American Journal of Biological Chemistry
2015; 3(3): 45-56
Published online July 30, 2015 (http://www.openscienceonline.com/journal/ajbc)

Open Science

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Essential Oil from *Hyptis spicigera* Leaves

Uraku A. J.[1, *], Offor C. E.[1], Itumoh E. J.[2], Ukpabi C. E.[3], Aja P. M.[1], Ebenyi L. N.[4], Azi S. O.[5], Emmanuel T. F.[6]

[1]Department of Biochemistry, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria
[2]Department of Chemistry, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria
[3]Department of Chemistry, Abia State Polytechnic, Aba, Abia State, Nigeria
[4]Department of Biotechnology, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria
[5]Department of Medical Laboratory, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria
[6]Department of Medical Biochemistry, Kogi State University, Anyigba, Kogi State, Nigeria

Email address

urakuaj@yahoo.com (Uraku A. J.), urakuaju@gmail.com (Uraku A. J.)

To cite this article

Uraku A. J., Offor C. E., Itumoh E. J., Ukpabi C. E., Aja P. M., Ebenyi L. N., Azi S. O., Emmanuel T. F.. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Essential Oil from *Hyptis spicigera* Leaves. *American Journal of Biological Chemistry.* Vol. 3, No. 3, 2015, pp. 45-56.

Abstract

Hyptis spicigera is a medicinal plant widely used in folkloric medicine of Africa and Asia in treatment of numerous ailments such as wound healing, gastrointestinal disorders, respiratory tract infections, colds, pain, fever, cramps and skin disease among others. The chemical constituent of methanol leaf extract of Hyptis spicigera was determined using Gas Chromatography - Mass Spectrometry (GC-MS). Six major chemical compounds were identified in the methanol leaf extract and they are; hexadecanoic acid (15.19%), 9 - octadecenoic acid (24.68%), octadecenoic acid (7.60%), 5 - hydroxylmethyl heptadecane (11.08%), eicosane aldehyde (30.06%) and octadecyl vinyl ether (11.39%). These relatively diverse chemical compounds may be responsible for the therapeutic potentials of *Hyptis spicigera* leaf.

Keywords

Hyptis spicigera Leaves, GS-MS Analysis, Methanol Extract and Chemical Constituent

1. Introduction

Nowadays, natural products gotten from plant part or parts thereof are being assessed for presence of new drugs with new mechanisms of pharmacological action (Gopalakrishnan and Udayakumar, 2014). Plants are used as medicine in many countries and also act as a source of many potent drugs. A large number of medicinal plants and their purified constituents have shown to posses very good therapeutic potentials. Natural remedies from medicinal plants have proved to be safe and effective in some conditions more than synthetic drugs (Uraku *et al*., 2015). Many plant species have been used and continued been used in folkloric medicine in treatment of numerous ailments despite flot of synthetic drugs in the market today (Uraku et al., 2015. Phytocompounds from these medicinal plants are essential in pharmaceutical industry for drug development and preparation of therapeutic agents (Gopalakrishnan and Udayakumar, 2014). The development of pharmaceuticals begins with identification of bioactive principles, detailed biological assays and dosage formulations followed by clinical studies to establish safety, efficacy and pharmacokinetic profile of the new drug (Gopalakrishnan and Udayakumar, 2014). There are approximately 500 000 plant species occurring worldwide, but only 1 % has been phytochemically investigated (Okwu and Ighodaro, 2010). There is great potential for discovering novel bioactive compounds from the rest of the plant kingdom.

Hyptis spicigera commonly known as bushmint in English is a member of lamiaceae family. It is a strong aromatic plant and may be herbaceous annual or perennial plant of 0.5-1m

high (Uraku *et al*., 2015). In Nigeria, *H. spicigera* is known as "Bunsuru fadama" or "Dai fadama" in Hausa, "Ogwu awunta" in Igbo and "Ogun efon" in Yoruba (Lambert *et al.,* 1985). The plant possesses very tiny brown and black seeds that clustered in groups of four, five or even more which are encased in each flowers that make-up inflorescence (Lambert *et al.,* 1985). *H. spicigera* is widespread in tropical North and South America as well as part of West Africa (Conti *et al*., 2011). Also, it is distributed in tropical and warm temperture region. It grows naturally in roadside, waste and damp places as well as in cultivated farmland. Globally, there are 300-400 species of the plant but in Nigeria, about fifty species are found.

Figure 1. *The Hyptis spicigera.*

In Latin America, *H. spicigera* are used tradomedically to heal wound and treat gastrointestinal disorders, respiratory tract infections, colds, pain, fever, cramps and skin disease. Infusions prepared with the leaves are used against cough and headaches (Jirovetz *et al*., 2000). The Bauju and Tyapp people of Southern Kaduna State of Nigeria used the inflorescence to cure headeache by sniffing (Jirovetz *et al*., 2000).The decoction of *Hyptis spicigera* is used as bath water or tea, as eupneic, or expectorant to treat bronchial secretions (Uraku *et al*., 2015). The powder obtained from the aerial organs is used as an antimigraine drug (Conti *et al*., 2011). Fresh inflorescences of this plant are used to treat headaches. In most Southern-Africa countries, this species is exploited by farmers to control grain infestations in storage, insect pests and to fight mosquitoes (Gabi *et al.,* 2012). The volatile oils from the plant are known for their antiseptics, bactericidal, virucidal, fungicidal and medicinal potentials (Jirovetz *et al*., 2000). The oils or some of their components are used in perfumes and make-up products, sanitory products, densitry, agriculture as food preservatives and additives and as natural remedies (Lambert *et al*., 2000). The essential oils are used frequently in aromatherapy and have been tested for their potentials as protective agents for human and livestock feeds (Lambert *et al*., 2000). The whole plant is used in traditional stores to protect cowpea against damage by *Callosobruhus* species. In some part of Africa region, *H. spicigera* leaves are used as a spray to keep and protect crops from various insect attacks and are placed in a layer below bundles of millet to keep away termites (Jirovetz *et al*., 2000).

Despite the popular use of *Hyptis spicigera* leaf for treatment of various disorders, there is limited data available regarding Gas Chromatography–Mass Spectrometry (GC-MS) analysis of the chemical constituents. This study therefore aimed to evaluate the Gas Chromatography–Mass Spectrometry (GC-MS) analysis of the chemical constituents of the methanol extract of *Hyptis spicigera* leaf from Abakaliki, Nigeria.

2. Materials and Methods

2.1. Collection and Identification of Plant Material

Fresh leaves of *Hyptis spicigera* were collected with hand in glove from Presco Campus of Ebonyi State University, Abakaliki, Nigeria. The plant samples were identified and authenticated by Prof. (Mrs) Nwosu, a taxonomist in the Department of Botany, University of Nigeria, Nsukka, Enugu State, Nigeria.

2.2. Preparation of Plant Material

The leaves of *Hyptis spicigera* were sorted and washed thoroughly with distilled water to remove dirt and debris. The washed plant materials were cut into smaller pieces before they were shade dried for 3 weeks at room temperature (28±3ºC). 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 polyethene bags protected from direct sunlight until required for use.

2.3. Plant Sample Extraction

Fouty (40) grams of the powdered leaves were extracted with 100 ml of 98% methanol overnight in a stopped bottle and with occasional stirring at room temperature (28±3ºC). 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 40ºC for 45 min in a rotary 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. The dry extract obtained was kept in a refrigerator at 4ºC until required for use.

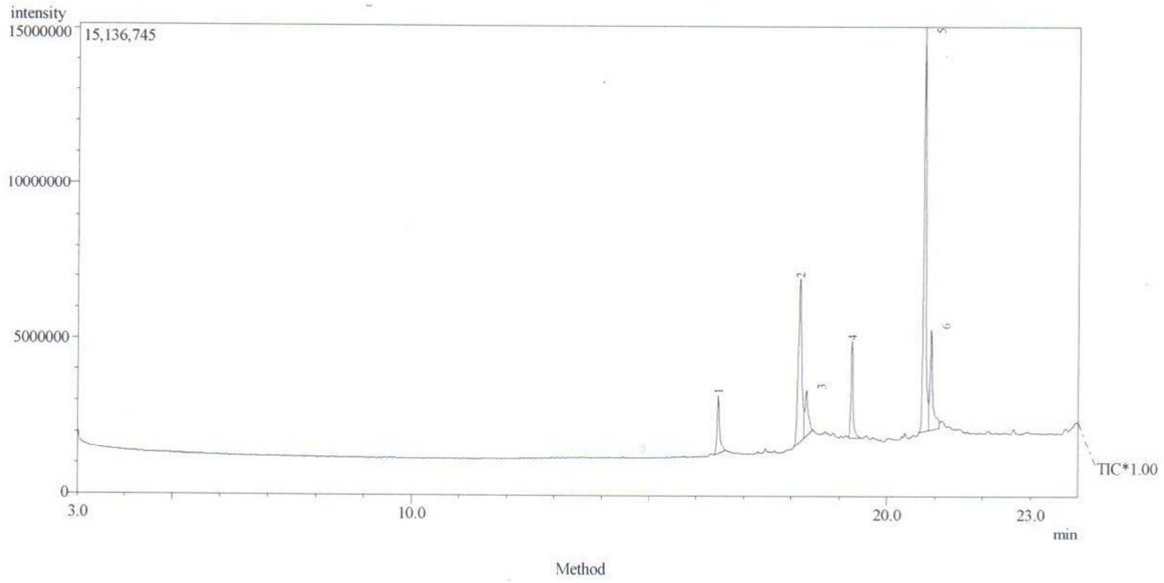
2.4. Gas Chromatography - Mass Spectrometry (GC-MS)

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 80ºC, Injection Temp 250ºC 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 200.00ºC and 250.00ºC 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 percentage with peak area normalization

2.5. 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.



Method

[Comment]

===== Analytical Line 1 =====

[AOC-20i]

# of Rinses with Presolvent	:4
# of Rinses with Solvent(post)	:4
# of Rinses with Sample	:3
Plunger Speed(Suction)	:High
Viscosity Comp. Time	:0.2 sec
Plunger Speed(Injection)	:High
Syringe Insertion Speed	:High
Injection Mode	:Normal
Pumping Times	:5
Inj. Port Dwell Time	:0.3 sec
Terminal Air Gap	:No
Plunger Washing Speed	:High
Washing Volume	:8uL
Syringe Suction Position	:0.0 mm
Syringe Injection Position	:0.0 mm
Use 3 Solvent Vial	:1 vial

[GC-2010]

Column Oven Temp.	:70.0 °C
Injection Temp.	:250.00 °C
Injection Mode	:Split
Flow Control Mode	:Linear Velocity
Pressure	:116.9 kPa
Total Flow	:40.8 mL/min
Column Flow	:1.80 mL/min
Linear Velocity	:49.2 cm/sec
Purge Flow	:3.0 mL/min
Split Ratio	:20.0
High Pressure Injection	:OFF
Carrier Gas Saver	:OFF
Splitter Hold	:OFF

Oven Temp. Program

Rate	Temperature(°C)	Hold Time(min)
-	70.0	0.00

Figure 2. *GS-MS Chromatogram of Hyptis spicigera methanol leaves extract.*

3. Results and Discussion

For thousands of years, plants have been used tradomedically. Traditional knowledge of medicinal plants has guided search for new drugs overtime (Achi and Ohaeri, 2015., Sampath and Rama, 2011). Authentication of medicinal plants in genetic and chemical level is a critical step in the use of these botanical materials for both research purposes and commercial preparations (Rajeswari, *et al.*, 2012). In spite of the advantage of modern high drug discovery and screening techniques, traditional medicinal knowledge have also given clues to the discovery of valuable drugs (Balamurugan *et al*., 2012). There is growing awareness in correlating the phytochemical compounds with their biological activities (Janakiraman *et al*., 2012).

In GC-MS analysis, six peaks were identified from the chromatogram of the methanol leaf extract of *H. spicigera*. These peaks (1-6) indicate the presence of six compounds (1-6) in the extract (Figure 2). 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 leaf extract comprises mainly; Hexadecanoic acid (15.19%), 9- Octadecenoic acid (24.68%), Octadecenoic acid (7.60%), 5- hydroxylmethyl heptadecane (11.08%), Eicosane aldehyde (30.06%) and Octadecyl vinyl ether (11.39%) as the major chemical constituents.

The nature of compound and biological activity of identified compounds of the leaves of *H. spicigera* was represented in Table 2. The biological activities of the phytocompound of *H. spicigera* mentioned in Table 2 are based on phytochemical and ethnobotanical database by Jim Duke of the Agricultural Research service/USDA.

The fragmentation patterns of the peaks and identified compounds of the plant were shown in Figure 3. This indicated disintegration of large fragments into small compounds giving rise to apperance of peaks at different m/z ratios.

It is very pertinent to identify the possible roles of these constituent compounds in the curative properties attributed to the plant by herbal medical practitioners. The study on the active principle of methanol fraction of *H. spicigera* revealed that the plant contained a wide range of GC/MS chemical constituents which may be contribute to its therapeutical value. The GC-MS analysis showed a fragmentation pattern characteristic of the presence of fatty acids such as Hexadecanoic acid, octadecanoic acid and 9 - octadecenoic acid. This result is in line with the result of Aja *et al.,* 2014 on GC/MS analysis of *Moringa oleifera* leaf and seed which revealed that 9 - octadecenoic acid (20.89%) constitutes the major constituent of the leaf extract while oleic acid (84%) is the major component of the seed extract. These compounds are known to have antimicrobial activity, antioxidant, hypercholesterolemic, anticancer and hepatoprotective activity (Ogunlesi *et al*., 2010, Charles *et al*., 2011 and Omotosho *et al*., 2014).

In addition, the methanol fraction of *H. spicigera* showed the presence of aldehyde compound, eicosanoic aldehyde. These are component of membranes and precursor of a group of hormones like prostaglandins, thromboxanes and prostacyclines which are important in regulation of verse physiological processes (Okwu and Ighodaro, 2010, Zibbu and Batra, 2011). The eicosanoids have been reported to possess anti-inflammatory properties (Achi and Ohaeri, 2015 and Omotoso et al., 2014). The bioactivities of these compounds may depend on the lipophilic properties of their functional groups. Many of the metabolites have been found to possess interesting biological activities and find to be of optimal important and applicable as pharmaceuticals, insecticides, dyes, flavors and fragrances among others (Abirami and Rajendran, 2011)).

4. Conclusion

GC-MS analysis revealed that *H. spicigera* leaf is rich in hexadecanoic acid (15.19%), 9-octadecenoic acid (24.68%), octadecenoic acid (7.60%), 5-hydroxylmethyl heptadecane (11.08%), eicosane aldehyde (30.06%) and octadecyl vinyl ether (11.39%) bioactive compounds and this confirms the application of *H. spicigera* in ethno-medicine

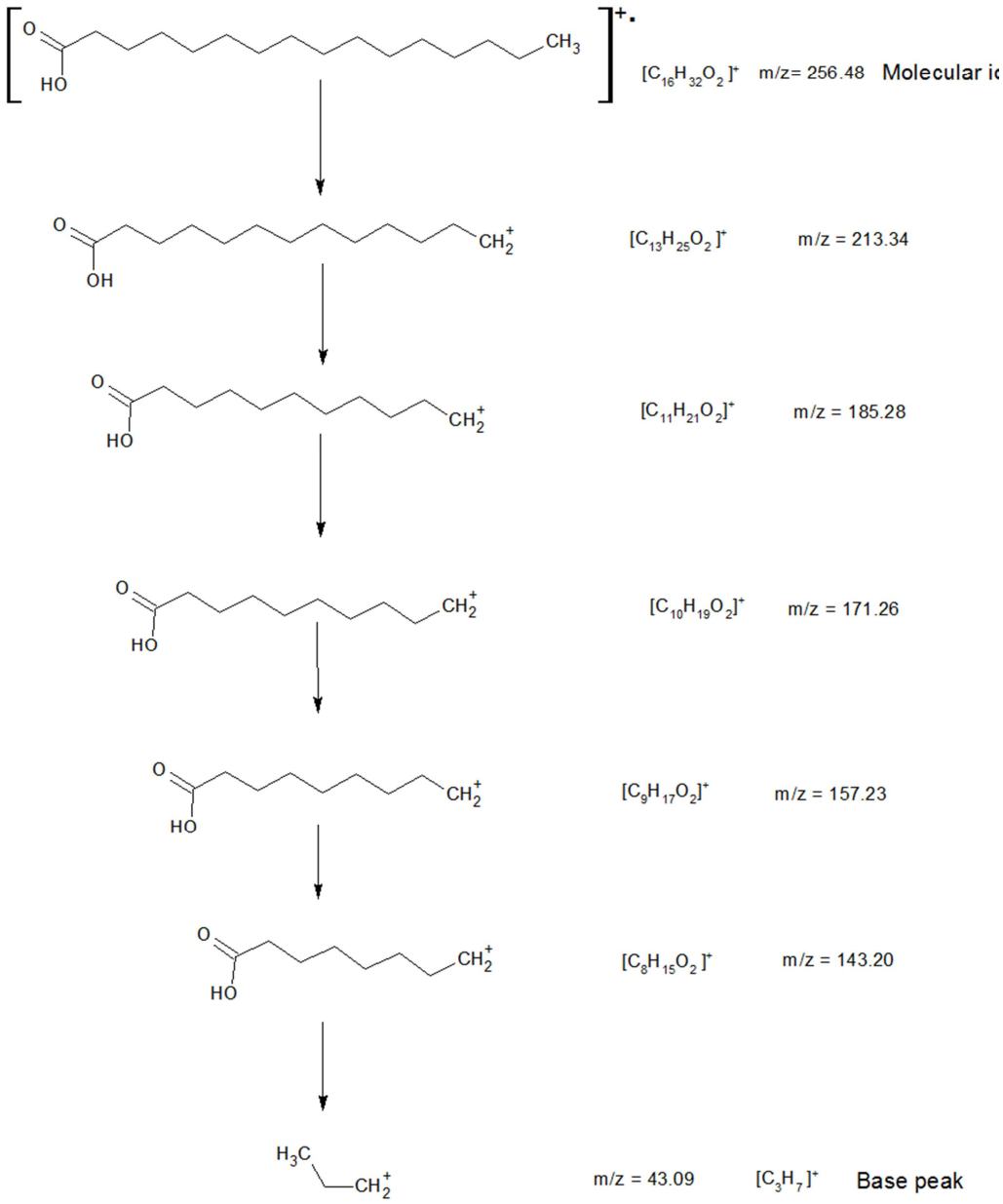
Table 1. *Chemicals identified from gc-ms analysis of methanol extract of H. spicigera leaves.*

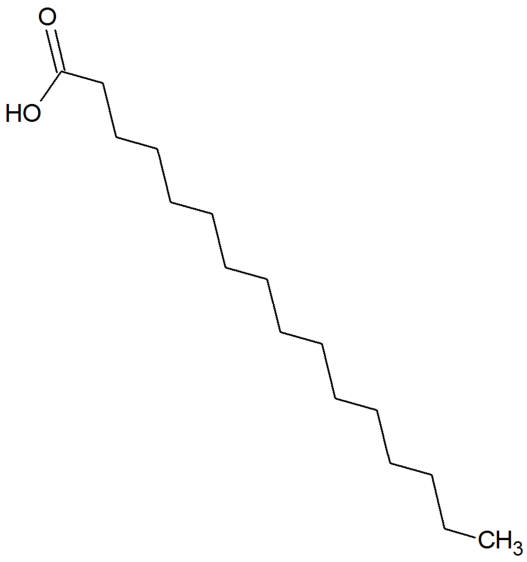
Peak no.	Retention time (RT) (S)	Name of compound	Molecular formular(MF)	Molecular weight (MW-g/mol)	Percentage content (%)	Peak area
1	16.47	Hexadecanoic acid	$[C_{16}H_{32}O_2]$	256.48	15.19	71
2	18.18	9- Octadecenoic acid	$[C_{19}H_{36}O]^+$	282.50	24.68	101
3	18.32	Octadecanoic acid	$[C_{18}H_{36}O_2]^+$	284.48	7.60	74
4	19.26	5-(Hydroxymethyl) heptadecane	$C_{18}H_{37}O]^+$	269.49	11.08	78
5	20.77	Eicosane aldehyde	$[C_{21}H_{41}O]^+$	309.59	30.06	158
6	20.92	Octadecyl vinyl ether	$[C_{20}H_{40}O]^+$	296.53	11.39	84

Table 2. *Biological actvity of phytocompounds identified from methanol extract of H. spicigera leaves.*

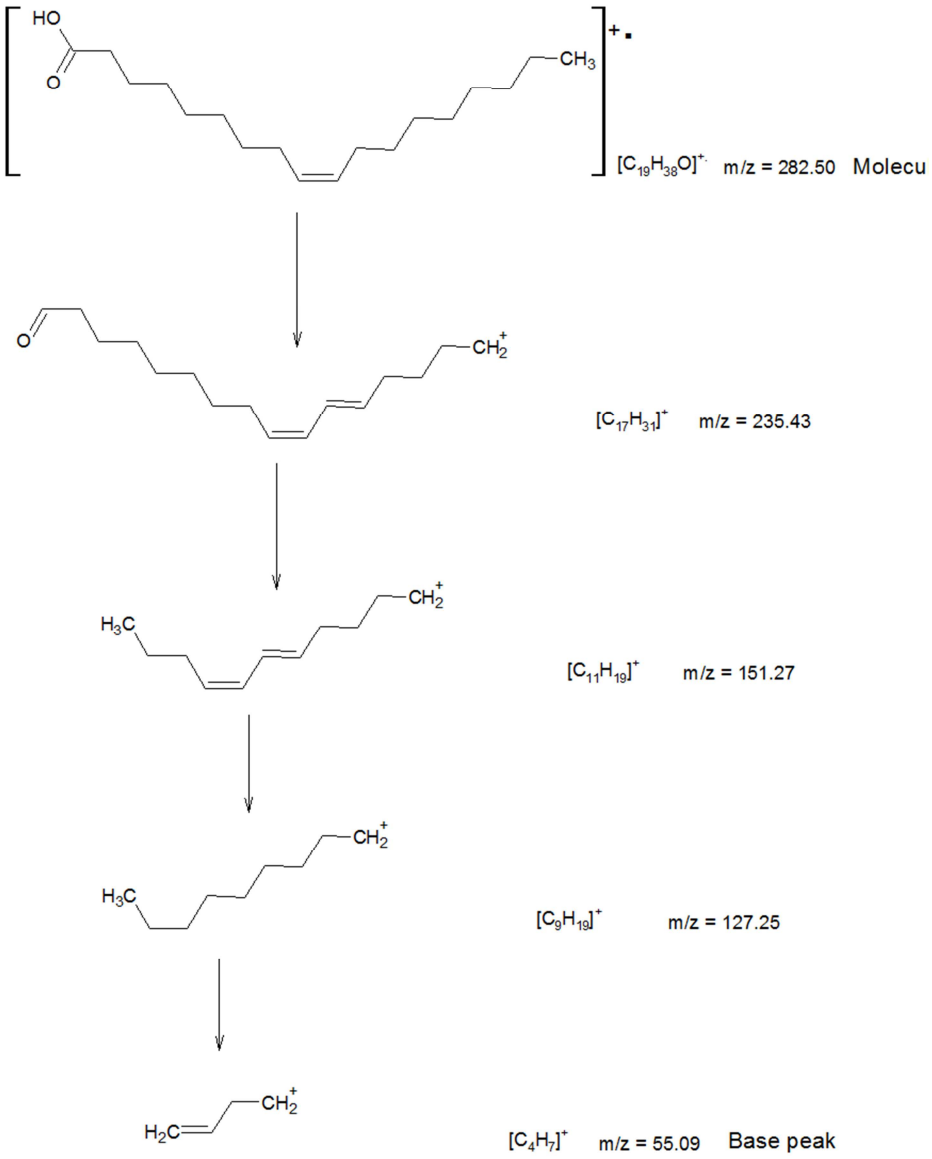
Peak no.	Name of Compound	Type of Compound	Biological Activity
1	Hexadecanoic acid	Palmitic acid	Antioxadant, hypocholesterolemic, lubricant, nematocide, and pesticide, antiandrrogenic and flavour
2	9- Octadecenoic acid	Oleic acid	Anti-inflammatory, Antiandrogenic, anemigenic, 5, α-reductase inhibitor, α- reductase inhibitor, lubricant, antitumor, choleretic, dermatitigenic, immunostimulant, anti-leucotriene-D4, lipoxygenase inhibitor, allergenic, flavour, hypocholesterolemic, insectifuge, irritant, percutaneo-stimulant, perfumery and propecic.
3	Octadecanoic acid	Stearic acid	Antiviral, antiinflammatory, 5-α-reductase inhibitor, hypocholesterolemic, propecic, suppository, flavour and cream formulation.
4	5-Hydroxymethyl heptadecane	Hydrocarbon	Nf
5	Eicosane aldehyde	Aldehyde	Antiinflammatory and anti-therogenic
6	Octadecyl vinyl ether	Alkoxyalkane or epoxide or ether	Nf

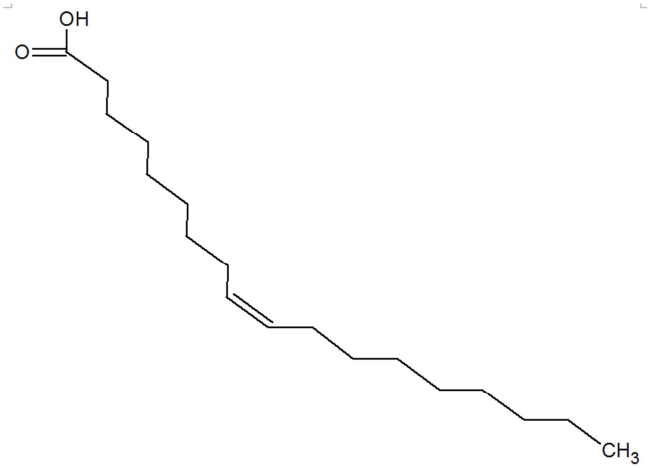
Nf: Not found. Source: Dr. Duke's phytochemical and ethnobotanical database.



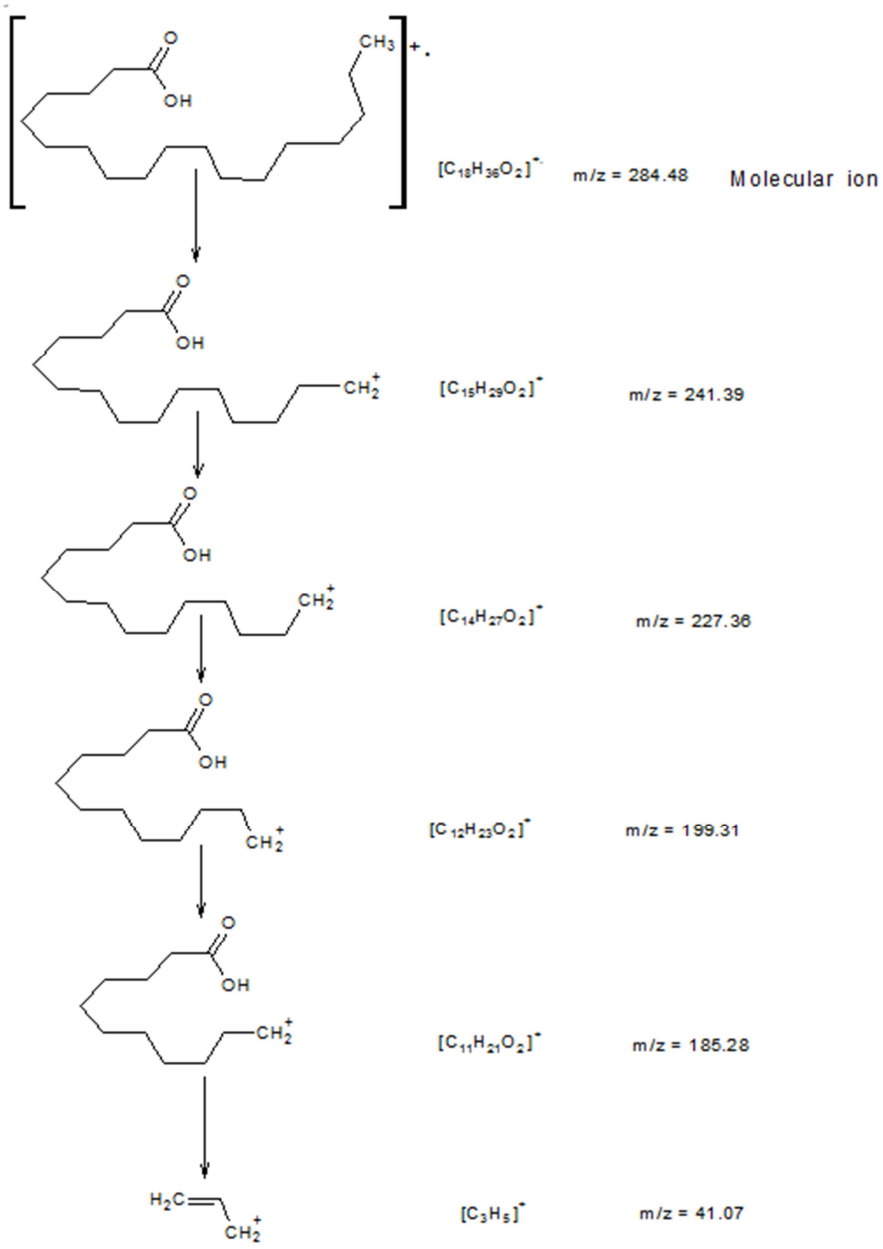


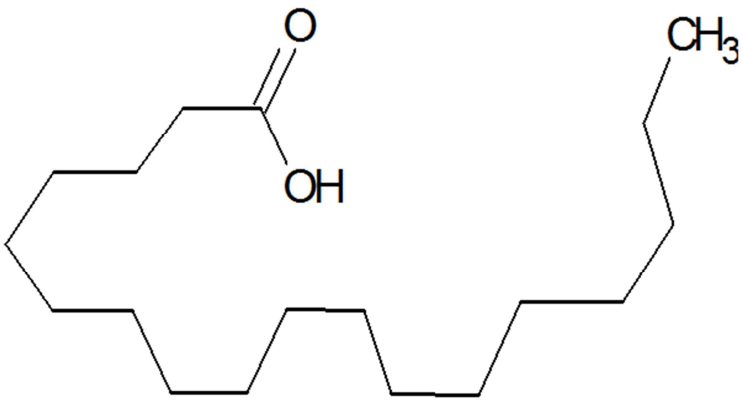
Hexadecanoic acid (Palmitic acid)



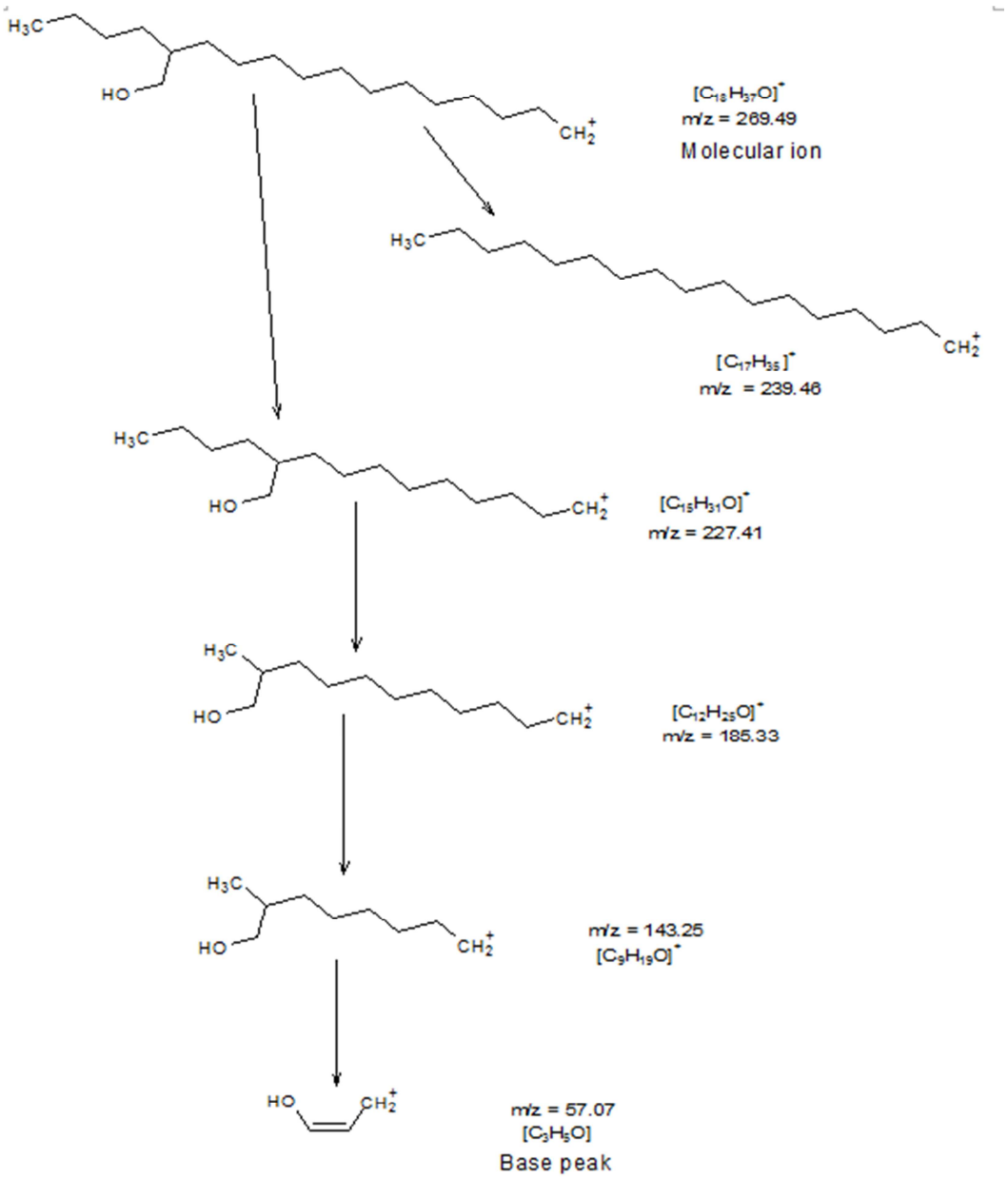


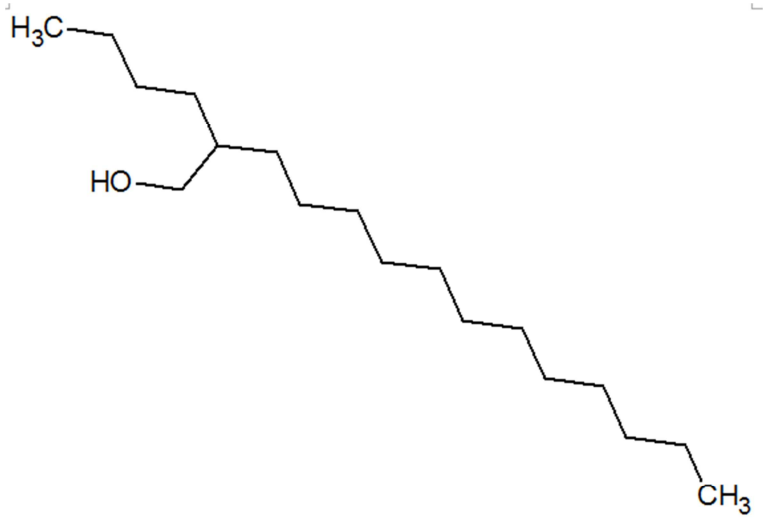
9-Octadecenoic acid (oleic acid)



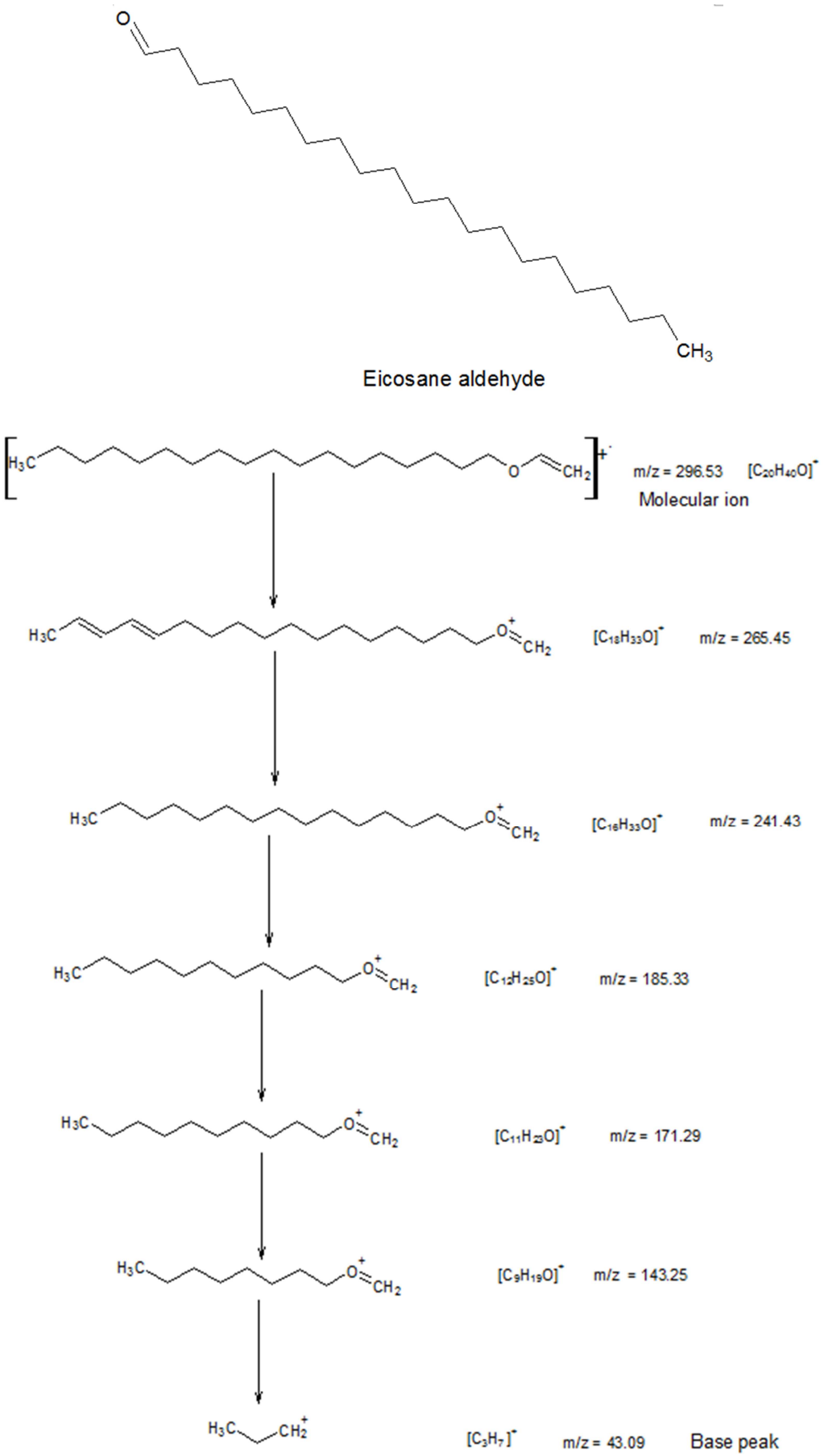


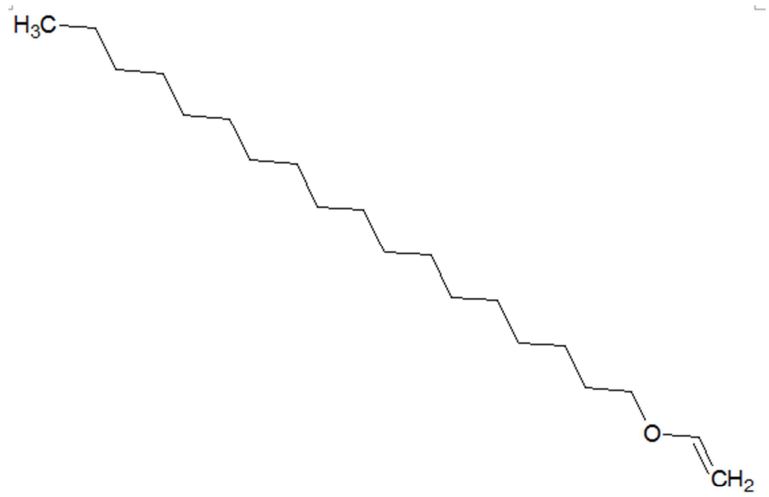
Octadecanoic acid (Stearic acid)





5-(Hydroxymethyl)heptadecane


Eicosane aldehyde
m/z = 296.53 [C20H40O]+
Molecular ion
[C18H33O]+ m/z = 265.45
[C16H33O]+ m/z = 241.43
[C12H25O]+ m/z = 185.33
[C11H23O]+ m/z = 171.29
[C9H19O]+ m/z = 143.25
[C3H7]+ m/z = 43.09 Base peak



Octadecyl vinyl ether

Figure 3. *The fragmentation patterns of the peaks and large fragments at different m/z ratios.*

Acknowledgement

Authors are grateful to Director of Research, Innovation and Commercialization (DRIC), EBSU and TETFund for financial support.

References

[1] Abirami, P., Rajendran, A (2011). GC-MS determination of bioactive compounds of *Indigofera aspalathoides. Journal of Natural Product and Plant Resources*, 1(4): 126-130.

[2] Achi, N. K. and Ohaeri, O. C. (2015). GC-MS determination of bioactive constituents of the methanol fractions of *Cnidoscolus aconitifolius. British Journal of Pharmaceutical Research, 5(3): 163-172.*

[3] Aja, P. M.**,** Nwachukwu, N., Ibiam, U. A., Igwenyi, I. O., Offor, C. E and Orji, U. O. 2014 .Chemical Constituents of *Moringa oleifera* Leaves and Seeds from Abakaliki, Nigeria, *AmericanJournal of Phytomedicine and Clinical Therapeutics 2 3 :310-321*

[4] Charles, A., Leo-Stanly, A., Joseph, M., Alex, R. V (2011). GC-MS analyses of bioactive components on the bark extract of *Alseodaphne semecarpifolia Nees* (Lauraceae) Asian *Journal of Plant Science Research* 1(4):25–32.

[5] Conti, B., Canale, A., Cioni, P. C., Flamini, G., Rifici, A. (2011). *Hyptis* and *Huptis spicigera* (Lamiacea) essential oil: quantitaive analysis, contact toxicity and repellent activity against *Sitophilus granaries* (L). *Journal of Pest Science,* 84: 219 - 228

[6] Duke"s Phytochemical and Ethnobotanical Database; 2014. Available: http://www.ars-grin.gov/cgibin/ duke/ethnobot/

[7] Gabi, B., Lawal, A. O. and Hauwa, B. S. (2012). Mosquito repellant activity and phytochemical characterization of essential oils from *Striga hermonthica, Hyptis spicigera* and *Ocimum basilicum* Leaf Extracts. *British Journal of Pharmacology and Toxicology,* 3(2): 43-48.

[8] Gopalakrishnan, K. and Udayakumar, R. (2014). GC-MS Analysis of Phytocompounds of Leaf and Stem of *Marsilea quadrifolia* (L.). *International Journal of Biochemistry Research and Review, 4(6): 517-526*

[9] Jirovetz, L., Buchbauer, G., Paschwwann, C., Ngassoum, M. B. (2000). Investigations of aromatic plants from Cameroon: analysis of the essential oils of flowers of *Hyptis spicigera* (L) by GC, GC/MS and olfactometry. *Journal of Essential Oil Bearing Plants*, 3: 71-77.

[10] Lambert, J., Arnason, J. T and Philogene, R. J. B (2000). Bruchid control wit traditionally used insecticidal plants, *Hyptis spicigera* and *Cassia nigricans*. *Insect Science Application*, 6:167-170.

[11] Okwu, E. D. and Ighodaro, U. B., (2010). GC-MS evaluation of bioactive compounds and antibacterial activity of the oil fraction from the leaves of *Alstonia boonei* De Wild *Der Pharma Chemica,* 2(1): 261-2.

[12] Ogunlesi, M., Okiei, W and Osibote, A. E., (2010) Analysis of the essential oil from the leaves of *Sesamum radiatum,* a potential medication for male infertility factor, by gas chromatography-mass spectrometry. *African Journal of Biotechnology,* 9(7): 1060-1067

[13] Rajeswari, G., Murugan, M and Mohan, V. R (2012). GC - MS analysis of bioactive components of *Hugonia mystax L.* (Linaceae). *Research Journal of Pharmaceutical, Biological and Chemical Sciences,* 3(4): 301-308.

[14] Zibbu, G., Batra, A (2011). GC-MS analysis of the desert plants of Apocynaceae family: *Neriumoleander L.* and *Thevetia peruviana* Schum. *International Journal of Pharmaceutical Research and Development*. 3(10): 49–62.

[15] Mahesh, B and Satish, S. (2008). Antimicrobial activity of some important medicinal plant against plant and human pathogens. *World Jounal Agricultural Science*, 4(S):839– 843.

[16] Janakiraman, N., Johnson, M., Sahaya, S. S. (2012). GC-MS analysis of bioactive constituents of *Peristrophe bicalyculata* (Retz.) Nees. (Acanthaceae). *Asian Pacific Journal of Tropical Biomedicine*, S46– S49.

[17] Balamurugan K, Nishanthini A, Mohan VR (2012). GC-MS analysis of *Polycarpaea corymbosa* (L.) Lam whole plant. *Asian Pacific Journal of Tropical Biomedicine*, S1289– S1292.

[18] Nisha K, Darshana M, Madhu G, Bhupendra MK (2011). GC-MS analysis and anti-microbial activity of *Psidium guajava* (leaves) grown in Malva region of India. *International Journal of Drug Development and Research*, 3(4):237– 245.

[19] Ncube NS, Afolayan AJ, Okoh AI (2008). Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *Afr J Biotechnol.,* 7(12):1797–1806.

[20] Prafulla, S., Lal, S. (2012). *Marsilea quadrifolia* linn. A valuable culinary and remedial fern in jaduguda, jharkhand, India. International *Journal of Life science & Pharmacological Reasearch.*, 2(3): 99–104.

[21] Oyagbemi, A. A., Odetola, A. A. (2010). Hepatoprotective effects of ethanolic extract of *Cnidoscolus aconitifolius* on paracetamol-induced hepatic damage in rats. *Pakistain Journal of Biological Science,* 13: 164-169.

[22] Selvamangai G, Bhaskar A (2012). GC-MS Analysis of phytocomponents in the methanlic extract of *Eupatorium triplinerve. Asian Pacific Journal of Tropical Biomedicine.* S1329 -S1343.

[23] Omotosho, A. E, Ezealisiji, K., Mkparu, K. I. (2014). Chemometric profiling of methanolic extract of *Cnidiscolus aconitifolius* (Euphorbiaceae) using UV-VIS, FTIR and GC-MS techniques. *Peak J. Med Plant Res.* 2(1):6-12.

[24] Omotoso, A. E, Eseyin, O. O, Suleiman, M (2014). Phytochemical Analysis of *Cnidoscolus aconitifolius* (Euphorbiaceae) leaf with Spectrometric Techniques. *Nig J. Pharm. Appl. Sc. Res*. 3(1):38-49.

[25] Sampath, K. S, Rama, K. N (2011). Pharmacognostic and Phytochemical investigation of *Naringi crenulata* (Roxb.) Niicols. *Journal of Life,* 31(1):17-21.

[26] *Uraku, A. J., Okaka, A. N. C., Ibiam, U. A., Agbafor., K. N., Ajah, P. M., Nweke, F. N (2015)*. Evaluation of chemical compositions of *Ocimum baslicum* and *Hyptis spicigera* leaves*. Ebsu Journal of Nature,* 1:21-33

[27] Uraku, A. J., Okaka, A. N. C., Ibiam, U. A., Agbafor, K. N., Obasi, N. A., Ibiam, Ajah, P. M., Obasi, O. U. and Nwalo, F. N. (2015). Antiplasmodial activity of ethanolic leaf extracts of *Spilanthes uliginosa, Ocimum basilicum (Sweet Basil), Hyptis Spicigera and Cymbopogon citratus on* mice exposed to *Plasmodium berghei* Nk 65. *International Journal of Biochemistry Research & Review,* 6(1): 28-36.