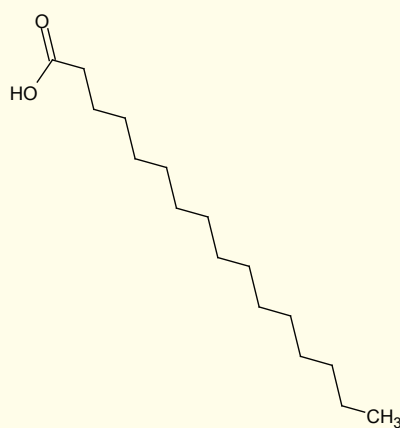
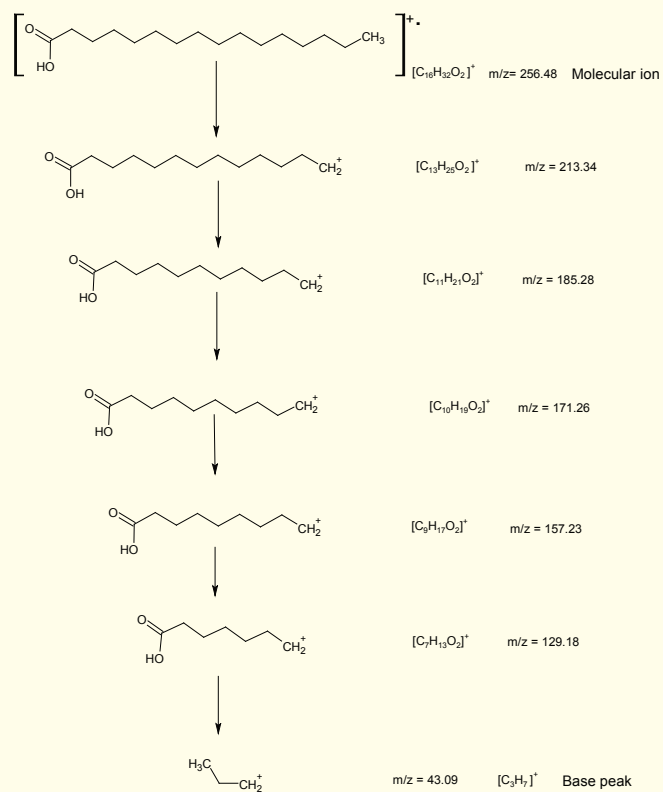
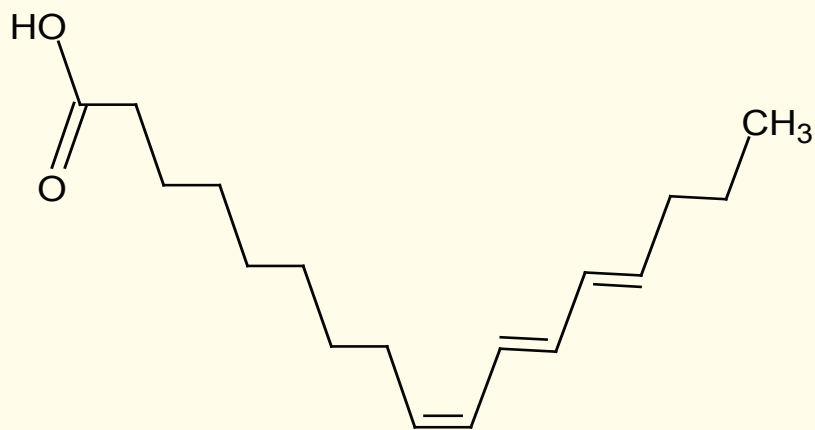
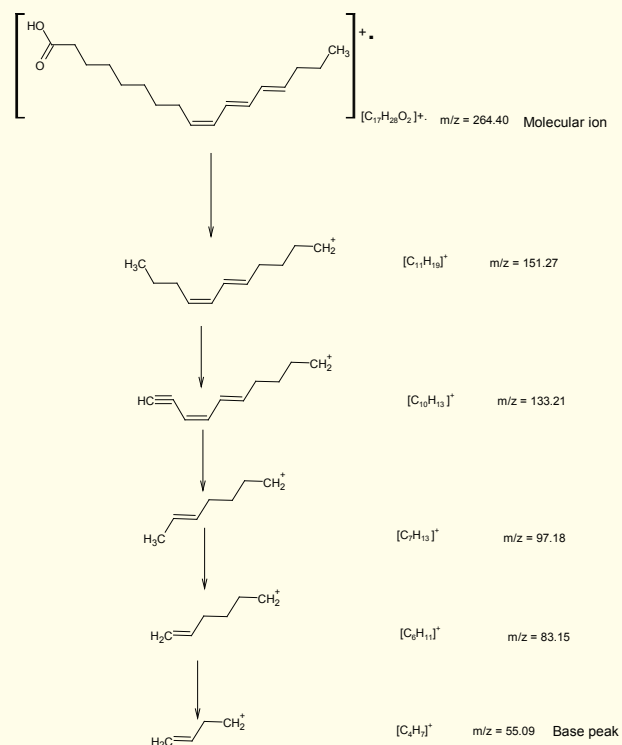


Figure: 3(a-I): Continue



Hepta-9,10,11-trienoic acid

Figure: 3(a-l): Continue

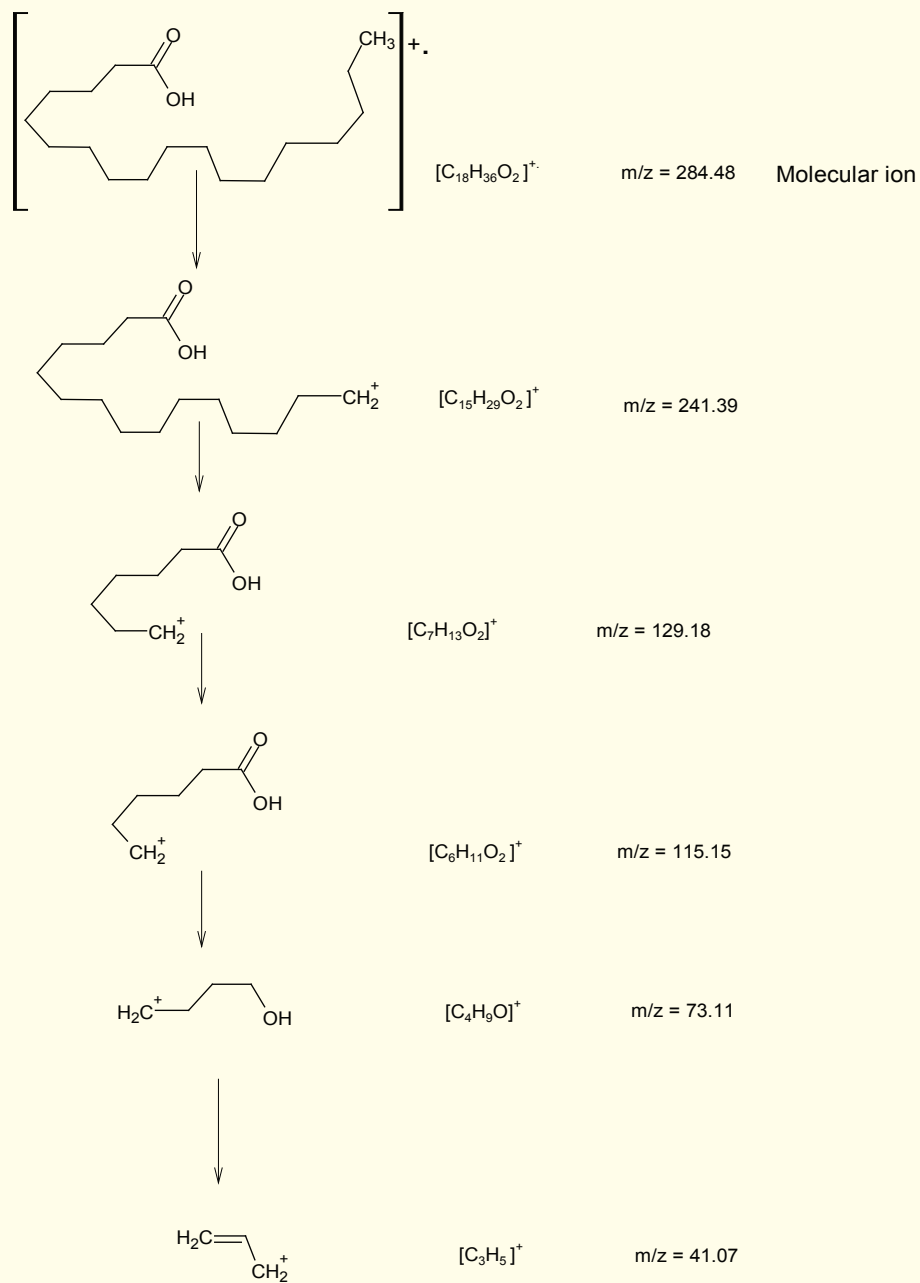
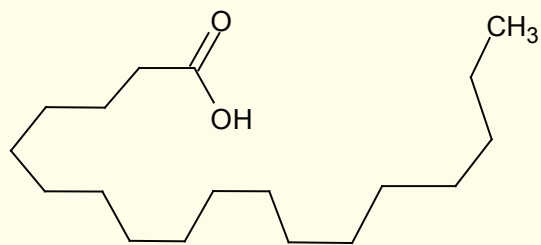


Figure 3(a-1): Continue



Octadecanoic acid (Stearic acid)

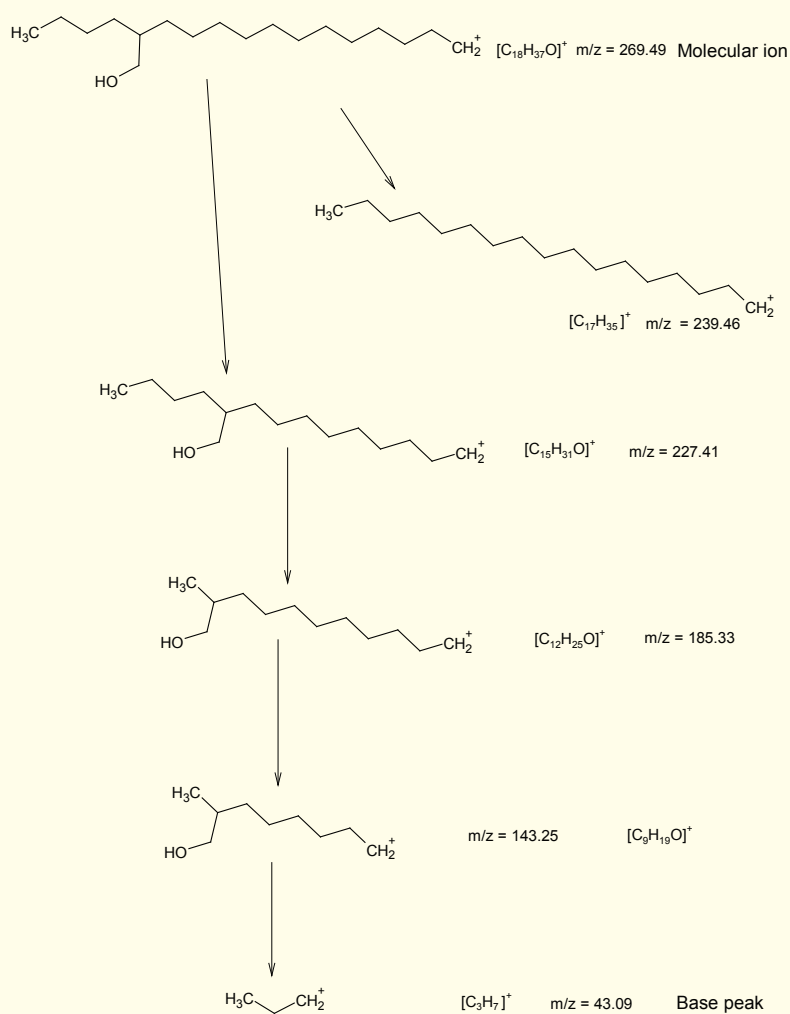
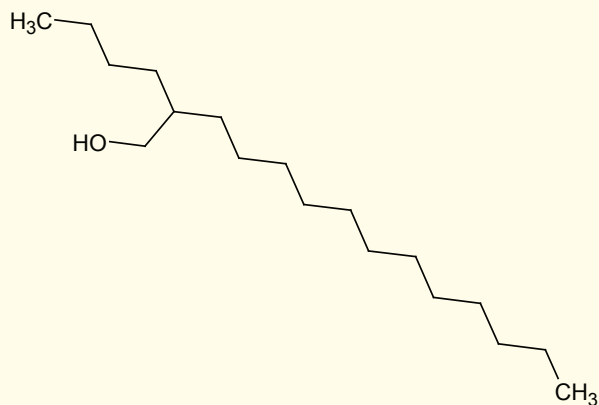


Figure: 3(a-l): Continue



5-(Hydroxymethyl)heptadecane

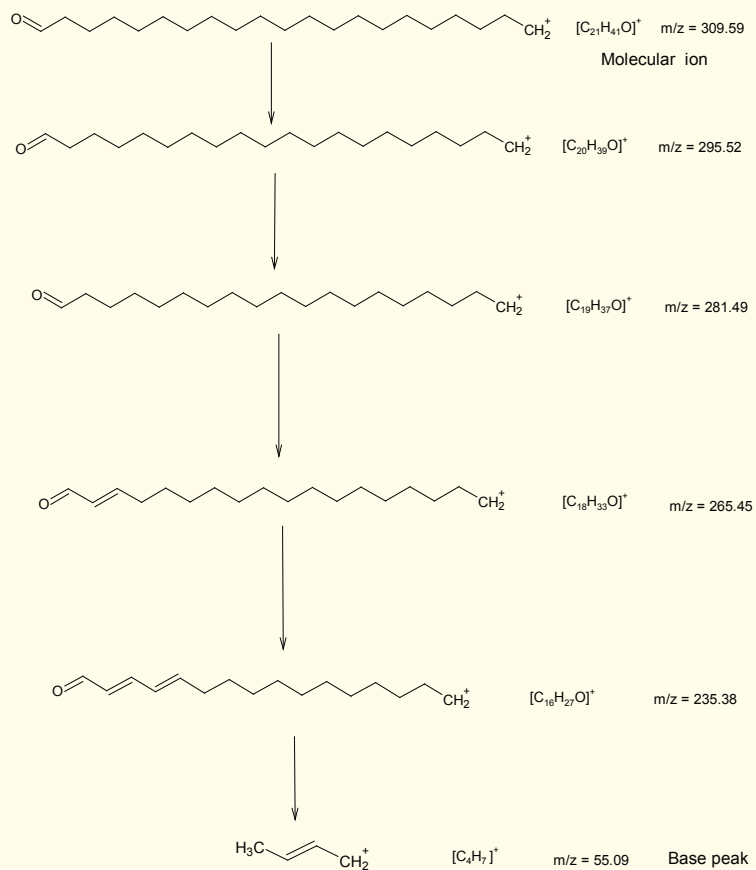


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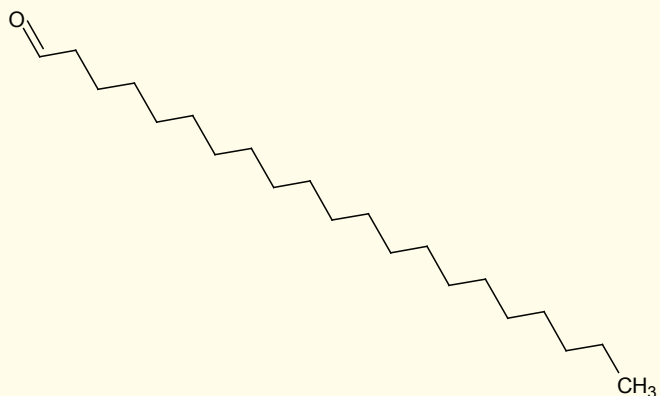
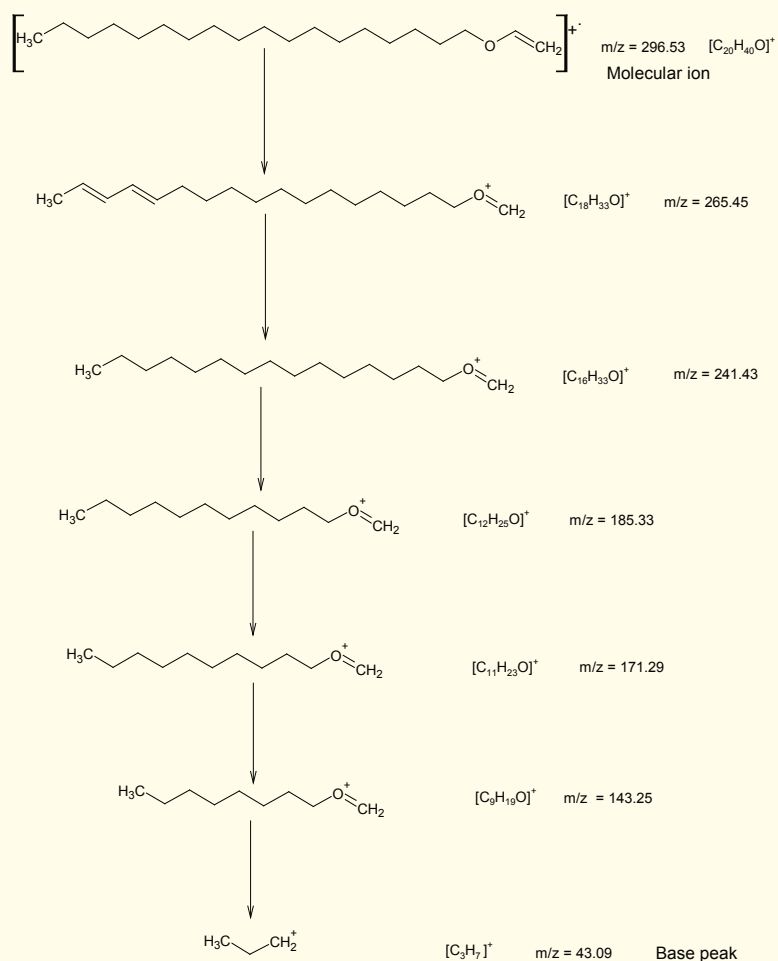
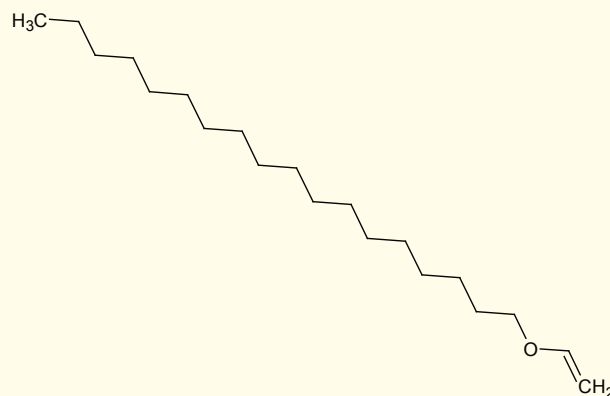


Figure: 3(a-l): Continue





Octadecyl vinyl ether

Figure 3(a-1): The fragmentation patterns of the peaks and large fragments at different m/z ratios.

Discussion

The result of GC-MS investigation showed that six peaks were identified from the chromatogram of the methanol leaf extract of *O. basilicum*. The peaks (1-6) indicate the presence of six phytocompounds (1-6) in the extract (Figure 1). The active principle, area of peak concentration (%), retention time (RT) molecular weight (MW) and molecular formula (MF) in the methanol extract as identified through the NIST database is listed in Table 1.

These compounds comprise mainly hydrocarbons, fatty acids, alcohols, esters and phenols. The composition of the extract comprises high amounts of eicosane aldehyde (37.36%), hepta-9, 10, 11- trienoic acid (17.04%), octadecyl vinyl ether (15.12%), 5- hydroxymethyl heptadecane (13.75%) with low amounts of hexadecanoic acid (8.37%) and octadecenoic acid (8.37%). The obtained result is in agreement the research of Uraku, *et al.* (2015) and Uraku, (2015) on GC/MS analysis of methanol leaves extracts of *Hyptis spicigera* and *Cymbopogon citratus*.

The nature of phytocompounds and their biological activities of acknowledged compounds of the leaves of *O. basilicum* were presented in table 2. The biological activities of the phytocompound of *O. basilicum* mentioned in table 2 are based on phytochemical and ethnobotanical database by Jim Duke of the Agricultural Research service/USDA. This is in line with the work of Omotosho *et al.* (2014).

The fragmentation patterns of the peaks and well-known phytocompounds of the plant leaves were exposed in figure 3. This indicated disintegration of large fragments into small compounds giving rise to appearance of peaks at different m/z ratios.

It is germane to categorize the likely functions of these voter compounds in the health-giving properties accredited to the plant by tradomedical practitioners.

The investigation on the energetic code of methanol fraction of *O. basilicum* discovered that the plant contained a spacious assortment of fatty acids which may donate to its therapeutical potentials. The GC-MS result showed that the lipid extract is mainly composed of esters and long chain fatty acids like hexadecanoic, octadecanoic and hepta-9, 10, 11-trienoic acid. These phytochemicals are known to have variable effects such as antimicrobial, hypocholesterolemic, pesticide, anti-inflammatory, antiandrogenic, antitumor, immunostimulant, antioxidant and Antiviral activity among others (Ogunlesi, *et al.* 2010, Charles, *et al.* 2011). The result of level of hexadecanoic acid disagree with the earlier report that it is the major component in leaf extracts of *Kigelia pinnata* (Grace, *et al.* 2002), *Senna*

alata L (Omotoyinbo and Sanni, 2015) and *Caralluma fimbriata* (Priya, *et al.* 2011). Also, the result disagree with the report that leaves extract of *Cleistanthus collinus* had n-hexadecanoic acid and octadecanoic acid as the major compounds (Parasuraman, *et al.* 2009).

Beside, the chromatogram of the plant showed the presence of eicosanoic aldehyde as the highest compounds contained in the plant. Eicosanoids work like hormones, but they do not like to travel and are such go by the nickname 'local hormones' because they act on cells close to their site of production. Eicosanoids also rapidly break down, so they are not able to travel very far. There are different types of eicosanoids, but the three most researched types are prostaglandins, thromboxanes, and leukotrienes which are primary in regulation of many physiological processes (Okwu and Ighodaro, 2010, Zibbu and Batra, 2011). Studies have shown that eicosanoids have anti-inflammatory and anti-thrombotic properties (Achi and Ohaeri, 2015). The bioactivities of these compounds may depend on the lipophilic properties of their functional groups.

The phytochemicals reported in this extract may account for the variety of pharmacological actions the leaves of parts thereof have power over. Infact, there is no study that can give a apparent inspiration and be accurate on the mode of action of the plant's essential oils. Given the complexity of their chemical composition, it is very likely that each of the constituents of the essential oils as obtained from *O. basilicum* leaf has its own mechanism of action. However, owing to the unpredictability of amounts and profiles of the components of essential oils, it is likely that their different medicinal activities is not due to a single mechanism, but to several sites of action at the cellular level and involving different modes of action. Thus, this type of GC/MS analysis is the first step towards understanding the nature of active principles in this medicinal plant and this type of study will be helpful for further detailed study in line with the biochemical and phytochemical functions mentioned above.

Conclusions

The result of the GC-MS analysis showed that the *O. basilicum* contained high amounts of eicosane aldehyde, hepta-9, 10, 11-trienoic acid octadecyl vinyl ether, 5- hydroxymethyl heptadecane with low amounts of hexadecanoic and octadecanoic acid. However, the pharmacological effects of this plant depend of the identified phytochemicals. Thus, there are need of further studies to isolate, spot and purify the specific phytochemical involved in preventing and treating ailments which may ultimately show the way to drug development.

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