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...
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, gonococci, 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], antiparasitoidal [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 Cameroon. These studies showed chemical variability according to the amounts of hydrocarbon monoterpenes ((E)–β-caryophyllene, α-pinene, β-pinene, terpinolene, limonene and δ-3-carene), hydrocarbon sesquiterpene (caryophyllene), oxygenated monoterpenes (β-citronellol, citronelllic acid, β-citronellal and geranyl acetate) and oxygenated sesquiterpenes ((E)–nerolidol, humuleno 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 METHODS

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-ethylene glycol) (60 m x 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-Elmer Autosystem 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 El-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%), caryophyllol (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 germacrene (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 Colombia

| No | Compounds                  | IRlα | Rlα | Rlp  | 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  | Sabine 65                  | 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-Terpine                  | 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-one             | 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-Terpene-7-one            | 1258 | 1771| -    | 0.1            | -      | -     | -     |        |
| 30 | Citronellyl acetate        | 1337 | 1331| 1657 | 0.1            | -      | -     | -     |        |
| 31 | β-Elemene                  | 1340 | 1337| 1467 | 0.3            | -      | 0.1   | -     |        |
| 32 | Neryl acetate              | 1342 | 1342| 1725 | 0.2            | -      | -     | 0.2   |        |
| 33 | α-Cubebeene                | 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-Guaiane                  | 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   | -     |        |
| No  | Compounds                          | IR<sub>I</sub> | IR<sub>A</sub> | IR<sub>P</sub> | Different parts |
|-----|-----------------------------------|--------------|-------------|--------------|----------------|
|     |                                   | Leaves       | Stems       | Roots        | Fruits         |
| 51  | Allo-aromadendrene                | 1462         | 1462        | 1638         | 0.4            | 0.2            | 0.3            | -              |
| 52  | Trans–β-ionone                    | 1468         | 1466        | 1936         | 0.2            | -              | -              | -              |
| 53  | 1/H, 7αH, 10β/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–Calamene                      | 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  | Eremophilia-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  | Elemone                           | 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  | ′-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–Murol–9–en–15–al            | 1672         | 2163        | 1.6          | 0.2            | 2.4            | -              | -              |
| 98  | Germacrene                        | 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 | Cembrone A*                       | 1962         | 1968        | -            | 2.2            | -              | -              | -              |
| 101 | Cis–9–Octadecen–1–ol              | 2052         | 2607        | -            | 4.6            | -              | -              | -              |
| 102 | (E)–Phytol                        | 2014         | 2105        | 2617         | 2.1            | -              | -              | -              |
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 (\((E)\beta\)-ocimene and \(\alpha\)-pinene). Moreover, leaf, stem and root oils are dominated by sesquiterpenes such as germacrene B, \(\beta\)-phellandrene, caryophyllene oxide and \(\beta\)-caryophyllene, germacrone, \(\gamma\)-elemene, elemenone, \(\beta\)-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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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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