

Volatiles from *Plicanthus hirtellus* (F. Weber) R.M. Schust. and *Radula boryana* (F. Weber) Nees (Hepaticae) grown in São Tomé e Príncipe Archipelago[†]

A. Cristina Figueiredo,^{a*} César Garcia,^{b,d} Manuela Sim-Sim,^{b,c} Cecília Sérgio,^{b,c} Luis G. Pedro^a and José G. Barroso^a

ABSTRACT: The main goal of this study was to isolate and characterize the volatiles from *Plicanthus hirtellus* and *Radula boryana* grown in the African Archipelago of São Tomé e Príncipe. The volatiles isolated by distillation–extraction from *Plicanthus hirtellus* and *Radula boryana* were analysed by GC and GC–MS. *Plicanthus hirtellus* volatiles were dominated by anastreptene (13%) and spathulenol (14%). *Radula boryana* volatiles were characterized by high amounts of *p*-cymene (17%) along with other monoterpene hydrocarbons and by the unusual presence of the oxygen-containing monoterpenes thymol and carvacrol (1% each). Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: *Plicanthus hirtellus*; *Radula boryana*; São Tomé e Príncipe Archipelago; bryophytes; liverworts; volatiles; GC; GC–MS

Introduction

São Tomé e Príncipe is an Archipelago located in the Gulf of Guinea, comprising two islands, São Tomé, the largest one, where the highest mountain is also located (2024 m), and Príncipe Island. The climate is tropical, hot and very humid, with small thermal amplitude. The natural forest of São Tomé e Príncipe was considered one of the most important in Africa, due to the high plant diversity, including many endemic plant species, namely bryophytes, and a rich fauna, especially birds.^[1–3]

Plicanthus hirtellus (F. Weber) R. M. Schuster [= *Jungermannia hirtella* F. Weber (Basionym), *Chandonanthus hirtellus* (Web.) Mitt., *Anthelia hirtella* (F. Weber) Stephani], is a robust liverwort belonging to the family Jungermanniaceae (Reichenb.), which grows on rocks or tree trunks and branches in full light habitats, occasionally on soil and even in wetter habitats.^[4] *P. hirtellus* has a broad distribution, being widespread in Africa, Asia, Australasia and Oceania.^[5]

Radula boryana (F. Weber) Nees [= *Jungermannia boryana* F. Weber (Basionym)] is a liverwort belonging to the family Radulaceae (Dumort.) Müll. Frib., which grows on boles and large branches of forest trees in humid habitats.^[5] *R. boryana* is abundant in many areas of tropical East Africa, frequently above 1100 m.^[5]

Previous chemical studies on *P. hirtellus* have used organic solvent extracts. Limonene, α -bisabolene and anastreptene were identified in *Chandonanthus hirtellus*, the latter being the main component in the ether extract of this species.^[6] The biflavone dicranolomin, luteolin and vanillic acid-4-*O*-neohesperidoside were isolated from the aqueous methanolic extract of *Chandonanthus hirtellus* ssp. *giganteus* collected in Zaire.^[7] Two isomeric diterpenoids of cembrane-type, chandonanthone and

isochandonanthone, were identified in *Chandonanthus hirtellus* collected in China.^[8] In addition to known terpenoids, Wang *et al.*^[9] identified a new bicyclogermacrene sesquiterpenoid, two new cembrane diterpenoids and five new dolabellane diterpenoids in the ether extract of the Malaysian *Chandonanthus hirtellus*.

Pulegone, *trans*- β -bergamotene, β -gurjunene, δ -elemene, bicyclogermacrene, cuparene, *trans*- β -farnesene, β -guaiene, β -himachalene and β -maaliene have been reported by *Spörle* from *Radula boryana*^[in 10].

Both species have a reasonable distribution on São Tomé Island, on rocks or on tree trunks, and their ecological affinities

* Correspondence to: A. C. Figueiredo, Universidade de Lisboa, Faculdade de Ciências de Lisboa, Departamento de Biologia Vegetal, Instituto de Biotecnologia e Bioengenharia, Centro de Biotecnologia Vegetal, C2, Campo Grande, 1749-016 Lisboa, Portugal. E-mail: acsf@fc.ul.pt

[†] This paper was originally presented at the 40th International Symposium on Essential Oils held at Savigliano, Italy, 6–9 September 2009, organized by Carlo Bicchi and Patrizia Rubiolo. See also the special issue on the same conference published in *Flavour and Fragrance Journal*, 2010, pp. 111–196.

^a Universidade de Lisboa, Faculdade de Ciências de Lisboa, DBV, Instituto de Biotecnologia e Bioengenharia, Centro de Biotecnologia Vegetal, C2, Campo Grande, 1749-016 Lisboa, Portugal

^b Universidade de Lisboa, Museu Nacional de História Natural, Jardim Botânico/CBA, Rua da Escola Politécnica 58, 1250-102 Lisboa, Portugal

^c Universidade de Lisboa, Faculdade de Ciências de Lisboa, DBV, Centro de Biologia Ambiental, C2, Campo Grande, 1749-016 Lisboa, Portugal

^d Universidad Autónoma de Madrid, Departamento de Biología (Botánica), C/ Darwin 2, E-28049 Madrid, Spain

Table 1. Percentage composition of the volatiles isolated by distillation-extraction from *Plicanthus hirtellus* and *Radula boryana* grown in São Tomé and Príncipe

Components	RI ^[16]		RI ^[17]		RI ^[18]		<i>Plicanthus hirtellus</i>		<i>Radula boryana</i>	
	DB-1	OV-101	DB-1	OV-101	OV-101	OV-101	[a]	[b]	[c]	[d]
Benzaldehyde	927	947	926	947	943				t	t
α -Pinene	930	940 [942]		942	926				t	t
1-Octen-3-ol	961	968	968				5.2	4.3		
3-Octanol	974	971	979				t	t		
α -Terpinene	1002		1008		1020				t	t
<i>p</i> -Cymene	1003	1015 [1020]	1010 [1014]	1020	1025				16.6	17.0
1,8-Cineole	1005	1028 [1028]		1027	1015		1.8	1.5		
Limonene	1009	1030	1020 [1024]	1030	1013 [1022]		t	t	8.2	8.4
γ -Terpinene	1035	1057 [1257]	1048 [1051]		1058		t	t	2.6	2.7
2-Methyl decane	1058								2.0	2.0
2,5-Dimethyl styrene	1059				1101				2.1	2.2
Terpinolene	1064	1082	1077						3.3	2.6
<i>p</i> -Cymen-8-ol	1148									
Carvone	1206	1228		1228	1210 [1236]		4.2	3.6		
Thymol	1275	1287		1287					1.4	1.2
Carvacrol	1286			1297					1.4	1.2
Africanene*	1287								1.9	2.0
δ -Elemene	1332				1323		t	t		
Anastreptene*	1377						12.8	14.1		
7- <i>epi</i> - α -Cedrene	1396				1395				3.7	3.8
α -Guirjunene	1400		1407 [1408]						1.2	1.2
Acora-3,5-diene*	1414								8.4	8.6
β -Acoradiene*	1463								7.1	7.3
Bicyclogermacrene	1487		1490		1500		8.2	9.1		
Cuparene	1491								2.7	2.8
β -Bisabolene	1500				1513				2.1	2.1
<i>cis</i> -Calamenene	1505				1549				2.5	2.6
Spathulenol	1551				1602		14.0	11.6		
Globulol	1566		1571				t	t		
UI Pha	1680						6.3	6.9		
Hexadecanoic acid (= palmitic acid)	1908						6.0	6.5	4.7	4.0
Linoleic acid	2125						11.5	12.4		
2,2-Dimethyl-5-hydroxy-7-(2-phenylethyl)-chromene*	2248								7.3	7.5

RI^a, calculated retention index relative to C₉-C₂₃ n-alkanes on the DB-1 column; RI^[16], RI^[17] and RI^[18], literature retention indices on DB-1 or similar phase column (100% dimethyl polysiloxane); in square brackets, additional RI, whenever more than one RI was listed by the same literature reference. t, trace (< 0.05%); UI, unidentified compounds.
*Identification based on mass spectra only.
[a], [c], the percentage composition of the oils was computed by the normalization method from the GC peak areas, calculated as mean values of two injections from each oil, using the response factors reported in the literature.^[12,13]
[b], [d], the percentage composition of the oils was computed by the normalization method from the GC peak areas, calculated as mean values of two injections from each oil, without using response factors.

and amplitudes are currently being investigated. To further typify these species, the aim of this study was to isolate and characterize the volatiles, isolated by distillation–extraction, from *Plicanthus hirtellus* and *Radula boryana* grown in São Tomé e Príncipe.

Materials and Methods

Plant Material

Collective samples of *Plicanthus hirtellus* and *Radula boryana* were collected on São Tomé Island at 1990 m near São Tomé peak and at 1430 m near the Carvalho peak, respectively. Voucher specimens have been deposited in the Herbarium of the University of Lisbon/National History Museum (LISU) under Voucher Nos LISU 234221 and LISU 234222, respectively.

Volatiles Extraction

The volatiles were isolated by distillation–extraction for 3 h using a Likens–Nickerson-type apparatus^[11] with distilled *n*-pentane (50 ml) as the organic solvent. The isolation procedure was run at a distillation rate of 3 ml/min. The oil recovered in pentane was concentrated, at room temperature, under reduced pressure on a rotary evaporator and collected in a vial and concentrated to a minimum volume, again at room temperature, under nitrogen flux. The volatiles were stored at –20°C in the dark until analysis.

Gas Chromatography

Gas chromatographic analyses were performed using a Perkin-Elmer Autosystem XL gas chromatograph (Perkin-Elmer, Shelton, CT, USA) equipped with two flame ionization detectors (FIDs), a data-handling system and a vapourizing injector port, into which two columns of different polarities were installed: a DB-1 fused-silica column (30 m × 0.25 mm i.d., film thickness 0.25 µm; J&W Scientific Inc., Rancho Cordova, CA, USA) and a DB-17HT fused-silica column (30 m × 0.25 mm i.d., film thickness 0.15 µm; J&W Scientific). The oven temperature was programmed from 45°C to 175°C at 3°C/min, subsequently at 15°C/min to 300°C, then held isothermal for 10 min; injector and detector temperatures, 280°C and 300°C, respectively; carrier gas, hydrogen, adjusted to a linear velocity of 30 cm/s. The samples were injected using a split sampling technique, ratio 1:50. The volume of injection was 0.1 µl pentane–oil solution. The percentage composition of the oils was computed by the normalization method from the GC peak areas, calculated as mean values of two injections from each oil, using the response factors reported in the literature^[12,13] (Table 1, [a] and [c]) and without using response factors (Table 1, [b] and [d]).

Gas Chromatography–Mass Spectrometry

The GC–MS unit consisted of a Perkin-Elmer Autosystem XL gas chromatograph equipped with a DB-1 fused-silica column (30 m × 0.25 mm i.d., film thickness 0.25 µm; J&W Scientific) and interfaced with a Perkin-Elmer Turbomass mass spectrometer (software version 4.1, Perkin-Elmer). Injector and oven temperatures were as above; transfer line temperature, 280°C; ion source temperature, 220°C; carrier gas, helium, adjusted to a linear velocity of 30 cm/s; split ratio, 1:40; ionization energy, 70 eV; ionization

current, 60 µA; scan range, 40–300 u; scan time, 1 s. The identity of the components was assigned by comparison of their retention indices, relative to C₈–C₂₄ *n*-alkane indices and GC–MS spectra from a laboratory-made library, constructed based on the analyses of reference oils, laboratory-synthesized components and commercially available standards.

Results and Discussion

Plicanthus hirtellus and *Radula boryana* samples afforded strong-smelling light-yellow oils. The identified oil components are listed in Table 1 in order of their elution on the DB-1 column. For comparison purposes, data reported in Table 1 are given after calculations with ([a], [c]) and without ([b], [d]) response factors.

P. hirtellus volatiles were dominated by anastreptene (13%) and spathulenol (14%). Carvone (4%) and 1,8-cineole (2%) were also detected in this species volatiles. Anastreptene was the main component identified by Asakawa *et al.*^[6] in the ether extract of *Chandonanthus hirtellus* collected in East Malaysia.

Interestingly, *R. boryana* volatiles were characterized by high amounts of *p*-cymene (17%), along with other monoterpene hydrocarbons, and by the unusual presence of the oxygen-containing monoterpenes, thymol and carvacrol. Sesquiterpenes and fatty acids were also present in *R. boryana* volatiles. This composition is markedly different from that usually found in *Radula* spp. organic-solvent extracts and from the volatiles of *Radula* spp. from Portugal.^[14,15]

P. hirtellus and *R. boryana* volatiles were here investigated in detail for the first time. The volatiles composition may support the delimitation of these species, together with ongoing distribution and morphological studies. However, further research, viz. analysis of the volatiles composition from other African *Plicanthus* and *Radula* spp., are required to ascertain the usefulness of this type of compound for systematic studies.

Acknowledgements

Partially funded by FCT under Research Contracts No. POCTI/AFR/58699/2004 and No. SFRH/BPD/22304/2005. The authors are grateful to the São Tomenses, Salvador Pontes, Aurélio Espírito-Santo, Estevão Soares, Francisco Álamo and Mr Lagoas for their help during the field work.

References

1. A. W. Exell. *Catalogue of the Vascular Plants of S. Tomé (with Príncipe and Annobon)*. British Museum: London, **1944**.
2. E. Figueiredo, A. Gascoigne. *Biodivers. Conserv.* **2001**, *10*, 45.
3. BirdLife International 2003. BirdLife's online World Bird Database: the site for bird conservation, version 2.0. BirdLife International: Cambridge, UK: <http://www.birdlife.org> [accessed 9 October 2009].
4. R. M. Schuster. *Nova Hedwigia* **2002**, *74*, 465.
5. M. J. Wigginton. *Scripta Bot. Bellica* **2004**, *30*, 1.
6. Y. Asakawa, X. Lin, K. Kondo, Y. Fukuyama. *Phytochemistry* **1991**, *30*, 4019.
7. L. Kraut, R. Mues. *Z. Naturforsch* **1999**, *54*, 6.
8. H. S. Shy, C. L. Wu, C. Paul, W. A. König, U. J. Ean. *J. Chinese Chem. Soc.* **2002**, *49*, 593.
9. Y. Wang, L. J. Harrison, B. C. Tan. *Tetrahedron* **2009**, *65*, 4035.
10. Y. Asakawa. In *Progress in the Chemistry of Organic Natural Products*, vol. 65, W. Hertz, G. W. Kirby, R. E. Moore, W. Steglich, Ch. Tamm (eds). Springer Verlag: Vienna, **1995**.
11. S. T. Likens, G. B. Nickerson. *Proc. Am. Soc. Brewing Chemists* **1964**, *5*.
12. R. Costa, B. d'A. Zellner, M. L. Crupi, M. R. De Fina, M. R. Valentino, P. Dugo, G. Dugo, L. Mondello. *Flavour Fragr. J.* **2008**, *23*, 40.

13. C. Bicchi, E. Liberto, M. Matteodo, B. Sgorbini, L. Mondello, B. d'A. Zellner, R. Costa, P. Rubiolo. *Flavour Fragr. J.* **2008**, *23*, 382.
14. A. C. Figueiredo, M. Sim-Sim, J. G. Barroso, L. G. Pedro, M. G. Esquivel, S. Fontinha, L. Luís, S. Martins, C. Lobo, M. Stech. *Flavour Fragr. J.* **2009**, *24*, 316.
15. M. Stech, M. Sim-Sim, M. G. Esquivel, L. Luís, S. Fontinha, C. Lobo, C. Garcia, S. Martins, C. Vieira, J. Barroso, L. G. Pedro, A. C. Figueiredo. *Systemat. Biodivers.* **2010** (in press).
16. T. Shibamoto. In *Capillary Gas Chromatography in Essential Oil Analysis*, P. Sandra, C. Bicchi (eds). Huethig: Heidelberg, **1987**; 259.
17. R. Mottram. The LRI and odour database. Flavour Research Group, School of Food Biosciences, University of Reading, UK: <http://odour.org.uk>
18. T. Acree, H. Arn. Flavornet. Cornell University, NYASES. New York: <http://www.flavornet.com>