AAS and GC-MS analysis of phytocomponents in the leaf, stem and root of *Azardiratcha indica*

ABSTRACT

Objective: To determine the mineral component and screen for the presence of bioactive phytoconstituents in hexane extract of *Azadirachta indica* leaf, stem and root using the GC-MS technique.
Methods: Mineral analysis was carried out using AAS**.** The leaf, stem and root hexane extracts of *A. indica* were prepared by standard procedure and concentrated at 40ºC using hot air oven. The concentrated hexane extracts were subjected to phytochemical analysis using GC-MS.
Results: The result of mineral analysis shows that Neem leaf, stem and root contain potassium, iron, copper, calcium, magnesium and sodium. The GC-MS analysis of the neem leaf, stem and root extract revealed the existence of the GC-MS chromatogram of twenty three peaks present. Ten chemical constituents was identified in the leaf of *A. indica*, six was found in the stem while seven was identified in the root of the plant by Gas Chromatogram Mass spectrometry (GC-MS) analysis.
Conclusion: The result of the analysis showed that the plant contains important minerals and many pharmacologically important bioactive compounds. The presence of various bioactive compounds justifies the uses of neem for various traditional medicines.

Key words: AAS, *Azadirachta indica*, GC-MS analysis, biological activities.

1. INTRODUCTION

The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds [1].
Neem (family. Meliaceae, genus. *Azaddirachta*) is an evergreen tree grown in Nigeria and other countries in Africa. It is one of the most well known plants indigenous to India and is cultivated in tropical and subtropical regions worldwide [2, 3]. Every part of the tree has been used as traditional medicine for household remedy against various human ailments, from antiquity [4-8]. Study has shown that the various chemical compounds, antioxidants, fatty acids, flavonoids, biological activities etc. in the various components of *Azadirachta indica* can be evaluated from the flower, leaves and barks [9]. Dholi *et al*., 2011 reported on its antidiabetic activity of the plant [10]. In addition, the aqueous extract of Neem leaves had shown a good therapeutic potential as anti- hyperglycemic agent [11]. Saseed *et al*. 2008 and El-Mahmood *et al*. 2010 supported the use of Neem seeds for treatment of infectious diseases especially those involving the eye and ear [12,13].. Antimicrobial activities of neem against human pathogenic bacteria have been studied [14-15]. The aim of this study was to determine the mineral components of *Azadirachta indica* and to screen the hexane extract of the plant using GC-MS technique with the possibility of discovering compound(s) of therapeutic value.

2. MATERIALS AND METHODS

2.1 Collection and identification of Plant extract

The *Azadirachta indica* plant was obtained from Ikorodu in Lagos State, Nigeria. The plant was authenticated from the department of Botany, University of Lagos, Lagos-Nigeria. Authentication number for the *A. indica* was given (6765).

2.2 Preparation of leaf, stem and root extract of *Azadirachta indica*

The leaf, stem and root of *A. indica* were washed separately, air dried under shade in the Biochemistry Laboratory, pulverised to coarse power using industrial blender. 2g each of the leaf, stem and root of the grounded *A. indica* plant material were placed in timble and later placed in a Soxhlet extractor with 30mls hexane and heated using heating mantle at 100^{o}C for 3 hours. The extracts were poured into separate beakers and concentrated with ultra sonic bath at 60^{o}C for one hour. The remaining extract was treated with anhydrous sodium sulphate to absorb the water in the samples and later treated with silica gel which helps to remove impurities in the samples. The extract was later used for GC-MS analysis.

2.3 Mineral Analysis of *Azadirachta indica*

The mineral composition of the plant was analyzed on aliquots of dry-ashing. 2g of the *A. indica* leaf, stem and root were separately weight into 250ml conical flasks, 10ml of aqua regia was added (HNO_3 and HCl in the ratio 1:3), the mixture was heated on porcelain crucible until the brown fumes disappeared leaving white fumes. It was later filtered with whatman filter paper into universal bottle; the mineral elements in the samples were determined by Atomic Absorption Spectrophotometer (Model PerkinElmer AAnalyst 400).

2.4 GC-MS analysis of the leaf, stem and root of *Azadirachta indica*

GC-MS analysis of the plant was carried out on an Agilent technology 7890 GC system equipped with a mass spectrometric detector (MSD). Ms model is agilent technology 5975 ms, the column used is HP-5MS agilent technology, length of the column is 30 m, internal diameter 0.320 mm, thickness of 0.25 μm. Volume of sample injected is 1μL. Oven temperature program with initial temperature of 80^{o}C to hold for 2 minutes at 10^{o}C/min to final temperature of 240^{o}C to hold for 6 minutes with injector temperature of 250^{o}C. The mobile phase is helium gas while the stationary phase is column.

2.5 Detection of components

Analysis of mass spectrum GC-MS was conducted by the database of National Institute Standard and Technique (NIST) having more than 62,000 patterns. The spectrum of the unidentified component was compared with the spectrum of the identified components stored in the NIST library. The name, molecular weight, structure of the components in the test material was ascertained (7,8,9 principle , strenhagen and Jennings).

3. RESULT

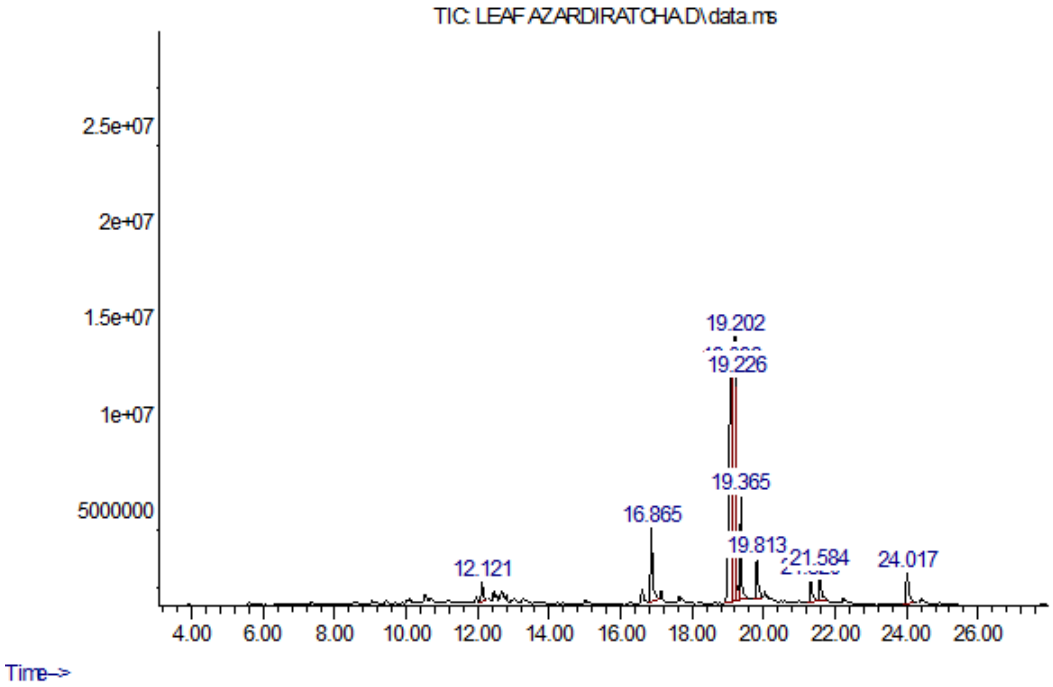
The macro and micro elements analysis of the leaf, stem and root of *Azadirachta indica* shows that the leaf contains higher concentration of more of the minerals, followed by the root while the stem has the least (Table 1).

Table 1. Mineral constituents of fresh leaf, stem and root of *Azadirachta indica*

Sample	Iron (mg/L)	Copper (mg/L)	Potassium (mg/L)	Calcium (mg/L)	Magnesium (mg/L)	Sodium (mg/L)
Leaf	**42.2198**	**0.2632**	**10.5667**	**8.1372**	**8.9112**	**8.6701**
Stem	**17.3061**	**0.0046**	**10.3578**	**8.1425**	**6.8883**	**5.8487**
Root	**35.8699**	**0.1616**	**10.3994**	**8.1292**	**7.9143**	**8.0981**

Twenty three compounds were identified in the *Azadirachta indica* plant by GC-MS analysis. The Peaks are indicating the presence of bio-active compounds. Ten, six and seven compounds were identified in the *A. indica* leaf, stem and root extract respectively by GC-MS analysis. The Peaks are indicating the presence of bio active compounds. The GC-MS chromatograms of the twenty three peaks of the bio compounds detected are shown in Figure 1, 2 and 3 respectively.

The bio activity components were identified and characterized and interpretation on mass spectrum GC–MS conducted using the database of National Institute Standard and Technology (NIST) which is having more than 62,000 patterns. The bioactive principles with their molecular formulae, molecular weight, Retention Time (RT), Peak area (%), are shown in Table 1, 2 and 3 respectively.



111 **Table. 2 Phytocomponents identified in the hexane leaf extract of *Azardiratcha indica***
112 **analysed by GC-MS.**

SN	Retention Time	Name of the compound	Molecular Formulae	Molecular Weight	Peak Area (%)	Activity
1	12.122	Caryophyllene oxide	$C_{15}H_{24}O$	220.35046 g/mol	1.62	Used as preservative in food, drugs and cosmetics, it as antifungal agent against dermatophytes
2	16.865	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.4507	6.92	Anti-oxidant, antimicrobial, decrease blood cholesterol, anti-inflammatory [19, 20].
3	19.097	9-Octadecenoic acid(Z)-, methyl ester	$C_{19}H_{36}O_2$	296.4879	25.49	Antioxidant, anti cancer [20, 21].
4	19.200	cis-13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.48794 g/mol	40.52	NF
5	19.229	9-Octadecenoic acid, methyl ester (E)	$C_{19}H_{36}O_2$	296.48794 g/mol	6.04	Antioxidant, anti cancer [20, 21].
6	19.366	Methyl stearate	$C_{19}H_{38}O_2$	298.5038	6.99	They are used as solvents or cosolvents, oil carrier in agricultural industry.
7	19.812	(E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	310.5145	3.68	NF
8	21.317	cis-11-Eicosenoic acid, methyl ester	$C_{21}H_{40}O_2$	324.5411 g/mol	2.29	NF
9	21.586	Methyl 18-methylnonadecanoate	$C_{21}H_{42}O_2$	326.5570	2.65	NF
10	24.018	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	354.6101	3.79	NF

113 NF=Not found

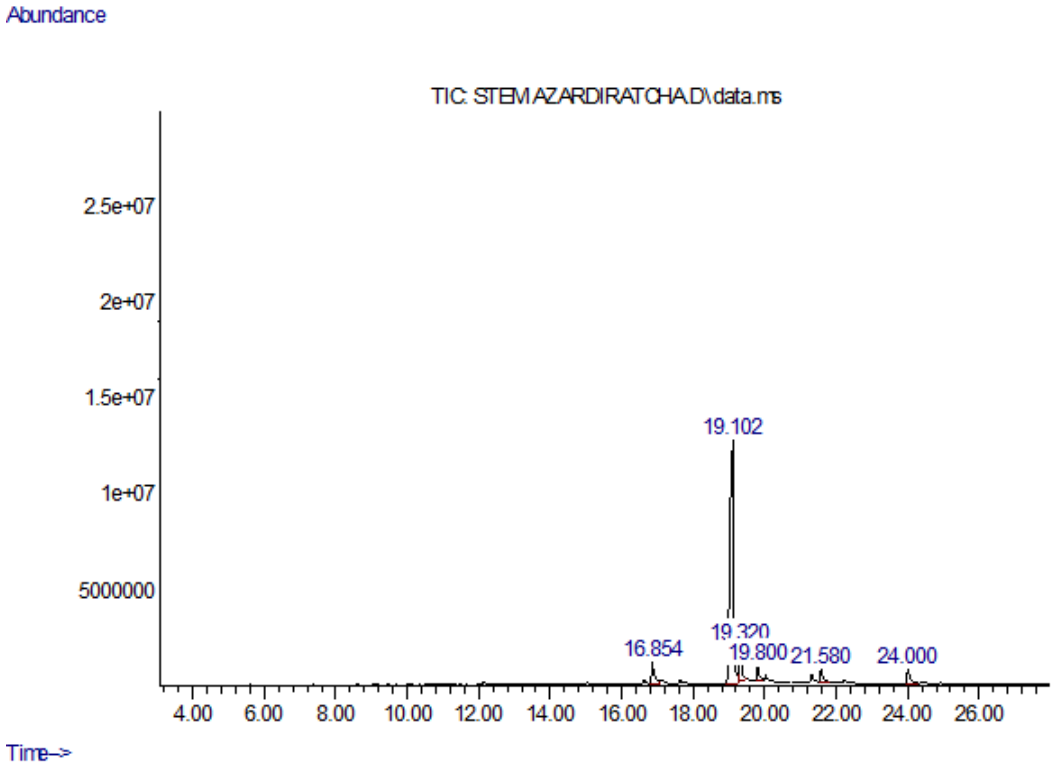


Figure.2 GC-MS Chromatogram of hexane stem extract of *Azardiratcha indica*

Table. 3 Phytocomponents identified in the hexane stem extract of *Azardiratcha indica* analysed by GC-MS.

	Retention Time	Name of the compound	Molecular Formulae	Molecular Weight	Peak Area (%)	Activity
1	16.854	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.4507	5.64	Anti-oxidant, antimicrobial, decrease blood cholesterol, anti-inflammatory [19,20].
2	19.102	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.4879	78.39	NF
3	19.320	Methyl stearate	$C_{19}H_{38}O_2$	298.5038 g/mol	6.29	
4	19.800	(E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	310.5145 g/mol	2.81	NF
5	21.580	Methyl 18-methylnonadecanoate	$C_{21}H_{42}O_2$	326.5570	2.62	NF
6	24.000	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	354.6101	4.24	NF

NF=Not found

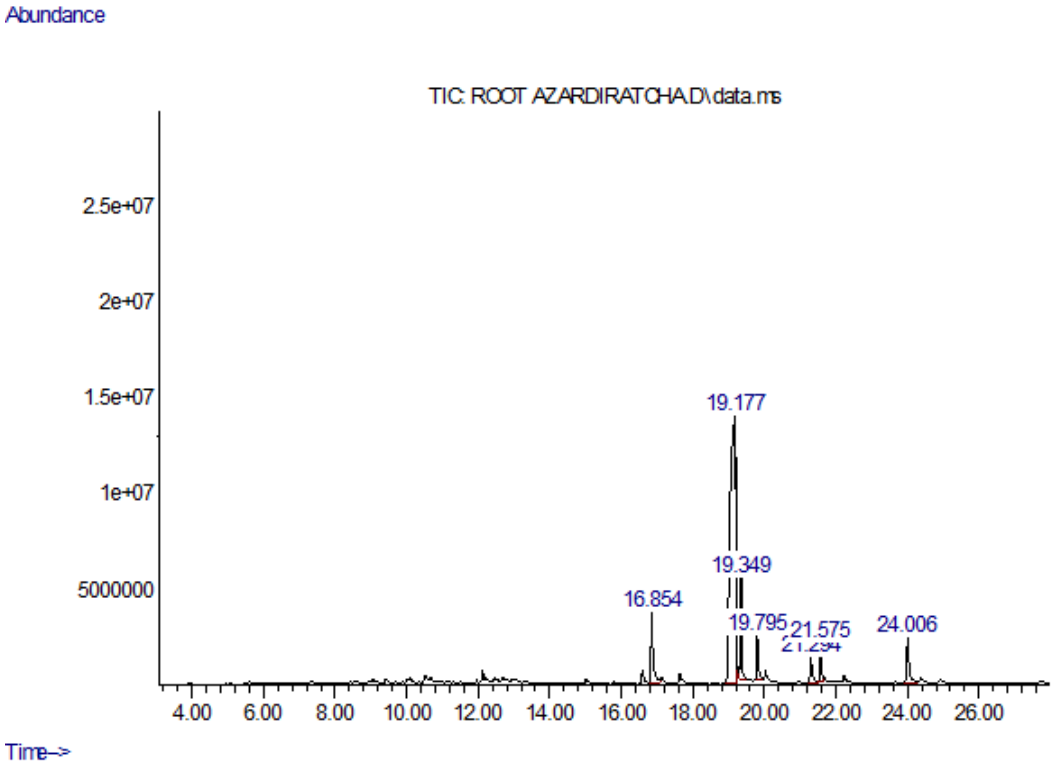


Figure 3 GC-MS Chromatogram of hexane root extract of *Azardiratcha indica*

Table. 4 Phytocomponents identified in the hexane root extract of *Azardiratcha indica* analysed by GC-MS.

SN	Retention Time	Name of the compound	Molecular Formulae	Molecular Weight	Peak Area (%)	Activity
1	16.854	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.4507	7.51	Anti-oxidant, antimicrobial, decrease blood cholesterol, anti-inflammatory [19, 20].
2	19.177	9-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.4879	70.14	Antioxidant, anti cancer [20, 21].
3	19.349	Methyl stearate	$C_{19}H_{38}O_2$	298.5038	6.78	They are used as solvents or cosolvents, oil carrier in agricultural industry.
4	19.795	(E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	310.5145	4.03	NF
5	21.294	cis-11-Eicosenoic acid, methyl ester	$C_{21}H_{40}O_2$	324.5411 g/mol	2.79	NF
6	21.575	Methyl 18-methylnonadecanoate	$C_{21}H_{42}O_2$	326.5570	3.04	NF
7	24.006	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	354.6101	5.72	NF

NF=Not found

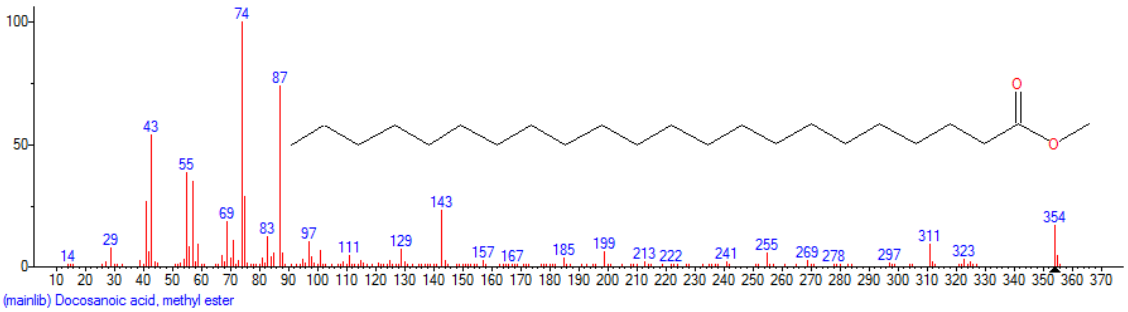


Figure 4. Mass spectrum of Docosanoic acid, methyl ester structure (3.79%, RT 24.018)

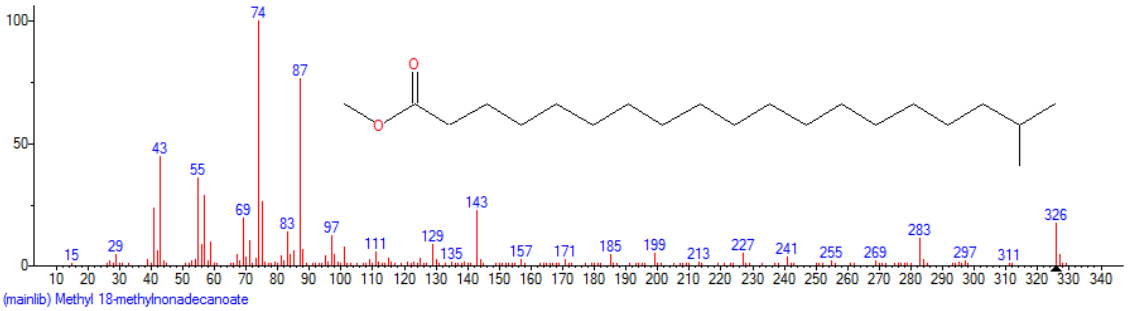


Figure 5. Mass spectrum of Methyl 18-methylnonadecanoate structure (2.65%, RT 21.586)

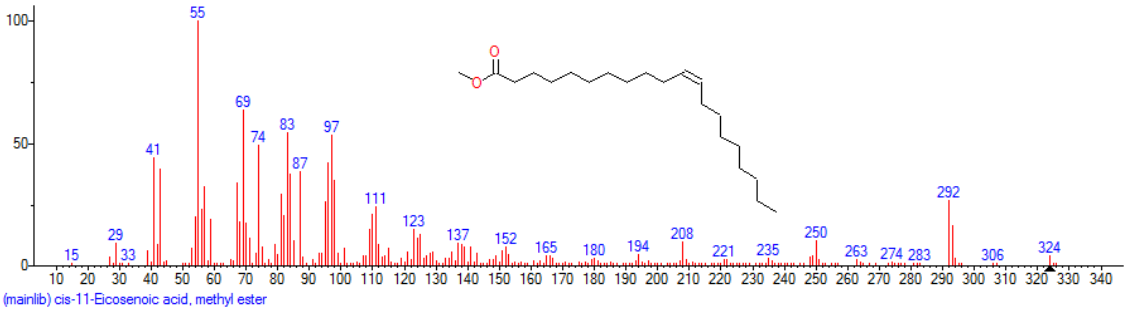


Figure 6. Mass spectrum of cis-11-Eicosenoic acid, methyl ester structure (2.29 %, RT 21.317)

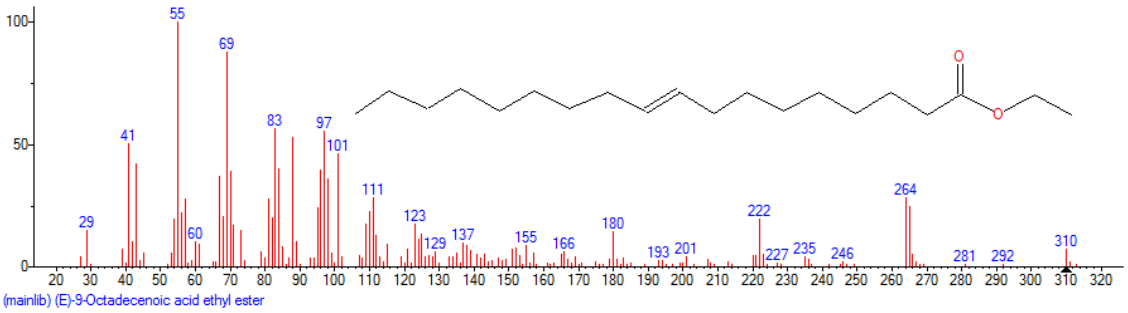


Figure 7. Mass spectrum of (E)-9-Octadecenoic acid ethyl ester structure (3.68 %, RT 19.812)

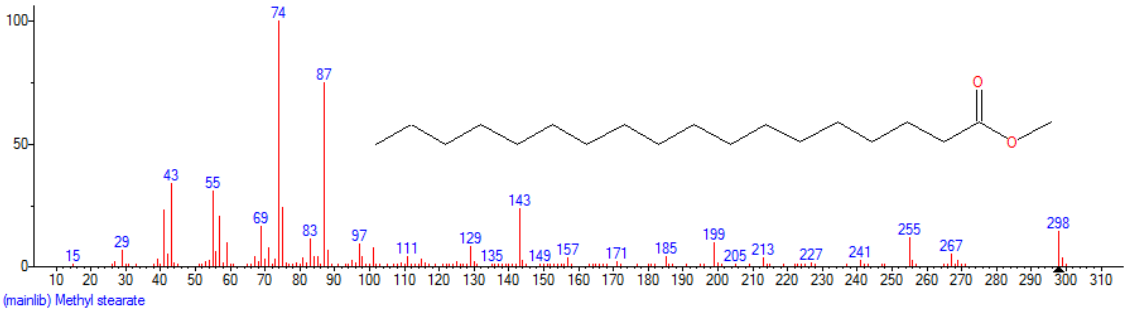


Figure 8. Mass spectrum of Methyl stearate structure (6.99%, RT 19.366)

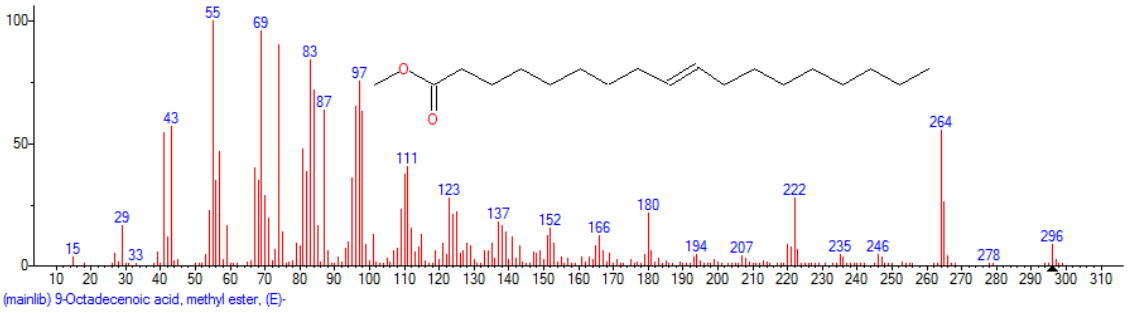


Figure 9. Mass spectrum of 9-Octadecenoic acid, methyl ester (E) structure (6.04 %, RT 19.229)

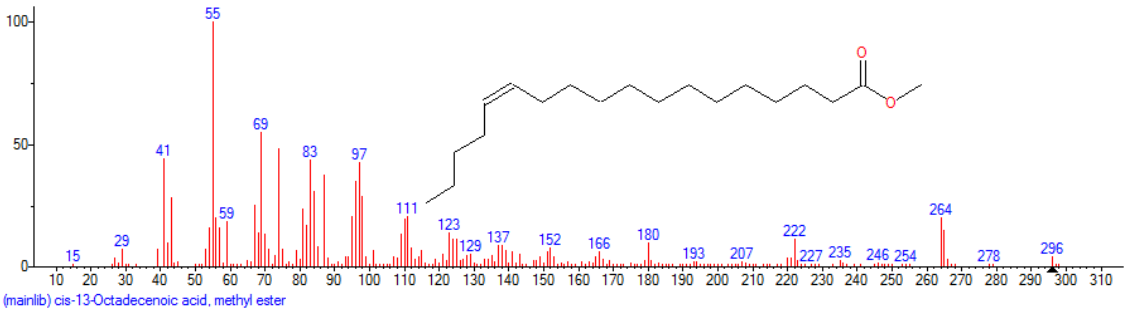


Figure 10. Mass spectrum of cis-13-Octadecenoic acid, methyl ester structure (40.52 %, RT 19.200)

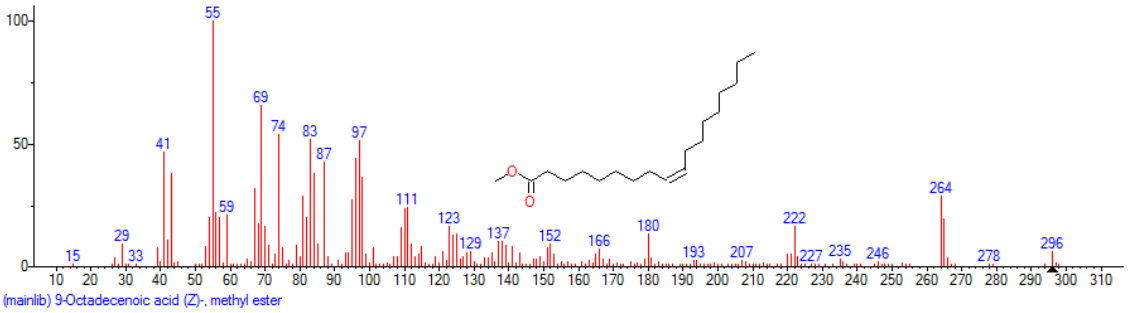


Figure 11. Mass spectrum of 9-Octadecenoic acid (Z)-, methyl ester structure (25.49 %, RT 19.097)

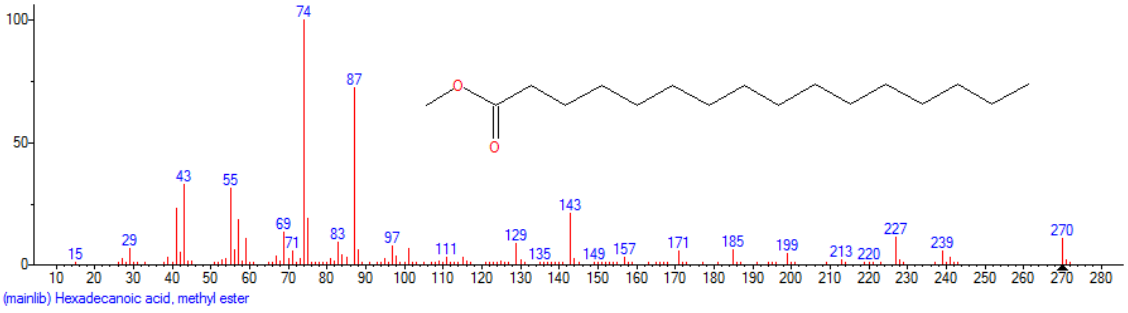


Figure 12. Mass spectrum of Hexadecanoic acid, methyl ester structure (6.92 %, RT 16.865)

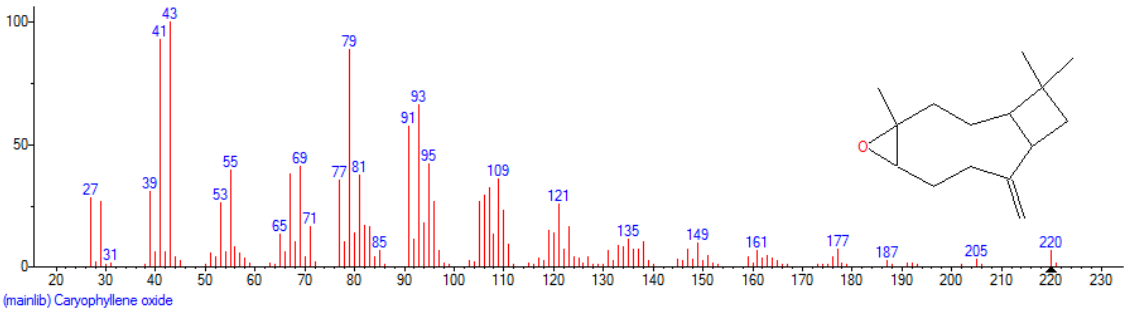


Figure 13. Mass spectrum of Caryophyllene oxide structure (1.62 %, RT 12.122)

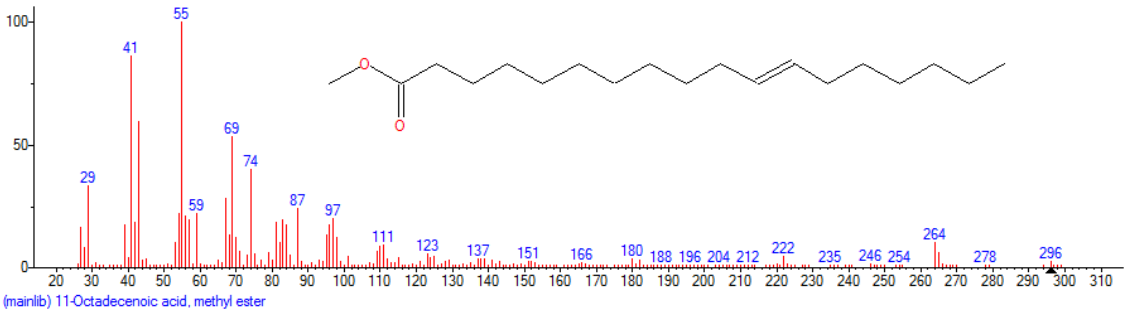


Figure 14. Mass spectrum of 11-Octadecenoic acid, methyl ester structure (78.39 %, RT 19.102)

Figure 4 -14 show the mass spectrograms of chemical bioactive compounds for Docosanoic acid, methyl ester, Methyl 18-methylnonadecanoate, cis-11-Eicosenoic acid, methyl ester, (E)-9-Octadecenoic acid ethyl ester, Methyl stearate, 9-Octadecenoic acid, methyl ester (E), cis-13-Octadecenoic acid, methyl ester, 9-Octadecenoic acid (Z)-, methyl ester, Hexadecanoic acid, methyl ester, Caryophyllene oxide and 11-Octadecenoic acid, methyl ester respectively.

4. DISCUSSION

The result shown of mineral analysis of fresh neem leaf, stem and root show the concentration of minerals such as Fe, Cu, Na, Mg, K, and Ca in the plants. Other research work also confirm the presence of vital minerals in *A.indica* plant [22]. The Ca^{2+}/ P ratio of *A. indica* plant is less than one, thus its consumption is likely to reduce the intestinal absorption of calcium. The Na+/k+ ration of *A. indica* leaf, stem and root is also less than one, based on daily recommendation which suggest that *A. indica* could be suitable for reducing high blood pressure. The mineral contents of *A. indica* in this study is lower than the percentage recommended by the Food and Agriculture Organization [23]. Sodium is 2400 mg, potassium (4700 mg) and calcium (1000 mg). This suggests the need to supplement a diet base on *A. indica* with a complementary mineral element source to make it more nutritious.

Gas chromatography coupled with mass spectrometry (GC-MS) is an established technique for reliable identification of bioactive compounds existing in medicinal plants including volatile matter, long chain and branched chain hydrocarbons, alcohols, acids, esters [24-27]

For quantitative determination, gas chromatography with flame ionization detector (GC-FID) and GC-MS are preferred [28,29]. The GC-MS analysis was based on the computer evaluation of mass spectra of samples through NIST by direct comparison of peaks and retention time with those for standard compounds, with eight peak index [30] and computer matching with the NIST. Besides that, the characteristic fragmentation patterns greatly helped in the identification of a particular class of compounds [30]. The identified compounds of the hexane extract of the leaf, root and stem of *Azardiratcha indica,* their retention time, peak area, molecular formulae, molecular weight, and their activities are given in the result. The GC-MS results showed the presence of twenty three compounds in the different parts of the plant. Out of which 10 compounds are found in the leaf: Caryophyllene oxide (1.62%), Hexadecanoic acid, methyl ester (6.92%), 9-Octadecenoic acid(Z)-, methyl ester (25.49%), cis-13-Octadecenoic acid, methyl ester (40.52%), 9-Octadecenoic acid, methyl ester (E) (6.04%), Methyl stearate (6.99%), (E)-9-Octadecenoic acid ethyl ester (3.68%), cis-11-Eicosenoic acid, methyl ester (2.29%), Methyl 18-methylnonadecanoate (2.65%) and Docosanoic acid, methyl ester (3.79%). The stem contain six compounds: Hexadecanoic acid, methyl ester (5.64%), 11-Octadecenoic acid, methyl ester (78.39%), (E)-9-Octadecenoic acid ethyl ester (2.81%), Methyl stearate (6.29%), Methyl 18-methylnonadecanoate (2.62%), Docosanoic acid, methyl ester (4.24%). Seven compounds was found in the root, they are: Hexadecanoic acid, methyl ester (7.51%), 9-Octadecenoic acid, methyl ester (70.14%), Methyl stearate (6.78%), (E)-9-Octadecenoic acid ethyl ester (4.03%), cis-11-Eicosenoic acid, methyl ester (2.79%), Methyl 18-methylnonadecanoate (3.04%) and Docosanoic acid, methyl ester (5.72%). The GC-MS of *A. indica* has also been reported by other research studies to contain bioactive compounds [31-33]. In the compounds obtained, six components were biological activities of anti-inflammatory, antifungal, antioxidant and anticancer all these compounds were promoting the wound healing process. This study helps to predict the formula and structure of active molecules which can be used as drugs. This result also enhances the traditional usage of *A. indica* which possesses a number of bioactive compounds.

5. CONCLUSION

This study helps to predict the formula and structure of active molecules in the plant that can be used as drugs. The result also enhances the traditional uses of the plant

REFERENCES

1. Hill RA. Terpenoids. In Thomson RH, (ed). Chemistry of Natural Products, Blackie Academic and Professional. London. 1985; 106-134.

2. Bailey LH, 1953. The Standard Cyclopedia of Horticulture", The Macmillan Company, New York.

3. Engler A, Melchoir H and Werdmann E. Syllabus der Pflanzenfamilien. Gebruder Borntraeger, Berlin, Germany; 1964.

4. Chopra RN, Nayer SL and Chopra IC. Glossary of Indian Medicinal Plants, CSIR, New Delhi.1956

5. Chopra RN, Chopra IC, Handa KL and Kapur LD, 1958. Indigenous Drugs of India, U.N. Dhur and Sons, Kolkata, 1958; pp. 51–595.

6. Kirtikar KR and Basu BD. Medicinal Plants (eds Blatter, E., Cains, J. F., Mhaskar, K. S.), Vivek Vihar, New Delhi, 1975; p. 536.

7. Koul O, Isman MB and Ketkar CM. Properties and uses of neem, *Azadirachta indica*. *Can. J. Bot.* 1990; 68, 1–11.

8. Chatterjee A and Pakrashi S. The Treatise on Indian Medicinal Plants,1994; 3, p. 76.

9. El-Hawary SS, El-Tantawy ME, Rabeh MA, Badr WK, *Intern Journ of Appl Res in Natural Products*, **2013** 6: 33.

10. Dholi SK, Ramakrishna R, Mankala SK and Nagappan K. *Invivo* Antidiabetic evaluation of Neem leaf extract in alloxan induced rats. *Journal of applied Pharmaceutical science*, 2011; 7, 100-105.

11. Sonia B and Srinivasan BP. Investigation into the Anti diabetic activity of *Azadirachta indica*. *Indian journal of pharmacology*, 1999; 31.138-141.

12. Saseed AK and Aslam J. Study on the effect of Neem (*Azadirachta indica*) leaves smoke in controlling airborne Bacteria in Residential premises. *Current research in Bacteriology*, 2008; 1 (2). 64-66.

13. EI- Mahmood AM, Ogbonna OB and Raji M.The antibacterial activity of *Azadirachta indica* (Neem) associated with eye and ear infections. *Journal of medicinal plant Research*, 2010; 4 (14).1414-142.

14. Maragathavalli S, Brindha S, Kaviyarasi NS, Annadurai B and Gangwar SK. Antimicrobial activity in leaf extract of neem (*Azadirachta indica* linn.) *I.j.s.n.,* 2012; vol. 3(1). 110-113.

15. Parmar Namitha, Rawat Mukesh, *Intern Res Journ of Pharma*, **2012**;3:31.

16. Principe P. Monetising the pharmacological benefits of plants. US Environmental protection Agency, Washington, D.C. 2005; pp. 1991.

17. Stenhagen E, Abrahamson S and McLafferty F. Registry of Spectral Data. J. Wiley and Sons, New York, NY, 1974.

18. Jennings W and Shibamoto T, Quality of Flavour and Fragrance Volatiles by Glass Capillary Gas Chromatography. Academic Press, New York, NY 1980.

19. Akpuaka A., Ekwenchi, MM, Dashak DA and Dildar A. Biological Activities of Characterized Isolates of n-Hexane Extract of *Azadirachta indica* A. Juss (Neem) Leaves. New York Sci. J, 2013; 6(6):119-124.

20. Hema, R, Kumaravel, S., Alagusundaram. *J. Am. Sci.* 7, 2011; 27.

21. Syeda FA, Habib-ur-Rahman AM, Khan, Choudahry M.I, Atta-Ur-Rahman. *Inter. J. Genetics Mol. Biol.* 3, 2011; 95.

22. Afolabi G, Oluwade A, Tunde O. Estimation of proximate and mineral composition of some tropical Crops. African Agricultural Journal. 1995; 21:103.

23. FAO. Law and sustainable development in Rio: Legal trends in agriculture and natural resource management. FAO Legislative Study. 2003;73.

24. Sermakkani M, Thangapandian V. GC-MS analysis of Cassia italic a leaf methanol extract. Asian Journal of Pharmaceutical and Clinical Research. 2012;5(2):90-94.

25. Kumar A, Kumari PS, Somasundaram T. Gas Chromatography-Mass Spectrum (GC-MS) analysis of bioactive components of the methanol extract of *Halophyte, Sesuvium portulacastrum L.* IJAPBC. 2014;3(3):766-772.

26. Cong Z, Meiling Q, Qinglong S, Shan Z, Ruonong F. J. Pharm. Biomed. Anal. 2007; 44:464.

27. Johnson M, Mariswamy Y, Gnaraj WF. Chromatographic finger print analysis of steroids in *Aerva lanasa* L. by HPTLC technique. Asian Pal. J. Trop. Biomedicine. 2011;1:428-433.

28. Haznagy-Radnal E, Czigle S, Mathe I. TLC and GC analysis of the essential oils of Stachys species. Journal of Planar Chromatography. 2007;20:189-196.

29. Lampronti I, Saab AM, Gambari R. Antiproliferative activity of essential oils derived from plants belonging to the Magnoliophyta division. Int. J. Oncol. 2006;29:989.

30. Mass Spectrometry Data Centre. Eight peak index of mass spectra: the eight most abundant ions in 31,101 mass spectra, indexed by molecular weight, elemental composition and most abundant ions (4 volume set). 2nd ed. Aldermaston: Mass Spectrometry Data Centre; 1974.

31. Akpuaka1 A, Ekwenchi1 MM, Dashak1 DA, Dildar A. Gas Chromatography-Mass Spectrometry (GC/MS) Analysis of Phthalate Isolates in n-Hexane Extract of *Azadirachta indica A. Juss* (Neem) Leaves. *J Am Sci* 2012;8(12):146-155.

32. Jessinta S, Nour AH, Tajuddin SN and Nour AH. Chemical Characterization and Biological Study of *Azadirachta* indica Extracts. European Journal of Academic Essays. 2014; 1(10): 9-16.

33. Chenganmal M and Yamalai M. Determination of bioactive components in *Azadirachta indica* flowers using GC-MS analysis. Asian Journal of Plant Science and Research, 2015, 5(8):61.