Chemical compositions of *Casuarina equisetifolia* L., *Eucalyptus toreliana* L. and *Ficus elastica* Roxb. ex Hornem cultivated in Nigeria

I.A. Ogunwande a,⁎, G. Flaminib, A.E. Adefuye a, N.O. Lawala, S. Moradeyoa, N.O. Avoseha

a Natural Products Research Unit, Department of Chemistry, Faculty of Science, Lagos State University, Badagry Expressway, P.M.B. 0001, Lasu Post Office, Ojo, Lagos, Nigeria

b Dipartimento di Scienze Farmaceutiche, sede Chimica Bioorganica e Biofarmacia, Universita di Pisa, Via Bonanno 33, 56126 Pisa, Italy

Received 10 March 2010; received in revised form 3 February 2011; accepted 4 February 2011

Abstract

Essential oils were obtained by separate hydrodistillation of three different plants cultivated in Nigeria and analysed comprehensively for their constituents by means of gas chromatography (GC) and gas chromatography-mass spectrometry (GC–MS). The leaf essential oil of *Casuarina equisetifolia* L. (Casuarinaceae) comprised mainly of pentadecal (32.0%) and 1,8-cineole (13.1%), with significant amounts of apiole (7.2%), α-phellandrene (7.0%) and α-terpinene (6.9%), while the fruit oil was dominated by caryophyllene-oxide (11.7%), trans-linalool oxide (11.5%), 1,8-cineole (9.7%), α-terpineol (8.8%) and α-pinene (8.5%). On the other hand, 1,8-cineole (39.4%) and α-terpinyl acetate (10.7%) occurred in large quantities in the essential oils of the leaf of *Eucalyptus toreliana* L. (Myrtaceae). The oil also features high levels of sabinene (5.9%), caryophyllene-oxide (4.7%) and α-pinene (4.2%). The main compounds identified in the leaf oil of *Ficus elastica* Roxb. ex Hornem. (Moraceae) were 6,10,14-trimethyl-2-pentadecanone (25.9%), geranylacetone (9.9%), heneicosene (8.4%) and 1,8-cineole (8.2%).

© 2011 SAAB. Published by Elsevier B.V. All rights reserved.

Keywords: 1,8-Cineole; 6,10,14-Trimethyl-2-pentadecanone; Caryophyllene-oxide; *Casuarina equisetifolia*; Essential oil; *Eucalyptus toreliana*; *Ficus elastica*; Pentadecal; trans-Linalool oxide; α-Terpinyl acetate

1. Introduction

*Casuarina* is a genus of 17 species in the family Casuarinaceae, native to Australasia, southeastern Asia, and islands of the western Pacific Ocean. *Casuarina equisetifolia* L. is a widespread seashore tree known as Common Ironwood, Beefwood, Bull-oak, and Whistling-pine and is often planted as a windbreak. This species is not a pine at all, but superficially resembles a conifer (Pinyopasrerk and House, 1993). The plant is a source of biologically active compounds such as catechin, ellagic acid, gallic acid, quercetin and lupeol, which are antioxidants (Aher et al., 2009), coumaroyl triterpenes (Takahashi et al., 1999) and d-gallocatechin (casuarin) (Nash et al., 1994). The plant is also known to store tannins (Li-Hua et al., 2009) and proline (Tani and Sasakawa, 2003) as well as being a nitrogen fixing plant (Li-Hua et al., 2009). *C. equisetifolia* also displays antimicrobial properties (Parekh et al., 2005).

*Eucalyptus* (Myrtaceae) is a large genus of trees and shrubs, which originates mainly from Australia. *Eucalyptus toreliana* L., is one of the known 500 species of *Eucalyptus* that produces terpenoids. The leaves of the *Eucalyptus* species have medicinal and flavouring properties. The essential oil-bearing *Eucalyptus* plants rank high both in quantity and frequency among the plants that are widely used all over the world (Ogunwande, 2001). Reports on the chemical constituents of some *Eucalyptus* species cultivated in Nigeria have been published (Ogunwande et al., 2003; Ogunwande et al., 2005; Jimoh et al., 2005).

*Ficus elastica* Roxb. ex Hornem, (also known as the rubber tree) is a common house plant, as it can grow in moderately...
luminous environments. As with other members of the genus *Ficus*, the flowers require a particular species of fig wasp to pollinate it in a co-evolved relationship. Rubber plants are not known to produce highly colourful or fragrant flowers to attract other pollinators (Busari, 2001). The edible fruit is a small yellow-green oval fig, one centimetre long and barely edible.

2. Materials and methods

2.1. Plant materials

Mature leaves and fruits of *C. equisetifolia* were collected from trees growing in front of Okunuga Hall, Faculty of Law, Lagos State University, Ojo, in March 2009. The leaves of *E. torreliana* were harvested from trees growing at Eleyele, a suburb of Ibadan, Oyo State, Nigeria, in April 2009, while those of *F. elastica* were obtained from the Botanical Garden, University of Lagos, Nigeria, in March 2009. The samples of *C. equisetifolia* and *F. elastica* were identified by Messrs. Ugbuoga and Shosanya at the Herbarium Headquarters, Forestry Research Institute of Nigeria (FRIN), Ibadan, where voucher specimens (FHI 107885 for *C. equisetifolia* and FHI 102907 for *F. elastica*), were deposited. *E. torreliana* was identified by Mr. Ogunduyile of the Herbarium, Department of Botany and Microbiology, University of Ibadan, Nigeria, where voucher specimen (UIH 222244) was kept for future reference.

2.2. Isolation of the volatile oils

Aliquots of air-dried and pulverised samples were hydrodistilled in an all glass Cleve-enger-type apparatus for 3 h according to established procedure (British Pharmacopoeia, 1990). Aliquots of leaves (1.0 kg) and fruits (0.40 kg) of *C. equisetifolia* afforded pale yellow oils at yields of 0.09 and 0.10% (v/w), respectively. In addition, 0.45 and 0.95 kg of *E. torreliana* and *F. elastica* afforded colourless and pale yellow oils, respectively, at yields of 0.34 and 0.11% (v/w).

2.3. Gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS)

GC analysis was accomplished with an HP-5890 Series II instrument equipped with HP-Wax and HP-5 capillary columns (both 30 m × 0.25 mm, 0.25 μm film thickness), with the following temperature programme: 60 °C for 10 min, rising at 5 °C/min to 220 °C. Both injector and detector temperatures were maintained at 250 °C; carrier gas nitrogen (2 mL/min); detector, FID; split ratio 1:30. The volume injected was 0.5 μL. The identification of the components was performed by comparison of their retention times with those of pure authentic samples and by means of their linear retention indices (LRI on HP-5 column) relative to the series of n-hydrocarbons. The relative proportions of the oil constituents were percentages obtained (% area) by FID peak-area normalisation without the use of response factor.

Gas chromatography–electron ionisation mass spectrometry analysis was performed with a Varian CP-3800 gas-chromatograph equipped with a HP-5 capillary column (30 m × 0.25 mm; 0.25 μm film thickness), with the same temperature programme, injector and detector temperatures, and carrier gas conditions. The relative proportions of the oil constituents were calculated by comparing their peak areas with those of pure authentic samples.
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 to 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/s.

Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear indices relative to the series of n-hydrocarbons, and on computer matching against commercially available spectral (Adams, 2005). Further identifications were also made possible by the use of self constructed spectral library built up from pure substances and components of known oils and MS literature data (Davies, 1990; Jennings and Shibamoto, 1980; Massada, 1975). Moreover, the molecular weights of all the identified substances were confirmed by gas chromatography–chemical ionisation mass spectrometry, using methanol as CI ionising gas.

### 3. Results and discussion

Table 1 lists the compounds identified in the leaf and fruit oils of *C. equisetifolia*. Seventy-six compounds comprising of monoterpane hydrocarbons (29.3%), oxygenated monoterpenoids (16.2%), sesquiterpene hydrocarbons (2.7%), oxygenated derivatives (1.0%), aliphatic (40.6%) and non-terpenoid (7.2%) compounds were observed in the leaf oils. The major compounds were pentadecanal (32.0%) and 1,8-cineole (13.1%). Significant quantities of α-phellandrene (7.0%), apiole (7.2%) and α-terpinene (6.9%) were present. The fruit oil was devoid of sesquiterpene hydrocarbon compounds. The main constituents were caryophyllene-oxide (11.7%), trans-linalool oxide (11.5%), 1,8-cineole (9.7%), α-terpinene (8.8%) and α-pinene (8.5%). All the eleven compounds identified in the oil occurred at levels between 3.4 and 11.7%. Both caryophyllene-oxide and trans-linalool oxide were absent in the leaf oil. The authors are not aware of any literature report on the constituents of the essential oil of the plant or of any Casuarina species and as such the present study may represent the first of its kind.

Monoterpenes (85.6%) and sesquiterpenes (12.5%), typical of *Eucalyptus* were the dominant classes of compounds among the forty-five constituents identified from the oil of *E. toreliana*. The aliphatic compounds made up 1.4% of the total oil content. 1,8-Cineole (39.4%) and α-pinene (10.7%) constituted sizeable proportion of the oil content (Table 2). Other noteworthy compounds were sabine (5.9%), caryophyllene-oxide (4.7%) and α-pinene (4.2%). In a previous report (Chalchat et al., 2000), 1,8-cineole, α-pinene, β-pinene and limonene were identified as

### Table 1

| Constituents       | LRI  | Percentage (%) |
|--------------------|------|----------------|
| **Leaves**         |      |                |
| trans-nerolidol    | 1566 | 0.1            |
| spathulenol        | 1578 | 4.4            |
| caryophyllene-oxide| 1583 | 11.7           |
| guaial             | 1595 | 6.4            |
| hexadecane         | 1600 | tr             |
| tetradecanal       | 1613 | 4.7            |
| γ-cadinol          | 1642 | tr             |
| α-cadinol          | 1655 | tr             |
| cadalene           | 1676 | tr             |
| apiole             | 1680 | 7.2            |
| heptadecane        | 1700 | tr             |
| pentadecanal       | 1719 | 32.0           |
| octadecane         | 1800 | 0.2            |
| hexahydrofarnesylacetone | 1848 | 0.9 |
| **Fruits**         |      |                |
| trans-nerolidol    | 1566 | 0.1            |
| spathulenol        | 1578 | 4.4            |
| caryophyllene-oxide| 1583 | 11.7           |
| guaial             | 1595 | 6.4            |
| Total identified   |      | 97.3%          |

Linear retention indices on HP-5 capillary column; tr, trace amount <0.1%.

### Table 2

| Constituents       | LRI  | Percentage (%) |
|--------------------|------|----------------|
| α-thujene          | 931  | tr             |
| α-pinene           | 939  | 4.2            |
| camphene           | 953  | tr             |
| sabine             | 976  | 5.9            |
| β-pinene           | 980  | 2.9            |
| myrcene            | 991  | 0.8            |
| α-phellandrene     | 1008 | 1.0            |
| β-terpinene        | 1021 | 0.8            |
| p-cymene           | 1029 | 1.0            |
| limonene           | 1034 | 2.0            |
| 1,8-cineole        | 1037 | 39.4           |
| α-terpinene        | 1064 | 0.6            |
| cis-sabinene hydrate | 1073 | tr             |
| dihydro myrcenol   | 1074 | 0.8            |
| terpinolene        | 1091 | tr             |
| linalool           | 1103 | 2.2            |
| trans-pinocarveol  | 1139 | tr             |
| borneol            | 1170 | 1.3            |
| δ-terpineol        | 1172 | tr             |
| 4-terpineol        | 1181 | 2.7            |
| α-terpineol        | 1193 | 3.9            |
| isobornyl acetate  | 1290 | tr             |
| α-terpinyl acetate | 1355 | 10.7           |
| neryl acetate      | 1370 | tr             |
| β-bourbonene       | 1385 | tr             |
| β-elemene          | 1394 | tr             |
| n-tetradecane      | 1400 | tr             |
| methyl eugenol     | 1409 | 1.6            |
| β-caryophyllene    | 1421 | 3.4            |
| trans-α-bergamotene| 1439 | tr             |
| (E)-isoegenol      | 1451 | tr             |
| α-humulene         | 1458 | 0.7            |
| germacren D        | 1483 | 1.6            |
| (E)-β-ionone       | 1487 | tr             |
| (E)-methyl isoegenol| 1495 | 2.4 |
| α-bulnesene        | 1506 | 0.5            |
| trans-nerolidol    | 1567 | tr             |
| spathulenol        | 1578 | 0.7            |
| caryophyllene-oxide| 1583 | 4.7            |
| n-hexadecane       | 1600 | tr             |
| eremoligenol       | 1631 | tr             |
| γ-eudesmol         | 1635 | tr             |
| β-eudesmol         | 1651 | tr             |
| pentadecanal       | 1719 | 1.4            |
| 14-hydroxy-α-muurolene | 1780 | 0.9 |
| **Total**          |      | 99.5%          |

Linear retention indices on HP-5 capillary column; tr, trace amount <0.1%.
the dominant constituents of the oil of *E. torelliana*. In addition, an earlier report (Jimoh et al., 2005) characterised by only GC–MS revealed the abundance of α-pine (21.7%), β-pine (10.3%), β-copaene (16.8%) and 1,8-cineole (32.8%) as constituents identified quantitative importance. The predominance of terpene compounds in the essential oil is typical of *Eucalyptus* species with concurrent variations in the medicinal properties of the oils (Ogunwande, 2001). 1,8-Cineole has been described as the most frequent major compound occurring in *Eucalyptus* oils (Chalchat et al., 2000; Ogunwande, 2001; Ogunwande et al., 2003; Ogunwande et al., 2005).

Aliphatic compounds (52.0%) occurred in highest proportions in the volatile oil of *F. elastica* (Table 3). Monoterpenes (32.6%) and oxygenated sesquiterpenes (11.0%) were also prominent. The main constituents include 6,10,14-trimethyl-2-pentadecanone (25.9%), geranyl acetone (9.9%), heneicosene (8.4%) and 1,8-cineole (8.2%). The other compounds of note were pentadecanal (6.1%), *trans*-nerolidol (4.2%), (E)-β-ionone (3.9%) and heptadecane (3.3%). The volatile oil composition of this plant has not been a subject of literature discussion. Previous studies on Nigerian grown *Ficus* species have revealed the abundance of 1,8-cineole (13.8%), (E)-phytol (13.7%) and p-cymene (11.4%) in *Ficus exasperata* (Sonibare et al., 2006); β-caryophyllene (37.0%), ethyl octanoate (14.9%) and methyl octanoate (8.3%) in *Ficus mucosa* (Ogunwande et al., 2009); acorenone (20.7%) and phytol (16.2%) in *Ficus lutea*, with *Ficus polist* consisting mainly of phytol (23.3%) and 6, 10, 14-trimethyl-2-pentadecanone (15.0%) and *Ficus thonninii* rich in 6, 10, 14-trimethyl-2-pentadecanone (18.8%) and phytol (14.7%) (Ogunwande et al., 2008). Both 6,10,14-trimethyl-2-pentadecanone and phytol have been described as marker components of the oils of Nigerian grown *Ficus* species (Ogunwande et al., 2008). In addition to 6,10,14-trimethyl-2-pentadecanone and 1,8-cineole, other constituents such as acorenone, (E)-6, 10-dimethyl-5, 9-undecadien-2-one, ethyl octanoate, methyl octanoate and phytol that are characteristic components of other Nigerian *Ficus* species, are conspicuously absent in *F. elastica*.

**Acknowledgement**

The authors are grateful to Prof. Jirovetz, of Vienna University, Austria, for literature information.

**References**

Adams, R.P., 2005. Identification of Essential Oils by gas Chromatography– Quadrupole Mass Spectroscopy. Allured Pub. Corp, Carol Stream, IL, USA.

Aher, A.N., Pal, S.C., Yadav, S.K., Patil, U.K., Bhattacharya, S., 2009. Antioxidant activity of isolated phytocomponents from *Casuarina equisetifolia* Frost (Casuarinaceae). Journal of Plant Science 4, 15–20.

British Pharmacopoeia, 1990. H.M. Stationery Office, P.A109.

Busari, A.O., 2001. Compendium of Plants Growing in Ogbomoso Area. Bayowa Press, Nigeria, p. 102.

Chalchat, J.-C., Gary, P., Sidibe, R.P., Harama, M., 2000. Aromatic plants of Mali (V): chemical composition of essential oils of four *Eucalyptus* species implanted in Mali: *E. camaldulensis*, *E. citriodora*, *E. torelliana* and *E. tereticornis*. Journal of Essential Oil Research 12, 698–701.

Davies, N.W., 1990. Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20 M phases. Journal of Chromatography 503, 1–24.

Jennings, W., Shibamoto, S., 1980. Qualitative Analysis of Flavour Volatiles by Gas Capillary Chromatography. Academic Press, New York.

| Constituents | LRI | Percentage (%) |
|--------------|-----|----------------|
| (E)-2-hexenal | 854 | 0.2 |
| 2-heptanone | 889 | tr |
| α-thujene | 931 | tr |
| α-pine | 939 | 1.2 |
| camphene | 953 | tr |
| benzaldehyde | 961 | 0.9 |
| sabinene | 976 | 1.0 |
| β-pine | 980 | 0.5 |
| 6-methyl-5-hepten-2-one | 985 | tr |
| 6-2-carene | 1001 | tr |
| α-phellandrene | 1007 | 1.4 |
| 6-terpinene | 1020 | 1.1 |
| p-cymene | 1028 | 1.1 |
| limonene | 1033 | 1.7 |
| 1,8-cineole | 1035 | 8.2 |
| benzene acetaldehyde | 1045 | tr |
| α-terpinene | 1063 | tr |
| acetophenone | 1067 | tr |
| nonanal | 1104 | 1.1 |
| naphthalene | 1181 | 1.9 |
| safranal | 1201 | tr |
| carvenone | 1252 | tr |
| (E)-2-decanal | 1261 | tr |
| 2-undecanone | 1293 | tr |
| β-caryophyllene | 1420 | tr |
| (E)-α-ionone | 1428 | 2.4 |
| cis-α-amaninol | 1438 | 0.2 |
| geranyl acetone | 1455 | 9.9 |
| (E)-β-ionone | 1486 | 3.9 |
| butylated hydroxy anisole | 1489 | tr |
| 2-tridecanone | 1496 | tr |
| pentadecane | 1500 | 1.0 |
| α-calcarene | 1543 | tr |
| cis-sesquisabinene hydrate | 1546 | tr |
| trans-nerolidol | 1566 | 0.8 |
| caryophyllene-oxide | 1582 | 4.2 |
| hexadecane | 1600 | 1.0 |
| humulene epoxide II | 1608 | tr |
| tetradecanl | 1613 | 1.4 |
| epoxy-αlloaromadendrene | 1641 | tr |
| selin-11-en-4-ol | 1654 | 1.6 |
| neo-intermedel | 1660 | tr |
| 5-iso cedranol | 1674 | 1.9 |
| acorenone | 1685 | 1.5 |
| heptadecane | 1700 | 3.3 |
| pentadecanl | 1719 | 6.1 |
| drimenol | 1759 | 1.0 |
| octadecane | 1800 | 1.1 |
| khusinol acetate | 1824 | tr |
| 6,10,14-trimethyl-2-pentadecanone | 1848 | 25.9 |
| nonadecane | 1900 | 1.1 |
| eicosane | 2000 | 0.9 |
| heneicosene | 2096 | 8.4 |
| heneicosane | 2100 | 0.2 |
| docosane | 2200 | 0.3 |
| Total | | 98.4% |

Linear retention indices on HP-5 capillary column; tr, trace amount <0.1%.
Jimoh, S.T., Ogunwande, I.A., Olawore, N.O., Walker, T.M., Schmidt, J.M., Setzer, W.N., Olaleye, N.O., Aboaba, S.A., 2005. *In vitro* cytotoxicity activities of essential oils of *Eucalyptus torreliana* F.v. Muell (leaves and fruits). Journal of Essential Oil-Bearing Plants 8, 110–119.

Li-Hua, Z., Gong-Fu, Y., Yi-Ming, L., Hai-Chao, Z., Qi, Z., 2009. Seasonal changes in tannin and nitrogen contents of *Casuarina equisetifolia* branchlets. Journal of Zhejiang University Science B 10, 103–111.

Massada, Y., 1975. Analysis of Essential Oils by Gas Chromatography and Mass Spectrometry. John Wiley & Sons, New York.

Nash, R.J., Thomas, P.I., Waigh, R.D., Fleet, G.W.J., Wormald, M.R., De Lilley, P.M.Q., Watkin, D.J., 1994. Casuarine: a very highly oxygenated pyrrolizidine alkaloid. Tetrahedron Letters 35, 7849–7852.

Ogunwande, I.A., 2001. Compositional patterns of the essential oils from the leaves of *Eucalyptus*, *Callitris*, *Thuja* and *Melaleuca* species growing in Nigeria, PhD Thesis, Department of Chemistry, Faculty of Science, University of Ibadan, p. 103.

Ogunwande, I.A., Olawore, N.O., Adeleke, K.A., Ekundayo, O., Koenig, W.A., 2003. Chemical composition of the essential oils from the leaves of three *Eucalyptus* species growing in Nigeria. Journal of Essential Oil Research 15, 297–301.

Ogunwande, I.A., Olawore, N.O., Adeleke, K.A., Ekundayo, O., 2005. Volatile constituents from the leaves of *Eucalyptus cloeziana* F. Muell and *Eucalyptus propinquua* Deane and Maiden from Nigeria. Flavour and Fragrance Journal 20, 637–639.

Ogunwande, I.A., Sonibare, M.A., Thang, T.D., Dung, N.X., Soladoye, M.O., Morohunfola, O.O., 2008. Comparative analysis of the oils of three *Ficus* species from Nigeria. Journal of Essential Oil Research 20, 387–389.

Ogunwande, I.A., Saroglou, V., Skaltsa, E., Ogunbinu, A.O., Kubmawara, D., 2009. Constituents of some essential oil-bearing plants from Nigeria. Journal of Essential Oil Research 21, 61–66.

Parekh, J., Jadeja, D., Chanda, S., 2005. Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. Turkish Journal of Biology 29, 203–210.

Pinyopusarerk, P., House, A.P.N., 1993. *Casuarina*: An Annotated Bibliography of *C. equisetifolia*, *C. junghuhniana* and *C. oligodon*. International Centre for Research in Agroforestry, Nairobi, Kenya, p. 296.

Sonibare, M.A., Ogunwande, I.A., Walker, T.M., Setzer, W.N., Soladoye, M.O., Essien, E., 2006. Volatile constituents of *Ficus exasperata* Vahl leaves. Natural Product Communications 1, 763–765.

Takahashi, H., Iuchi, M., Fujita, Y., Minami, H., Fukuyama, Y., 1999. Coumaroyl triterpenes from *Casuarina equisetifolia*. Phytochemistry 51, 543–550.

Tani, C., Sasakawa, H., 2003. Proline accumulates in *Casuarina equisetifolia* seedlings under salt stress. Soil Science and Plant Nutrition 52, 21–25.

Edited by AM Viljoen