AAS and GC-MS analysis of phytocomponents in the leaf, stem and root of *Azadirachta indica A. Juss* (**Dongoyaro**)

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ABSTRACT

Objective: To determine the mineral component using AAS and screen for the presence of bioactive phytoconstituents in hexane extract of *Azadirachta indica A. Juss* 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 *Azadirachta indica A. Juss* 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 were identified in the leaf of *A. indica*, six were found in the stem while seven were 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 A. Juss*, 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 *Azadirachta*) is an evergreen tree grown in Nigeria and other countries in Africa. In Nigeria, neem is locally called Dongoyaro and is used for the treatment of malaria. 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 the

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 A. Juss* 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 (6967).

2.2 Mineral Analysis of *Azadirachta indica A. Juss*

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).

2.3 Preparation of leaf, stem and root extract of *Azadirachta indica A. Juss*

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 30 ml hexane and heated using heating mantle at 100°C for 3 hours. The extracts were poured into separate beakers and concentrated with ultra sonic bath at 60°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.

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

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°C to hold for 2 minutes at 10°C/min to final temperature of 240°C to hold for 6 minutes with injector temperature of 250°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 (16, 17, 18 principle , strenhagen and Jennings).

3. RESULT

The macro and micro elements analysis of the leaf, stem and root of *Azadirachta indica* A. Juss 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* A. Juss

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 bioactive components were identified and characterized and interpreted 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.
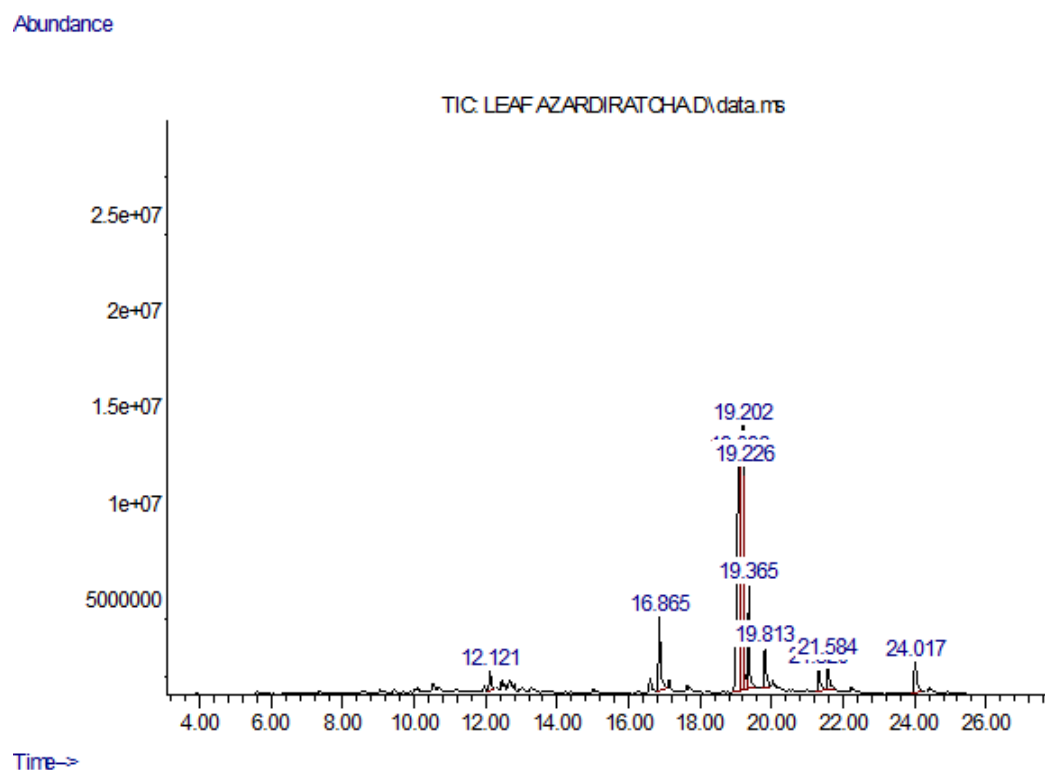
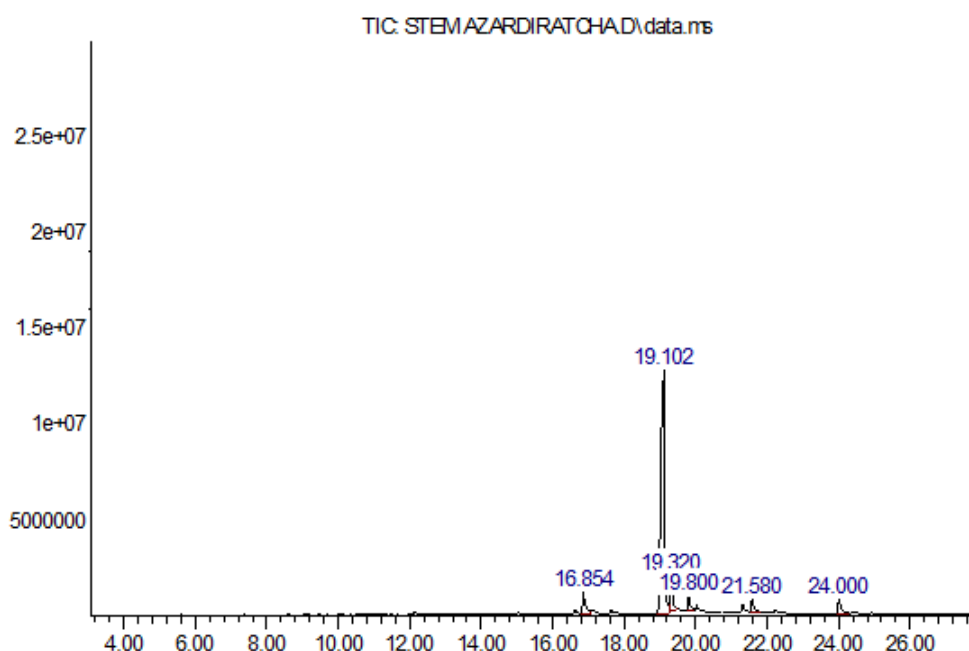**Figure 1. GC-MS Chromatogram of hexane leaf extract of *Azadirachta indica A. Juss***

Table. 2 Phytocomponents identified in the hexane leaf extract of *Azadirachta indica A. Juss* 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	$C_{20}H_{38}O_2$	310.5145	3.68	NF

		acid ethyl ester				
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

NF=Not found

Abundance

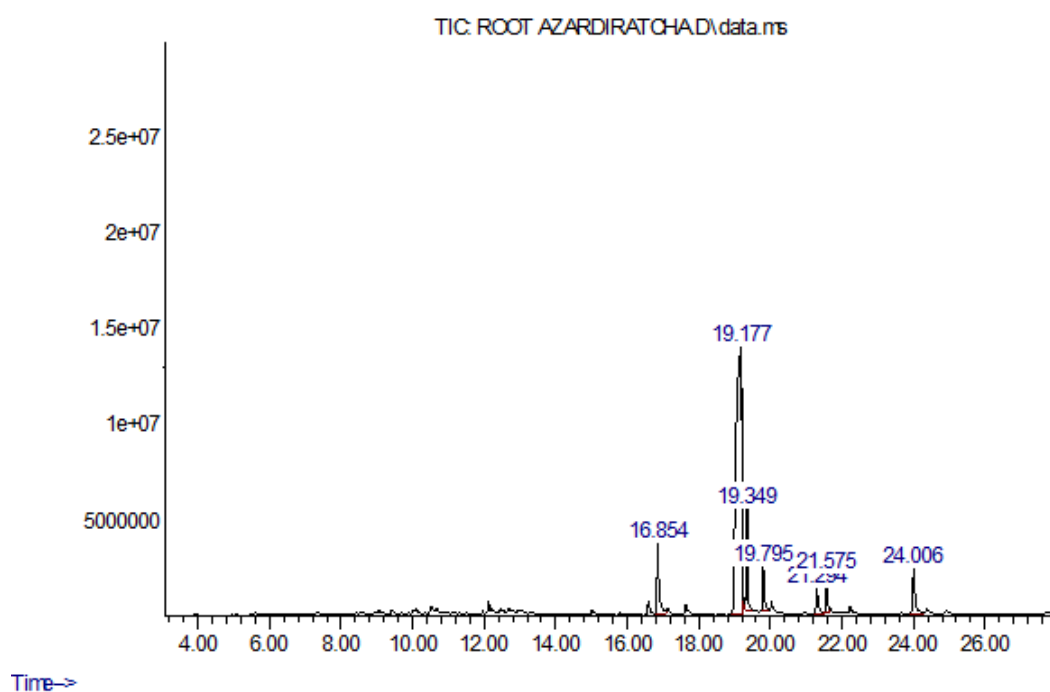TIC: STEM AZARDIRATCHA D\data.ms

Figure 2. GC-MS Chromatogram of hexane stem extract of *Azadirachta indica A. Juss*

Table. 3 Phytocomponents identified in the hexane stem extract of *Azadirachta* indica *A. Juss* 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	5.64	Anti-oxidant, antimicrobial, decrease blood cholesterol, anti-

SN	Retention Time	Name of the compound	Molecular Formulae	Molecular Weight	Peak Area (%)	Activity
						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 <mark>Azadirachta</mark> indica <mark>A. Juss</mark>

Table. 4 Phytocomponents identified in the hexane root extract of <mark>Azadirachta</mark> indica <mark>A. Juss</mark> 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,	$C_{19}H_{36}O_2$	296.4879	70.14	Antioxidant, anti cancer

		methyl ester				[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 -14 below 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.
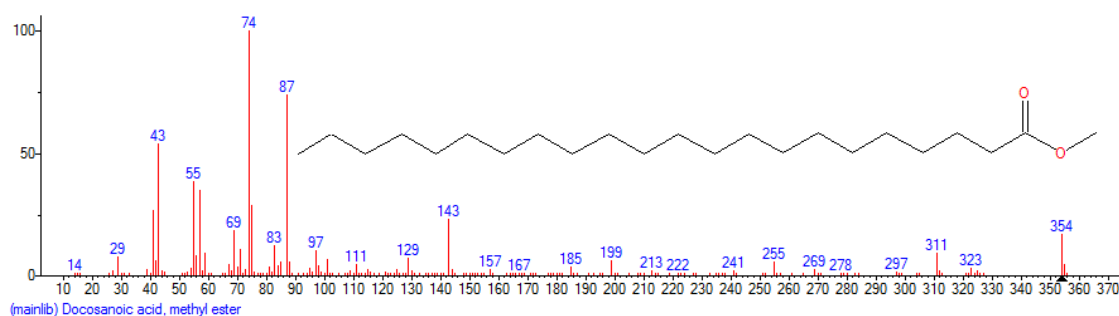
Figure 4. Mass spectrum of Docosanoic acid, methyl ester structure (3.79%, RT 24.018).
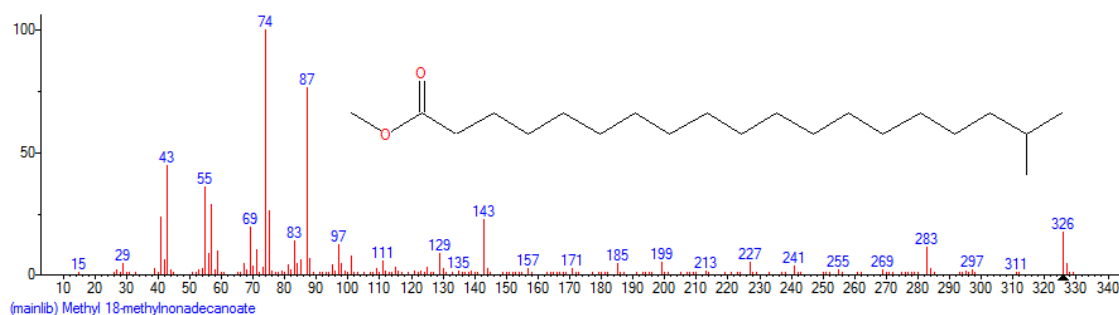
Figure 5. Mass spectrum of Methyl 18-methylnonadecanoate structure (2.65%, RT 21.586).
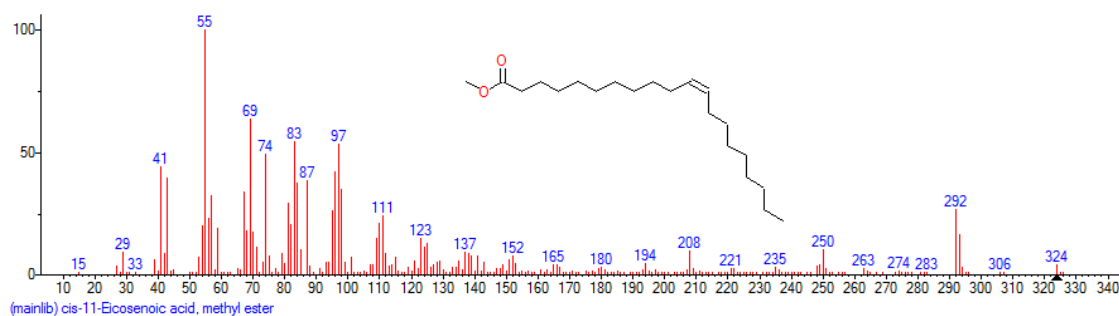
Figure 6. Mass spectrum of cis-11-Eicosenoic acid, methyl ester structure (2.29 %, RT 21.317).
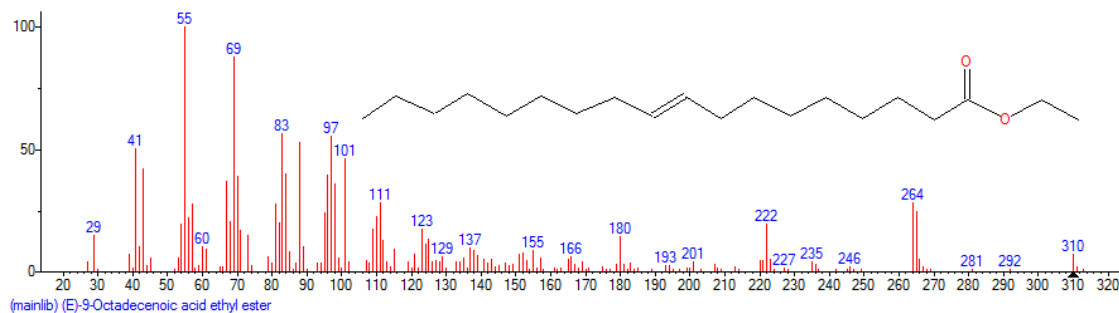

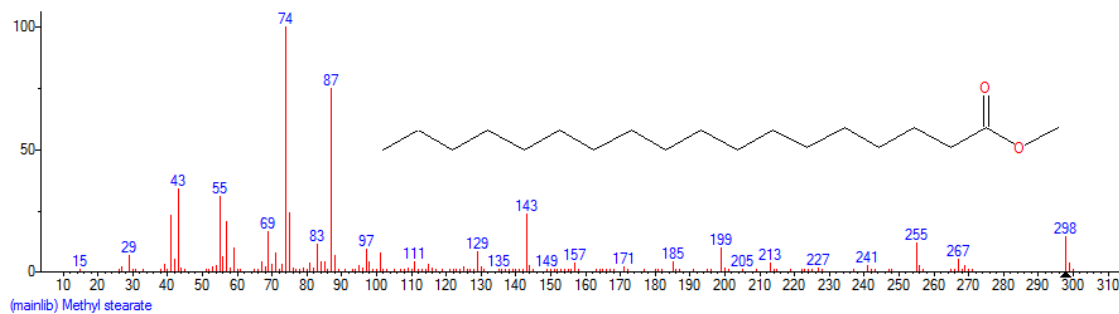Figure 7. Mass spectrum of (E)-9-Octadecenoic acid ethyl ester structure (3.68 %, RT 19.812).

Figure 8. Mass spectrum of Methyl stearate structure (6.99%, RT 19.366).
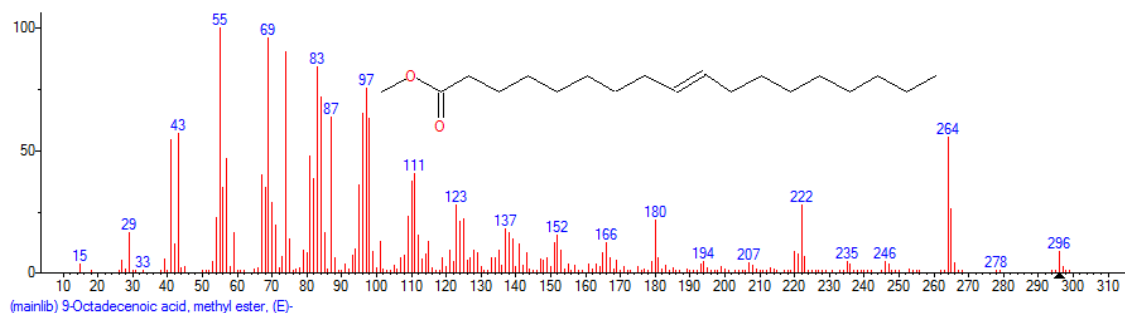
Figure 9. Mass spectrum of 9-Octadecenoic acid, methyl ester (E) structure (6.04 %, RT 19.229).
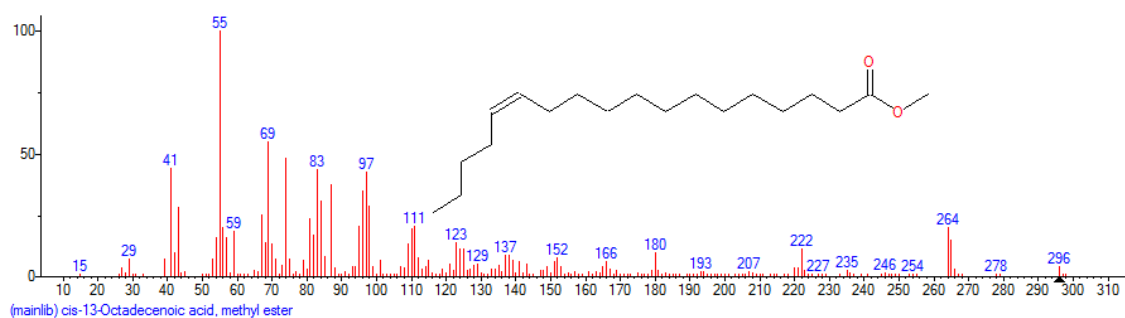

Figure 10. Mass spectrum of cis-13-Octadecenoic acid, methyl ester structure (40.52 %, RT 19.200).
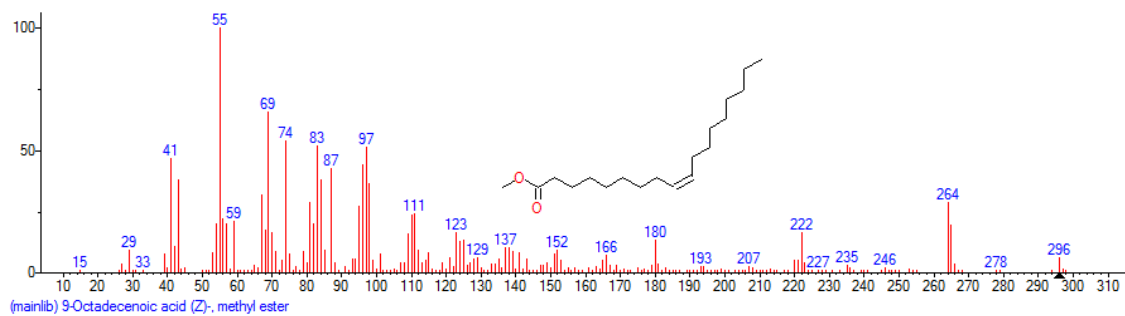

Figure 11. Mass spectrum of 9-Octadecenoic acid (Z)-, methyl ester structure (25.49 %, RT 19.097).
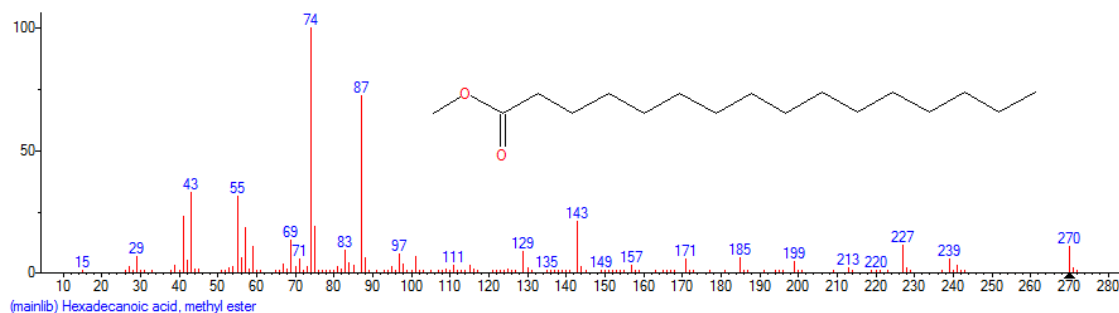
Figure 12. Mass spectrum of Hexadecanoic acid, methyl ester structure (6.92 %, RT 16.865).
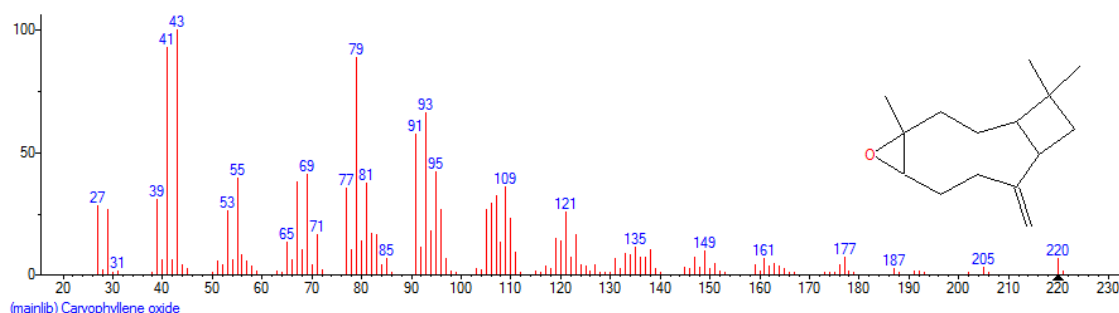

Figure 13. Mass spectrum of Caryophyllene oxide structure (1.62 %, RT 12.122).
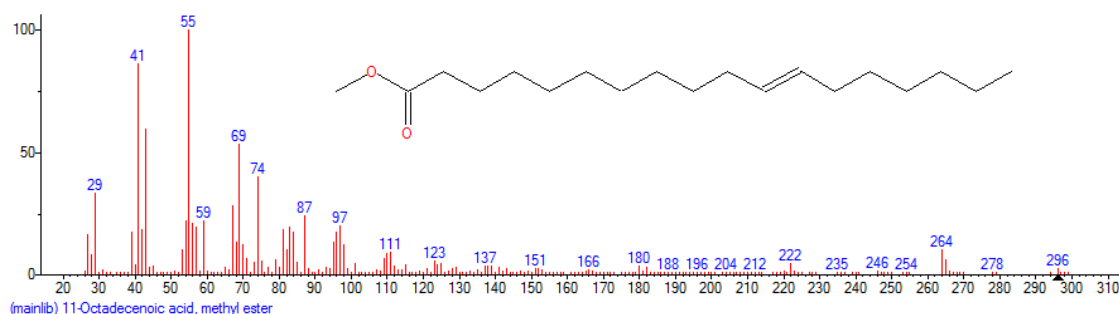

Figure 14. Mass spectrum of 11-Octadecenoic acid, methyl ester structure (78.39 %, RT 19.102).

4. DISCUSSION

The result for 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 confirms the presence of vital minerals in *A.indica* plant [22]. 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 are 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 complementary mineral elements 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 *Azadirachta indica* **A. Juss,** 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 were 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 are important in the formulation of different medicines. This study shows the formulae and structures of active compounds which may be used in the synthesis of drugs. This result also enhances the traditional usage of *A. indica* which possesses a number of bioactive compounds.

5. **CONCLUSION**

This study shows the mineral components, formulae and structures of active compounds found in the *Azadirachta indica A. Juss*

CONFLICT OF INTEREST STATEMENT
We declare that we have no conflict of interest.

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