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Essential Oil of *Phyllanthus reticulatus* Poiret from Nigeria*

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Abstract

The chemical composition of the essential oil obtained by hydrodistillation from the leaves of *Phyllanthus reticulatus* Poiret (Euphorbiaceae) growing in Nigeria has been studied. The constituents of the oil were analyzed by means of gas chromatography (GC-FID) and gas chromatography coupled with mass spectrometry (GC-MS). Monoterpenes (64.9%) were the dominant class of compounds, followed by sesquiterpenes (23.0%). The major constituents were *α*-pinene (6.0%), sabinene (7.6%), *β*-pinene (18.1%), linalool (6.9%) and camphor (7.7%), among the monoterpenes, and *β*-caryophyllene (11.9%) and germacrene D (8.6%) among the sesquiterpenes. This is the first report on the volatile constituents of *Phyllanthus reticulatus*.

Keywords

***Phyllanthus reticulatus*, Essential Oil Composition, Monoterpenes, Sesquiterpenes**

1. Introduction

Phyllanthus reticulatus Poiret., (Family Euphorbiaceae) is a many branched deciduous shrub or small tree sometimes partially scrambling, usually 1 - 5 m high. *P. reticulatus* usually has a distinct smell that is emitted by the minute flowers when they open towards the early evening. The bark is light reddish-brown or grey-brown with

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hairy stems when young, which become smooth with age. The leaves alternate along slender branches. They are up to 25 cm long and appear as leaflets of large pinnate leaves. The leaves are thinly textured, usually hairless. It flowers from September to October, but the flowering season can extend from July onwards. *P. reticulatus* has very small, roundish berry like fruits that are green at first, turning purple-black, 4 - 6 mm in diameter [1]. Extracts of the plant is known to possess both analgesic and anti-inflammatory activities [2]-[4]. Aqueous extract of *P. reticulatus* can be utilized for prevention of atherosclerosis in hypercholesterolemic patients [5]. There are reports which describe the antiviral [6], antibacterial [7], hepatoprotective 8], antioxidant [9], potential RNase H inhibition and protection against the viral cytopathic effects of HIV-1 [10], antidiabetic [11] and hypoglycemic [12] activities.

Some biologically active compound such as 2-*α*-hydroxyfriedel-4(23)-en-3-one and other triterpenoids [13], purine, 3-(3-methylbut-2-en-1-yl)isoguanine and cleistanthane-type diterpenoid glucoside, 19-hydroxyspruceanol 19-*O*-*β*-D-glucopyranoside [14], (5R, 6R)-4,6-Dimethoxycarbonyl-5-[2',3',4'-trihydroxy-6'-(methoxycarbonyl) phenyl]-5,6-dihydro-2H-pyran-2-one, 3,4,3'-tri-*O*-methylellagic acid and methyl gallate [15], reticulatusides A and B [16], cytotoxic scopoletin [17] and flavonoid glycosides [18] [19] have been isolated from this plant. The isolation of lupeol, stigmasterol and lupeol acetate from the plant have been reported [20]. Regarding the volatile constituents, there appears to be no published work.

The objective of the present work was to examine the volatile constituents of this plant for future exploration. Our finding into the volatile oils of some poorly studied Nigerian flora was recently published [21].

2. Materials and Methods

2.1. Plant Sample

Leaves of *P. reticulatus* were collected from a location in Ibefun, Odogbolu, Ogun State, Nigeria, on March, 2012. Botanical identification was performed by Messrs Ugbogu O.A and Shasanya, O.S., at the Herbarium Headquarters, Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria, where voucher specimen (FHI 109586) has been deposited for future reference.

2.2. Extraction of Essential Oil

Aliquots (400 g) of the air-dried and pulverized plant sample were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus in accordance with the British Pharmacopoeia specification [22] to produce a pale yellow essential oil.

2.3. Analysis of the Oil

GC analysis was accomplished with a HP-5890 Series II instrument equipped with a HPWax and HP-5 capillary columns (both 30 m × 0.25 mm, 0.25 μm film thickness), working with the following temperature program: 60˚C for 10 min, rising at 5˚C/min to 220˚C. The injector and detector temperatures were maintained at 250˚C; carrier gas nitrogen (2 mL/min); detector dual, FID; split ratio 1:30. The volume injected was 0.5 μL. The relative proportions of the oil constituents were percentages obtained by FID peak-area normalization without the use of response factor.

GC-EIMS analysis was performed with a Varian CP-3800 gas-chromatograph equipped with a HP-5 capillary column (30 m × 0.25 mm; film thickness 0.25 μm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions: injector and transfer line temperature 220˚C and 240˚C respectively; oven temperature programmed from 60˚C - 240˚C at 3˚C/min; carrier gas was helium at a flow rate of 1 mL/min; injection of 0.2 μL (10% hexane solution); split ratio 1:30. Mass spectra were recorded at 70 eV. The acquisition mass range was 30 - 300 m/z at a scan rate of 1 scan/sec.

2.4. Identification of the Constituents

Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear retention indices (LRI) relative to the series of n-hydrocarbons, and on computer matching against commercially available spectral [23]-[25]. Further identifications were also made possible by the use of homemade library mass spectra built up from pure substances and components of known oils and MS literature data. Moreover, the molecular weights of all the identified substances were confirmed by GC-CIMS, using

MeOH as CI ionizing gas.

3. Results and Discussion

P. reticulatus yielded low content of essential oil 0.12% (v/w) on a dry weight basis. **Table 1** shows the identities of 84 compounds identified in the oil of *P. reticulatus*, accounting for 99.7% of the total oil contents. **Figure 1** depicts the GC chromatogram of the essential oil. The classes of compounds identified in the oil were monoterpene hydrocarbons (42.0%), oxygenated monoterpenoids (23.5%), sesquiterpene hydrocarbons (29.4%), oxygenated sesquiterpenoids (3.6%) and non-terpene derivatives (1.2%). The major oil constituents were *α*-pinene (6.0%), sabinene (7.6%), *β*-pinene (18.1%), linalool (6.9%) and camphor (7.7%), among the monoterpenes; and *β*-caryophyllene (11.9%) and germacrene D (8.6%) among the sesquiterpenes. This may represent the first analysis of the oil of this species.

Regardless of *Phyllanthus* being large family, with about 1000 species, the essential oils of *P. reticulatus* has not been investigated. However, the volatile constituents of few species grown have been reported. Phytol (21.5%), *β*-citronellol (17.7%), *trans*-geraniol (13.5%), *cis*-3-hexenol (12.6%) and 1-hexanol (11.3%) were the major constituents of *Phyllanthus salviaefolius* H.B.K. [26]. However, linalool (36.4%) and phytol (13.0%) dominated the oil of *Phyllanthus amarus* Sch. and Thonn [27]. Volatile compounds have been isolated from *P. acidus* (L.) Skeels fruits fermented for 1, 3 and 6 months. Among the 46 compounds identified, acids and alcohols dominated the volatiles profile; acids particularly characterized the quantitative profile of the volatile compounds after 6 months of fermentation. Other significant changes were in the sesquiterpenes, with increments of *δ*- and *α*-cadinene after 3 months of fermentation, and *α*-cadinol and *τ*-muurolol after 6 months [28]. *Phyllanthus arenarius* Beille in Lecomte has *n*-hexadecanoic acid (14.0%), 1,2-benzene dicarboxylic acid, bis (2-methylpropyl) ester (12.7%) and di-*n*-octyl phthalate (10.3%) as its main compounds [29] while *Phyllanthus urinata* L., was rich in 3,3,5-trimethylcyclohexanone (17.2%) and *n*-hexadecanoic acid (12.4%). The abundant of 3,3,5-trimethylcyclohexanone (12.4%) and 3,7-dimethyl-1,6-octadien-3-ol (10.2%) was reported in the oil of *Phyllanthus niriru* L. [29]. The essential oil of *Phyllanthus emblica* L. contained high amounts of *β*-caryophyllene, *β*-bourbonene, 1-octen-3-ol, thymol, and methyleugenol [30]. Another investigation reported that *β*-bourbonene, heptadecanol, pentadecanone, thymol, *β*-caryophyllene, *β*-neoclovene, nerol and borneol were the major compounds were the main oil contents of the plant [31]. (*E*)-Isoelemicin (36.40%) was the main compounds of *Phyllanthus muellerianus* (Kuntze) Excel which also showed antimicrobial property [32].

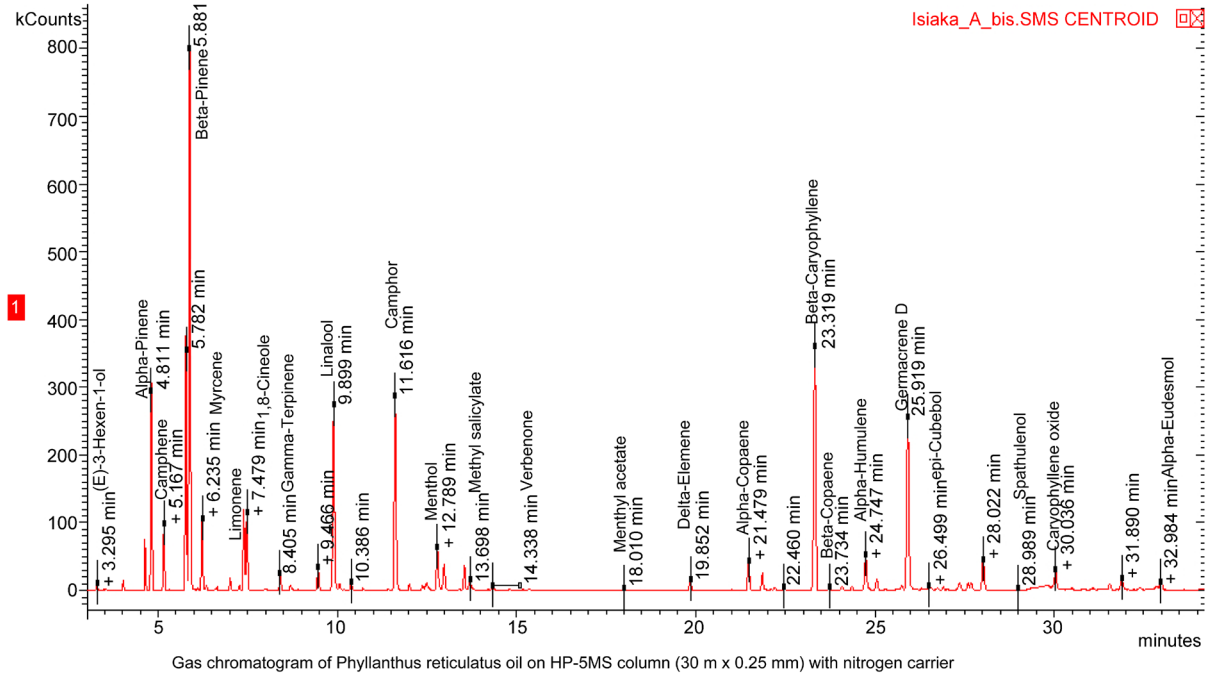

Figure 1. Gas chromatogram of *Phyllanthus reticulates* oil on HP-5MS column (30 m × 0.25 mm) with nitrogen carrier.

Table 1. Essential oil constituents of *Phyllanthus reticulates*.

Compounds[a]	Retention Times[b]	RI[c]	RI[d]	Percentage (%)
(*E*)-Hex-3-en-1-ol	3.29	854	850	0.4
Hexan-1-ol	3.48	872	863	Tr
n-Nonane	4.02	900	900	0.3
Tricyclene	4.55	928	921	Tr
α-Thujene	4.64	931	924	1.3
α-Pinene	4.81	940	932	6.0
Camphene	5.16	954	946	1.9
Thuja-2,4(10)-diene	5.29	958	953	Tr
Benzaldehyede	5.45	962	962	Tr
Sabinene	5.78	977	969	7.6
β-Pinene	5.88	980	974	18.1
Octan-3-one	6.11	988	979	Tr
Myrcene	6.23	992	988	2.4
Octan-3-ol	6.34	995	988	Tr
α-Phellandrene	6.64	1005	1002	Tr
α-Terpinenne	7.01	1018	1014	0.4
p-Cymene	7.26	1027	1020	0.2
Limonene	7.39	1031	1024	2.6
1,8-Cineole	7.48	1034	1026	2.7
(*Z*)-***β***-Ocimene	7.69	1041	1032	Tr
(Z)-Oct-3-en-1-ol	7.97	1047	1047	Tr
(*E*)-***β***-Ocimene	8.04	1051	1044	Tr
γ-Terpinene	8.41	1062	1054	0.6
cis-Sabinene hydrate	**8.70**	1070	1065	0.2
cis-Linalool oxide (furanoid)	8.89	1075	1067	Tr
Non-1-en-3-ol	9.16	1083	1088	Tr
Terpinolene	9.47	1089	1086	0.9
Linalool	9.90	1099	1095	6.9
n-Nonanal	10.07	1103	1100	0.2
Octen-1-ol acetate	10.39	1107	1110	0.3
cis-*p*-Menth-2-en-1-ol	10.72	1122	1118	Tr
trans-*p*-Menth-2-en-1-ol	11.43	1125	1136	Tr
Camphor	11.62	1144	1141	7.7
Menthone	12.01	1155	1158	0.3
Pinocarvone	12.37	1163	1160	0.2

Continued

Borneol	12.49	1166	1165	0.5
Menthol	12.79	1174	1167	1.9
Terpinen-4-ol	12.97	1178	1174	1.1
(*Z*)-Hex-3-enyl butanoate	13.41	1186	1184	Tr
α-Terpineol	13.54	1190	1186	1.0
Methyl salicylate	13.70	1192	1190	0.6
n-Decanal	14.21	1205	12.01	Tr
Verbenone	14.34	1208	1204	0.3
β-Cyclocitral	14.80	1218	1217	Tr
Nerol	15.18	1228	1227	Tr
3-Methyl-hex-3-en-1-yl butanoate	15.36	1236	1232	Tr
Piperitone	16.24	1253	1249	Tr
Menthyl acetate	18.01	1294	1294	0.1
δ-Elemene	19.85	1340	1335	0.5
α-Cubebene	20.36	1351	1345	Tr
Eugenol	20.74	1358	1356	Tr
α-Copaene	21.48	1376	1374	1.5
β-Bourbonene	21.58	1384	1387	1.0
β-Cubebene	22.11	1390	1387	Tr
β-Elemene	22.28	1392	1389	0.2
Cyperene	22.46	1398	1398	0.2
Isocaryophyllene	22.79	1405	1408	Tr
Dodecanal	22.97	1408	1408	Tr
β-Caryophyllene	23.32	1418	1417	11.9
β-Copaene	23.73	1429	1430	0.2
α-Guaiene	24.08	1439	1437	0.2
Aromadendrene	24.35	1441	1439	0.2
α-Humulene	24.75	1455	1452	1.8
allo-Aromadendrene	25.05	1461	1458	0.5
cis-Muurola-4(14),5-diene	25.29	1463	1465	Tr
γ-Muurolene	25.74	1477	1479	1.8
Germacrene D	25.92	1480	1484	8.6
(*E*)-***β***-Ionone	26.18	1485	1487	Tr
cis-***β***-Guaiene	26.31	1490	1492	Tr
epi-Cubebol	26.50	1494	1493	0.4

Continued

trans-***β***-Guaiene	26.75	1500	1502	Tr
α-Bulnesene	26.90	1505	1509	0.3
δ-Amorphene	27.02	1512	1511	Tr
Cubebol	27.35	1515	1514	0.5
δ-Cadinene	27.69	1524	1522	0.4
Germacrene B	28.83	1556	1559	0.1
Spathulenol	28.99	1576	1577	0.4
Caryophyllene oxide	30.04	1581	1582	1.2
Humulene epoxide II	31.07	1606	1608	Tr
1,10-di-*epi*-Cubenol	31.54	1614	1618	0.3
τ-cadinol	32.39	1641	1639	0.2
Cubenol	32.56	1647	1645	Tr
α-Cadinol	32.86	1652	1652	0.2
α-Eudesmol	32.98	1654	1652	0.4
		TOTAL		99.7
		Monoterpene hydrocarbons		42.0
		Oxygenated monoterpenes		23.5
		Sesquiterpene hydrocarbons		29.4
		Oxygenated sesquiterpenes		3.6
		Others		1.2

[a]Elution order on HP-5MS column; [b]Retention time in order with respect to the chromatogram (**Figure 1**); [c]Retention indices on HP-5 MS capillary column; [d]Literature retention indices (References 23 - 25); Tr, Trace amounts < 0.1%.

The main compounds of *P. salviaefolius* could not be identified in *P. reticulatus*. Except for linalool, the quantitatively significant compounds of *P. amarus* were conspicuously absent from *P. reticulatus*. Also, the low contents of δ-cadinene and α-cadinol, and the absence of the sesquiterpenoid compounds such as α-cadinene and τ-muurolol in this study makes the composition differ from that found in *P. acidus*. Also, the major compounds in the oils of *P. arenarius*, *P. urinate* and *P. niriru* were absent in *P. reticulatus*. In addition, the oil of *P. reticulatus* could be distinguished from those of *P. embelica* by its lack of thymol, methyleugenol, heptadecanol, pentadecanone and nerol. Notably, (*E*)-isoelemicin, the main compound of *P. muellerianus* was not identified in *P. reticulatus*.

4. Conclusion

The chemical constituents of essential oil obtained from *P. reticulatus* grown in Nigeria are being reported for the first time. In addition, a comparison of the chemical composition was made with the other known essential oils from *Phyllanthus* plants. It could be seen that the essential oils of *Phyllanthus* plants exhibit high chemical variability. Each species has its own compositional pattern different from other. The very high content of compounds identified in the oil of *P. reticulatus* may be an important chemical and economic characteristic of the oil sample.

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