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Characterization and identification of the constituents of the essential oil of the leaves *Agelaea pentagyna* (Lam.) Baill. Connaraceae, endemic plant of Madagascar

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Abstract

Agelaea pentagyna (Lam.) Baill. is an endemic plant of Madagascar belonging to the Connaraceae family. The essential oil from leaves of this plant was extracted by hydrodistillation and analyzed by gas chromatography coupled with mass spectrometry (GC/MS), in order to determine the chemical composition of the volatile fraction and to identify the chemotypes of the species. The essential oil has a yellowish color and has a no odor; the yield was 0.05%. A total of fifty-nine compounds, 99.93% of its compounds are identified in the sample. The main compounds in the oil were δ-cadinene (16.284%), caryophyllene (10.151%), and benzylbenzoate (09.57%).

Keywords: *Agelaea pentagyna;* Connaraceae; Essential oil; Leaves; GC/MS

1. Introduction

The Connaraceae family brings together plants comprising only lianas and shrubs or small trees. They are divided into 24 genera in tropical regions. In Madagascar, it includes 4 genera: *Ellipanthus*, *Agelaea*, *Rourea*, *Cnestis* [1].

The *Agelaea* genus includes around fifty species, most of which are in western and central tropical Africa, 2 in eastern tropical Africa and 3 in Madagascar: *Agelaea thouarsiana* H. Baill., *Agelaea pentagyna* (Lam.) Baill. and *Agelaea baronii* Schellenb [1].

Agelaea pentagyna has a large vine of 25 m with sarmentose, brownish branches. The leaves are alternate with 3 oval, elliptical leaflets; more or less acuminate at the top and trinerved at the base with a robust petiole 2 mm long. Inflorescences flowers whitish when mature, brownish when in bud, terminal, subterminal or axillary, in paniculate racemes, ample, multiflorous; rachis of inflorescences reaching 30 cm, covered with fascicle hairs sometimes dense or scattered or even glabrescent, but the ramifications become more and more tomentose towards the outside of the inflorescence; pedicels 1 mm long, densely hairy, linear lanceolate bracts 3 mm long, trifid at apex, silky; sepals 4-5 mm long, lanceolate-acute, pubescent on both sides, with glandular margins and short ciliate; petals 5-6 mm long, thin, glabrous, oblong; stamens fused into a tube at the base, the epipetals longer, anthers oval, a little cordate at the base and very short apiculate at the top; carpels densely pubescent.

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Follicles bright red when mature, 10-12 mm long and 6 mm thick, ovoid-oblique, pericarp to tomentose yellowish-brown on the dry outside, glabrous inside, calyx persistent at the base. Seeds about 8 mm long and 4-5 mm thick, shiny black, are arillate and ventrally fissured [1,2,3].

According to the traditional utilization, this endemic plant of the big island known by the vernacular names vahijôby, ravinamainty, vahimainty, bebaka, vahimenty, sefamainty, soandro is used to treat diarrhea, fatigue, high blood pressure and fever [2].

The aim of this study is to isolate and identify the compounds in the essential oil of *Agelaea pentagyna* leaves.

To our knowledge, no in-depth chemical study has yet been undertaken on this species. This is the first extraction of volatile oil from the leaves of the *Agelaea* species in Madagascar.

2. Materials and method

2.1. Plant material

The leaves of *Agelaea pentagyna* used for the extraction of essential oil are harvested during the year 2014, in Joffre ville, in the northern region of the island, district of Antsiranana II. This plant was identified by botanists (Mrs. Razafinirina Lydia and Mr. Letsara Rokiman) at the Tsimbazaza Botanical and Zoological Park in Antananarivo, Madagascar.

2.2. Essential oil extraction

Hydrodistillation was used to extract the volatile compounds contained in leaves (20 g) of the species *Agelaea pentagyna* with a Clevenger type apparatus, for 4 hours. The essential oil obtained was stored in the dark at 4 °C until use.

2.3. Thin Layer Chromatography (TLC)

The essential oil sample from the leaves obtained was analyzed qualitatively by thin layer chromatography (TLC).

A ready-made silica plate [size 20 x 20 cm, silica gel 60F254, Merck] was used. It is placed in a tank containing the following mobile phase: toluene/ethyl acetate (9:1) v/v. After migration, the solvent is evaporated by drying using a dryer then the plate is sprayed with a solution of sulfuric vanillin reagent [4].

2.4. Gas Chromatography – Mass Spectrometry (GC/MS)

The essential oil sample from the leaves obtained was analyzed quantitatively by gas chromatography coupled with mass spectrometry (GC/MS).

1 µL of the oil extract to be analyzed is dissolved in 20 mL of cyclohexane. The chemical analysis of essential oils was also made by GC/MS (Agilent Technologies 5975C MS, 7890A GC system, series 7683B) equipped with a HP-5 (5% phenyl, 95% methyl-polysiloxane) capillary column (30 m × 25µm i.d., 0.25 µm film thickness). The injector and interface were operated at 150 ˚C and 250 ˚C, respectively. The oven temperature was programmed to rise from 60˚C to 240 ˚C (3˚C/min). Helium was the carrier gas, 1µl injection volume and a split ratio of 1:15. In mass spectrometry electron-impact ionization was performed at electron energy of 70 eV. The constituents of essential oils were identified based on their Kovats Index, calculated in relation to the retention time of a series of n-alkanes as standard. The compounds were identified by comparison of retention indices with those reported in the literature and by comparison of their mass spectra with the NIST and WILEY databases [5,6].

3. Results

3.1. Essential oil extraction

The essential oil from the leaves of *Agelaea pentagyna* is in the form of a mobile liquid, yellowish in color and odorless. The essential oil yield from the leaves of *Agelaea pentagyna* is 0.05% (w/w).

3.2. Thin Layer Chromatography (TLC)

The most effective and convenient method for quick qualitative analysis is the thin layer chromatography. We have employed this method for preliminary analysis of the essential oil. When the TLC plate was treated with vanillin sulfuric reagent and heated to 110 °C for a few minutes, there is a faint pink, blue, yellow and brown colour spots that shows the presence of essential oils (terpene hydrocarbons).

3.3. Gas Chromatography – Mass Spectrometry (GC/MS)

The identification of peaks by their retention times and the comparison of the corresponding Kovats indices as well as their masses allowed the identification of the constituents of the essential oil. The analysis results (Table 1) indicate that the essential oil of the leaves is made up of 59 compounds representing 99.93% of the constituents.

Table 1 Composition of the essential oil from leaves of *Agelaea pentagyna*

N°	Constituents	RT (min)	RI Calculated	KI	(%)	Formulas
1	3-Methyl-apopinene	5.647	-	948	0.075	$C_{10}H_{16}$
2	Linalol	11.108	1113	1109	1.964	$C_{10}H_{18}O$
3	Bicyclohexane	19.789	1323	1304	1.496	$C_{12}H_{22}$
4	α-Cubebene	21.963	1375	1345	1.137	$C_{15}H_{24}$
5	Cycloisosativene	22.63	1391	1125	0.042	$C_{15}H_{24}$
6	Copaene	23.11	1403	1375	2.426	$C_{15}H_{24}$
7	β-Bourbonene	23.504	1413	1386	0.196	$C_{15}H_{24}$
8	β-Cubebene	23.744	1419	1400	0.302	$C_{15}H_{24}$
9	β-Elemene	23.827	1421	1373	0.269	$C_{15}H_{24}$
10	Caryophyllene	25.012	1450	1442	10.151	$C_{15}H_{24}$
11	β-Cubebene	25.408	1460	1400	0.351	$C_{15}H_{24}$
12	α-Guaiene	25.819	1470	1426	0.256	$C_{15}H_{24}$
13	α-Cubebene	26.323	1482	1345	0.760	$C_{15}H_{24}$
14	α-Caryophyllene	26.45	1485	1448	3.449	$C_{15}H_{24}$
15	Alloaromadendrene	26.757	1493	1460	1.052	$C_{15}H_{24}$
16	Epi-bicyclosesquiphellandrene	26.853	1495	1470	0.148	$C_{15}H_{24}$
17	β-Cadinene	27.316	1507	1529	1.671	$C_{15}H_{24}$
18	τ-Muurolene	27.441	1510	1486	0.940	$C_{15}H_{24}$
19	Germacrene D	27.623	1515	1464	0.494	$C_{15}H_{24}$
20	Eudesma-4(14),11-diene	27.837	1521	1469	0.289	$C_{15}H_{24}$
21	Epi-bicyclosesquiphellandrene	28.081	1527	1470	1.233	$C_{15}H_{24}$
22	β-Gurjunene	28.202	1530	1401	0.975	$C_{15}H_{24}$
23	Elixene	28.278	1532	1431	0.540	$C_{15}H_{24}$
24	α-Muurolene	28.441	1536	1495	1.736	$C_{15}H_{24}$
25	τ-Cadinene	29.015	1551	1471	3.397	$C_{15}H_{24}$
26	δ-Cadinene	29.424	1561	1497	16.284	$C_{15}H_{24}$

27	Isopropyl-1,6-dimethyl-1,2,3,4,4a,7-hexahydronaphthalene	29.762	1570	1546	0.824	$C_{15}H_{24}$
28	Isopropyl-4,7-dimethyl-1,2,4a,5,6,8a-hexahydronaphthalene	29.969	1575	1516	0.460	$C_{15}H_{24}$
29	α-Calacorene	30.19	1581	1590	0.582	$C_{15}H_{20}$
30	Caryophyllene oxide	30.581	1591	1561	0.280	$C_{15}H_{24}O$
31	Spathulenol	31.602	1618	1576	4.042	$C_{15}H_{24}O$
32	Caryophyllene oxide	31.83	1629	1561	4.988	$C_{15}H_{24}O$
33	Epi-bicyclosesquiphellandrene	32.025	1633	1470	0.191	$C_{15}H_{24}$
34	1H-Cycloprop(e)azulene,decahydro-1,1,7-trimethyl-4-methylene	32.164	1636	1386	0.201	$C_{15}H_{24}$
35	Ledene oxide(I)	32.26	1640	1890	0.214	$C_{15}H_{24}O$
36	5-Azulenemethanol,1,2,3,4,5,6,7,8-octahydro-α,α,3,8-tetramethyl-	32.419	1646	1614	1.136	$C_{15}H_{26}O$
37	Globulol	32.635	1652	1571	1.181	$C_{15}H_{26}O$
38	Diepicedrene-1-oxide	32.872	1655	1281	1.029	$C_{15}H_{24}O$
39	Cubenol	32.977	1658	1645	0.312	$C_{15}H_{26}O$
40	Cubenol	33.094	1666	1645	0.347	$C_{15}H_{26}O$
41	2-(4a,8-dimethyl-2,3,4,4a,5,6-hexahydro-naphthalene-2-yl)-prop-2-en-1-ol	33.405	1669	1727	0.111	$C_{15}H_{22}O$
42	Gurjunene	33.514	1672	1509	0.512	$C_{15}H_{24}$
43	Cubenol	33.622	1676	1645	4.530	$C_{15}H_{26}O$
44	Cubenol	33.77	1680	1645	0.246	$C_{15}H_{26}O$
45	Tetracyclo(6.3.2.0(2,5)0.(1,8))tridecan-9-ol,44-dimethyl-	33.925	1682	1490	0.242	$C_{15}H_{24}O$
46	Spathulenol	34.002	1687	1576	0.465	$C_{15}H_{24}O$
47	tau-Muurolol	34.164	1691	1635	6.286	$C_{15}H_{26}O$
48	δ-Cadinol	34.324	1699	1646	1.822	$C_{15}H_{26}O$
49	α-Cadinol	34.643	1713	1632	3.904	$C_{15}H_{26}O$
50	Bulnesol	35.138	1716	1651	0.362	$C_{15}H_{26}O$
51	Isoaromadendrene epoxide	35.26	1721	1579	0.558	$C_{15}H_{24}O$
52	Cadalene	35.421	1725	1735	0.241	$C_{15}H_{18}$
53	Murolan-3,9(11)-diene-10-peroxy	35.569	1732	1729	0.192	$C_{15}H_{24}O_2$
54	7-Methyl-4-(1-methylethylidene)bicyclo(5.3.1)undec-1-en-8-ol	35.842	1761	1754	0.248	$C_{15}H_{24}O$
55	Pentadecanal	36.896	1813	1694	0.148	$C_{15}H_{30}O$
56	Benzylbenzoate	38.775	1898	1738	9.570	$C_{14}H_{12}O_2$
57	Benzylsalicylate	42.466	1890	1824	0.434	$C_{14}H_{12}O_3$

58	n-Hexadecanoic acid	45.658	1890	1973	0.180	$C_{16}H_{32}O_2$
59	Tetracosamethyl-cyclododecasiloxane	54.369	1870	2338	2.529	$C_{24}H_{72}O_{12}Si_{12}$
	Total (%)				99,925	

RT: Retention time, **RI** : Retention indices relative to n-alkanes on a HP-5 column, **KI** : Kovats index, **(%)** : percentage.

4. Discussion

Hydrodistillation of dry leaves of *Agelaea pentagyna* gave an oil without odor and has a very low yield of 0.05%. Indeed, the yield of essential oil depends on the nature of the parts of the plant used, the equipment used for extraction and the extraction method, as well as the origin of the plants and the harvest period.

The chromatogram of the essential oil of *Agelaea pentagyna* allowed us to identify 59 compounds of which the majority compounds are δ-cadinene (16.284%), caryophyllene (10.151%), benzylbenzoate (09.57%) and tau-muurolol (6.286%).

Sesquiterpene hydrocarbons (51.109%) are the most dominant followed by oxygenated sesquiterpenes (32.495%), followed by esters (10.004%), then an oxygenated monoterpene (1.964%), a monoterpene hydrocarbon (0.074%) and the other compounds (2.709%). The whole represents 99.925% of the total compound of the essential oil.

Composition of the essential oil from the leaves of *Agelaea pentagyna* is particularly rich in sesquiterpenoids (83.604%). The oxygenated terpene compounds found in this oil have interesting biological and pharmacological activities [7], in particular a strong antimicrobial power such as for example spathulenol (4.042%) and copaene (2.426%) have a strong antimicrobial activity.

5. Conclusion

This study aims to contribute to the knowledge of the essential oil of *Agelaea pentagyna* leaves. The essential oil obtained by hydrodistillation was analyzed by TLC and GC/MS.

The GC/MS technique was used for the identification of the essential oil of *Agelaea pentagyna* leaves. Fifty-nine compounds have been highlighted, the fact that the leaf oil contains a greater number of molecules such as sesquiterpenes are the most abundant, which could explain the diverse biological activity of this plant [8].

This is therefore the first time that research has been carried out on the volatile compounds of dry *Agelaea pentagyna* leaves.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declared no conflict of interest.

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