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CHEMICAL COMPOSITION OF ESSENTIAL OIL OF *AZADIRACHTA INDICA* A. JUSS ROOT FROM DIKKO, NIGER STATE, NIGERIA

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ABSTRACT: *Azadirachta indica* (*A. indica*) belongs to the family Meliaceae. Various parts of the plant, including root, are used as a drug for various ailments, including malaria. The purpose of this study was to determine the chemical composition of essential oil (EO) of the root of *A. indica* collected from North Central Nigeria. The root essential oil of *Azadirachta indica* was obtained by using hydrodistillation method. Gas Chromatography, coupled to mass spectrometry, was used to analyze and determine the chemical composition of the essential oil. Mass spectra were searched against Mass Spectrometry databases, and twenty-eight components were identified. The main constituents citronellic acid (29.60%), 1-bromotriacontane (8.59%), totara-8,11,13-triene-7.beta.,13-diol (8.26%) and 4,8,12,15,15-pentamethyl-bicyclo[9.3.1]pentadeca-3,7-dien-12-ol (5.07%).

INTRODUCTION: Plants contain chemical substances known as phytoconstituents, which are naturally occurring products. Amongst the various classes of naturally occurring plant products, volatile oils make up an important class responsible for the essence and characteristic smell or odor of plants. This oil of essence is embedded in various parts of plants such as leaves, flowers, stems, herb, roots and seeds among others, occurring in the intracellular spaces of the plant tissues.

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Essential oils have been harnessed for several medicinal purposes for many decades. Interest in aromatherapy of essential oils has increased in recent decades due to their health benefits. Essential oils find applications in pharmaceuticals, perfumes, cosmetics, soaps, cleaning products and as a flavor for foods and drink [1].

The genus *Azadirachta* has three species of plants with Indo-Malayan origin, characterized in detail [2]. The genus *Azadirachta* belongs to a family known as Meliaceae which comprises 600 species in 52 genera. *Azadirachta indica* A. Juss commonly called neem tree is naturalized in most of the subtropical and tropical countries, native to India and distributed worldwide [3, 4]. Plants in this genus exhibit pharmacological effects and are used in folkloric medicine for the remediation of various

ailments. They have been applied in remedying headache, fever colds, malaria, general weakness and infections. *A. indica* possesses many medicinal activities such as immunostimulatory, anti-inflammatory, antioxidant, antipyretic, analgesic, hypoglycaemic, cardiovascular, antimicrobial, anti-malarial, anthelmintic, insecticidal, herbicidal, antifungal activities [5, 6]. Insecticidal, anticancer and antimalaria terpenoids have also been obtained from the extracts of plants belonging to this genus [7, 8]. *Azadirachta* is a rich reservoir of phytochemicals including polyphenols, phenolic acids, flavonoids, anthocyanins, diterpenoids, sesquiterpenoids and triterpenes [9, 10]. The total flavonoid content of the root bark extracts of *Azadirachta indica* prepared using varying extraction techniques has been reported [11]. The essential oil of *A. indica* flower contained α-cubebene, δ-cadinene, copaene, humulene and sesquiterpenes [12]. Essential oil components of *Azadirachta indica* leaves included γ-elemene, trans-caryophyllene, hexadecanal, methyl linoleat, and germacrene-B among another compound [13].

Extracts from neem possess high medicinal values; they are used to cure a range of diseases. The bark extract of *A. indica* had the potential ability to control gastro-duodenal ulcers [14]. The oil obtained from *Azadirachta indica* has ample beneficial effects including; mosquito repellant, insecticidal activity, tumor cells, cancers, *etc*. The injection of the extract obtained from neem leaves on solid cancerous tumors helps to limit growth and reduce the risk of the spread of the disease, especially skin cancer which response very well to the treatment with neem.

It has emerged as a highly potent bio-pesticide with the component azadirachtin responsible for pesticidal and antifeedant activities [15, 16, 17]. A large number of biologically active compounds/ principles are present in *A. indica* including gedunin, azadirone, promeliacin, limonoids, vilasinin, C-secomeliacins, nimbin, azadirachtin, salanin and other non-isoprenoids as well as polysaccharides, sulphurous compounds protein / amino acids, polyphenolics such as flavonoids, tannins, coumarins, glycosides, dihydrochalcone, aliphatic compounds amongst others. Azadirachtin is reportedly to be the component with the most bioactivity in *A. indica* [18].

The neem seed oil can produce soap, even without being mixed with other fats because neem contains a little quantity of free fatty acids which results in a high saponification index and low index of acidity and indicates a high proportion of saponin. The essential seed oil of neem is made up of fatty acids, which oleic acid, linoleic acid, stearic acid, and palmitic acid are the four major components. It is also made up of amino acids, steroids, tocopherol, glycerides amongst others [19].

The phytochemical components of the seed oil of neem revealed gedunin, meliacin, and Mahmood in as major constituents [20, 21]. There has been no reported information on the chemical composition of the root essential oil of *Azadirachta indica* (*A. indica*). Therefore, the objectives of the present are to identify the chemical composition of the root of *Azadirachta indica* collected from Northern Nigeria using Gas chromatography coupled with mass spectrometry (GC-MS).

MATERIALS AND METHODS:

Plant Material: The underground parts of *Azadirachta indica* were collected in July from Dikko (a village in Niger State in Northern Nigeria), and consequent authentication carried out by a taxonomist at the Herbarium of the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria.

Isolation of Essential Oil: The volatile oil from the plant was obtained from fresh plant material by hydrodistillation method employing Clevenger-type apparatus. The extraction process took 4 h. To improve their essential oil recovery, n-hexane was used to take up the oil which was subsequently dried over anhydrous sodium sulfate to remove traces of water and then stored in a dark glass bottle properly sealed at the temperature 4 °C until GC-MS analyses. The yield of the volatile oil obtained was 1.2% (w/w).

Analysis of Essential Oil by GC-MS: The essential oil analysis was performed using a Shimadzu QP-2010 Gas Chromatograph coupled to Shimadzu QP-2010 plus mass detector and employing an HP-5 column (30 m length, 0.25mm ID, 0.25 μm stationary phase thickness). An electron ionization system, with ionization energy of 70 eV was employed for GC-MS detection.

Helium (99.999%) was the carrier gas, at a flow rate of 1.61 mL/min with the pressure of 100 Pa and split injection with a split ratio of 1:100. The gas chromatography conditions were set as the follows: column temperature, 2 min in 60 °C, 60-180 °C at 10 °C/min and 180-280 °C at 15 °C/min; held for 4 min in 280 °C; injector temperature, 250 °C, and 1.0 µL of volume injection of the essential oil. The mass spectrometry operating parameters were as follows: ionization potential, 70 eV; ion source temperature, 200 °C, interface temperature, 250 °C; solvent delay, 2 min; scan speed, 2 000 amu/s; scan range, 35-450 amu.

Identification of Compounds: The chemical constituents of the volatile root oil obtained from the neem plant was identified using their retention times and Mass Spectra library data (NIST 11). The percentage composition of the volatile oil was computed from the GC peak areas.

RESULTS AND DISCUSSION: Essential oils are chemically characterized as complex mixtures of low molecular weight compounds which may possess flavors or aromas [22]. The essential oil obtained from the root of *Azadirachta indica* was light yellow with an aromatic odor. The percentage oil yield of *A. indica* root was 1.2% (w/w).

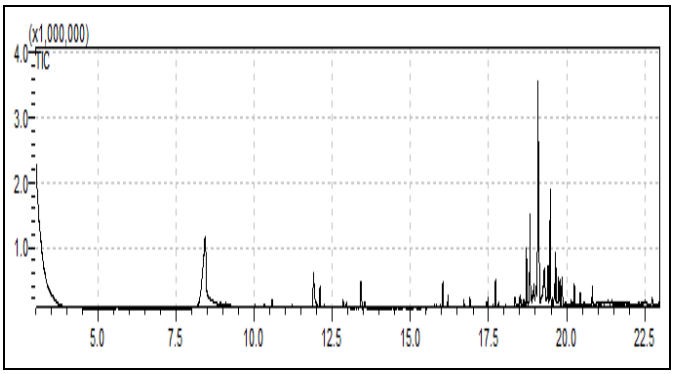

FIG. 1: CHROMATOGRAM OF THE ESSENTIAL OIL OF *AZADIRACHTA INDICA* ROOT

TABLE 1: THE ESSENTIAL OIL CONSTITUENTS OF *AZADIRACHTA INDICA* ROOT

Peak#	Retention Time	Compound	% Composition
1	8.341	Citronellic acid	29.60
2	10.333	Clovene	0.27
3	10.571	α-Funebrene	0.57
4	12.105	Epiglobulol	1.75
5	12.844	α-cadinol	0.72
6	12.952	[-]-Globulol	0.56
7	13.420	Isolongifolen-9-one	2.55
8	13.541	Pentadecanal-	0.58
9	16.036	Cis,cis,cis-7,10,13-Hexadecatrienal	2.34
10	16.199	Benzenamine,4,5-dimethyl-2[4-pyridymethoxy]-	0.97
11	16.708	1,3-Dimethyladamantine	0.68
12	16.904	Cembrene A	0.64
13	17.428	5-methoxy-2-methyl-4-oxo-1,2,3,4-tetrahydro-1,10-phenanthroline	0.36
14	17.721	Phenanthrene	2.17
15	17.820	Myristyl alcohol	0.28
16	18.712	Bicyclo[9.3.1]pentadeca-3,7-dien-12-ol,4,8,12,15,15-pentamethyl-	5.07
17	18.910	Abietyl alcohol	1.09
18	19.015	4,8,13-duvatriene-1,3-diol	1.72
19	19.280	1-Bromotriacontane	8.59
20	19.402	1,4-Dimethyl-8-isopropylidenetricyclo[5.3.0.0[4,10]]decane	4.21
21	19.475	Totara-8,11,13-triene-7.beta,13-idol	8.26
22	19.608	Ferruginol	1.51
23	19.735	Oleamide	2.00
24	19.781	Nimbiol	1.88
25	19.849	Fischer's aldehyde	2.73
26	20.246	Thunbergene	1.45
27	20.438	Sugiol	1.36
28	22.738	Farnesyl acetone	0.06
	Total		83.97%

The present work involved the isolation and GC-MS analysis of essential oil from the root of *Azadirachta indica* collected from North Central Nigeria. The fresh root of *Azadirachta indica* was hydrodistilled to obtain the essential oil (EO) using Clevenger apparatus. The chromatogram of the

essential oil is shown in **Fig. 1**. Twenty-eight compounds were identified, representing 83.97% of the essential oil composition **Table 1**. Essential oils grouped chemically as complex mixtures of compounds with low molecular weight which may possess flavors and aromas [22]. The essential oil prepared from the root of *A. indica* root extract was light yellow with an aromatic odor. The percentage oil yield of *A. indica* root was 1.2%. The present work deals with the isolation and GC-MS analysis of essential oil from the root of *Azadirachta indica* (A. Juss). The fresh root of *Azadirachta indica* was hydrodistilled to obtain the essential oil (EO) using Clevenger apparatus by hydrodistillation. Twenty-eight compounds were identified, representing 83.97% of the total oil composition.

Citronellic acid (29.60%), also known as 3,7-dimethyl-6-octenoic acid being the most abundant constituent in the essential oil from the root of *Azadirachta indica* is a well-known acyclic monoterpene carboxylic acid. Citronellic acid is a flavor and fragrance agent. It is an intermediate used in the commercial production of (S)-(+)-4-methyl-3-heptanone, an alarm pheromone found in three other ant genera of the subfamily myrmicinae and the principal alarm pheromone of the leaf-cutting ant *Atta texana*. (S)-(+)-4-methyl-3-heptanone is a component of the defensive secretion of the "daddy longlegs" *Leiobunum vittatum* (Opiliones) and is produced by the elm bark beetles *Scolytus scolytus* (F.) and S. multi-striatus [23, 24, 25, 26]. Ferruginol is a natural phenol and a diterpenoid. Ferruginol also possesses antioxidant activity against linoleic acid oxidation under non-solvent condition [27].

α-Funebrene is a sesquiterpene. It is biologically active as an inhibitor of α-amylase, α-glycosidase inhibitor [28]. Epiglobulol, a sesquiterpenoid, shows antibacterial activity in molecular docking studies against bacterial proteins [29]. In a study, globulol, from *Eucalyptus globulus* Labill fruit belonging to the family Myrtaceae obtained by bioassay-guided isolation possesses antimicrobial activity [30]. Nimbiol, an aromatic dicyclic diterpene, have been found to possess insecticidal activity [31]. α-Cadinol is also a sesquiterpenoid that acts as an antifungal agent [32]. Pentadecanal, a long chain fatty aldehyde has been confirmed to be an anti-biofilm molecule [33]. Myristyl alcohol has been reported to have phagodeterrent effect on aphids [34]. Oleamide has been confirmed to have anti-inflammatory and antibacterial activities [35]. E, E-farnesyl acetone presented moderate antibacterial activity against *Aeromonas sp.* in study [36].

Sugiol is a diterpene, which has anti-inflammatory properties. Though sugiol shows a low inhibitory activity against the DPPH radical, it can reduce intracellular reactive oxygen species (ROS) production effectively in lipopolysaccharide (LPS)-stimulated macrophages [37]. Cembrene diterpenoids are a large and structurally diverse group of naturally occurring products obtained from both marine and terrestrial organisms which strongly inhibits tumor promoters [38]. The composition of the essential leaf oil of *Azadirachtha indica* revealed that the major components of the oil were: γ-elemene (20.8%) being the most abundant, germacrene-B (20.3%), trans-caryophyllene (13.5 %); hexadecanal (12.8%), and methyl linoleat (10.5%) [39]. Kurose and Yatagai (2005) [40] studied components of the seed essential oils obtained from three species of *Azadirachta*. Seed oil components of *A. indica* were hexadecanoic acid (34.0 %), oleic acid (15.7%), 5,6-dihydro-2,4,6-triethyl-(4H)-1,3, 5-dithiazine (11.7%), methyl oleate (3.8%), and eudesm-7(11)-en-4-ol (2.7%).

The major components of A. siamensis seed oil were hexadecanoic acid (52.2%), tricosane (10.5 %), tetradecanoic acid (6.8%), pentacosane (4.9%) and oleic acid (4.9%). Seed oil from *A. excelsa* was more abundant in components such as oleic acid (31.3%), followed by hexadecanoic acid (14.2%), octadecanoic acid (13.0%), 4-octylphenol (9.7%), and O-methyloximedecanal (6.8%) as its major constituents. The essential oils of gotten from the seed of *A. siamensis, A. indica* and *A. excelsa* were found to contain fatty acids (52.6% - 72.3%) as major components. The minor components of the oils were n-alkanes, esters, nitrogen compounds, aromatics sulphur and terpenoids [40]. Cis- and trans-3,5-diethyl-1,2,4-trithiolanes were identified as major components on analysis of neem seed oil obtained using steam distillation reported by Mubarak and Kulatilleke [41]. Sesquiterpenes, fatty acids, fatty acid esters, steroids and hydrocarbons were identified from the hexane soluble fraction of the fresh flowers of neem (*A. indica*) plants. The flower essential oil of neem plant has also been

obtained using steam distillation with the resulting volatile oil comprising mainly of δ-cadinene (9.43%) α-copaene (7.03%), humulene (3.7%), δ-cubebene (3.04%), and some sesquiterpenes. Globulol (0.65%) was found as one of its constituents [42], in the present study globulol was 0.56%. The stem essential oil of *Aglaia odorata* Lour yielded Germacrene D (20.3%), α-humulene (17.1%), α-himachalene (12.7%) and β-caryophyllene (10.2%) as the major components.

The leaf essential oil of *Aglaia odorata* Lout comprised linalool, hendecane, α-copaene, β-elemene, β-caryophyllene, α-humulene, aromadendrene, γ-cadinene, α-himachalene, δ-cadinene, β-guaiene, γ-gurjunene, γ-elemene, , β-elemene-9β-ol, β-humulene-7-ol, nerolidol, earyophyllenol-1, farnesol, β-santalol, elemol; none of these constituents was found in the present study [43, 44]. The main compounds identified in the essential oil of *Guarea kunthiana* A. Juss were α-zingiberene (34.48%), β-sesquiphellandrene (22.90%), and α-curcumene (16.17%) [45]. The essential oil of *Cedrela odorata* L. bark from S. Tomé and príncipe yielded majorly; α-copaene (14.4%), α-cadinol (11.2%), β-eudesmol (9.4%) and δ-cadinene (9.2%) [46].

Chemical components of essential oil of *Toona sinensis* (A. Juss.) Roem leaf from China, contained mainly caryophyllene (19.51%), humulen-(v1) (16.04%), himachala-2, 4-diene (5.71%), seychellene (4.82%), longifolene-(V4) - (4.42%), caryophyllene oxide (4.03%), and aristolene (3.18%); isolongifolene was found in the present study [47]. The essential oil of *Toona sinensis* (A. Juss.) Roem roots contained majorly tridecane (15.54%), tetradecane (7.00%), 9-octadecenoic acid, methyl ester (6.19 %), 13-docosenoic acid, methyl ester (5.13 %), (Z,Z)-9,12-octadecadienoic acid (4.53 %), 1-hexadecyne (3.79%), and α-cubebene (3.07%); none of these constituents were found in the present study [48]. GC and GC-MS analysis of the root volatile oil of *Trichilia connaroides* revealed the presence of β-chamigrene (31.1%) as the most bundant component followed by α-cadinol (7.5%), δ-cadinene (5.3%), cis-calamenene (5.6%), β-caryophyllene (3.5%), β-eudesmol (3.5 %) and occidentalol (3.0 %); α-cadinol (0.72%) was reported in the present study [49]. The essential oil from the stem bark essential oil of *Khaya grandifoliola* were α-pinene (10.56%), limonene (1.25%), β-carophyllene (3.87%), β-pinene (7.80%), α-phellandrene (7.45%), and citronellol (5.10%); citronellic acid (29.60%) was reported in the present study [50]. The essential oil obtained by hydrodistillation of *Carapa guianensis* Aubl. (Meliaceae) leaves contained bicyclogermacrene (28.5%), α-humulene (17.2%), germacrene B (11.9%), and trans-beta-caryophyllene (9.9%) [51].

CONCLUSION: Essential oil of the root of *Azadirachta indica* analysed by GC-MS revealed 28 compounds with citronellic acid (29.60%) as the most abundant. Other major constituents were totara-8, 11, 13-triene-7.beta, 13-idol (8.26%), 4, 8, 12, 15, 15-pentamethyl- bicyclo[9.3.1]pentadeca-3,7-dien-12-ol (5.07%).

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CONFLICT OF INTEREST: No conflict of interest.

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