PHYTOCHEMICAL AND GAS CHROMATOGRAPHY-MASS SPECTROMETRIC (GC-MS) ANALYSES OF *ZANTHOXYLUM ZANTHOZYLOIDES* LEAF

P. M. Aja[1]., N. B. Nwokporo[1]. L. Nweke[2], and C. O. Okoro[1]

1. Department of Biochemistry, Faculty of Sciences, Ebonyi State University Abakaliki, Nigeria

2. Department of Medical Biochemistry, Faculty of Basic Medicine, Ebonyi State University Abakaliki, Nigeria

Abstract

Zanthoxylum zanthoxyloides is a medicinal plant widely used in folkloric medicine of Africa and Asia for the treatment of ailments such as inflammation, cancer, anemia and liver damage. The phytochemical and GC-MS analyses of *Zanthoxylum zanthoxyloides* leaf were carried out using standard methods. Qualitative phytochemical revealed the presence of flavonoids, alkaloids, saponins, cardiac glycosides and tannins from the sample. Twelve (12) chemical constituents were identified from GC-MS analysis of the sample which include ethylcyclohexane (0.43%), 1,3-cyclopentadiene (0.60%), 5-(1-methylethylidene) (0.91%), heptane (2.94%), octa-1,3,5,7-tetraene (1.60%), isopropyl benzene (cumene) (2.64%), 3,5-dimethylhepta-3,-dien-l-yne (6.601%), oct-2-ene (12.517%), oct-2-ene (12.62%), heptanoic acid (16.44%), hepta-3,5-dien-l-ol (30.53%), and hept-3-en-1-ol(12.17%). Result obtained showed that the leaf extract of *Zanthoxylum zanthoxyloides* has hepta-3, 5-dien-l-ol (30.53%) as the highest and ethylcyclohexane (0.43%) as the lowest chemical compound. These relative diverse chemical constituents may be responsible for the medicinal properties of *Zanthoxylum zanthoxyloide leaf*.

Key Words: GC-MS analysis, Chemical constituents, *Zanthoxylum zanthoxyloides* leaf, ethanol extract.

Introduction:

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi, and herbivorous mammals (Tapsell *et al.,* 2006). At least 12,000 of such compounds have been isolated so far a number estimated to be less than100% of the total (Tapsell *et al.,* 2006). Chemical compounds in plants mediate their effect on human body through processes identical to those already well understood for the chemical compound in conventional drugs. Thus herbal medicines do not differ from conventional drugs in terms of how they work. This enables herbal medicine to be as effective as conventional medicines but also give them same potential to cause harmful side effects (Sofowora, 1993).

Zanthoxylum zanthoxyloides is a far root plant species in the genus *zanthoxylum* (Adesina , 2005). It is a deciduous shrub and tree of the family *Rutaceae* which comprises 250 species that are native to warm temperate and sub tropical region of the world. In Nigeria *Z. zanthoxyloides* is a common component of the rainforest vegetation of southern Nigeria and is also distributed in African countries (Adesina, 2005). *Zanthoxylum zanthoxyloides* is commonly called Senegal prickly-ash, Candle wood in English, "Ata" in Yoruba, "Fasakwari" in Hausa, "Uzazi" in Igbo and "Nka" in Ebonyi State(Adesina , 2005).

Despite numerous researches work done in *Zanthoxylum zanthoxyloides* no documented data/information has been done on the GC-MS constituents of the leaf. Though, with recent drop in the price of crude oil in the international market and its attendant effect on economy of less developed nations like Nigeria, it has become vivid that medicinal plants/ staple crops will play increasing role in the food, nutrition and health security of the rural populace and the increasing urban poor. As popular as *Zanthoxylum zanthoxyloides* is in Nigeria, there is a paucity of information on GC-MS analysis of the leaf .This study therefore evaluates GC-MS analysis of the ethanol leaf extract of *Zanthoxylum zanthoxyloides* leaf.

Figure 1: *Zanthoxylum zanthoxyloides* Leaves

Materials and Methods

Materials

Plant Collection:

The fresh leaves of *Zanthoxylum zanthoxyloids* were collected form Ezza North Local Government Area of Ebonyi State. And were identifies by taxonomist in the Department of Applied Biology Ebonyi State University, Abakaliki, Nigeria. Apart was also deposited in the hebarium for reference purpose.

Preparation of Sample

The leaves were destalked, washed and shook dried at ambient temperature with constant turning averts fungal growth. The seeds were destalked and dried through the same process. The leaves were later milled to obtained the vegetable leaf meals (VLMS) using an electric blender and were stored in 4^{o}C temperature in refrigerator in well labeled air tight containers for analysis.

Preparation of Extract

Exactly 40mg of dried powdered leaves of *Zanthoxylum zanthoxyloides* were extracted successively with 300ml of ethanol in an orbital shaker for 24hours at room temperature. The extracts were filtered using whatman No.1 filter paper to remove extractable sustenance(s) at every 3hrs interval. The combined extracts were then evaporated with rotary evaporated and the dried extracts were stored at $4^{o}C$ in two different sterile containers.

Methods

Preliminary Phytochemical Analysis

The preliminary phytochemical screening for the presence of tannins, sapanins, alkaloids, cardiac glycosides and flavonoids were carried out on the extract of *Zanthoxylum zanthoxyloides*.

Test for the Presence of Tannins: This was carried out by the method of Harborne (1973)

Principle: Tannins are secondary metabolites of plant species and consist of sugar and non-sugar parts. They are capable of undergoing hydrolysis when inserted into dilute acids or boiling water to give rise to products such as polyhydroxyl phenolic compounds. They are reactive following the possession of functional groups called hydroxyl group (OH). They participate in redox reaction to give characteristics colour change on the reagent applied.

Procedure: One milliliter (1ml) of crude extract of the sample was collected using syringe and dispensed into test tube. Then, one milliliter (1ml) of ferric chloride ($FeCl_3$) was added to the test tube. A dirty green precipitate was observed which showed the presence of tannins.

Test for the Presence of Saponins: This was carried out by the method of Harborne (1973)

Principle: Saponins are glycosides with distinctive foaming characteristics. They consist of a polycyclic aglycone that is either a choline steroid or triterpeniod attached through C_3 and an ether bond to a sugar side chain. The aglycone is referred to as the sapogenin and steroid saponins are called saraponins. The ability of saponins to foam is caused by the combination of the non-polar sapogenin and the water –soluble side chain (hydrophilic part), which have hydroxyl groups (¯OH) as functional groups that is why the frothing test is carried out.

Procedures:

Frothing Test

Two milliliters (2mls) of the extract were diluted with 5ml of distilled water in a test tube. The mixture was stirred vigorously for about 5mins and was allowed to stand for 30minutes. Frothing which persisted for this duration indicated the presence of saponins.

Emulsion Test:

An emulsion is any thick liquid in which tiny drops of oil or fat are evenly distributed. Two to Five (2-5) drops of olive oil were added to 3mls of the sample in a test tube, stirred vigorously and allowed to stand for 30mins. Emulsification that was observed for this duration indicated the presence of saponins.

Test for Presence of Alkaloids: This was carried out by the method of Trease and Evans (1989)

Principle: Alkaloid can be detected as loose complexes following their ability to react with some reagents by producing characteristics colour changes depending on the type of reagent used. Alkaloids have an amino group (NH_2) as their functional group as in nicotine.

Procedure: Two milliliters (2mls) of the extract was collected using syringe and was dispensed into a test tube, the test tube was heated for 2mins and 5mls of hydrogen (HCl) was added and heated again and allowed to cool. The mixture was divided into A and B. To A, 2 drops of Meyer's reagent was added and white precipitate was observed which showed the presence of Alkaloids. To B, 2 drops of Dragendroff's reagent was added and the formation of red precipitate was observed which confirmed the presence of alkaloids.

Test for the Presence of Flavonoids: This was carried out by the method of Harborne (1973).

Principle: Flavonoids are colourless or pale yellow glycosides that are not soluble in non- polar solvents. They are compound that are oxidize by ethyl-acetate. They react with polar solvent to produce colour changes in accordance with the level of redox reactions that are likely to take place. Flavonoids also reacts with sodium hydroxyl group (NaOH) to form a yellow colour following the reaction of the hydroxyl group ($^-$OH) with the ketone functional group.

Procedure: Five milliliters (5ml) of the extract was collected using syringe and was dispensed into a test tube. Exactly 10mls of distilled water, 5mls of dilute ammonium hydroxide (NH_4OH) and few drops of tetraoxosulphate (VI) acid (H_2SO_4) were added in the test tube. A yellow colouration was observed which showed the presence of flavonoids.

Test for the Presence of Cardiac Glycoside This was carried out by the method of Harborne (1973)

Principles: Cardiac glycosides are organic compounds that are capable of undergoing hydrolysis in the presence of dilute acids, alkali or enzymes.

Procedure: Two milliliters (2mls) of the extract was collected into a test tube and 5ml of glacial acetic acid was added and then 2mls of $FeCl_3$ and 2mls of concentrated ferric acid were added too. A brown ring formation at inter phase of the mixture indicated the presence of deoxy sugar characteristics of cardiac glycosides.

GC-MS Analysis:

Procedures:

GC-Ms analysis of the ethanol extract of *Zanthoxylum zanthoxyloides* leaf was performed using Shimadzu Japan gas chromatography QP2010 plus with a fused gas chromatography (GC) column (2010) coated with poly-methyl silicon (0.25nm x 50m) and the conditions were as follows: Temperature programming from 80-200^{o}C held at 80^{o}C for 1minute, rate 5^{o}C/min and at 200^{o}C for 20min. Field ionization detector (FID) Temperature of 300^{o}C, injection temperature of 220^{o}C, carrier gas nitrogen at a flow rate of 1ml/min, split ratio of 1:75. Gas chromatography mass spectrum was conducted using GCMS-QP 2010 plus Shimadzu Japan with injector temperature of 220^{o}C and carrier gas presence of 116.9kpa. The column length is 30m with a diameter of 0.25mm and flow rate of 50ml/min. Elutes were automatically passed into a mass spectrometer

with a dictator voltage set at 1.5 Kv and sampling rate of 0.2 sec. The mass spectrum was also equipped with a computer fed mass spectra bank. German Hermlez 233M-Z centrifuge was used.

Component Identification

Chemical constituent of the extract was identified by matching the peak with computer Wiley Ms libraries and confirmed by those comparing mass spectra of the peaks and those from literature

Results

Result of qualitative phytochemical Analysis of *Zanthoxylum zanthoxyloides leaf extract*

The qualitative phytochemical screening on the ethanol-leaf extract of *Zanthoxylum zanthoxyloids* revealed the presences of alkaloids, flavonoids, glycosides, tannins and saponins as shown in Table 1.

Table1: Phytochemical screening of *Zanthoxylum zanthoxyloids* Ethanol Leaf-Extract.

Phytochemicals	**Remarks**
Alkaloid	**Positive**
Cardiac glycosides	**Positive**
Flavonoids	**Positive**
Saponins	**Positive**
Tannins	**Positive**

Result of GC-MS Analysis of *Zanthoxylum zanthoxyloides* Leaf

The ethanol extract of the leaves of *Zanthoxylum zanthoxyloides* showed twelve peaks (Fig.2). These peaks indicate the presence of twelve compounds (1-12) in the extract (Table 2). The composition of the extract comprises of ethlycyclohexane, 1,3-cyclopentadines, 5-(1-methylethylidene), heptanes, acta-1, 3,5,7-teraene, isopropylberizene (Cumene), 3,5-dimethylhepta-3, 5-dien-1-yne, act-2-ene, heptamoin acid, hepato-3-5-dien-1-ol and hept-3-en-1-ol as the major chemical constituents (Table 2).

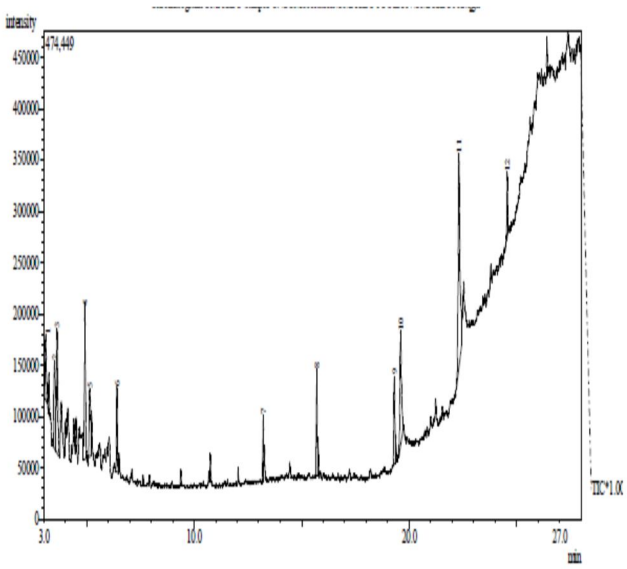


Figure 2: Gas Chromatogram of ethanol leaf extract of *Zanthoxylum zanthoxyloides.*

Table 2: Result of GC-MS Analysis and Mass Spectral Data of *Zanthoxylum zanthoxyloides* Leaf Extract Showing Molecular Formula, Molecular Weight, Percentage Content, Retention Time and Base Peak.

Peak	Compound	Molecular Formula	Molecular Weight	Percentage Content	Mass Peak	Retention Time
1	Ethylcyclohexane	C_8H_{15}	111	3.091	0.427	19
2	1, 3-Cyclopentadiene, 5- (1-methylethlidene)	C_8H_9	105	3.501	0.600	22
3	Heptane	C_7H_{18}	100	3.618	0.907	17
4	Octa-1, 3,5,7- tetraene	C_8H_9	105	4.911	2.936	22
5	5. Isoprpylbenzene (cumene)	$C_{12}H_{12}$	156	5.142	1.601	18
6	3,5-dimethylhepta-3,5-dien-l-yne	$C_{11}H_{11}$	143	6.403	2.642	19
7	Oct-2-ene	C_8H_{15}	111	13.214	6.605	18
8	Oct-2-ene	C_8H_{15}	111	15.702	12.517	23
9	Oct-2-ene	C_8H_{15}	111	19.19.316	12.623	24
10	Heptanoic acid	$C_7H_{13}O_2$	129	19.606	16.440	34
11	Hepta-3,5-dien-1-01	$C_7H_{11}O$	111	22.316	30.531	47
12	Hept-3-en-1-01	$C_7H_{13}O$	113	24.567	12.170	35

Discussion and Conclusion:

Discussion:

The phytochemical analysis of *Zanthoxylum zanthoxyloides* leaf showed the presence of alkaloids, flavonoid, sapponin, tannin and cardiac glycoside. The result of this study correlated with the report of Aja *et al.* (2010) which revealed the presence of these bioactive compounds in *Talinum triangulare* leaf in both dry and wet samples. Offor *et al*. and Aja *et al.* (2015) also reported the presence of all the phytochemicals in various concentrations in *Terminalia catappa* leaf, *Cajanus cajan* leaf and seed respectively. These phytochemicals found in the leaf extract of *Zanthoxylum Zanthoxyloides* showed several medicinal benefits. Alkaloids and the synthetic derivatives are used as the basic medicinal agent because of their analgesic anti-spasmodic and anti-bacteria properties (Dubes *et al.*,, 1990). Glycoside may inhibit the growth of many RNA and DNA viruses, thereby inactivating herpes simplex virus particles irreversibly. Some glycosides have inhibitory effects on enzymes such as 11-beta-hydroxysteroid dehydrogenase, which convert active cortisol to inactive cortisol in the kidney (Mishra *et al.*, 1991).

The presence of the chemical, heptanoic acid reduces prostaglandin production and COX-2 expression in colon cancer cells inflammation and subsequent elevation of the enzyme cyclooxygenase-2 (COX-2) are two of such factors involved in the development of colon cancer and inhibition of these process could be important target for chemoprevention (Ranjan, 2009). It down regulates TNFa from Lps-stimulated monocytes, shorten the half life of COX-2 m RNA in a dose dependent fashion, which resulted in net reduction of PG $E_{2.}$ And this is in accordance with the chemicals constituent of *Moringa oleifera* leaves by Aja *et al.* (2014) and also according to Charles and Ophardt (2003) in chemical constituents of *Zanthoxylum zanthoxyloids*. Ethyl-cyclohexane plays roles in the treatment of acute myeloid leukemia and can booster the production of a protein anticancer agent called L-asparaginase (Ranjan, 2009). In the 1950's a biochemical difference in metabolism related to the amino acid asparagines was found. Normal cells apparently can synthesize asparagines while leukemia cells cannot. If leukemia cells are deprived of asparagines, they will eventually die (Ranjan, 2009). So if the enzyme L-asparaginase is given to humans, various types of leukemia's can be controlled since l-asparaginase is an enzyme that destroys asparagines external to the cell. Normal cell are able to make all the asparagines they need internally where as tumor cells become depleted rapidly and die. This enzyme converts asparagines in the blood into aspartic acid by a deamination reaction. The leukemia cells are thus deprived of their supply of asparagines and will die (Charles and Ophardt, 2003).

Conclusion

The result obtained showed that the ethanol leaf -extract of *Zanthoxylum zanthoxyloids has* hepta-3, 5-dien-l-ol (30.53%) as the highest and ethylcyclohexane (0.43%) as the lowest chemical compound. This may be responsible for the health benefits of *Zanthoxylum zanthoxyloides* leaf.

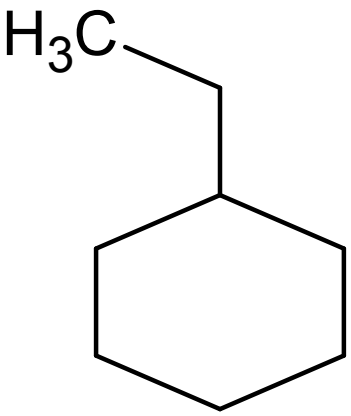
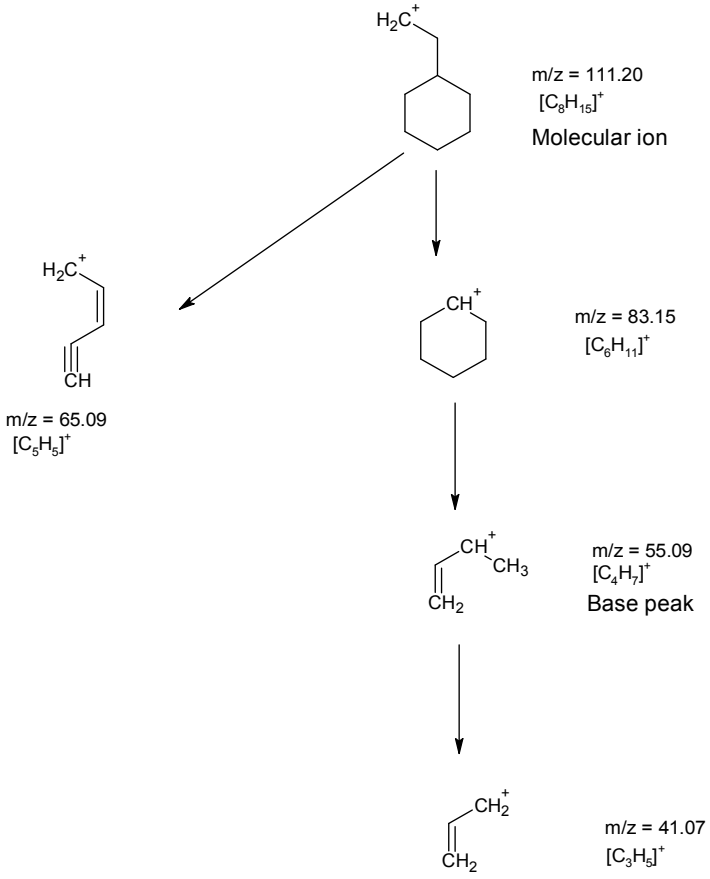


Figure 1: Ethylcyclohexane



Scheme 1: Fragmentation pattern for Ethylcyclohexane

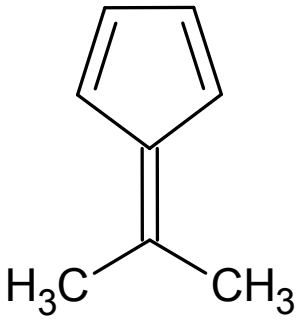
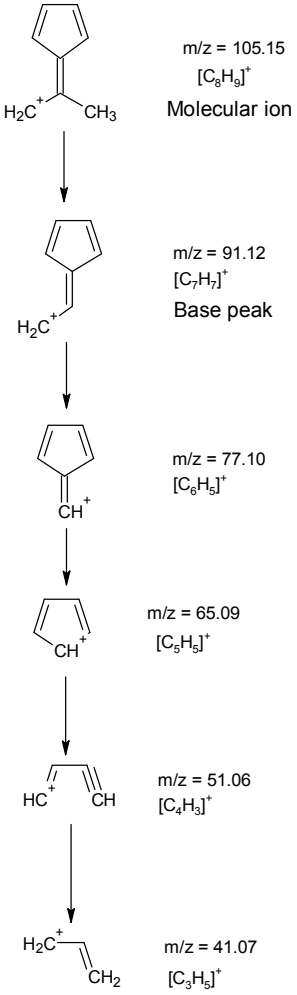


Figure 2: 1,3-Cyclopentadiene, 5-(1-methylethylidene)



Scheme 2: Fragmentation pattern for 1,3-Cyclopentadiene, 5-(1-methylethylidene)

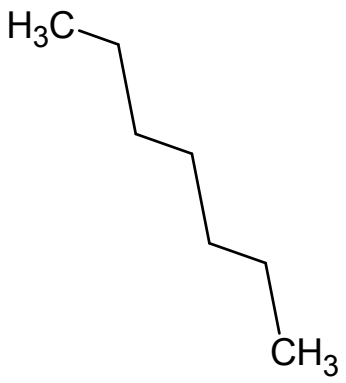
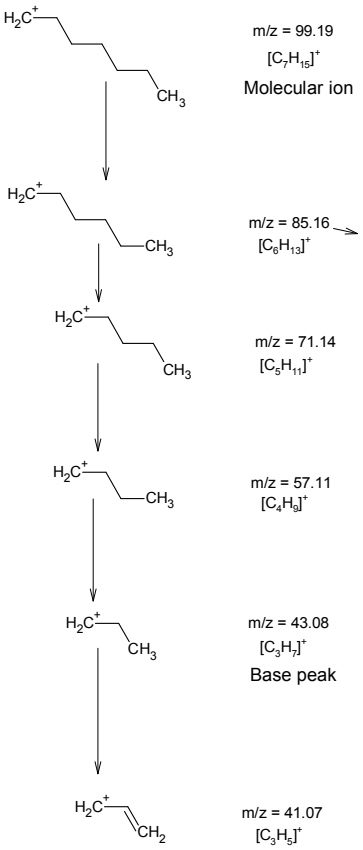


Figure 3: Heptane



Scheme 3:Fragmentation pattern for Heptane

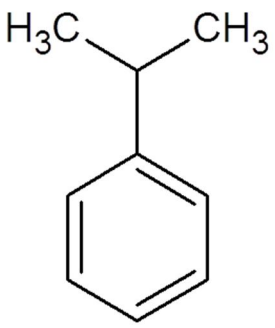
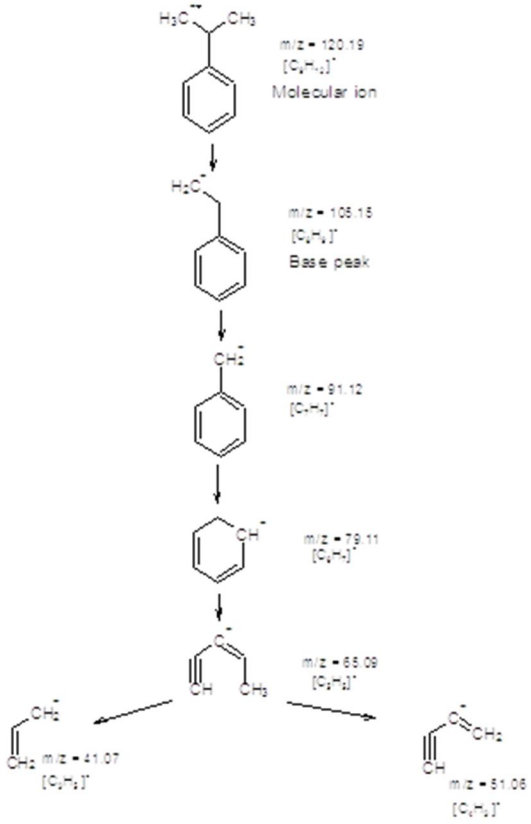


Figure 5:Isopropylbenzene (Cumene)



Scheme 5: Fragmentation pattern for Isopropylbenzene (Cumene)

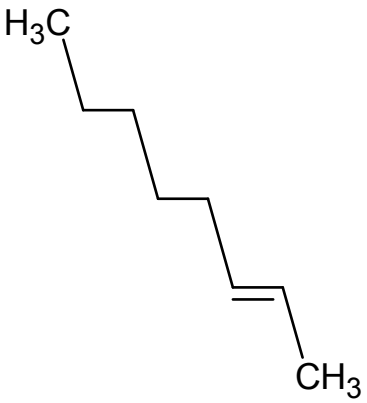
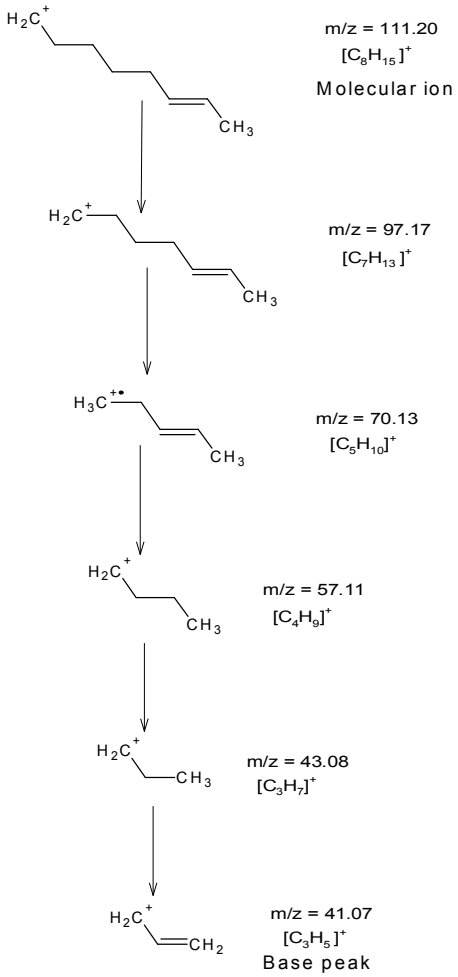


Figure 7: Oct-2-ene



Scheme 7: Fragmentation pattern for Oct-2-ene

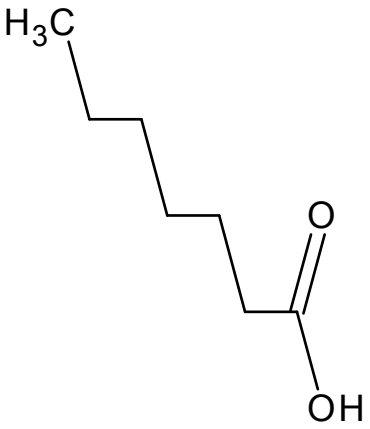
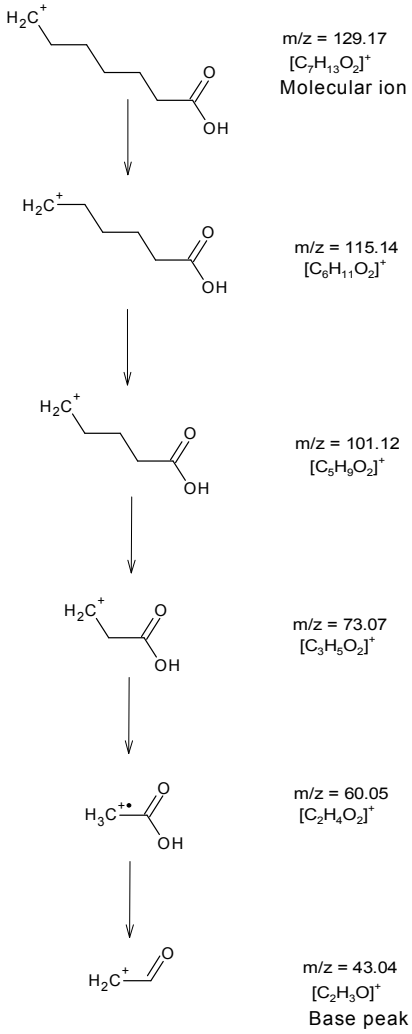


Figure 10: Heptanoic acid



Scheme 10: Fragmentation pattern for Heptanoic acid

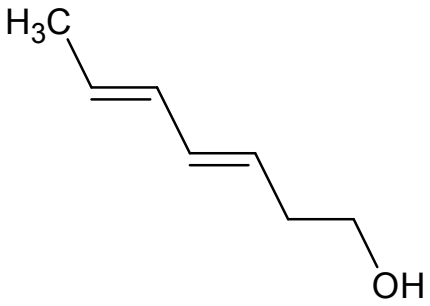
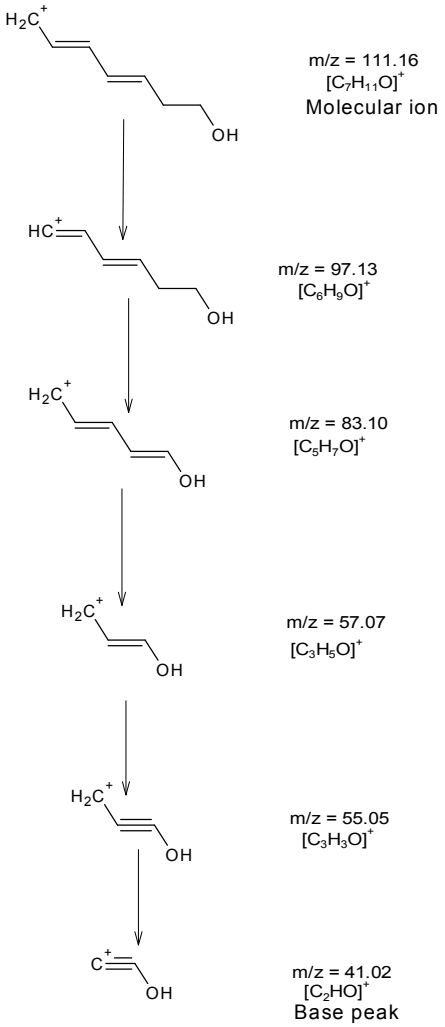


Figure 11: Hepta-3,5-dien-1-ol



Scheme 11: Fragmentation pattern for Hepta-3,5-dien-1-ol

Reference:

1. Adesina, S. K., (2005). The Nigerian Zanthoxylum., chemical and biological values. African Journal of Traditional Complementary and Alternative Medicines, 2(3): 282-301.
2. Aja, P.M., A.N.C. Okaka, P.N. Onu, U.A. Ibiam and A.J. Uraku, 2010. Phytochemical Composition of Talinum triangulare (Water Leaf) Leaves. Pakistan Journal of Nutrition, 9(6): 527-530.
3. Aja, P.M., Alum, E.U., Ezeani, N. N., Nwali, B. U and Edwin, N.(2015). Comparative Phytochemical Composition of Cajanus cajan Leaf and Seed, International Journal of Microbiological Research 6 (1): 42-46
4. 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, American Journal of Phytomedicine and Clinical Therapeutics 2(3):310-321
5. Dubes, Kumar, A. and Tripathi, S. C. (1990). Anti-fungal and insect-Repellant activity of essential oil of Zanthoxylum alatum. Annals of Botany, 65: 457-459.
6. Charles, E. and Ophardt, C. (2003). Anticancer drugs, drug action, enzyme inhibitions and other antibiotics. Elmhurst College, 50(1): 21-36.
7. Harborne, J.B. (1973). Text book of Phytochemical Method, 1st Edition, Champmaan and Hall Ltd. London, 110-113.
8. Mishra, D. N., Dixit, V. and Mishra, A. K. (1991). Mycotoxic Evaluation of some Higher Plants against Ringworm causing Fungi. India Drugs, 28 (3):300-3.
9. Offor, C. E., Ugwu, Okechukwu, P. C., Aja,, P. M. and Igwenyi, I. O. (2015). Proximate and Phytochemical Analyses of Terminalia catappa Leaves, European Journal of Applied Sciences, 7 (1): 09-11
10. Ranjan, R., Swamp, D., Patra, C. R., and Vikas, C. (2009). Tamarindus indica L. and Moringa oleifera Extracts administration ameliorates fluoride toxicity in rabbits, Indian Journal of Experimental Biology, 49:900-905.
11. Tapsell, L. C., Hemphill, I. and Cabiacl, A. (2006). Health benefits of herbs and spices. The part, the present the future. Medical Journal of Australia, 185 (4): 14-24.
12. Trease, G. E. and Evans, W. C. (1989). Pharmacognosy, 11th Edition, Macmillian, Brailliae Tiridal Can.
13. Sofowora, E. A. (1993). Medicinal plants and Traditional medicine in Africa. Spectrum Books limited, Ibadan, Oweri-Kaduna-Lagos. 5: 195-238.