Comparative analysis of leaf essential oil constituents of *Piliostigma thonningii* and *Piliostigma reticulatum*

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Hydro-distilled essential oils from leaves of *Piliostigma thonningii* and *P. reticulatum* (Caesalpiniaceae) were studied by combined gas chromatography and gas chromatography-mass spectrometry for the first time. Both qualitative and quantitative differences existed in the composition of the two oils. While *P. thonningii* oil is composed of sesquiterpenes and monoterpene hydrocarbons, monoterpenes and sesquiterpene hydrocarbons represented the chief class of terpenoids in *P. reticulatum.* Major constituents in *P. reticulatum* leaf oil were γ-muurolene (10.3%), α-pinene (9.4%), tricyclene (7.2%), δ-cadinene (5.6%), α-terpineol (5.3%) and β-caryophyllene (4.2%). However, the sesquiterpene-rich *P. thonningii* oil was characterized by β-myrcene (13.3 %), limonene (8.6%), α-pinene (7.6%), β-caryophyllene (7.1%), γ-muurolene (6.1%), caryophyllene oxide (5.2%) and spathulenol (4.0%).

Key words: Essential oil, *Piliostigma thonningii*, *Piliostigma reticulatum*

INTRODUCTION

Piliostigma thonningii (Schum.) Milne-Rech (Caesalpiniaceae) is a small tree of the savannah, often of crooked growth, with dark brown to black fissured bark. It has large two-lobed simple leaves and without thorns or spines.[1] It attains 5-10 m height, and sometimes found as a climber. This species is very similar to *P. reticulatum* (DC.) Hochst except that the leaves are less deeply slit, slightly larger, having a ferrugineous pubescence underneath, which is absent in *P. reticulatum***,** lobes are wide open while they are closed in *P. reticulatum*,[1] Pods look similar except that in *P. thonningii* there is a compact dark ferrugineous pubescence on the epidermis which is absent in *P. reticulatum***.**

In Nigerian traditional medicine, *P. thonningii* is used in the treatment of dermatosis and malaria.[2] The medicinal uses are similar to those of *P. reticulatum*, while its leaves as well as those of *P. reticulatum* are browsed by cattle during the dry season.[1] The stem bark of this plant has been studied for antibacterial and antioxidant properties.[3] Furthermore, the anti-inflammatory and antibacterial principle, piliostigmin and other flavonoid derivatives have been isolated from both plants.[4,5] Leaves of *P. reticulatum* are used for flavouring drinks while tea made from the leaves is a remedy for colds. Also, leaves and bark are employed for their haemostatic and antiseptic properties, cure for ulcers, boils, wounds and syphilitic cancer.[1] Other medical uses are against coughs, bronchitis, malaria, hepato-biliary ailments, hydropsy, sterility, rachitis and kwashiorkor.

Presently, there are no literature reports on the essential oil constituents of these two plants. However, in line with our investigations into the aromatic flora of Nigeria to create a database, we compared the constituents of the essential oils of *P. thonningii* and *P. reticulatum* leaves growing in Nigeria, and herein report our findings.

MATERIALS AND METHODS

Plant Material and Extraction of Oil

Fresh leaves of *P. thonningii* were harvested from trees growing at Awo village/Iwo Road junction, Osun State, Nigeria, while those of *P. reticulatum* were collected at Ikire in Osun State. Authentication was done by Mr. A T Oladele of the Faculty of Pharmacy, Obafemi Awolowo University (OAU) herbarium (voucher specimen nos.: *P. thonningii* OAU 14885; *P. reticulatum* OAU 3173). The leaves were hydro-distilled separately for essential oils (*P. thonningii*: Pale yellow oil, 0.04% fresh wt; *P. reticulatum*: Yellow oil, 0.01% fresh wt) in a Clevenger-type apparatus and stored in separate screw-capped vials, and refrigerated at 4°C until needed.

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Gas Chromatographic and Gas Chromatographic-Mass Spectroscopic Analyses

The oils were analysed using an Agilent 6890 series gas chromatograph interfaced with an Agilent 5973 *N* mass selective detector (Agilent Technologies, Little Falls, DE, USA) and a vaporization injector operating at 250°C in the split mode (1:100). A fused silica capillary column, 30 m×0.25 mm i.d.×0.25 μm film thickness (TRB-5MS; 5% diphenyl 95% dimethyl polydimethylsiloxane, Teknokroma, Spain) was used. The oven temperature was programmed from 45°C for 1 minute and then increased at 5°C/min to 240°C, and held isothermally for 5 minutes. High purity helium was used as carrier gas at 30 cm/s. Electron ionization mass spectra in the scan mode acquisition ranging from 35 to 550 Da was recorded at 70 eV, with an ionization current of 39.6 μA. The quadrupole, source and transfer line temperatures were maintained at 150, 230 and 280°C, respectively. A solvent delay of 5 minutes and a turbo molecular pump (10^{-5} torr) were used. All data were recorded using MS ChemStations (Agilent Technologies). The identity of each compound was determined by comparison of its retention index (RI) relative to standard mixture of *n*-alkanes,[6] as well as of its spectra with the Wiley library spectral data bank (Agilent Technologies). For semi-quantification, the normalized peak area of each compound was used without any correction factor to establish abundance. For each oil, the RI and the peak area percentages were calculated as mean values of three injections.

RESULTS AND DISCUSSION

A comparative analysis of the leaf essential oils of the two plants revealed higher yield for *P. thonningii* (0.04%) than *P. reticulatum* (0.01%). A total of 53 and 58 compounds were detected in the total ion chromatograms of *P. thonningii* and *P. reticulatum* respectively [Table 1], and this accounted for 74.8% and 87.2%, respectively, of their total oils. While the monoterpenes (38.3%) and sesquiterpene hydrocarbons (35.8%) dominated *P. reticulatum* oil, the sesquiterpenes (37.3%) and monoterpene hydrocarbons (32.2%) largely constituted *P. thonningii* oil. It is interesting however to note that both oils have similar proportions of the monoterpene (38.3% and 33.4% respectively for *P. reticulatum* and *P. thonningii*) and sesquiterpene class of compounds (39% and 37.3% respectively for *P. reticulatum* and *P. thonningii*). On a quantitative scale, γ-muurolene (10.3%), α-pinene (9.4%), tricyclene (7.2%), δ-cadinene (5.6%), α-terpineol (5.3%) and β-caryophyllene (4.2%) represented the major constituents of *P. reticulatum* leaf oil. The sesquiterpene-rich *P. thonningii* oil was characterized by β-myrcene (13.3%), limonene (8.6%), α-pinene (7.6%), β-caryophyllene (7.1%), γ-muurolene (6.1%), caryophyllene oxide (5.2%) and spathulenol (4.0%). Limonene rich-*Citrus* species have been shown to possess typical flavouring,[7] insecticidal[8] and antimicrobial[9] properties of respective oils. In certain studies, the sesquiterpenes have been shown to exhibit antioxidant[10] and insecticidal[11,12] properties. It is however evident that the two *Piliostigma* oils shared similarities in composition particularly with respect to some of their major constituents (α-pinene, β-caryophyllene and γ-muurolene). Both α-pinene and β-caryophyllene are useful as antimicrobial, anesthetic and anti-inflammatory agents,[13] while germacrene D exhibits insecticidal properties.[14] Furthermore, among the six major components detected in *P. reticulatum* leaf oil, tricyclene and α-terpineol were absent from *P. thonningii* oil, while spathulenol was the only predominant compound of *P. thonningii* oil undetected in *P. reticulatum* leaf oil.

Sesquiterpene-rich *Pseudocedrela kotschyi* oil was described to possess anti-oxidant activity.[10] Such a biological activity previously reported for *P. thonningii*[3] could also be attributed to its high sesquiterpene content. In addition, anti-ulcer and anti-inflammatory activities which are reputed for sesquiterpene-rich leaf oil of *Casearia sylvestris*[15] could also be predicted for these *Piliostigma* species. Both qualitative and quantitative differences existed in the composition of the minor constituents of the two oils. Present in *P. reticulatum* oil were camphene, β-myrcene, o-cymene, limonene, terpinen-4-ol among the monoterpenes; and germacrene D and α-muurolene (sesquiterpene hydrocarbons), as well as non-terpenes like o-allyltoluene and hexenyl acetate in 1.8-2.7% yields. Notable minor constituents in *P. thonningii* oil present in 1.3 to 2.7% yields were β-pinene, α-selinene, δ-cadinene, γ-muurolene and α-cadinol.

Other compounds belonging to diterpenes, a triterpene, aliphatic compounds and a fatty acid were also found in both oils, but in twice a yield in *P. reticulatum* (9.9%). These were typified by hexenyl acetate, 3-hexenyl benzoate, *o*-allyltoluene and hexadecanoic acid (0.9–2.9%) in *P. reticulatum*, and by *n*-nonanal, nonacosane and kaur-16-ene (0.5-1.8%) in *P. thonningii* oil.

CONCLUSION

The Caesalpiniaceae is a frequently unreported family of essential oils, but of anthraquinones. This study is therefore significant in that it reports for the first time the constituents of leaf essential oils of both *P. reticulatum* and *P. thonningii* that have no known flavouring or perfumery properties. Nevertheless, these potentials can be predicted for the two oils except for the relatively low yields. Both oils are predominantly rich in hydrocarbons. While *P. thonningii* oil was characterized by oxygenated sesquiterpenes, *P. reticulatum* was comparatively richer in oxygenated monoterpenes.

Table 1: Composition of *Piliostigma reticulatum* and *P. thonningii* leaf oils

Compound	RI[a]	%[b]	
		P. reticulatum	*P. thonningii*
Tricyclene	888	7.2	-
α-Thujene	891	-	t
α-Pinene	898	9.4	7.6
Camphene	909	2.3	0.6
Verbenene	915	0.1	0.1
Linaloyl oxide	931	0.2	0.1
β-Pinene	935	0.2	1.7
β-Myrcene	952	2.1	13.3
α-Phellandrene	964	0.1	-
Hexenyl acetate	967	2.1	-
1,4-Cineole	974	1.0	-
α-Terpinene	976	0.9	-
o-Cymene	984	2.7	0.2
Limonene	989	2.2	8.6
γ-Terpinene	1022	0.9	0.1
α-Terpinolene	1053	-	0.2
o-Allyltoluene	1055	2.9	-
Linalool	1070	0.8	0.4
n-Nonanal	1071	-	0.7
Fenchol	1079	-	0.1
Chrysanthenone	1090	-	0.1
1-Terpineol	1106	0.8	-
trans-Pinocarveol	1106	-	0.1
1,5,8-p-Menthatriene	1114	0.5	-
Exo-methyl-camphenilol	1116	-	t
cis-Pinocarveol	1132	-	0.1
Borneol	1141	0.1	-
4-Terpineol	1153	-	0.2
Terpinen-4-ol	1156	2.2	-
α-Terpineol	1173	5.3	-
γ-Terpineol	1181	0.9	-
Naphtalene,1,2,3,4,tetrahydro-1,1,6-trimethyl-	1196	0.2	-
β-Cyclocitral	1202	-	0.2
3-hexenyl-2-methylbutanoate	1226	0.3	-
1-Cyclohexene-1-acetaldehyde,2,6,6-trimethyl	1246	-	t
Vitispirane	1274	0.7	-
Cyclohexene,4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl),(3-*trans*)-	1345	0.2	-
α-Cubebene	1359	0.3	0.1
α-Ylangene	1383	0.1	0.1
α-Copaene	1388	1.4	0.5
β-Bourbonene	1397	1.6	1.0
β-Elemene	1406	0.8	0.8
α-Gurjunene	1409	-	0.1
β-Caryophyllene	1435	4.2	7.1
γ-Elemene	1452	0.1	-
Aromandrene	1461	0.2	0.1
α-Humulene	1471	1.1	0.8
Allo-Aromadendrene	1479	0.7	1.0
γ-Muurolene	1497	10.3	6.1
Germacrene-D	1502	1.8	-
β-Selinene	1507	1.1	1.0
Epicyclo sesquiphellandrene	1512	0.4	-
α-Selinene	1516	1.0	1.5
α-Muurolene	1523	2.6	0.3
Germacrene-A	1526	0.4	-
γ-Cadinene	1537	0.6	0.7
Δ-Cadinene	1548	5.6	1.6
Naphtalene,1,2,3,4,4a,5,7-hexahydro-1,6-dimethyl-4 (1methylethyl)	1556	0.5	0.1

Contd...

Contd...

Compound	RI[a]	%[b]	
		P. reticulatum	*P. thonningii*
6,10-dimethyl-3 (1methylethylidene)-1-cyclodecane	1570	-	0.4
Germacrene B	1579	0.5	-
3-Hexenyl benzoate	1597	0.9	-
Spathulenol	1601	-	4.0
Caryohyllene oxide	1604	0.4	5.2
Salvial-4-(14)-en-1-one	1613	-	1.1
γ-Muurolol	1662	-	1.3
α-Muurolol	1669	1.0	-
α-Cadinol	1677	0.8	2.7
Sandaracopimaradiene	1935	-	t
Hexadecanoic acid	1967	1.4	-
Kaur-16-ene	2011	-	0.5
Phytol	2086	0.6	-
Linoleic acid	2107	0.1	-
Oxazepam	2150	-	0.2
Di (2-ethylhexyl) adipate	2294	0.3	-
Pentacosane	2362	-	t
Eicosane	2473	0.1	-
Heptacosane	2468	-	0.2
Octacosane	2511	-	t
Squalene	2526	0.1	-
Nonacosane	2550	-	1.8
Heneicosane-n	2554	0.7	-
Octadecane	2584	-	t
Hentriacontane	2618	-	0.1
Monoterpene hydrocarbons	-	27.9	32.2
Oxygenated monoterpenes	-	10.4	1.2
Sesquiterpene hydrocarbons	-	35.8	23.0
Oxygenated sesquiterpenes	-	3.2	14.3
Others	-	9.9	4.1
Total identified	-	87.2	74.8

[a]Retention index relative to standard mixture of *n*-alkanes on TRB-5MS capillary column; [b]Values (area %) represent averages of three determinations (t: trace, $<$0.05 %)

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