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Composition and Antioxidant Activities of Leaf and Root Volatile Oils of *Morinda lucida*

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Morinda lucida (L.) Benth. (Rubiacae) is used in traditional medicine in many West African countries for the treatment of various human diseases. The leaves and roots of this plant were subjected to hydro-distillation to obtain volatile oils which were analyzed by high resolution GC/MS. Fifty compounds were identified in the leaf volatile oil and the major compounds were α–terpinene (17.8%) and β–bisabolene (16.3%). In the root oil, 18 compounds were identified, the major constituents being 3-fluoro-*p*-anidine (51.8%) and hexadecanoic acid (12.0%). Antioxidant activities of the oils were examined using the DPPH, ABTS, reducing power and lipid peroxidation assays. All assays were concentration dependent with varying antioxidant potentials. The antioxidant activity of the root volatile oil of *M. lucida* was similar to that of the standard drugs used.

Keywords: Antioxidant activities, β–bisabolene, 3-fluoro-*p*-anidine, *Morinda lucida,* α–terpinene, volatile oil.

Morinda lucida (L.) Benth. (Rubiacae), widely known as Brimstone tree, is called *Oruwo* in south-west Nigeria. *Morinda* is comprised of about 80 species and occurs throughout the tropical regions [1]. In Africa, the five common species are *M . lucida, M. citrifolia, M. geminata, M. longiflora* and *M . morindodide* [2]. *M. lucida* is a medium-sized tree about 15 m tall that is widely used as a medicinal plant in West Africa, especially in Nigeria, Côte d'Ivoire, Ghana and DR Congo [3]. The leaf decoction is used for treatment of fevers and diabetes [4-5]. In West Africa, the plant is used traditionally for the treatment of arthritis, rheumatism, leprosy, pulmonary troubles and as a vermifuge [1]. The ethanol extracts of both leaf and root were reported as strong stimulants and used for cancer treatment [6]. Moreover, the leaf and root of *M. lucida* have a relaxant effect on isolated uterine vascular smooth muscle of both non-pregnant and pregnant mice [7]. The leaf extract has also been shown to have antibacterial and antimalarial properties [8]. The extract of *M. lucida* was also documented to possess antispermatogenic, antihypertensive and antifungal activities [9-10].

The stem bark essential oil of *M. lucida* has strong antioxidant activity in the β-carotene assay [11]. In the last two decades much emphasis has been placed on natural antioxidants to reduce free radicals generated in the body, which play a vital role in damaging various cellular macromolecules. The generation of free radicals, such as superoxide (O_2•−), hydroxyl (OH•), peroxyl (RO_2•), hydroperoxyl (HO_2•) alkoxyl (RO•), nitric oxide (NO•) and lipid peroxyl (LOO•) beyond the antioxidant capacity of a biological system gives rise to oxidative stress. Oxidative stress has been implicated in the pathogenesis of a variety of human diseases, such as diabetes mellitus, hypertension, inflammation, cancer, atherosclerosis and AIDS [12]. Therefore, both the naturally occurring nutritive and non-nutritive antioxidants have become a major area of scientific research. Essential oils have earlier been considered as natural antioxidants and proposed as potential substitutes for synthetic antioxidants due to the volatile and lipophilic nature of the oils, and considering the fact that essential oils have been reported to penetrate tissue 100 times faster than water and 10,000 times faster than salts [10,13]. However, information is scanty on the antioxidant activity of the leaf and root essential oils of *M. lucida*, as well as its volatile compounds. Hence, this present study was conducted to investigate the volatile compounds and the antioxidant activities of the essential oils of *M. lucida* hoping to give credence to its medicinal values.

The yields of the leaf and root oils were 0.17% and 0.53%, respectively. The compounds of the volatile oils identified from the leaves and root of *M. lucida* are listed in Table 1. GC/MS analysis identified 50 out of 52 compounds in the leaf oil, which accounted for 91.4% of the total oil. Of these, 21 compounds are monoterpenoids (46.2% of the total oil composition), and 24 sesquiterpenoids, (42.1% of the total oil). The major monoterpenes are α–terpinene (17.9%) and α–pinene (10.2%), while β –bisabolene

Table 1: Composition of leaf and root essential oil of *M. lucida*.

S/N	RI	Compound[a]	Composition (%)	
			Leaf	Root
1	916	α-Pinene	10.2	-
2	922	Camphene	0.8	-
3	939	β- Myrcene	0.3	-
4	939	β-Phellandrene	0.2	-
5	957	α-Phellandrane	0.3	-
6	963	α-Terpinene	17.9	-
7	971	Limonene	0.2	-
8	980	*trans*-Ocimene	0.2	-
9	1004	Disulfide-2-propenyl	0.5	-
10	1015	Terpinolene	1.4	-
11	1033	Linalool	3.0	-
12	1076	Terpinene 1-o1	0.4	-
13	1089	β-Terpineol	0.5	-
14	1094	NI	-	2.6
15	1101	NI	-	1.3
16	1105	Nonanal	0.6	-
17	1130	Menthol	0.1	-
18	1136	Camphor	-	1.5
19	1152	Terpinen-4-o1	1.8	-
20	1161	*Z*-2-Nonenal	-	1.8
21	1165	Borneol	0.2	3.4
22	1182	β-Fenchyl alcohol	7.9	1.5
23	1192	Iso-terpinolene	0.7	-
24	1202	Decanal	0.7	-
25	1210	2-Hydroxy-methyl benzoic acid	-	3.4
26	1215	Citral	0.1	-
27	1256	1-Chloro-1-ethylcyclo-propane	0.2	-
28	1260	NI	-	1.6
29	1274	Tridecane	0.2	-
30	1275	Carvacrol - 2-methyl-5-(1-methylethyl) phenol	-	3.8
31	1277	NI	0.2	-
32	1281	Tetradecane	-	1.5
33	1345	Neryl propionate	0.1	-
34	1375	Geranyl propionate	0.1	-
35	1385	β-Elemene	0.6	-
36	1405	Tridecanal	0.1	-
37	1410	α-Bergamotene	0.5	-
38	1419	β-Caryophyllene	5.3	1.0
39	1423	Neryl acetone	-	0.7
40	1425	3-Fluoro-*p*-anidine	-	41.8
41	1427	*trans*-α-Bergamotene	14.0	-
42	1432	Zingiberene	0.1	-
43	1445	Ui	0.4	-
44	1457	β-Seliene	0.2	-
45	1461	Germacrene-D	0.7	-
46	1463	*trans*-B-Farnesene	0.3	-
48	1468	Cadinene	0.7	-
49	1472	(*E*,*Z*) Farnesene	0.2	-
50	1474	Pentadecane	0.1	-
50	1477	*trans*-α-Bisabolene	0.6	-
51	1482	β-Bisabolene	16.3	-
52	1487	γ-Bisabolene	0.4	-
53	1493	δ-Cadinene	0.1	-
54	1507	α-Gurjunene	0.3	-
55	1513	*cis*-α-Bisabolene	0.3	-
56	1536	Germacrene D	0.8	-
57	1575	Dodecanoic acid	-	8.5
58	1582	NI	-	1.4
59	1611	Tetradecanal	-	5.4
60	1614	Viridiflorol	0.1	-
61	1653	β-Tumerone	0.2	-
62	1665	α-Bisabolol	0.2	-
63	1768	Tetradecanoic acid	-	2.5
64	1831	2-Dodecanone	0.1	-
65	1845	1,2-Benzenedicarboxylic acid	-	0.9
67	1860	NI	-	2.6
68	1925	9-Octadecenoic acid	-	1.2
69	1941	Hexadecanoic acid	-	5.0
70	2111	9,17-Octadecadienal	-	3.9
Identified compounds (%)			91.4	97.3

RI:Retention indices relative to C8-C22 *n*-alkanes on HB-5 column.
NI: Not identified.
[a] Compounds listed in order of elution from HB-5 column and identified using retention index and mass spectra.

Table 2: Antioxidant activities of the volatile oils of Morinda lucida. IC_{50} (mg/mL)

Activity	Leaf Oil	Root Oil	Vit C	Rutin	BTH
DPPH	7.82±0.03	7.82±0.04	1.50±0.01	0.54±0.04	-
ABTS	6.20±0.02	8.82±0.01	0.03±0.01	0.02±0.01	-
Lipid peroxidation	0.08±0.03	0.02±0.04	0.17±0.03		0.12±0.01

Values are mean ±SD, n = 3.

(16.3%), *trans* α–bergamotene (14.0%) and β-caryophyllene (5.33%) are the predominant sesquiterpenes. Other important components of the leaf oil are β –fenchyl alcohol (8.0%), linalool (3.0%), terpinen-4-ol (1.8%), terpinolene (1.4%), germarcrene D (0.8%), cadinene (0.7%) and β–elemene (0.6%).

The root essential oil consisted of 22 compounds, of which 17 were identified. The major monoterpenoids are carvacrol (3.8%) and borneol (3.4%), while β-caryophyllene (1.0%) and neryl acetone (0.7%) are the major sesquiterpenoids identified. The predominant components in the root oil were non-terpenoids and fatty acids, accounting for 75.9% of the total oil; these include 3-fluoro-*p*-anidine (41.8%), hexadecanoic acid (5.0%), dodecanoic acid (8.5%), 2-hydroxy-methyl benzoic acid (3.4%), tetradecanoic acid (2.5%), 9-octadecanoic acid (1.2%) and 1,2-benzedicarboxylic acid (0.9%). Aliphatic hydrocarbons identified in the root oil were tetradecanal (5.4%), 9, 17-octadecadienal (3.9%) and tetradecane (1.5%). The high quantity of 3-fluoro-*p*-anidine observed in the root oil is noteworthy as the compound is a primary precusor in the synthesis of antitrypanasonomal and anti-inflammatory agents [14-15]. This dominant compound in the root oil of *M.lucida* has not been reported before as a component of the plant.

Various concentrations of the oils were investigated for their DPPH, ABTS and lipid peroxidation radical scavenging activities. The results obtained from ABTS and DPPH assays indicate some antioxidant activity of both the leaf and root oils of *M .lucida.* The percentage inhibition of the ABTS and DPPH radicals were concentrations dependent, as shown in Figures 1 and 2, respectively. The IC_{50} values for the ABTS radical scavenging activity of the leaf and root essential oils of *M.lucida* were 6.20±0.02 and 8.82±0.01 mg/mL respectively, whereas the concentrations required for inhibiting the DPPH radical by 50% by the oils were 7.82±0.03 and 7.82±0.04 mg/mL, respectively.

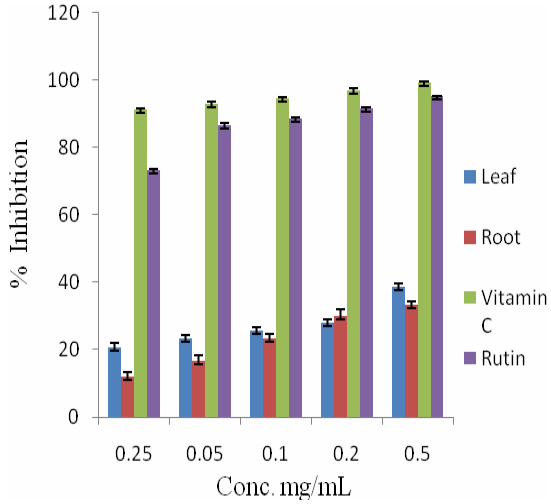

Figure 1: Antioxidant effect of the volatile oils from leaves and root of *M. lucida* by using ABTS.

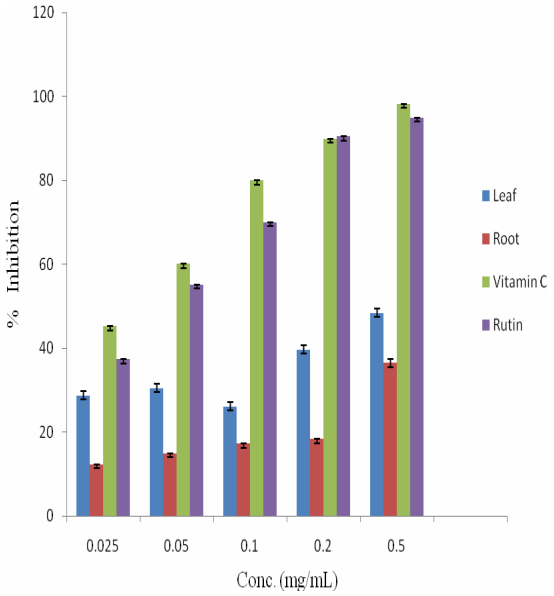

Figure 2: Antioxidant effect of the volatile oils from leaves and root of *M. lucida* by using DPPH.

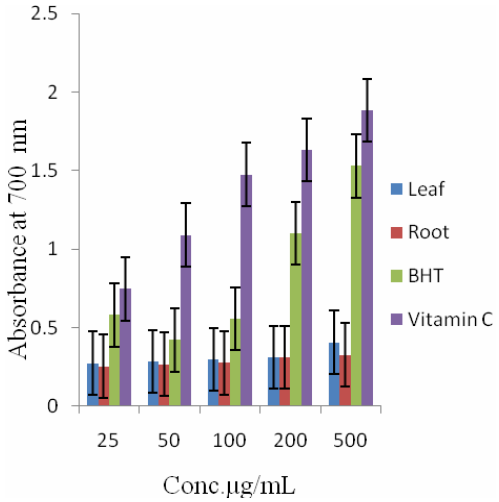

Figure 3: Antioxidant effect of the volatile oils from leaves and root of *M. lucida* by using reducing power free radical.

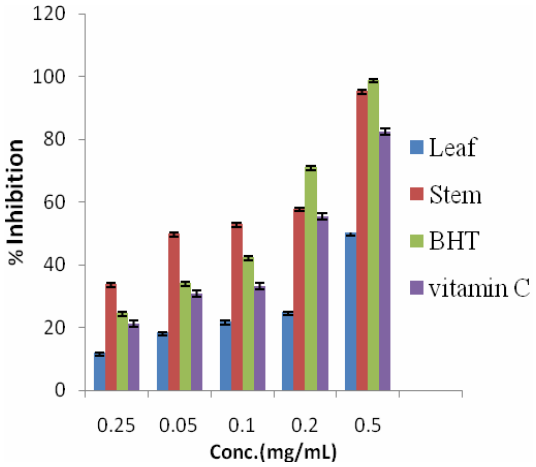

Figure 4: Antioxidant effect of the volatile oils from leaves and root of *M. lucida* by using lipid peroxidation.

The radical scavenging capacities of the oils were lower than the standard drugs at all concentrations. The antioxidant activity of the leaf oil was higher in the ABTS and DPPH assays than of the root oil at all concentrations (Figures 1 and 2). However, it is interesting to note that the oils possess lipid peroxidation inhibitory activity.

The leaf and root oils of *M. lucida* inhibited lipid peroxidation induced by ferrous sulfate in egg-yolk homogenates in a dose-dependent manner with IC_{50} values 0.8±0.03 and 0.2±0.04 mg/mL, respectively. While the IC_{50} values of the standard drugs were 0.12±0.01 and 0.17±0.03 mg/mL for BHT and Vitamin C, respectively.

The scavenging of H_2O_2 by the oils may be attributed to their phenolics, which donate electrons to H_2O_2, thus reducing it to water. The oils were capable of scavenging hydrogen peroxide in a concentration dependent manner. Moreover, the two phenolic compounds carvacrol (3.8%) and 2-hydroxy methyl benzoic acid (3.4%), and the presence of oxygenated terpenes, such as borneol (3.4%), camphor (1.5%) and β-fenchyl alcohol (1.5%) may have enhanced the H_2O_2 scavenging potential of the root oil in the lipid peroxidation assay. Gianni *et al* [16], documented the relationship between the chemical composition and biological activities of essential oils. The chemical profile of the leaf oil was comprised mainly of monoterpenes, such as α-terpinene (17.8%) and α–pinene (10.2%), as well as sesquiterpenes, such as β–bisabolene (16.3%), α–bergamotene (14.0%) and β–caryophyllene; these have been reported in several studies as strong antioxidant agents [17]. Beta-bisabolene has also been documented as an antioxidant compound in the essential oil of *Teucrium marum* [18], and β-caryophyllene was reported as the most scavenging compound in *M. pergrinum* essential oil [19].

Reducing power has been used as an important measure of the antioxidant capability of medicinal herbs [20]. Reductive ability was determined by monitoring the Fe^{3+} to Fe^{2+} transformation in the presence of both oils; increase in absorbance of the reaction mixture indicated reducing power. As shown in Figure 3, both oils demonstrated moderate concentration-dependent reducing power, ranked in a similar order to DPPH and ABTS radical scavenging activity. The reducing powers of the leaf and root oils were similar. However, BHT and vitamin C (standard drugs) reducing powers were superior to both oils.

The antioxidant activity of essential oils may be improved by other components even in small amounts, indicating possible synergistic interaction between the constituents [21]. In addition the main component, 3-fluoro-*p*-anisidine (41.8%), identified in the root oil for the first time could have reacted with peroxyl radicals through various mechanisms suggested by Foti *et al*. [22]. Five compounds were unidentified in the root oil, three of which are monoterpenoids, while two are sesquiterpenoids. Moreover, the root oil scavenging power compares well with that of BHT and vitamin C (Figure 4). This indicates the potency of the plant in preventing the joining of nucleosides in the DNA, and possible breakage leading to carcinogenesis and cytotoxicity [23].

Conclusion: This study shows that besides the traditional uses of the plant extract, the volatile oils extracted from *M. lucida* leaves and root have good antioxidant potential and can be used to produce natural antioxidants as well as natural food preservatives.

Experimental

Chemicals: Potassium persulfate (PPS), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2,2[1]-azinobis-(3-ethylbenzothiazolin-6-sulfonic acid) diammonium salt (ABTS) and 2,6-di-tert-butyl-4-methylphenol (butylated hydroxytoluene, BTH) were procured from Sigma -Aldrich (St Louis, USA). Methanol was obtained from Fluka Chemicals (Buchs, Switzerland). All chemicals used were analytical grades.

Plant material: The plant materials were collected at the Forest Research Institute of Nigeria (FRIN), Ibadan, Nigeria and authenticated at the Botany Department, University of Lagos by Mr T. K. Odewo; a voucher specimen (LUH2011) was deposited in the Herbarium of the Department of Botany.

Isolation of volatile oil: The leaf and root samples were air dried for 5 – 8 days, then powdered, and samples of each hydrodistilled for 3 h, using a modified Clevenger-type apparatus [24]. The volatile oils obtained were extracted into *n*-hexane and dried over sodium sulfate. The volatile oils were preserved in sealed vials at 4°C until further analysis. The yields of the leaf and root oils were 0.17% and 0.53%, respectively

Gas chromatography/Mass spectrometry (GC/MS): The GC/MS analyses of the oils were conducted on a Hewlett-Packard HP 5973 mass spectrometer interfaced with a HP 6890 gas chromatograph equipped with a HB-5 column. The following column and temperature conditions were used; initial temperature70°C, equilibration time 3.00min, ramp 4°C/min, final temperature 240°C; inlet: splitless, initial temperature 220°C, pressure 8.27 psi, purge flow 30 mL/min, purge time 0.20 min, helium gas; column: capillary, 30 m x 0.25 mm i.d; 0.25 μm, film thickness 0.7 mL/min, average velocity 32 cm/sec; MS: The ion source was set at 240°C and electron ionization at 70 eV. Helium flow rate was at 1 mL/min. The scanning range was 50 to 500 amu.

Identification of compounds: The volatile oil compounds were identified by matching their MS data with those of authentic standards held in the computer library (Wiley 275, New York) and by comparison of the calculated retention indices relative to C8-C22 *n*-alkanes injected under the same conditions as the samples. The percentage composition was calculated from summation of the peak areas of the total oil composition [25].

Antioxidant assays: The antioxidant activities were determined by the ABTS free radical decolorization assay, DPPH radical-scavenging test, reducing power and lipid peroxidation scavenging activities.

DPPH assay: The DPPH test of the essential oils was carried out as previously described [26]. The IC_{50} value was calculated by plotting inhibition percentages against concentrations of the oils.

ABTS assay: In the ABTS free radical assay, the method of Witayapan *et al.* was adopted, with minor modification (ABTS stock solution diluted in methanol) [27]. All measurements were carried out in triplicate. The percentage inhibition of ABTS radical by the oils was calculated as described in the DPPH assay.

Lipid peroxidation assay: The thiobarbituric acid-reactive species (TBARS) assay was used to measure the lipid peroxidation, as described previously [28]. All measurements were made in triplicate and mean values were calculated.

Statistics: Data were calculated as means ±SD. Pearson's correction analysis (SPSS15.0 for windows, SPSS Inc) to test for the significance of the relationship between the concentration and percentage inhibition.

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