Available online at http://ajol.info/index.php/ijbcs

Int. J. Biol. Chem. Sci. 8(4): 1808-1818, August 2014

ISSN 1997-342X (Online), ISSN 1991-8631 (Print)

International Journal of Biological and Chemical Sciences

Original Paper

http://indexmedicus.afro.who.int

Constituents of leaf, stem bark and root volatile oils of *Anogeissus leiocarpus* DC. Guill. & Perr.

Dorcas Olufunke MORONKOLA[1*] and Oluyemisi Folasade KUNLE [2]

[1]*Chemistry Department, University of Ibadan, Ibadan. Nigeria.*
[2]*Department of Medicinal Plants Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development, Abuja, Nigeria.*
[*]*Corresponding author; E-mail: funkemoronkola@yahoo.com; Tel: +2348023416336, +2349032442392*

ABSTRACT

We report volatile compounds in Anogeissus leiocarpus, which is scarce in literature. Here in Nigeria leaf, stem bark and root essential oils were obtained by hydro-distillation using Clevenger-type apparatus, analyzed by GC and GC-MS. 88% leaf oil comprised of eleven compounds, with abundance of z-9-octadecenoic acid (29.0), n-hexadecanoic acid (21.4), n-octadecanoic acid (12.7), methyl-7E-7-octadecenoate (8.5), and methylhexadecanoate (5.4). Thirteen compounds amount to 80% of the stem bark oil, its significant compounds being z-9-octadecenoic acid (22.8), n-hexadecanoic acid (20.8), methyl-9z-octadecenoate (12.4), methylhexadecanoate (7.8) and eicosane (4.1). Fourteen compounds make-up 91% of root oil, dominated by methyl-7E-7-octadecenoate (18.1), n-hexadecanoic acid (17.6), methyl linoleate (16.2), z-9-octadecenoic acid (15.7) and methylhexadecanoate (14.2). Leaf, stem bark and root oils are characterized by the following classes of compounds respectively (%): fatty acids [65.8, 43.6, 36.7]; esters [17.9, 20.2, 49.9]; hydrocarbons [3.2, 15.7, 1.7]; leaf and root oils contain terpenoids [1.1, 1.1]; dl-arabinose (sugar) is in stem bark oil. Methylhexadecanoate and hexadecanoic acid are common to the three oils. They can serve as chemo-taxonomic markers characteristic for this species. Compositions of *Anogeissus leiocarpus* three oils vary, are unique and of different chemo-types. This report is first of its kind in literature for 'axlewood' plant.

Keywords: Combretaceae, Anogeissus leiocarpus, essential oil, GC-MS.

INTRODUCTION

Anogeissus leiocarpus DC. Guill. & Perr. is a Combretaceae commonly called 'axlewood', locally referred to in Nigeria as 'Ayin, Orin-odan, Marike and Atara'. It is an evergreen tall tree found in savannah region of Tropical Africa, especially West and East Africa through tropical Southeast Asia (Steentoft, 1988; Odugbemi and Akinsulire, 2008). It is typically found growing at altitudes of 450 to 1900 m, and do grow on a range of soil types including compact clay soils (Vertisols) (Moctar and Sidi, 2007).

A. leiocarpus flowers in the rainy season, from June to October, its winged seeds [samaras], are mostly dispersed by ant (Steentoft, 1988). *A. leiocarpus* have many social applications. It is utilized in carving wood constructions because of its insect and termite resistance property; for tanning leather

DOI : http://dx.doi.org/10.4314/ijbcs.v8i4.38

and fabrics; for producing firewood and charcoal; root is used as chewing stick and is one of the plants for making traditional Malian mud cloth referred to as 'bogolanfini'. Yellow dye is obtained from its leafy small branches (Moctar and Sidi, 2007).

Anogeissus leiocarpus has been utilized in the treatment of many ailments and diseases in folk medicine. It possesses antiplasmodial activity and largely used by traditional healers in sub-Saharan Africa. In Burkina Faso, the leaf extract was evaluated *in vitro* using the multi-resistant strain (W2) of *Plasmodium falciparum* (Kabore et al., 2010). The extract displayed high *in vitro* antiplasmodial activity, as well as low levels of cytotoxicity against K562S cells. Hence *A. leiocarpus* is good in treatment of malaria (Kabore et al., 2010; Akanbi et al., 2012). Methanol extract of *A. leiocarpus* was reported to have high antimalarial activity, high antioxidant property, and capable of boosting HDL level in *Plasmodium bergheii*-infected organisms (Akanbi et al., 2012).

A. leiocarpus is one of the eight plants with strong anthelmintic activity on screening of sub-Saharan African plants (Waterman et al., 2010). Bark of the tree has anthelmintic properties and is utilized in the treatment of worm and protozoan diseases of livestock (Bizimana, 1994).

A. leiocarpus was reported to have high antimicrobial activities in many chemotherapeutic applications, hence its continued use in the treatment of bacterial infections (Mann et al., 2009; Mann et al., 2010; Mann, 2012). Antibacterial activity of alcohol extracts of leaf, stem and root bark of *Anogeissus leiocarpus* showed higher activity against *Staphylococcus aureus* than other test organisms. *In vitro* investigation of extracts of *A. leiocarpus* for antifungal activities against *Aspergillus niger*, *A. fumigatus, Penicillium* species, *Microsporum audouinii* and *Trichophyton rubrum* using radial growth technique displayed depression on rats (Mann et al., 2008a; Mann et al., 2008b).

The plant, widely used in the treatment of parasitic diseases, has superior leishmonicidal activity (Shuaibu et al., 2008). It is effective in the traditional treatment of trypanosomiasis (Wurochekke and Anyanwu, 2012).

A. leiocarpus is suitable for use in the therapeutic management of asthma, and has been developed to single tablet dosage form (Emeje et al., 2011). It possesses wound healing activity in a dose-dependent manner and provides a scientific rationale for the traditional use of it in the management of wounds (Barku et al., 2013).

Literature reports suggest that the aqueous extract of *A. leiocarpus* could be used, with some degree of safety, by oral route (Agaie et al., 2007; Olabanji et al., 2007). In Nigeria, the inner bark, which possesses antibacterial activities, is used as chewing stick. It was assessed as one of sixteen medicinal plants used for cleaning teeth in southwestern Nigeria. It affects the formation, growth, development, and protection of human teeth. The PIXE (particle-induced X-ray emission) technique was used (Olabanji et al., 2007). Reports of toxicity studies on the plant extracts showed it had no toxic effect on the liver, and its consumption is safe at a dose up to 200 mg/kg body weight (Ahmad and Wudil, 2013).

Castalagin was isolated from stem bark of *A. leiocarpus* along with other hydrolysable tannins (Shuaibu et al., 2008). The plant contains other important classes of bioactive constituents such as glycosides, phenols, tannins, saponins, alkaloids, steroids, flavonoids, ellagic acids and anthraquinones, which may be responsible for its medicinal uses and activities (Barku and Abban, 2013; Adamu et al., 2013).

In continuation of our quest to study chemical compositions of rarely studied plants with ethno-medicinal and industrial values, we have this report on the composition of the volatile oils obtained from leaf, stem bark and

root of *Anogeissus leiocarpus*, which has not yet been documented in literature.

MATERIALS AND METHODS

Plant material

Fresh samples of *A. leiocarpus* were collected from Ago-Iwoye, Ogun State, Nigeria, late 2009. The plant was authenticated by Soladoye M.O. (Plant taxonomist) as well as Prof E. Abiodun Ayodele, staff of the herbarium, Department of Botany, University of Ibadan, Ibadan, where some voucher samples have been deposited, with voucher number UIH – 22397.

Extraction of essential oils

500 gm of each plant part [leaf, stem bark and root] was crushed and subjected to hydro-distillation for 2½ hours using an all-glass Clevenger-type apparatus designed to British Pharmacopeia (1980) specifications. 0.5 ml of distilled n-hexane was added for better yield, which was removed afterwards and the oils refrigerated. The percentage yields were 0.03 to 0.05%. Oils procured were a little cloudy and had distinct characteristic pleasant smell. The oils were stored in appropriate containers and refrigerated before use.

Gas Chromatography

Each of the essential oils was analyzed on GC-2010[AOC-20i] gas chromatograph. Column oven temperature was 60 ^{o}C, injection temperature was 250 ^{o}C; split injection mode, at 100.2 kPa; column flow of 1.61 ml min^{-1} and total flow of 6.2 ml min^{-1}; 1.0 split ratio; oven temperature programming was 60 ^{o}C (for 5mins), increasing at the rate of 5 ^{o} min^{-1} to 140 ^{o}C, then 15 ^{o}min^{-1} to 280 ^{o}C.

Gas Chromatography-Mass Spectrometry

The GC-MS analyses were performed on GC-MS QP2010 Plus. Ion source temperature was 200 ^{o}C; interface temperature, 250 ^{o}C; solvent cut time 2.5 min; relative detector gain mode and threshold, 3000; detector, FID; mass range of m/z 40-400.

Identification of components

The components of the oils were identified based on their retention indices (determined with a reference to homologous series of n-alkanes), by computer matching against the library spectra built up from authentic compounds and mass spectrum literature data (Massada, 1976; Joulain and Koenig, 1998).

RESULTS

Volatile oils obtained from hydro-distillation of the three parts leaf, stem bark and root of *Anogeissus leiocarpus* were obtained in good yields of 0.03 to 0.05% and had distinct characteristic pleasant smells (Table 1). Each of the oils was analyzed using GC and GC-MS. Their chromatograms are shown in Figures 1-3. Compositions of the essential oils of the leaf, stem bark and root are presented in Tables 2-4.

Table 1: Yields of essential oils procured from leaf, stem bark and root of *Anogeissus leiocarpus.*

Plant Parts	Weight of sample (g)	Weight of volatile oil procured (g)	% Yield of essential oil procured	Physical Characteristics
Leaf	500	0.25	0.05	Milky with leafy herbal smell
Stem bark	500	0.20	0.04	Colourless oil with herbal odour
Root	500	0.15	0.03	Whitish oil with herbal smell

Table 2: Chemical composition of leaf essential oil of *Anogeissus leiocarpus.*

Peak No[a]	MS [Base peak+most abundant peaks][b]	Identified compound[c]	% TIC[d]	Retention time [mins[e]	Calculated RI[f]
1	57,71,43,85,41,56,70,69,42,55,	Dodecane	1.6	22.02	1970
2	105,83,69,57,55,97,41,43,56,70,91,	Nopol	1.1	24.81	2367
3	57,105,71,85,43,41,56,99,70,42,55,79,91,97,	tridecane	1.6	26.79	2415
4	74,87,43,55,75,41,57,69,59,101,227,	methylhexadecanoate	5.4	27.62	2748
5	73,60,43,57,55,41,71,69,83,85,87,256,61	n-hexadecanoic acid	21.4	27.87	2754
6	55,69,74,83,41,96,97,98,84,87,43,67,81,57	methyl-7E-7-octadecenoate	8.5	28.81	2778
7	55,69,83,97,41,43,84,98,96,57,67,70,56,82	z-9-octadecenoic acid	29.0	29.05	2784
8	73,43,57,55,60,41,69,71,83,85,87,97,284	n-octadecanoic acid	12.7	29.19	2787
9	67,81,95,82,79,96,55,68,280,	z,z-9,12-octa decadienoic acid	2.7	29.50	2795
10	57,71,14970,43,113,41,167,279,55,104,	di-n-octylphthalate	2.7	31.39	2842
11	74,87,75,43,55,57,382,101,69,283,339,	methyltetracosanoate [382]	1.3	32.83	3179

[a]According to the % TIC and retention time from GC [Figure 1]; [b][m/e] values of base peak 1st stated, and other most prominent ions; [c]see identification of components;
[d]Total ion concentration in %; [e]retention time in minutes; [f]Retention Index determined with reference to homologous series of n-alkanes.

Table 3: Chemical composition of stem bark essential oil of *Anogeissus leiocarpus*.

Peak No[a]	MS [Base peak+most abundant peaks][b]	Identified compound[c]	% TIC[d]	Retention time [mins][e]	Calculated RI[f]
1	57,71,43,85,41,44,55,99,56,69,70,	3,7-dimethylnonane	1.2	24.93	2370
2	73,60,69,71,83,43,41,55,57,	dl-arabinose	0.5	26.32	2403
3	83,97,57,69,55,43,41,70,56,71,111,	3E-3-icosene	1.4	26.71	2413
4	74,87,43,55,75,41,69,57,101,227,59,83,	methylhexadecanoate	7.8	27.63	2415
5	73,60,43,57,55,41,71,69,83,85,87,213	n-hexadecanoic acid	20.8	27.87	2754
6	57,71,85,43,99,70,56,41,42,55,84,82,	n-nonadecane	1.4	28.25	2764
7	55,55,69,74,83,97,84,96,98,41,87,43,81,	methyl-9z-octadecenoate	12.4	28.81	2778
8	55,69,83,41,97,67,81,43,82,57,96,	z-9-octadecenoic acid	22.8	29.04	2784
9	57,71,85,43,99,113,41,55,77,94,107,	eicosane	4.1	29.51	2795
10	57,71,85,43,99,113,41,55,69,83,97,110,	Heneicosane	2.1	30.10	2810
11	57,71,85,43,99,55,113,41,69,83,97,70,	Docosane	2.1	30.61	2823
12	57,71,149,70,167,43,104,41,112,113	Tricosane	2.3	31.39	2842
13	57,71,85,43,113,99,110,83,55,82,41,	Tetracosane	1.1	31.93	3155

[a]According to the % TIC and retention time from GC [Figure 2]; [b][m/e] values of base peak 1st stated, and other most prominent ions; [c]see identification of components;
[d]Total ion concentration in %; [e]retention time in minutes; [f]Retention Index determined with reference to homologous series of n-alkanes.

Table 4: Chemical composition of root essential oil of *Anogeissus leiocarpus*.

Peak No[a]	MS [Base peak+most abundant peaks][b]	Identified compound[c]	% TIC[d]	Retention time [mins][e]	Calculated RI[f]
1	57,43,41,55,56,70,69,67,44,81,82,68,	n-tridecane	0.4	12.94	1117
2	98,126,43,41,70,67,95,55,81,53,69,	1-(4-cycloocten-1-yl) ethanone	1.0	22.90	1991
3	73,60,55,41,43,57,69,71,85,87,	n-undecanoic acid	1.5	24.31	2355
4	57,71,85,43,41,69,99,56,70,	n-pentadecane	0.6	24.93	2370
5	82,67,81,83,96,68,95,55,41,56,97,	1,14-tetradecanediol	0.6	25.90	2393
6	73,60,43,57,55,41,87,69,71,85,83,	n-pentadecanoic acid	1.9	26.32	2403
7	83,97,55,57,69,43,41,56,70,71,111,	bornyl angelate	1.1	26.71	2413
8	74,87,43,55,75,41,69,57,101,227,	methylhexadecanoate	14.2	27.63	2748
9	73,60,43,57,55,41,71,69,83,85,87,256	n-hexadecanoic acid	17.6	27.88	2754
10	67,81,95,82,55,68,79,96,41,109,69,54,	methyl linoleate	16.2	28.76	2776
11	55,69,83,74,97,96,84,98,41,87,43,67,81	methyl-7E-7-octadecenoate	18.1	28.81	2778
12	55,69,83,41,97,67,81,43,82,84,96,57,70,	z-9-octadecenoic acid	15.7	29.04	2784
13	57,71,85,43,99,113,55,41,69,83,70,97	n-docosane	0.7	30.61	2823
14	57,71,70,149,104,41,43,113,167,112	di-n-octyl phthalate	1.4	31.39	2842

[a]According to the % TIC and retention time from GC [Figure 3]; [b][m/e] values of base peak 1st stated, and other most prominent ions; [c]see identification of components; [d]Total ion concentration in %; [e]retention time in minutes; [f]Retention Index determined with reference to homologous series of n-alkanes.

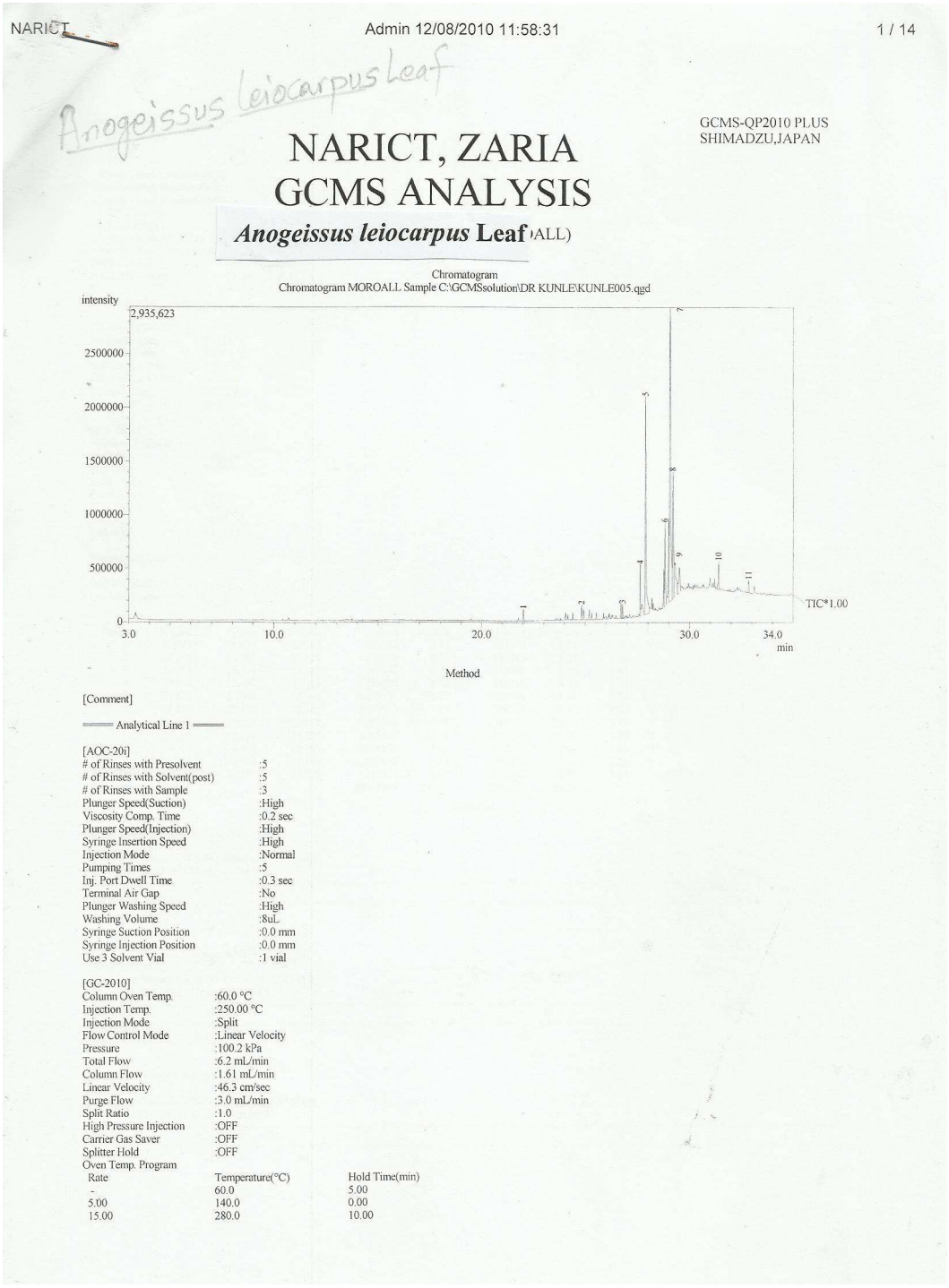

Figure 1: Gas chromatogram of the leaf essential oil of *Anogeisus leiocarpus* using GC-2010[AOC-20i] chromatograph. [Column 60 ^{0}C, injection temp. 250 ^{0}C, split injection mode at 100.2kPa; flow 1.61ml/min and total flow 6.2ml/min; 1.0 split ratio; oven temperature programming is 60 ^{0}C (for 5ins), m and at the rate of 50/min till 140 ^{0}C, 150/min till 280 ^{0}C. [For peak numbers refer to Table 2].

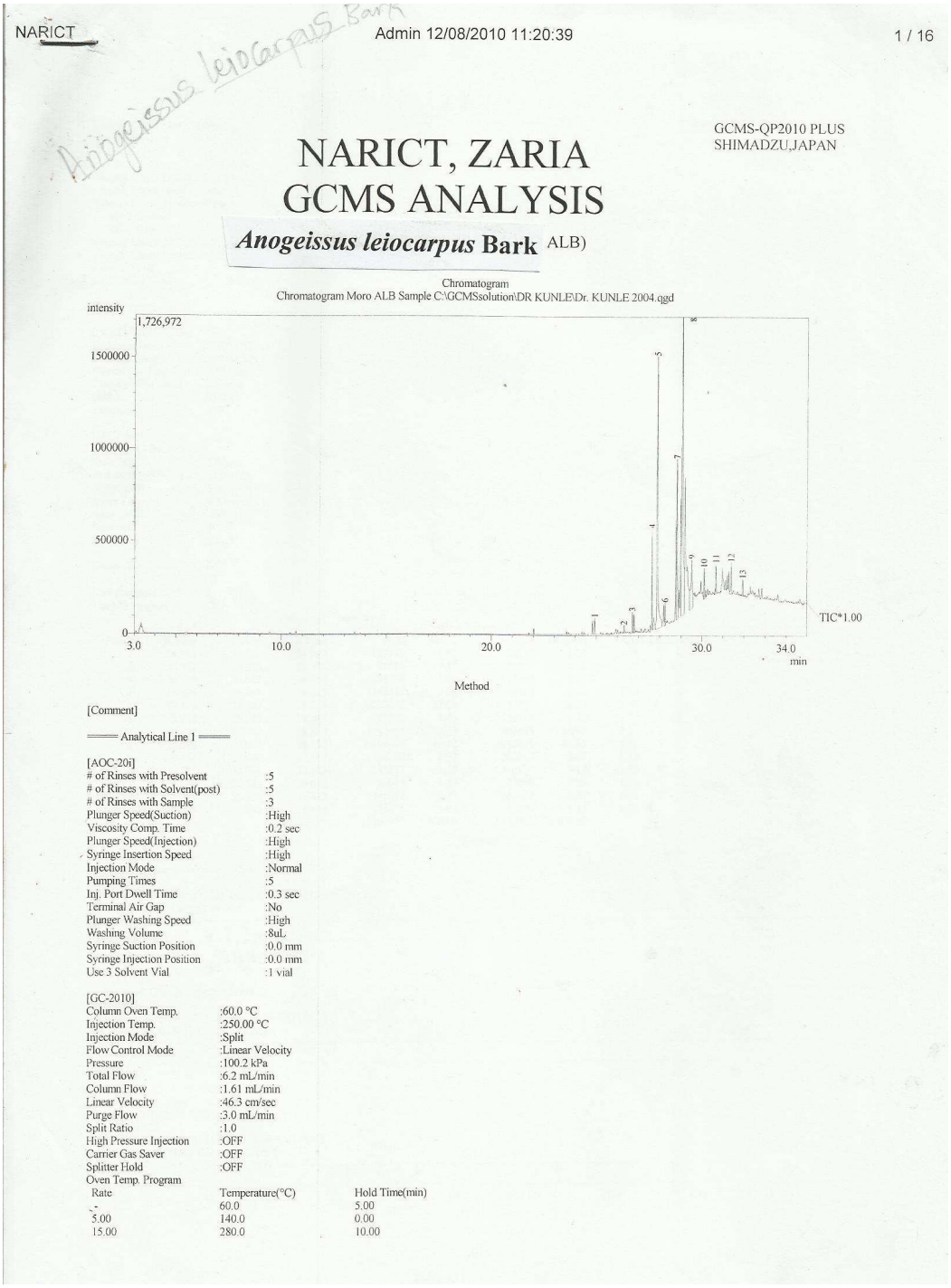

Figure 2: Gas chromatogram of the stem bark essential oil of *Anogeisus leiocarpus* using GC-2010[AOC-20i] chromatograph. [Column 60 ^{0}C, injection temp. 250 ^{0}C, split injection mode at 100.2kPa; flow 1.61ml/min and total flow 6.2ml/min; 1.0 split ratio; oven temperature programming is 60 ^{0}C (for 5ins), m and at the rate of 50/min till 140 ^{0}C, 150/min till 280 ^{0}C. [For peak numbers refer to Table 3].

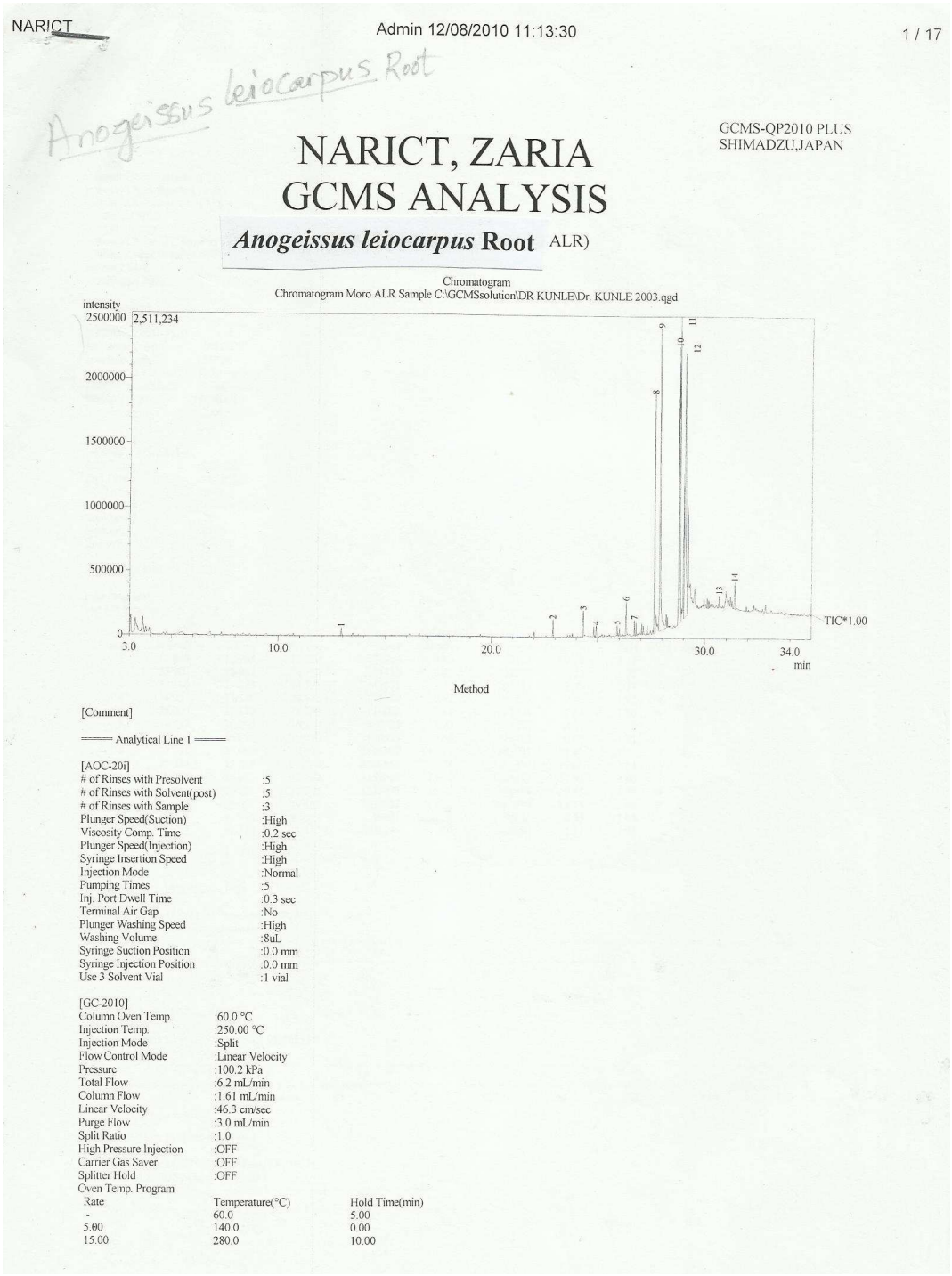

Figure 3: Gas chromatogram of the root essential oil of *Anogeisus leiocarpus* using GC-2010[AOC-20i] chromatograph. [Column 60 ^{0}C, injection temp. 250 ^{0}C, split injection mode at 100.2kPa; flow 1.61ml/min and total flow 6.2ml/min; 1.0 split ratio; oven temperature programming is 60 ^{0}C (for 5ins), m and at the rate of 50/min till 140 ^{0}C, 150/min till 280 ^{0}C. [For peak numbers refer to Table 4].

DISCUSSION

From Nigerian sample of *Anogeissus leiocarpus* DC. Guill. & Perr. (Combretaceae) were obtained by hydro-distillation three oils from its leaf, stem bark and root. They were analyzed for their constituents by means of gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC-MS). 88% of the leaf oil comprised of eleven compounds, the most abundant was z-9-octadecenoic acid (29.0%). Other important compounds in it were n-hexadecanoic acid (21.4%), n-octadecanoic acid (12.7%), methyl-7E-7-octadecenoate (8.5%), and methylhexadecanoate (5.4%). Six other compounds are reported in Table 2. Thirteen compounds made up 80% of the stem bark oil, the significant compounds being z-9-octadecenoic acid (22.8%), n-hexadecanoic acid (20.8%), methyl-9z-octadecenoate (12.4%), methylhexadecanoate (7.8%) and eicosane (4.1%). Eight other compounds in the stem bark oil are presented in Table 3. Fourteen compounds made up 91% of the root oil and is dominated by methyl-7E-7-octadecenoate (18.1%), n-hexadecanoic acid (17.6%), methyl linoleate (16.2%), z-9-octadecenoic acid (15.7%) and methylhexadecanoate (14.2). Nine other compounds in the root essential oil are shown in Table 4.

The three oils showed the presence of the following classes of compounds in the leaf, stem and root respectively: fatty acids [65.8%, 43.6%, 36.7%]; esters [17.9%, 20.2%, 49.9%]; hydrocarbons [3.2%, 15.7%, 1.7%]. The leaf and root oils contained terpenoids [1.1%, 1.1% respectively]. The leaf and stem bark oils were dominated by acids [65.8% and 43.6% respectively], followed by esters (17.9% and 20.2% respectively). On the other hand, root oil contained more esters (49.9%) than acids (36.7%). The presence of a sugar, (dl-arabinose), was unique to the stem oil. Homologous series of hydrocarbons were also observed in the stem oil. Two compounds, methylhexadecanoate and hexadecanoic acid, were common to the three oils. These can be used as chemo-taxonomic markers for this plant species. Further studies on other species of *Anogeissus* can confirm the taxonomic importance of these two compounds for the genus. These results represent the first time in literature for the volatile oil composition of 'axlewood' plant '*Anogeissus leiocarpus*'.

Conclusion

Essential oils of *Anogeissus leiocarpus* have acids dominate the leaf and stem bark oils (65.8% and 43.6% respectively), followed by esters (17.9% and 20.2% respectively). Root oil rather has esters as most abundant (49.9%), followed by acids (36.7%). Notable is the unique presence of sugar (dl-arabinose) in stem oil. Methylhexadecanoate and hexadecanoic acid are common to the three oils, hence are chemo-taxonomic compounds. They can serve as taxonomic compounds characteristic of this specie. Further studies on other species of *Anogeissus* will confirm these compounds for taxonomic identification of the genus. The compositions of the three oils are generally interesting, and may also contribute to the wide ethno-medicinal uses and social applications of the axlewood plant. Our present contribution on essential oils of *Anogeissus leiocarpus* represents the first of its kind in literature.

ACKNOWLEDGEMENTS

We acknowledge the assistance of Orodiran Seyi in the collection and hydro-distillations of plant samples.

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