Original Research Article

Chemical variability of essential oils from *Zanthoxylum leprieurii* Guill. et Perr (Rutaceae) according to plant organs

ABSTRACT

Zanthoxylum leprieurii belonging to Rutaceae family, is a medicinal and aromatic plants widely used in ethnopharmacology. Like other plants of Rutaceae family such as *Citrus* sp., the essential oils of this species could be used in cosmetic and pharmaceutical products. Thus, the essential oils of *Z. leprieurii* from different plant organs (fruit, leaf, stem and root) were investigated by using GC and GC–MS. The volatile composition of *Z. leprieurii* fruits exhibited relative high amounts of hydrocarbons monoterpenes (90.9%) such as (*E*)–*β*-ocimene (50.9%) and *α*–pinene (30.4%). The chemical composition of fruit oils was compared with volatile fractions of leaves, stems and roots from the same plant station. Germacrene B (9.0%), *β*–phellandrene (7.6%), caryophyllene oxide (7.2%) and *β*–caryophyllene (5.3%) were identified as the major constituents of leaves whereas the essential oil composition of the roots dominated by sesquiterpenes (83.3%) such as germacrone (23.5%), germacrene B (19.1%), *γ*–elemene (6.0%), elemenone (4.0%) and *β*–elemene (2.4%) in the essential oil of the stems, *β*–Phellandrene (12.7%), germacrene B (5.0%), germacrene D (4.7%) and *cis*–9–Octadecen–1–ol (4.6%) have been reported as majority compounds.

Keywords: Essential oils, Zanthoxylum leprieurii and GC-MS

1. INTRODUCTION

Belonging to the Rutaceae family, *Zanthoxylum leprieurii* is a deciduous aromatic tree distributed in Central and West Africa [1]. It is used in herbal medicine for the treatment of malaria, urinary infections, rheumatic pain, skin infections, intestinal parasites, gonococcies, sickle cell anemia, stomach disorders and dysentery [1–3]. In Cameroon, the dried fruits are traditionally used as a spice in soups [4]. Literature has also shown that extracts of this plant possess a potential antimicrobial [5–9], insecticidal [10], antiplasmodial [11], cytotoxic [12,13], anti-inflammatory [14] and antioxidant activities [14–16].

Previous phytochemical studies on the secondary metabolites of *Z. leprieurii* showed the presence of diterpene [17], alkaloids [11,12,18–22], flavonoids [1], amides [22] and coumarins [7,11,22] in solvent extracts from various organs (root, stem, bark, leaf and fruit). Moreover, some papers were reported the chemical composition and biological activities of fruit essential oils [5–7,14,23–27] from various geographical origins such as Nigeria and Cameroun. These studies showed chemical variability according to the amounts of hydrocarbon monoterpenes ((*E*)–β–ocimene, *α*–pinene, *β*–pinene, terpinolene, limonene and δ–3–carene), hydrocarbon sesquiterpene (caryophyllene), oxygenated monoterpenes (*β*–citronellol, citronellic acid, *β*–citronellal and geranyl acetate) and oxygenated sesquiterpenes ((*E*)–nerolidol, humulenol and elemol).

However, to the best of our knowledge, to date no study has been carried out on the chemical composition of essential oils from *Z. leprieurii* growing wild in Senegal. Therefore, the present study was performed to characterize the chemical compositions of *Z. leprieurii* oils from different plant organs (fruit, leaf, stem and root) by using GC and GC-MS.

2. MATERIALS AND METHOD

2.1. Plant material

The fruit (ripe), leaf (young), stem and root samples of *Z. leprieurii* were harvested in October 2015 from only tree, growing wild in one Senegalese locality, Colomba (12°46'N, 16°14'O). The plant material was identified by Dr William Diatta from the Department of botanical and pharmacognosy of University Cheikh Anta Diop of Dakar.

2.2. Essential Oil Isolation

Plant material were air-dried for 14 days at room temperature. Samples were hydrodistilled (6 h) using a Clevenger-type apparatus according to the method recommended in the European Pharmacopoeia [28]. The yields of essential oil (w/w, calculated on dry weight basis) were given in the table 1.

2.3. GC and GC/MS Analysis

Analyses were carried out using a Perkin-Elmer Autosystem XL GC apparatus (Walthon, MA, USA) equipped with dual flame ionisation detection (FID) system and fused-silica capillary columns, namely, Rtx-1 (polydimethylsiloxane) and Rtx-wax (poly-ethyleneglycol) (60 m × 0.22 mm i.d; film thickness 0.25 µm). The oven temperature was programmed from 60 to 230°C at 2°C/min and then held isothermally at 230°C for 35 min: hydrogen was employed as carrier gas (1 mL/min). The injector and detector temperatures were maintained at 280°C, and samples were injected (0.2 µL of pure oil) in the split mode (1:50). Retention indices (RI) of compounds were determined relative to the retention times of a series of n-alkanes (C5–C30) by linear interpolation using the Van den Dool and Kratz (1963) equation with the aid of software from Perkin-Elmer (Total Chrom navigator). The relative percentages of the oil constituents were calculated from the GC peak areas, without application of correction factors.

Samples were also analysed with a Perkin-Elmer Turbo mass detector (quadrupole) coupled to a Perkin-ElmerAutosystem XL, equipped with fused-silica capillary columns Rtx-1 and Rtx-Wax. The oven temperature was programmed from 60 to 230°C at 2°C/min and then held isothermally at 230°C (35 min): hydrogen was employed as carrier gas (1 mL/min). The following chromatographic conditions were employed: injection volume, 0.2 µL of pure oil;

injector temperature, 280°C; split, 1:80; ion source temperature, 150°C; ionisation energy, 70 eV; MS (EI) acquired over the mass range, 35–350 Da; scan rate, 1 s.

Identification of the components was based on: (a) comparison of their GC retention indices (RI) on non-polar and polar columns, determined from the retention times of a series of n-alkanes with linear interpolation, with those of authentic compounds or literature data; (b) on computer matching with commercial mass spectral libraries [29–31] and comparison of spectra with those of our personal library; and (c) comparison of RI (Retention indices) and MS spectral data of authentic compounds or literature data.

3. RESULTS AND DISCUSSION

It is known that in Rutaceae, the fruits are more aromatic than the other organs and that we want to check if it is the same for *Z. leprieurii*. Thus, individualized samples of fruits, leaves, stems and root bark were collected on the same tree in Colomba.

In comparison with essential oil yield from fruits collected in Colomba (1.64%), hydrodistillation of the leaves, root and stems of *Z. leprieurii* harvested on the same tree afforded drastically lower oil yields of 0.22%, 0.75% and 0.08%, respectively (Table 1). As for other plants of Rutaceae family, this difference confirms that fruits are the most aromatic organs relative to root barks, stems and leaves.

Combined analysis of the essential oils from separated plant organs led to the identification of 102 components, accounting for 80.7 to 96.2% of the total oils (Table 1). Ninety-two compounds were identified by comparing their Electronic Impact-mass spectra and their retention indices with those of laboratory-made library. Ten constituents (with an asterisk in Table 1) were identified by comparison of their EI-mass spectra and their apolar retention indices with those of commercial libraries.

GC and GC-MS analysis of *Z. leprieurii* fruit allowed identifying 29 compounds, amounting to 96.2% of total oil composition. The fruit oils are dominated by hydrocarbon monoterpenes (90.9%) with (*E*)–β–ocimene (Entry 15; 50.9%) and *α*–pinene (Entry 2; 30.4%) as main components. The richness of fruit essential oils in (*E*)–β–ocimene has also been described in the literature, whose relative percentages are between 29.4% and 80.7% [5,7,14,25,26]. However, the combination of the two isomers, (*E*)–β–ocimene and α-pinene in significant proportions have never been described in the literature to our knowledge.

Sixty-five volatile components were detected in leaf essential oils of this taxon, accounting for 87.6% of the total chemical composition. In contrast to fruits, the essential oil of the leaves is rich in sesquiterpenes (75.6%) such as germacrene B (Entry 79; 9.0%), *β*–phellandrene (Entry 13; 7.6%), caryophyllene oxide (Entry 83; 7.2%) and *β*–caryophyllene (Entry 41; 5.3%). The chemical composition of the essential oil of leaves from Senegal is different from those described in the literature. In Cameroon, three chemotypes have been reported for the essential oils from the leaves. The former is characterized by the presence of terpinolene (49.8%), *δ*–3–carene (17.9%) and geranyl acetate (8.5%) [25]. The second is largely dominated by limonene (94.9%) [27] while the latter is rich in *β*-ocimene, myrcene and thujanol [14]. For their part, leaf essential oils from Nigeria are rich in *β*–pinene (22.9%), caryophylleol (24.6%) and caryophyllene (13.6%) [24].

This is the first report on the essential oil composition of the roots of *Z. leprieurii*. It is mainly dominated by sesquiterpenes (83.3%) such as germacrone (Entry 98; 23.5%), germacrene

B (Entry 79; 19.1%), γ–elemene (Entry 42; 6%), elemenone (Entry 84; 4.0%) and β–elemene (Entry 37; 2.4%).

This high content of sesquiterpenes has also been reported in the essential oil of the stems. β–Phellandrene (Entry 13; 12.7%), germacrene B (Entry 79; 5.0%), germacrene D (Entry 56; 4.7%), *cis*-9-Octadecen–1–ol (Entry 101; 4.6%) and finally *β*–caryophyllene (Entry 41; 4.3%) have been reported as majority compounds. This composition is different from those described in the literature. Essential oils reported by Oyedeji et al. [27] had as major constituents (*E*)-nolidolidol (23.0%), humulenol (17.5%) and elemol (5.7%).

Table 1. Comparison of volatiles compositions of separated organs (leaf, stem, root and fruit) of *Z. leprieurii* from Colomba

No[a]	Compounds	IRIa[b]	RIa[c]	RIp[d]	Different parts			
					Leaves	Stems	Roots	Fruits
1	*α*–Thujene	932	922	1023	-	0.1	-	0.3
2	*α*–Pinene	936	931	1022	0.1	0.3	0.4	30.4
3	Camphene	950	943	1066	-	-	-	0.4
4	Sabinene	973	964	1120	0.1	0.4	-	2.7
5	*β*–Pinene	978	970	1108	-	-	-	0.9
6	Myrcene	987	979	1159	0.3	0.5	-	1.3
7	Octanal	981	980	1290	-	0.1	-	0.2
8	*α*–Phellandrene	1002	997	1164	-	0.4	-	-
9	*α*–Terpinene	1013	1008	1178	-	0.4	-	0.4
10	*p*–Cymene	1015	1011	1268	-	-	-	0.5
11	Limonene	1025	1020	1201	0.9	1.5	-	0.6
12	1,8–Cineole	1024	1020	1209	-	-	-	0.5
13	*β*–Phellandrene	1025	1020	1215	7.6	12.7	-	-
14	*(Z)–β*–Ocimene	1029	1024	1230	-	tr	-	0.7
15	*(E)–β*–Ocimene	1041	1034	1247	0.1	1.2	0.1	50.9
16	*γ*–Terpinene	1051	1047	1243	0.3	1.0	-	0.7
17	Octanol	1063	1065	1531	-	-	-	0.1

18	Terpinolene	1082	1078	1280	-	0.3	-	0.4
19	Linalool	1086	1081	1544	0.6	0.3	-	tr
20	Nonanal	1076	1083	1394	tr	0.1	-	0.1
21	*p*–Mentha–1,3,8–triene	1123	1122	1443	-	0.3	-	0.7
22	(*E*)–Ocimenoxide	1125	1125	1482	-	0.2	-	0.1
23	Cryptone	1160	1157	1667	0.2	0.5	-	-
24	Terpinen–4–ol	1164	1161	1600	0.9	1.2	-	1.8
25	α–Terpineol	1176	1179	1700	0.2	0.1	-	0.4
26	Decanal	1180	1185	1498	-	-	-	0.1
27	Cuminaldehyde		1217	1782	-	0.3	-	-
28	Phellandral		1251	1708	0.4	1.5	-	-
29	α–Terpinen–7–al		1258	1771	-	0.1	-	-
30	Citronellyl acetate	1337	1331	1657	0.1	-	-	-
31	δ–Elemene	1340	1337	1467	0.3	-	0.1	-
32	Neryl acetate	1342	1342	1725	0.2	-	-	0.2
33	α–Cubebene	1355	1350	1452	0.1	0.1	-	-
34	Geranyl acetate	1362	1361	1752	0.2	0.7	-	0.4
35	Cyclosativene	1378	1373	1484	1.2	0.2	0.5	-
36	α–Copaene	1379	1379	1488	0.7	0.6	0.3	-
37	β–Elemene	1389	1388	1589	2.7	0.7	2.4	-
38	Sativene	1394	1397	1529	0.4	0.3	0.2	-
39	α–Gurjunene	1413	1413	1524	0.1	-	0.1	-
40	α–Cedrene	1418	1417	1562	-	0.1	-	-
41	β–Caryophyllene	1421	1424	1591	5.3	4.3	1.5	0.5
42	γ–Elemene	1429	1429	1638	2.4	1.4	6.0	-
43	β–Gurjunene	1437	1439	1591	0.2	0.2	-	-

44	*α*–Guaiene	1440	1440	1583	0.5	0.3	0.2	-
45	*β*–Copaene	1430	1444	1581	-	-	0.2	-
46	Aromadendrene	1443	1447	1611	-	-	0.4	-
47	(*E*)–*β*–Farnesene	1446	1448	1661	0.2	0.1	-	-
48	Isogermacrene D*	1445	1449	1627	0.3	0.2	-	-
49	*α*–Humulene	1455	1450	1660	1.9	1.8	0.7	0.1
50	Selina–4(15), 7-diene	1457	1457	1728	-	0.1	0.3	-
51	Allo-aromadendrene	1462	1462	1638	0.4	0.2	0.3	-
52	Trans–*β*–Ionone	1468	1466	1936	0.2	-	-	-
53	1*β*H, 7*α*H, 10*β*H-Guaia-4,11-diene		1467	1660	-	-	0.2	-
54	*γ*–Muurolene	1474	1468	1681	1.9	1.1	0.2	-
55	*γ*–Gurjunene	1472	1470	1654	-	-	0.2	-
56	Germacrene D	1479	1476	1704	2.7	4.7	0.3	-
57	*α*–Amorphene	1477	1480	1694	-	-	0.8	-
58	*β*–Selinene	1486	1483	1712	2.3	0.9	2.1	-
59	*Cis*–Eudesma–6,11–diene	1484	1488	1713	0.4	0.2	0.4	-
60	Valencene	1494	1489	1719	-	0.8	0.2	-
61	*γ*–Amorphene*	1492	1492	1710	1.0	0.4	-	-
62	Bicyclogermacrene	1494	1494	1727	0.1	-	0.2	-
63	α–Selinene	1494	1495	1720	1.4	-	0.7	-
64	*α*–Muurolene	1496	1496	1719	0.9	0.6	0.3	-
65	*β*–Bisabolene	1503	1504	1720	0.8	-	-	-
66	*δ*–Amorphene*	1499	1505		-	0.6	0.6	-
67	γ–Cadinene	1507	1511	1752	1.9	1.7	-	-

68	Nootkatene	1512	1511	1812	-	-	1.5	-
69	*Cis*–Calamenene	1517	1512	1816	0.4	-	-	-
70	δ–Cadinene	1520	1516	1752	3.9	2.9	1.0	-
71	Spirovetiva–1(10), 7(11)–diene	1523	1520	1738	0.8	0.5	1.1	-
72	Cadina-1,4-diene	1523	1523	1763	0.6	0.3	-	-
73	Eremophila-1(10), 7(11)-diene	1527	1525	1738	0.5	0.3	0.6	-
74	Selina-4(15), 7(11)-diene*	1534	1535	1775	1.4	1.4	-	-
75	Elemol	1541	1536	2072	-	-	2.1	-
76	Selina-3,7(11)-diene*	1542	1540	1775	0.9	0.7	1.5	-
77	(*E*)-Nerolidol	1553	1546	2037	3.2	0.8	-	0.1
78	β–Vetivenene	1552	1552	1857	-	1.0	1.2	-
79	Germacrene B	1552	1552	1828	9.0	5.0	19.1	-
80	Spathulenol	1572	1572	2119	1.4	0.9	0.4	-
81	β–Copaen-4-α-ol		1575	2141	0.2	-	-	-
82	Globulol	1589	1575	2074	-	0.8	0.4	-
83	Caryophyllene oxide	1578	1576	1980	7.2	2.2	-	0.7
84	Elemenone	1589	1585	2082	0.8	-	4.0	-
85	Scapanol*	1586	1586		-	0.9	-	-
86	Viridiflorol	1592	1591	2089	-	-	0.9	-
87	Copaborneol	1595	1592	2159	-	-	0.6	-
88	Humulene epoxide II	1602	1598	2044	2.3	0.7	-	-
89	Torilenol*	1599	1602	2268	1.1	0.8	-	-
90	Myliol*	1617	1619		-	1.1	-	-
91	Epicubenol	1623	1624	2059	0.3	-	-	-
92	*t*–Cadinol	1633	1625	2169	1.6	1.0	-	-
93	Pogostol*	1647	1645		-	1.1	1.5	-

94	7–*Epi*–*α*–eudesmol	1653	1651	2221	-	2.5	-	-
95	14–Hydroxy–9–*epi*–(*E*)–Caryophyllene		1656	2316	1.7	-	-	-
96	Eudesma–4(15),7–dien–1β–ol	1671	1667	2347	0.5	1.4	1.0	-
97	4–*β*–H–Muurol–9–en–15–al		1672	2163	1.6	0.2	2.4	-
98	Germacrone	1684	1678	2216	3.4	1.1	23.5	-
99	Eudesma–7–11–en–4–α–ol	1676	1690	2284	0.2	0.5	1.1	-
100	Cembrene A*	1962	1968		-	2.2	-	-
101	*Cis*–9–Octadecen–1–ol		2052	2607	-	4.6	-	-
102	(*E*)–Phytol	2014	2105	2617	2.1	-	-	-
	Hydrocarbon Compounds				57.2	55.7	45.9	91.5
	Oxygenated Compounds				30.4	25.0	37.9	4.7
	Hydrocarbon Monoterpenes				9.4	19.1	0.5	90.9
	Oxygenated Monoterpenes				2.6	4.2	0.0	3.4
	Hydrocarbon Sesquiterpenes				47.8	34.4	45.4	0.6
	Oxygenated Sesquiterpenes				27.8	16.0	37.9	0.8
	Oxygenated non-terpenic Compounds				0.0	4.8	0.0	0.5
	Hydrocarbon Diterpenes				0.0	2.2	0.0	0.0
	Total identified				87.6	80.7	83.8	96.2
	Yelds (m/m vs materiel vegetal sec)				0.22	0.08	0.75	1.64

[a] *Order of elution is given on apolar column (Rtx-1). Bold types refer to main compounds.*

[b] *Retention indices of literature on the apolar column (IRIa) [30].*

[c] *Retention indices on the apolar Rtx-1 column (RIa).*

[d] *Retention indices on the polar Rtx-Wax column (RIp).*

[*] *Compound identified with commercial library [29–31].*

tr: trace (%<0,05%)

4. CONCLUSION

The present study was performed to characterize the chemical compositions of *Z. leprieurii* oils from different plant organs. The fruit oils are dominated by hydrocarbon monoterpenes ((*E*)–*β*–ocimene and *α*–pinene). Moreover, leaf, stem and root oils are dominated by sesquiterpenes such as germacrene B, *β*–phellandrene, caryophyllene oxide and *β*–caryophyllene, germacrone, *γ*–elemene, elemenone, *β*–elemene and germacrene D. Like other plants of Rutaceae family, *Z. leprieurii* fruit oils could be used in cosmetic and/or food products for their flavouring properties.

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