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Identification of Sec-Butylamine and Methenamine and Other Bioactive Compounds from Edible *Azadirachta indica* Methanolic Extracts Using GC-MS

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Abstract

Background: *Azadirachta indica* (Neem) is a medicinal plant that is traditionally known for its antimicrobial properties. The present study was aimed to determine the bioactive components of the *A. indica* leaves using GC-MS. **Results:** Results revealed presence of 35 different active compounds in different percentage composition successions. Bioactive compounds listed include; Methenamine, Hexadecanoic acid, methyl ester; Phenol; Dodecanoic acid, methyl ester; Methyl tetradecanoate; sec-Butylamine; 2-Propanone, 1-methoxy-; 10-Octadecenoic acid, methyl ester; Heptadecanoic acid, 16-methyl-; Phenol, 2,6-dimethoxy-; Phenol, 2-methoxy-; n-Hexadecanoic acid. Their molecular weight and formula was observed while their biological activities were also illustrated. **Conclusions:** The findings of this study suggest that *A. indica* leaf collected from the study area contain sec-Butylamine and Methenamine that have not been identified from other studies which are important bioactive compounds that can be used in several biological activities.

Subject Areas

Medicinal Chemistry

Keywords

Azadirachta indica, Neem, GC-MS, Bioactive, Sec-Butylamine, Methenamine

1. Introduction

Gas chromatography-mass spectrometry (GC-MS) is a well-established technol-

ogy for identifying bioactive chemicals in medicinal plants, such as volatile matter, long chain and branch chain volatiles (BCVs). Gas chromatography with flame ionization detector (GC-FID) and gas chromatography mass spectrometry (GC-MS) are recommended for quantitative analysis [1].

Azadirachta indica (neem) belonging to Meliaceae family is a very important medicinal plant which is traditionally used to treat different diseases. The evergreen tree neem (family Meliaceae, genus Azadirachta) is used to treat malaria in Nigeria and other African nations. It's one of India's most well-known plants, and it is grown in tropical and subtropical climates all over the world [2]. From antiquity, every component of the tree has been employed as a traditional medicine for domestic remedies against a variety of human maladies [3].

Several studies have shown that neem contains a number of beneficial triterpenoids from the limonoids class. The primary non-volatile chemicals include Azadirachtin (Azadirachtin A), Nimbidiol, 3-tigloylazadirachtol (Azadirachtin B), Salannol, Salannin, Nimbinin, Nimbin, and 1-tigloyl-3-acetyl-11-hydroxymeliacarpin (Azadirachtin D) [4]. Neem also contains sulfur-modified fatty substances such loeic acid (50% - 60%), palmitic acid (13% - 15%), stearic acid (14% - 19%), linoleic acid (8% - 16%), and arachidic acid (1% - 3%).

A visit to various herbalists in Imo State, Nigeria revealed a recognized and fascinating consistency in their regular usage of indigenous medicinal herbs for the treatment of skin and wound infections such as Erythrasma, impetigo, ecthyma, folliculitis, erysipelas, and cellulitis (which are generally known to the herbalist as Ocha-ere). The bioactive constituent of this plant in the region were reached were investigated to determine the variety of compounds it posses and their medicinal importance using GC-MS methods.

2. Methods

2.1. Sample Collection and Preparation

Fresh leaves of *Azadirachta indica* were collected from farms from the study area. The plants were identified, validated and recognized by a Plant Taxonomist at Imo State University, Department of Crop Science, based on their taxonomic classification and further deposited in the herbarium Department of Crop Science, Imo State University Owerri. The leaves were air dried for 6 days at 27˚C. The dried leaves were pulverized into powder.

2.2. Soxhlet Extraction of Materials

After air drying, the leaves were subsequently dried and sterilized in an oven for 30 minutes in a 500 ml clean boiling flask at 110˚C. After that, it was placed in a desiccator and left to cool. 100 g of each sample were weighed and poured into the soxhlet thimble. To help filter the extract, the extraction thimble was lightly blocked with cotton wool, and the boiling flask was filled with 300 ml of ethanol. The soxhlet apparatus was put together and left to reflux for 4 hours at 600˚C. The thimble was carefully removed, and the extract was placed into a volumetric

flask to cool. To separate the solvent (n-hexane) from the oil, the contents of the volumetric flask were transferred to a rotatory evaporator [5].

2.3. Preparation of Stock Solution

One gram (1 g) of extract after soxhlet extraction was collected, weighed and placed in a test tube, along with 25 ml of ethanol. The test tube was placed on a hot plate at 600˚C for 90 minutes to respond. The reaction product in the test tube was transferred to a separatory funnel when the reaction period had passed. The tube was successfully cleansed with 20 ml ethanol, 10 ml cold water, 10 ml hot water, and 3 ml hexane, all of which were passed to the funnel. The extracts were mixed and rinsed three times with a 10 ml ethanol aqueous solution containing 10% v/v ethanol. The solvent was evaporated after the solution was dried with anhydrous sodium sulfate. The sample was dissolved in 1000 µ pyridine, 200 µl of which was transferred to a vial for GC-MS examination [6].

2.4. The GC-MS Analysis

Agilent Technologies GC-7890A/MS-5975C a high-performance gas chromatography (GC) system manufactured by Agilent Technologies, based in Santa Clara, CA, USA equipped with a HP-5MS column, which is a type of high-performance liquid chromatography (HPLC) column that is commonly used in the analysis of volatile and semi-volatile organic compoundswere used to analyze bioactive chemicals from the various extracts. An electron ionization device using high energy electrons was used for spectroscopic detection by GC-MS (70 eV). The carrier gas was pure helium gas (99.995% purity) at a flow rate of 1 mL/min. The starting temperature was set at 50˚C - 150˚C, with a 3˚C/min increase rate and a 10-minute hold duration. Finally, the temperature was raised to 300˚C at a rate of 10˚C per minute. In a splitless mode, one microliter of the prepared 1 percent of the extracts diluted with corresponding solvents was injected. Based on the peak area obtained in the chromatogram, the relative quantity of chemical compounds contained in each of the extracts was represented as a percentage [5].

2.5. Identification of Chemical Constituents

Based on GC retention time on HP-5MS column and spectra matching with computer software data of standards (Replib and Mainlab data of GC-MS systems), bioactive chemicals isolated from various extracts were identified. The National Institute of Standards and Technology (NIST) database, which contains over 62,000 patterns, was used to analyze the mass spectrum GC-MS. The spectra of the unknown components were compared to the spectrum of the known components in the NIST collection. The components in the test sample were identified by their name, molecular weight, and structure [7].

2.6. Calculation of Retention Time

Retention time was calculated using the following principles below;

I^T was calculated using a quasi-linear equation for temperature programmed retention index:

$$I^T = \frac{100(t_x - t_n + n)}{t_{n+1} - t_n}$$

where I^T is the temperature-programmed retention index of the intriguing compound; t_n, $t_n + 1$, and t_x are the retention durations (in minutes) of the two standard *n*-alkanes with *n* and *n* + 1 carbons, respectively, and the compound of interest [7].

3. Results

From the HP-5MS column and spectra matching with computer software data of standards (Replib and Mainlab data of GC-MS systems), the following results were obtained.

Table 1 presents the bioactive principles with their molecular formulas, molecular weight, retention time (RT), and percentage content (Percent).

The peak indicates the existence of bio-active chemicals, according to the analysis. GC-MS study of the *Azadirachta indica* leaf extract revealed thirty-five components. 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 has more than 62,000 patterns.

Figures 1-12 show the mass spectrograms of twelve of the thirty-five compounds with the highest percentage of bioactive compounds, including; Methenamine (26.29%, RT 8.451); Hexadecanoic acid, methyl ester (14.54%, RT 15.790); Phenol (8.01%, RT 5.216); Dodecanoic acid, methyl ester (7.05%, RT 11.857); Methyl tetradecanoate (5.02%, RT 13.920); sec-Butylamine (4.88%, RT 6.638); 2-Propanone, 1-methoxy- (3.19%, RT 5.414); 10-Octadecenoic acid, methyl ester (3.12%, RT 17.283); Heptadecanoic acid, 16-methyl- (3.03%, RT 17.481); Phenol, 2,6-dimethoxy- (2.39%, RT 9.959); Phenol, 2-methoxy- (2.12%, RT 6.550); n-Hexadecanoic acid (2.08%, RT 16.152).

4. Discussion

Many bioactive elements found in plants are recognized to be physiologically active molecules that can be exploited in medication development. There is scientific proof in this study that the plants harvested with the advice and instructions of certain herbalists in the study region contain bioactive components that researchers have demonstrated to have bioactive actions against a variety of diseases and infections. The selection of these natural resources was motivated by herbalists' advice for using these materials to treat a variety of clinical and community illnesses, including upper respiratory infections, skin and urine infections, and sexually transmitted infections. All of these served as an encouragement to conduct a phytochemical study utilizing GC-MS as a method to look into bioactive chemicals.

By GC-MS analysis, 35 chemicals were found in *Azadirachta indica* leaf

Table 1. Phytocomponent identities of the hexane GC-MS chromatogram analyses of *Azadirachta indica* leaf.

S/N	Parameters	Retention time	Molecular Formulae	Molecular weight (g/mol)	% composition
1.	Phenol	5.216	C_6H_6O	94.11	8.01
2.	2,4,6-cycloheptatrien-1-one	5.304	$C_{10}H_{12}O_2$	164.20	1.14
3.	2-Propanone, 1-methoxy-	5.414	$C_4H_8O_2$	88.11	3.19
4.	Methylvalerate	5.529	$C_6H_8O_2$	116.16	0.31
5.	3-methylcyclopentane-1,2-dione	5.718	$C_6H_8O_2$	112.13	1.54
6.	2-Pyrrolidinone	6.358	C_4H_7NO	85.11	0.65
7.	Phenol, 2-methoxy-	6.550	$C_7H_8O_2$	85.11	2.12
8.	sec-Butylamine	6.638	$C_4H_8N_4$	112.13	4.88
9.	1H-Imidazole, 1-methyl-4-nitro-	6.755	$C_4H_5N_3O_2$	127.10	0.81
10.	Octanoic acid, methyl ester	6.936	$C_9H_{18}O_2$	158.24	1.08
11.	4(1H)-Pyridone	7.136	C_5H_5NO	95.10	0.53
12.	3-Pyridinecarboxylic acid, 4-hydro	7.209	$C_6H_5NO_3$	139.11	1.50
13.	3(2H)-Isoxazolone, 4,5-dimethyl-	7.894	$C_5H_7NO_2$	113.11	0.68
14.	2-Hexenal, (E)-	7.977	$C_6H_{10}O$	98.14	0.78
15.	1,4:3,6-Dianhydro-.alpha.-d-glucop yranose	8.250	$C_6H_8O_4$	144.1253	1.12
16.	Methenamine	8.451	$C_6H_{12}N_4$	140.186	26.29
17.	1,2-Benzenediol, 3-methoxy-	8.961	$C_7H_8O_3$	140.1366	0.72
18.	Decanoic acid, methyl ester	9.545	$C_{11}H_{22}O_2$	186.29	1.77
19.	Phenol, 2,6-dimethoxy-	9.959	$C_8H_{10}O_3$	154.16	2.39
20.	Undecanoic acid, methyl ester	10.732	$C_{13}H_{26}O_2$	214.34	0.28
21.	3,5-Dimethoxy-4-hydroxytoluene	11.076	$C_9H_{12}O$	168.1898	0.38
22.	Dodecanoic acid, methyl ester	11.857	$C_{13}H_{26}O_2$	214.34	7.05
23.	5-tert-Butylpyrogallol	11.961	$C_{10}H_{14}O_3$	182.22	0.20
24.	Tridecanoic acid, methyl ester	12.911	$C_{16}H_{32}O_2$	256.42	0.41
25.	Methyl tetradecanoate	13.920	$C_{15}H_{30}O_2$	242.40	5.02
26.	Pentadecanoic acid, methyl ester	14.872	$C_{16}H_{32}O_2$	256.42	0.71
27.	(Z)-Methyl hexadec-11-enoate	15.606	$C_{17}H_{32}O_2$	268.4348	0.54
28.	Hexadecanoic acid, methyl ester	15.790	$C_{17}H_{34}O_2$	270.45	14.54
29.	n-Hexadecanoic acid	16.152	$C_{16}H_{32}O_2$	256.4	2..08
30.	Heptadecanoic acid, methyl ester	16.649	$C_{19}H_{38}O_2$	298.5	0.34
31.	10-Octadecenoic acid, methyl ester,	17.283	$C_{19}H_{36}O_2$	296.5	3.12
32.	11-Octadecenoic acid, methyl ester	17.331	$C_{19}H_{36}O_2$	296.5	1.10
33.	Heptadecanoic acid, 16-methyl-	17.481	$C_{19}H_{38}O_2$	298.5	3.03
34.	trans-13-Octadecenoic acid	17.638	$C_{18}H_{34}O_2$	282.5	0.25
35.	Diisooctyl phthalate	20.640	$C_{24}H_{38}O_4$	390.6	0.26

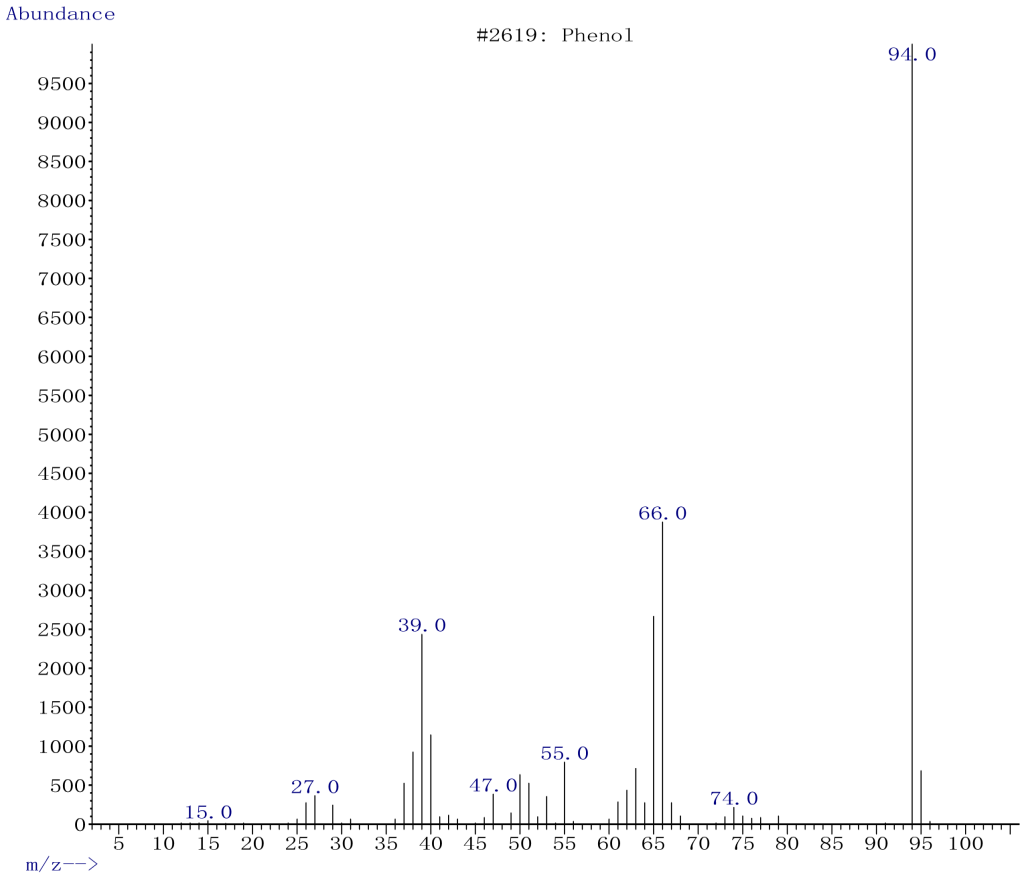

Figure 1. Mass spectrum of Phenol (8.01%, RT 5.216).

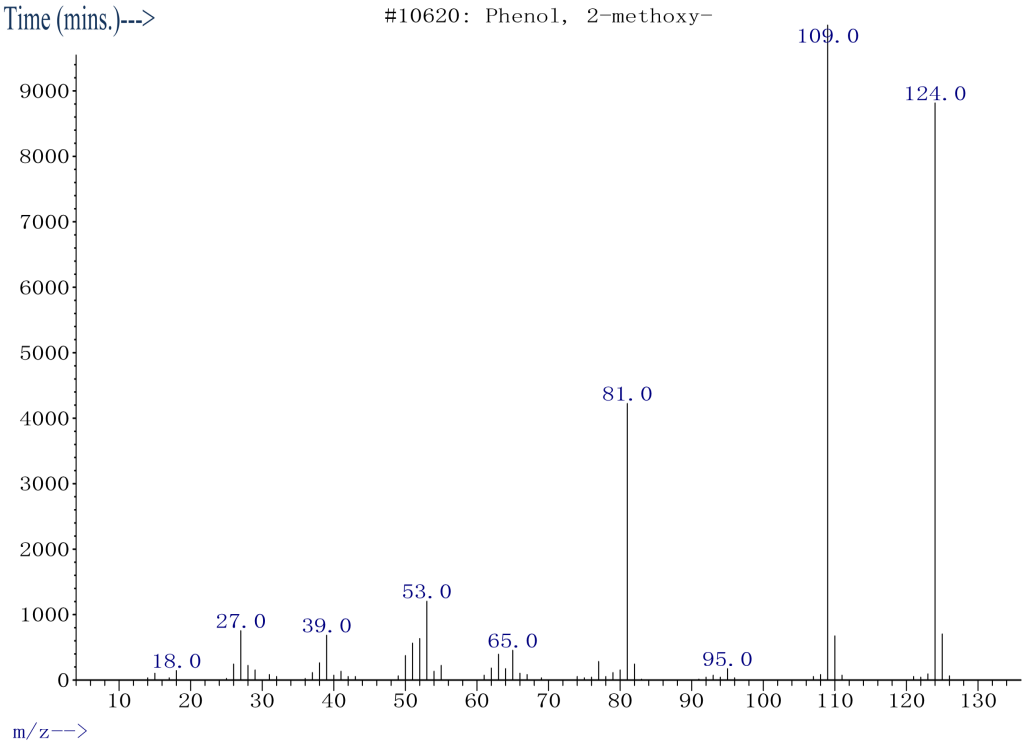

Figure 2. Mass spectrum of Phenol, 2-mthoxy- (2.125%, RT 6.550).

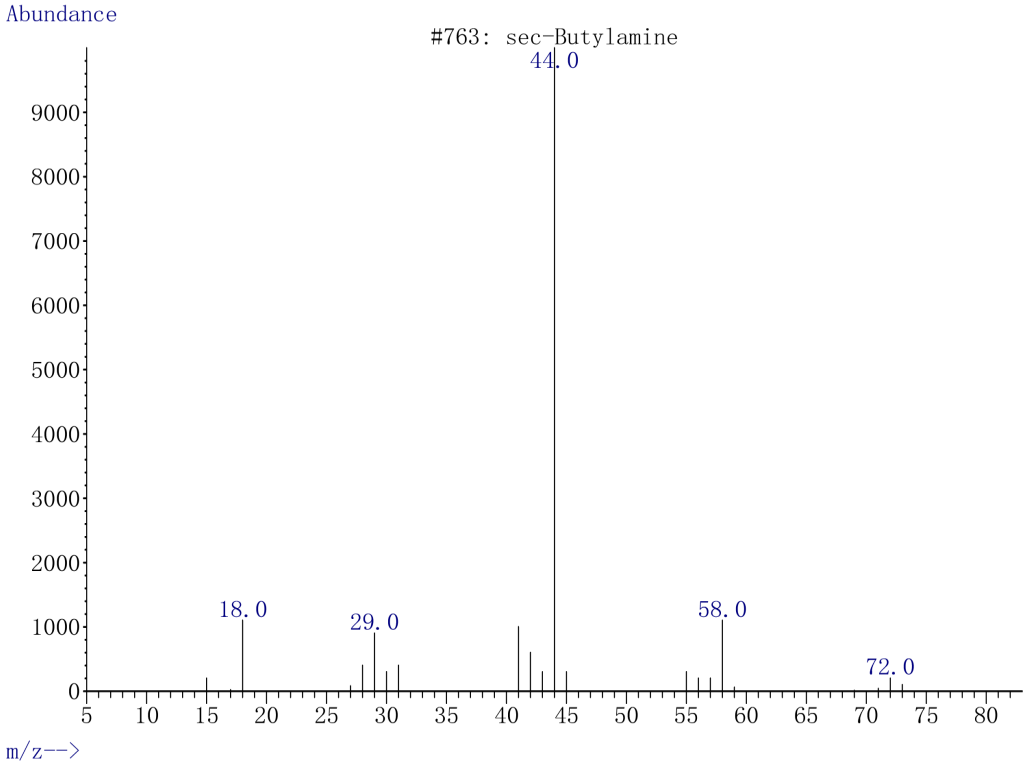

Figure 3. Mass spectrum of sec-Butylamine (4.88%, RT 6.638).

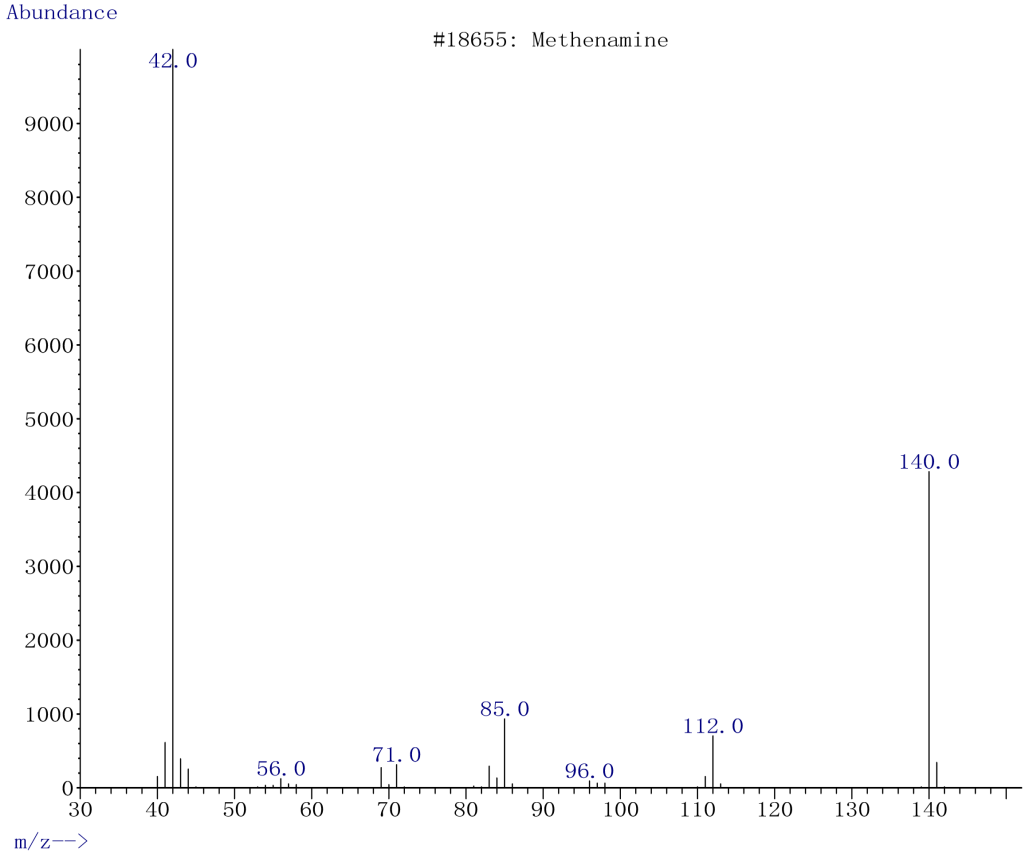

Figure 4. Mass spectrum of Methenamine (26.29%, RT 8.451).

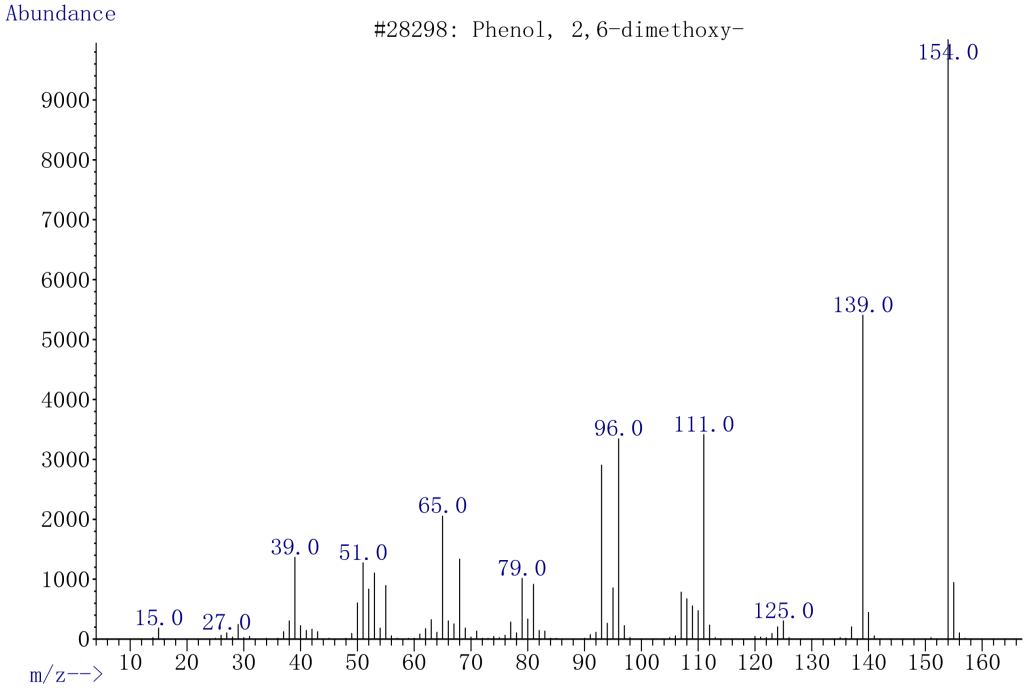

Figure 5. Mass spectrum of Phenol, 2,6-dimethoxy- (2.39%, RT 9.959).

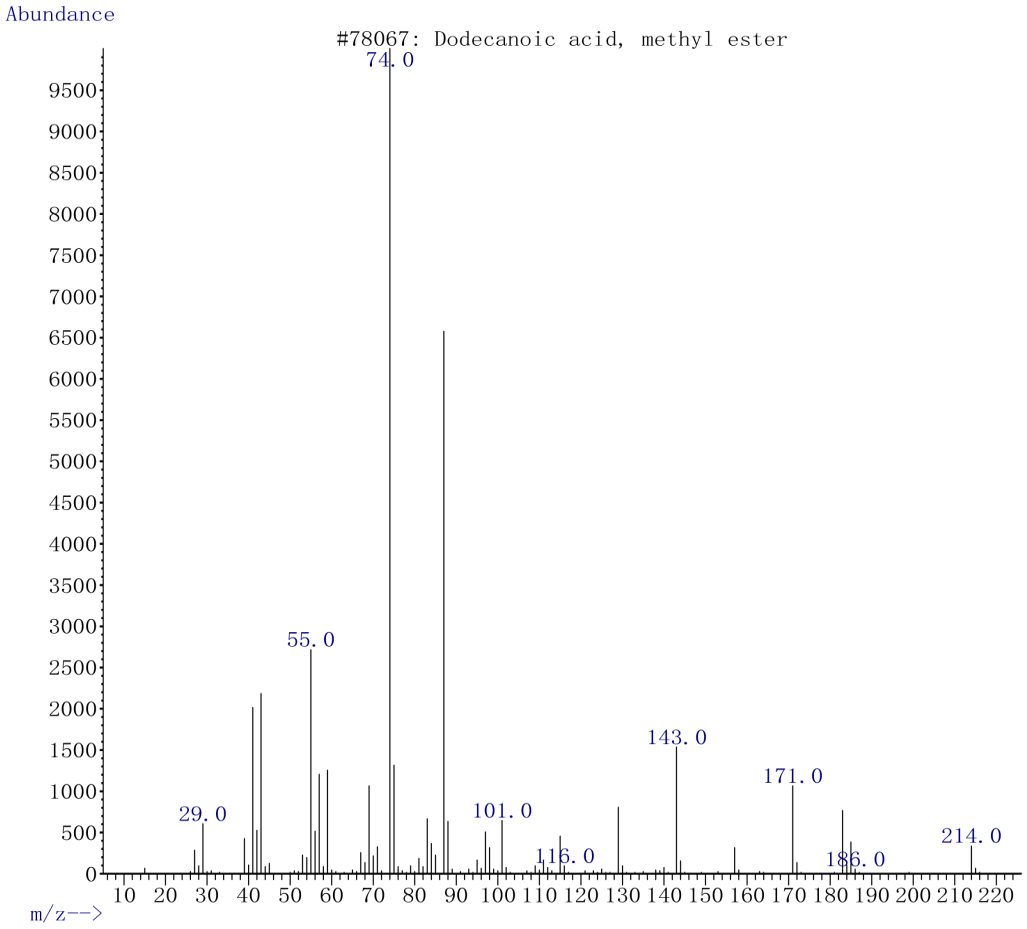

Figure 6. Mass spectrum of Decanoic acid, methyl ester (1.77%, RT 9.545).

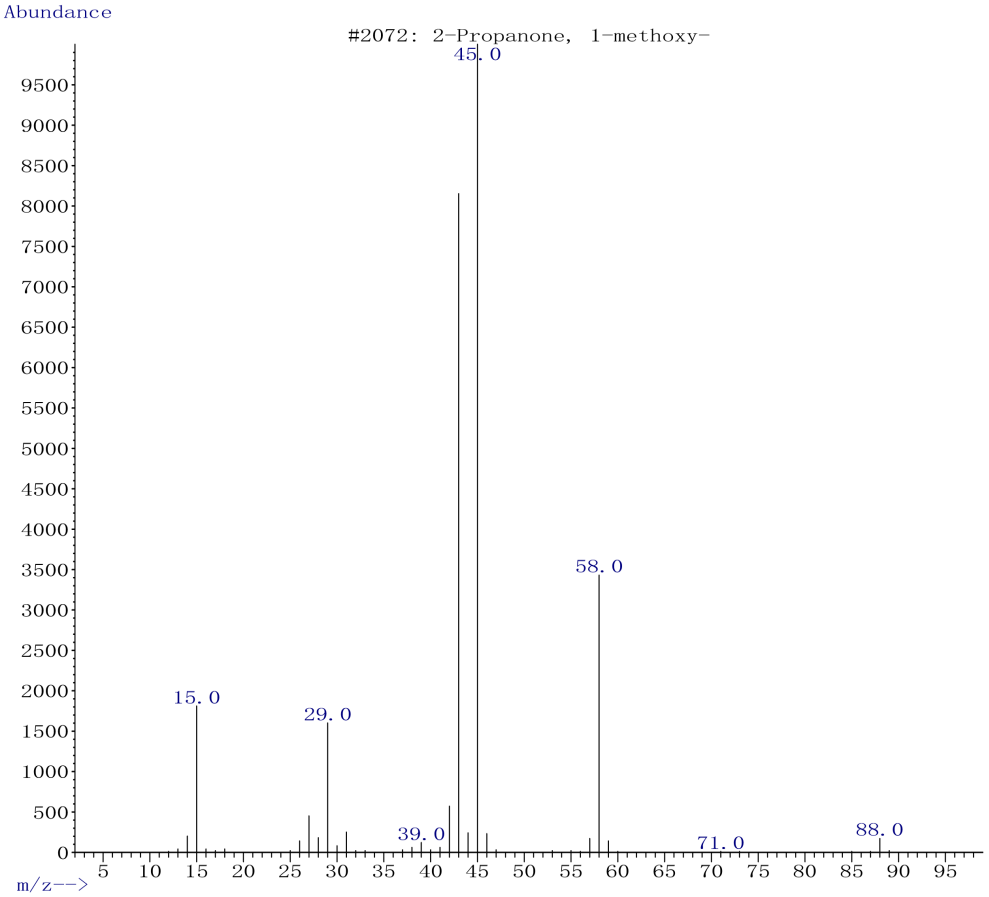

Figure 7. Mass spectrum of 2-Propanone, 1-methoxy (3.19%, RT 5.414).

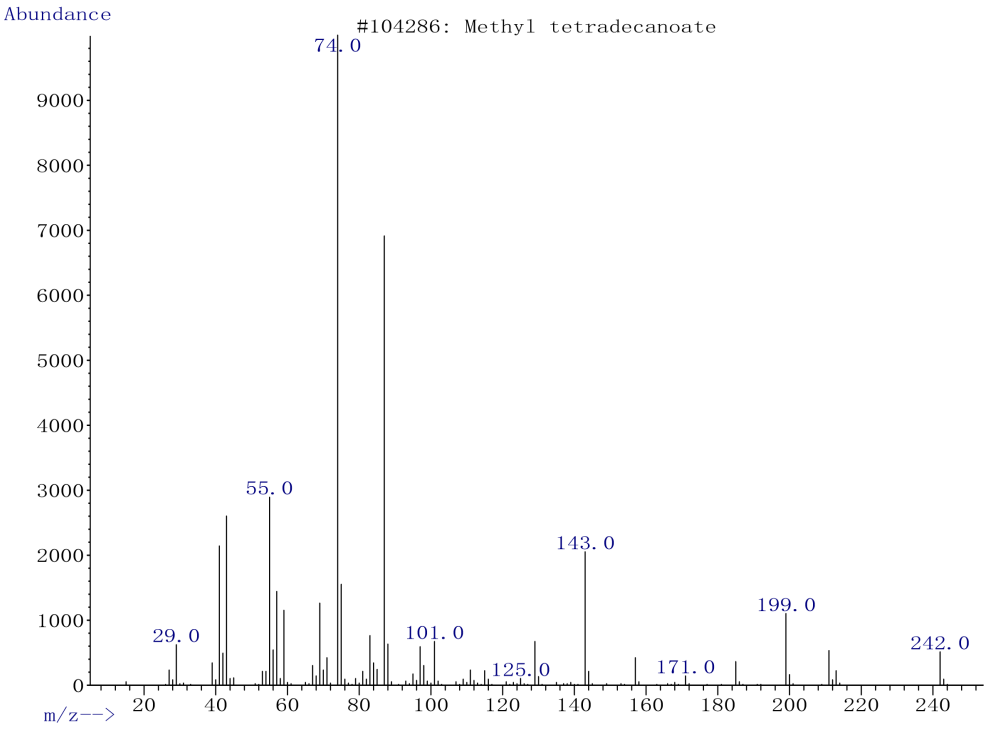

Figure 8. Mass spectrum of Methyl tetradecanoate (5.02%, RT 13.920).

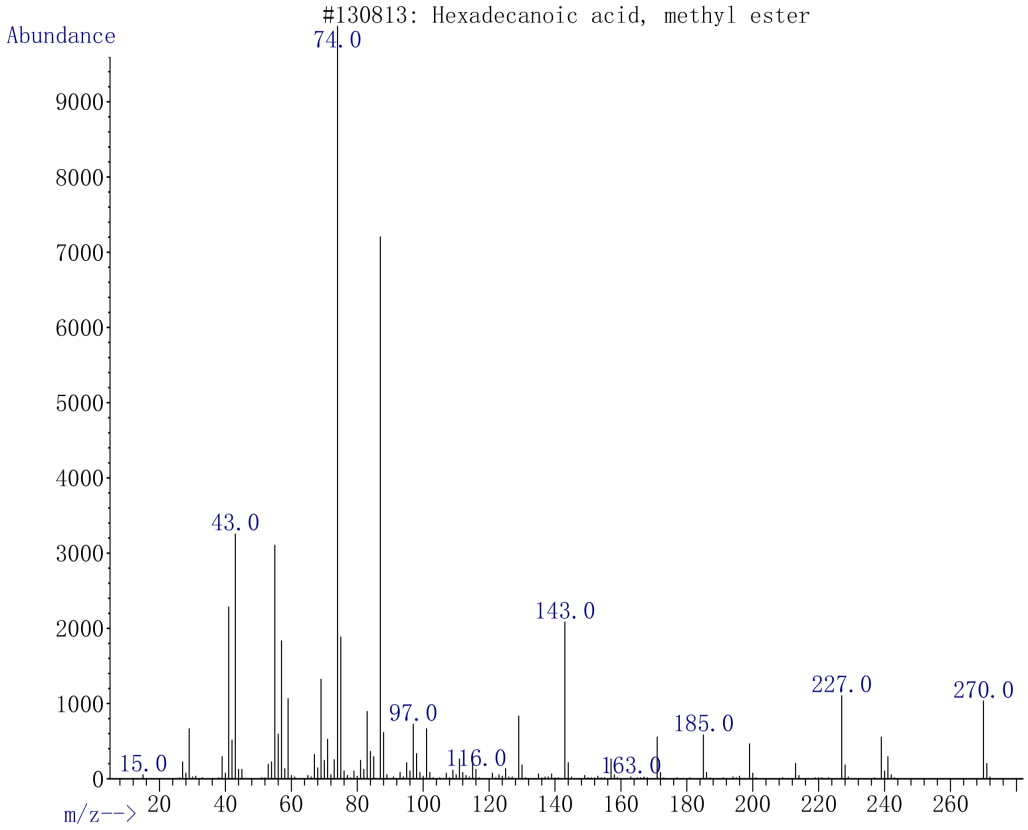

Figure 9. Mass spectrum of Hexadecanoic acid, methyl ester (14.54%, RT 15.790).

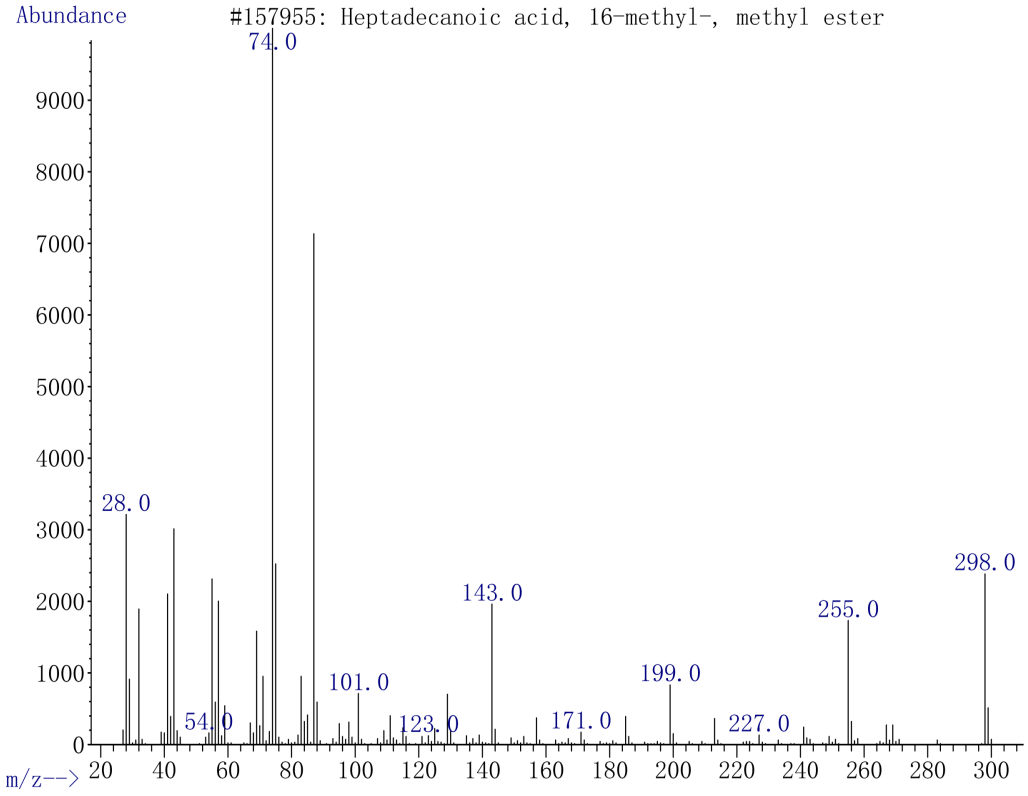

Figure 10. Mass spectrum of Heptadecanoic acid, 16-methyl- (3.03%, RT 17.481).

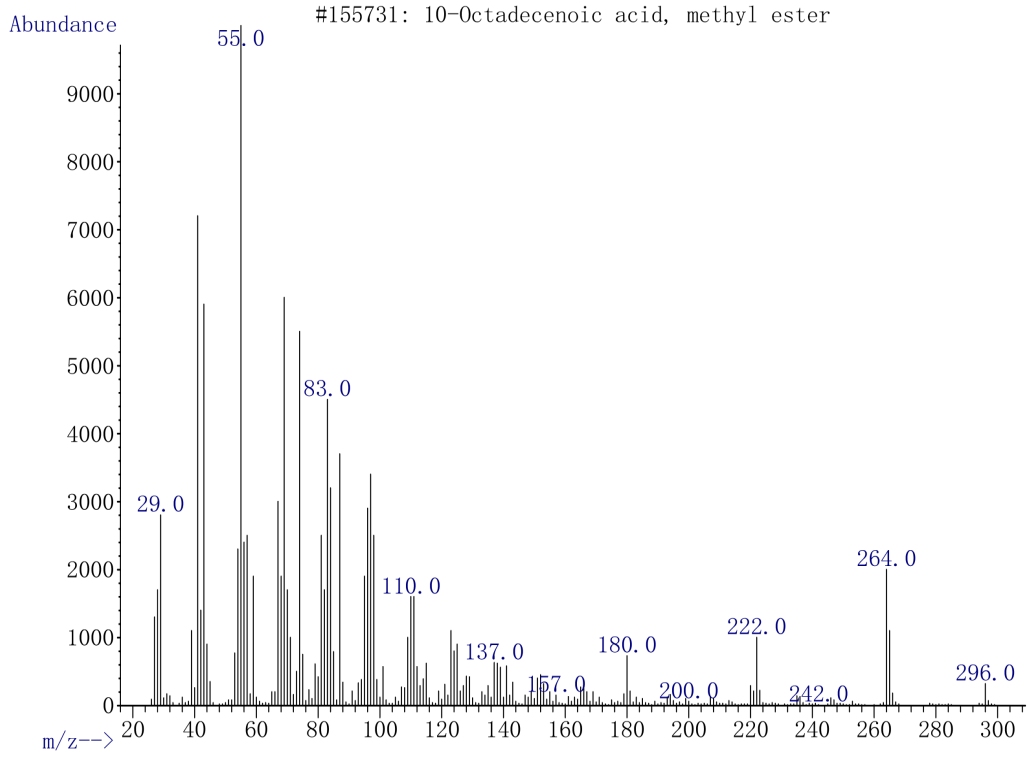

Figure 11. Mass spectrum of 10-Octadecenoic acid, methyl ester (3.12%, RT 17.283).

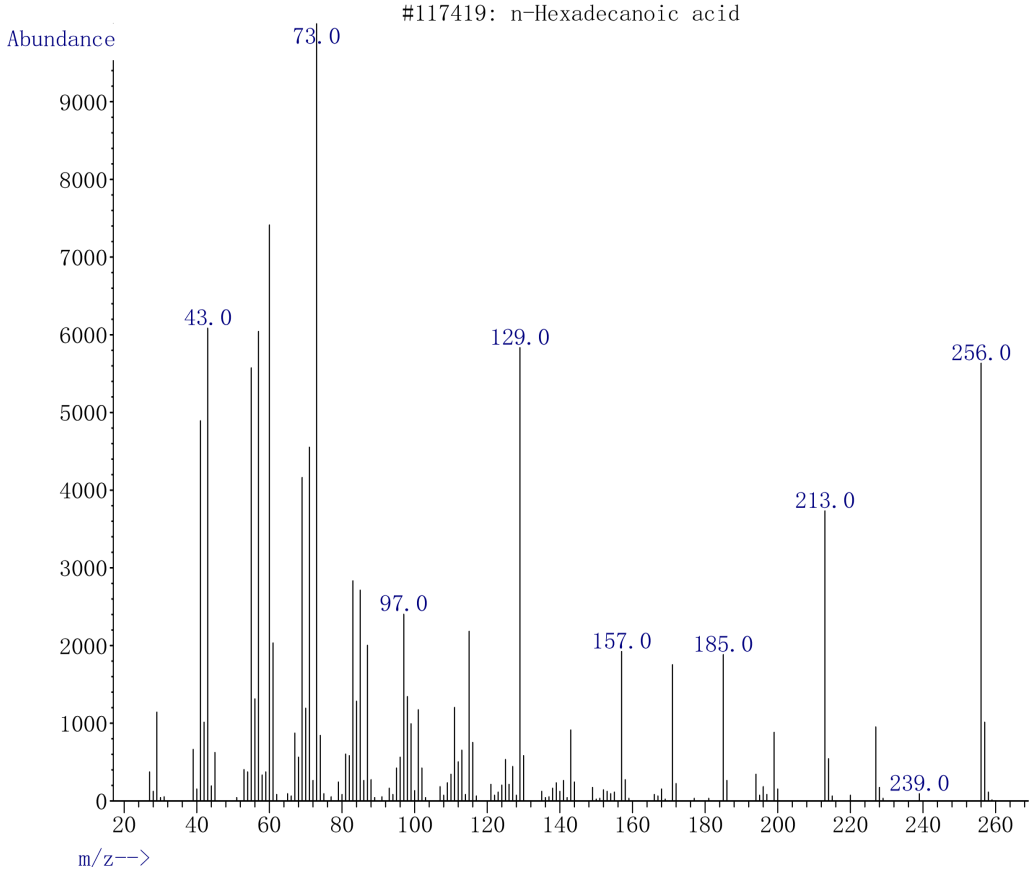

Figure 12. Mass spectrum of n-Hexadecanoic acid (2.08%, RT 16.649).

extract. The bioactive components were identified, characterized and interpreted on mass spectrum GC-MS conducted using the database of National Institute Standard and Technology (NIST) which has more than 62,000 patterns. Aside from that, the distinctive fragmentation patterns aided in the identification of a certain class of chemicals [8]. **Table 1** indicate the bioactive principles with their molecular formulas, molecular weight, retention time (RT), and percentage content (percent) of the plant material. The following is the distribution of bioactive identities from the hexane GC-MS chromatogram of *Azadirachta indica* leaf. Methenamine, Hexadecanoic acid, methyl ester; Phenol; Dodecanoic acid, methyl ester; Methyl tetradecanoate; sec-Butylamine; 2-Propanone, 1-methoxy-; 10-Octadecenoic acid, methyl ester; Heptadecanoic acid, 16-methyl-; Phenol, 2,6-dimethoxy-; Phenol, 2-methoxy-; n-Hexadecanoic acid. This is consistent with the findings of Yuvarajan *et al.* [9], who found several comparable phytochemicals and derivatives in low and high percentage proportions, respectively. Some of the phytochemical with high percentage composition determined in this study is in agreement with the study. They include; phenol, [10], 2-Propanone, 1-methoxy- [11], Phenol, 2-methoxy [12], Hexadecanoic acid, methyl ester [13], Dodecanoic acid, methyl ester, Hexadecanoic acid, methyl ester, and 10-Octadecenoic acid, methyl ester have been described by Bolade *et al.* [14]. The current study reviewed that bioactive compounds such as sec-Butylamine and Methenamine with elevated composition obtained in the extracts examined in this study had not previously been determined by other researchers. This could be due to the plants' biosynthetic pathway, which is heavily influenced by many ecological factors such as temperature, humidity, soil type and nutrients, and atmospheric pressure. Furthermore, the catalytic enzymes may differ. sec-Butylamine and Methenamine has antibacterial properties [15]. There is a significant difference in the outcomes of bioactive chemicals determined from the current investigation.

Zih-Rou *et al.* [16]; Shi *et al.* [17]; Nayak *et al.* [18]; Bartnik *et al.* [19]; Yong *et al.* [20]; Madrona *et al.* [21]; Pant *et al.* [22]; Saloni *et al.* [23]; Meechaona *et al.* [24]; Adsul *et al.* [25]; Oon *et al.* [26]; Kasture *et al.* [27] and Scortichini *et al.* [28] explained the medicinal important of *Azadirachta indica* (Neem) as follows: anti-inflammatory, hepatoprotective effect, wound healing effect, antidiabetic activity, antinephrotoxicity effect, neuroprotective effects, antimicrobial effect, immunomodulatory and growth promoting effect and that *A. indica* mouth rinse is equally effective in reducing periodontal indices which justifies the result obtained in the present study. All of these phytochemicals play a significant role in the formulation of various medicinal products. As antioxidants, phenol, sec-Butylamine, methyl tetradecanoate, hexadecanoic acid, and methyl ester have been employed [15] [29] [30] [31].

5. Conclusion

This research has revealed that *Azadirachta indica* possess vital bioactive com-

pounds that have been found to have a wide range of biological properties. The structure of active chemicals that might be exploited in drug production was also shown. Our findings also support the traditional use of *Azadirachta indica*, which contains a variety of bioactive components as antimicrobial agents. There is therefore need for a single fractionalization and separation of active compounds for a single slope antibacterial activity, which can aid in the utilization of these compounds in manufacture of pharmaceutical products.

Ethics Approval

The plants were identified, validated and recognized by a Plant Taxonomist at Imo State University, Department of Crop Science, based on their taxonomic classification and further deposited in the herbarium Department of Crop Science, Imo State University Owerri with the voucher number DPS/IMSU/0621.

Consent for Publication

The authors have declared that neither the manuscript nor any parts of its content are currently under consideration or published in another journal and all authors have approved the manuscript and agree with its submission and publication in Bulletin of the National Research Centre.

Availability of Data and Material

Data is contained within the article.

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Author Contributions

IFC, OSI, NRI and CIC, designed the work. OSI, NRI and CIC supervised the study. IFC and AII conducted the sampling and survey. IFC analyzed the data. IFC and CIC wrote the paper. All authors read and approved the final manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

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List of Abbreviations

GC-MS: Gas Chromatography-Mass Spectrometry

BCVs: Branch Chain Volatiles

GC-FID: Gas Chromatography with Flame Ionization Detector

NIST: The National Institute of Standards and Technology