ORIGINAL ARTICLE

Comparative analysis of essential oils from the leaf, fruit and stem bark of *Harungana madagascariensis*

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A comparative analysis of hydro-distilled essential oils of the leaf, stem bark and fruit of *Harungana madagascariensis* was carried out by gas chromatography-mass spectroscopy (GC-MS) for the first time. Both qualitative and quantitative differences existed in the composition of the three oils which comprised mainly sesquiterpene hydrocarbons (66.8-69.6%). β-caryophyllene (32.4% and 18.4% respectively, for leaf and fruit oils only), α-humulene (10.4%, 9.8% and 7.3% respectively for leaf, stem bark and fruit oils), germacrene D (8.7% for leaf oil only), and α-farnesene (37.4% and 10.4% respectively, for stem bark and fruit oils only) were the predominant constituents.

Key words: Essential oil, gas chromatography-mass spectroscopy, *Harungana madagascariensis*, Hypericaceae, sesquiterpenes

INTRODUCTION

Harungana madagascariensis Lam. ex Poir. (Hypericaceae) is a shrub up to 6-10 m high. It is a sun-loving secondary species widely spread in all inter-tropical Africa and Madagascar.[1] The fruits are small, drupe-like, yellow or red at maturity. In the African traditional medicine, the leaves and stem bark are used in treating anemia, while the stem bark is also an ingredient of antimalarial herbal medicines.[1] The orange red resinous sap from the bark has been associated with wound healing properties.[2] In one instance, leaf aqueous extract of the plant was reported to show antimicrobial activity[3] while a diminution of oral bacteria was also reported by Moulari *et al*.[4] in an *in vitro* study. However, in a separate study by Moulari *et al.*,[5] a flavanone, astilbin was isolated as the antibacterial principle. Other biological activities including antioxidant;[6] antiamoebic, antidiarrhoeal and spasmolytic;[7] as well as antiplasmodial and anti-trichomonal[8] activities have been also documented.

In spite of previous phytochemistry on this plant, there has been no report on the essential oil constituents. Therefore, in line with our search for plants with medicinal and olfactive purposes, we considered it necessary to investigate the constituents of the leaf, fruit and stem bark essential oils from *H. madagascariensis* growing in Nigeria.

MATERIALS AND METHODS

Plant Material and Extraction of Oil

Fresh leaves, fruits and stem bark of *H. madagascariensis* were obtained from trees growing in the neighbourhood of the Obafemi Awolowo University campus (OAU, Ile-Ife), and authenticated at the Forestry Research Institute of Nigeria, Ibadan (FRIN) herbarium (voucher no. FHI 95563). Essential oils were obtained separately from the leaf (yellow, 0.01%), fruit (yellow, 0.004%) and chopped stem bark (dark yellow, 0.03%) by hydro-distillation in a Clevenger-type apparatus. The oils were stored in separate screw-capped vials and refrigerated at 4°C until needed.

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

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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,[9] 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

Essential oils were obtained from the leaf, stem bark and fruits of *H. madagascariensis* with 0.1%, 0.03%, and 0.004% yields, respectively. The constituents of these essential oils analysed by gas chromatography (GC) and gas chromatography-mass spectroscopy (GC/MS) are presented in Table 1. Sesquiterpene hydrocarbons constituted the chief class of terpenoids in all the three oils in similar proportions (66.8-69.6%). In all, 29 compounds each in the leaf and stem bark oils, and 42 compounds in fruit oil accounting for 93.2%, 91.6% and 85.6%, respectively, for leaf, stem bark and fruit total oils were detected. In addition, monoterpene hydrocarbons (11.5% and 23% for leaf and stem bark oils,

Table 1: Composition of leaf, fruit and stem bark essential oils of *Harungana madagascariensis*

Compound	RI[a]	%[b]		
		Leaf	Stem bark	Fruit
Thujenel	892	-	t	-
α-Pinene	897	0.2	19.7	0.1
Camphene	908	-	0.1	-
β-Pinene	934	-	1.9	t
β-Myrcene	950	-	0.2	-
α-Terpinene	976	-	0.2	-
o-Ocimene	984	-	0.1	-
Limonene	987	-	0.5	-
cis-Ocimene	1000	-	-	0.2
trans-Ocimene	1011	-	0.1	4.1
β-Ocimene	1012	11.3	-	-
γ-Terpinene	1021	-	0.1	-
α-Terpinolene	1053	-	0.1	-
Linalool	1069	2.2	-	0.9
Fenchol	1078	-	0.1	-
exo-Methyl-camphenilol	1116	-	t	-
Borneol	1138	-	t	-
4-Terpineol	1155	-	0.1	-
α-Terpineol	1169	-	0.4	-
Decanal	1189	-	-	3.1
Vitispirane	1272	-	-	0.3
Tridecane	1305	-	-	0.1
Longipinene	1355	-	0.1	-
α-Cubebene	1359	0.2	-	0.4
Cyclosativene	1374	-	t	0.1
Yiangene	1380	-	-	0.1
α-Copaene	1388	1.4	0.2	2.6
β-Bourbonene	1398	0.3	-	0.7
β-Elemene	1406	2.2	1.6	2.9
Ciperene	1412	0.3	-	-
α-Gurjunene	1425	0.2	-	-
trans-Carophyllene	1434	-	8.0	-
β-Caryophyllene	1437	32.4	-	18.4
α-*trans*-Bergamotene	1455	0.5	-	0.6
α-Himachalene	1463	-	-	0.1
Aromadendrene	1464	-	0.1	-
α-Humulene	1472	10.4	9.8	7.3
β-Farnesene	1478	-	0.3	-
Allo-aromandrene	1479	0.6	-	0.8
β-Acoradiene	1483	-	0.4	2.1
γ-Muurolene	1498	1.1	-	2.0
Germacrene D	1502	8.7	-	4.4
β-Selinene	1507	1.3	4.0	3.0
Valencene	1510	-	-	0.3
α-Selinene	1515	-	4.0	4.4
Viridiflorene	1517	2.6	-	-
Epizonarene	1518	-	-	1.4
8-Isopropenyl-1,5-dimethyl-cyclodeca-1,5-diene	1522	-	-	1.2
α-Muurolene	1523	0.5	-	-
Germacrene A	1526	1.6	-	-
Azulene 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethyl)-(1S(1α,7α, 8aβ))	1526	-	0.9	-
α-Farnesene		-	37.4	10.4
7-epi-α-Selinene	1539	2.1	-	-
Δ-Cadinene	1547	2.0	0.4	2.8
α-Cadinene	1558	-	-	2.5
Germacrene B	1579	0.7	-	0.7
Spathulenol	1597	-	-	0.1
Caryophyllene oxide	1604	3.8	0.6	1.6
Viridiflorol	1614	0.5	-	0.6
β-Costol	1643	-	-	0.4
Naphtalene,1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	1651	0.5	-	0.5
γ-Muurolol	1661	-	-	1.1
epi-α-Muurolol	1665	1.4	-	-
Torreyol		-	-	0.4
α-Muurolol	1669	0.5	-	-
α α-Cadinol	1676	3.6	-	2.3
Heptadecadiene	1693	-	-	0.5
Juniper camphor	1712	-	t	-
Nonadecane	1905	-	-	0.1
Palmitic acid	1962	-	0.1	-
Ethyl palmitate	1983	-	0.1	-
Heneicosane	2070	-	-	0.1
Ethyl oleate	2120	-	t	-
Tricosane	2222	-	-	t
Pentacosane	2549	-	-	0.5
Eicosane	2554	0.1	t	-
Monoterpene hydrocarbons		11.5	23.0	4.5
Oxygenated monoterpenes		2.2	0.6	0.9
Sesquiterpene hydrocarbons		69.6	66.8	69.3
Oxygenated sesquiterpenes		9.8	0.7	6.3
Others		0.1	0.4	4.6
Total identified		93.2	91.6	85.6

[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 %)

respectively) and oxygenated sesquiterpenes (6.3% for fruit oil) were also detected. Although Hypericaceae is an uncommon source of essential oils, all the three oils investigated in this case exhibited both qualitative and quantitative differences in composition. In the sesquiterpene category, β-caryophyllene (32.4% and 18.4%, respectively, for leaf and fruit oils only), α-humulene (10.4%, 9.8% and 7.3%, respectively, for leaf, stem bark and fruit oils), germacrene D (8.7% for leaf oil only), α-farnesene (37.4% and 10.4%, respectively, for stem bark and fruit oils only) were the predominant constituents. Elufioye and Agbedahunsi[10] have reported antimalarial activities for sesquiterpene compounds in *Tithonia diversifolia* and *Crossopteryx febrifuga*. In other studies, the sesquiterpenes have been shown to exhibit antioxidant[11] and insecticidal[12,13] properties. Beta-ocimene (11.3%) and α-pinene (19.7%) represented the chief monoterpene hydrocarbons for the leaf and stem bark oils, respectively. Sesqiterpenes have also been reported as dominant constituents in typical plant oils of the Meliaceae,[11] Annonaceae[12] and Lamiaceae[13] which have contributed to their various biological properties. In addition, antiulcer and anti-inflammatory activities which are reputed for sesquiterpene-rich leaf oil of *Casearia sylvestris*[14] could also be predicted for *H. madagascariensis*.

Constituents common to the three oils included α-pinene, α-copaene, β-elemene, α-humulene, β-selinene, δ-cadinene and caryophyllene oxide in different quantities. Both α-pinene and β-caryophyllene are useful as antimicrobial, anesthetic and anti-inflammatory agents,[15] while germacrene D exhibits insecticidal properties.[16] Among the less frequently occurring monoterpenes, linalool (2.2% in leaf), β-pinene (1.9% in the stem bark) and *trans*-ocimene (4.1% in fruit) were noticed. The minor sesquiterpenes in 1.4-4.4% yields found in the oils were α-copaene, β-elemene, viridiflorene, germacrene A, 7-epi-α-selinene, δ-cadinene, caryophyllene oxide, epi-α-muurolol and α-α-cadinol for leaf; β-pinene, β-elemene, β-selinene, α-selinene for stem bark; and *trans*-ocimene, α-copaene, β-elemene, β-acoradiene, γ-muurolene, germacrene D, β-selinene, α-selinene, epizonarene, α-and δ-cadinene, caryophyllene oxide and α-α-cadinol for fruit. Non-terpene components in the aliphatic category (0.1-3.1%) occurring largely in the fruit oil, and fatty acid derivatives (0.1%) in the stem bark oil were also present.

CONCLUSION

In this study, the constituents of the essential oils from various morphological parts of *H. madagascariensis* have been reported for the first time. The plant is a rich source of sesquiterpenes with both qualitative and quantitative differences for the plant parts, which may possibly account for the various reported biological activities of the plant.

REFERENCES

1. Adjanohoun E, Ahiyi MRA, Ake-Assi L, Dramane K, Elewude JA, Fadoju SO, *et al*. Traditional Medicine and Pharmacopoeia. Contribution to Ethnobotanical and Floristic Studies in Western Nigeria, Lagos: OAU/STRC Publications; 1991. p. 149.
2. Dalziel JM. The Useful Plants of West Tropical Africa. London: Crown Agents; 1937.
3. Okoli AS, Okeke MI, Iroegbu CU, Ebo PU. Antibacterial activity of *Harungana madagascariensis* leaf extracts. Phytother Res 2002;16:174-9.
4. Moulari B, Lboutounne H, Chaumont JP, Guillaume Y, Millet J, Pellequer Y. Potentiation of the bactericidal activity of *Harungana madagascariensis* Lam. ex Poir. (Hypericaceae) leaf extract against oral bacteria using poly (D, L-lactide-co-glycolide) nanoparticles: *In vitro* study. Acta Odontol Scand 2006;64:153-8.
5. Moulari B, Pellequer Y, Lboutounne H, Girard C, Chaumont JP, Millet J, *et al.* Isolation and *in vitro* antibacterial activity of astilbin, the bioactive flavanone from the leaves of *Harungana madagascariensis* Lam. ex Poir. (Hypericaceae). J Ethnopharmacol 2006;106:272-8.
6. Kouam SF, Ngadjui BT, Krohn K, Wafo P, Ajaz A, Choudhary MI. Prenylated anthronoid antioxidants from the stem bark of *Harungana madagascariensis*. Phytochemistry 2005;66:1174-9.
7. Tona L, Kambu K, Ngimbi N, Mesia K, Penge O, Lusakibanza M, *et al.* Antiamoebic and spasmolytic activities of extracts from some antidiarrhoeal traditional preparations used in Kinshasa, Congo. Phytomedicine 2000;7:31-8.
8. Iwalewa EO, Omisore NO, Adewunmi CO, Gbolade AA, Ademowo OG, Nneji C, *et al.* Anti-protozoan activities of *Harungana madagascariensis* stem bark extract on trichomonads and malaria. J Ethnopharmacol 2008;117:507-11.
9. Adams RP. Identification of Essential Oil Components by Gas Chromatographic/Quadrupole Mass Spectrometry. USA: Carol Stream, IL: Allured Publ. Corp; 2001.
10. Elufioye TO, Agbedahunsi JM. Antimalarial activities of *Tithonia diversifolia* (Asteraceae) and *Crossopteryx febrifuga* (Rubiaceae) on mice *in vivo*. J Ethnopharmacol 2004;93:167-71.
11. Boyom FF, Fotio D, Zollo A, Agananiet H, Menut C, Bessiere JM. Aromatic plants of Tropical Central Africa. Part XLIV. Volatile component from *Pseudocedrela kotschyi* (Schweinf.) Harms growing in Cameroon. Flav Fragr J 2003;18:296-8.
12. Ee GCL, Seow BT, Neoh BK, Taufiq-Yap YH, Sukari MA, Rahmani M. Larvicidal terpenoids from *Polyalthia jenkensii* and *Polyalthia sumatrana*. J Trop Med Plants 2001;2:27-30.
13. Sundufu AJ, Shoushan H. Chemical composition of the essential oil of *Lantana camara* L. occurring in China. Flav Fragr J 2004;19:229-32.
14. Esteves I, Souza IR, Rodrigues M, Cardoso LGV, Silva Santos LS, Sertie JAA, *et al.* Gastric antiulcer and anti-inflammatory activities of the essential oil from *Casearia sylvestris.* Sw. J Ethnopharmacol 2005;101:191-6.
15. Hmamouchi M, Hmamouchi J, Zouhdi M. Chemical and antimicrobial properties of essential oils of five Moroccan Pinaceae. In: Lawrence BM, editor. The antimicrobial/biological activity of essential oils. Carol Stream: IL Allured; 2005. p. 368-72.
16. Mozuraitis R, Stranden M, Ramirez MI, Borg-Karlson A.-K, Mustaparta H. (–)-Germacrene D increases attraction and oviposition by the tobacco budworm moth *Heliothis virescens*. Chem Senses 2002;27:505-9.

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