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Essential oil of *Ayapana triplinervis* from Reunion Island: A good natural source of thymohydroquinone dimethyl ether

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ABSTRACT

Three specimens of *Ayapana triplinervis* (Vahl) R.M. King & H. Rob from Reunion Island (Indian Ocean) collected at two distant locations (North of the island; samples 1 and 2, South of the island; sample 3), in different growth phases (flowering; samples 1 and 3, vegetative; sample 2) were investigated for their leaf essential oil composition. This study reports the chemical character of this species on the island and investigates the relationship between essential oil composition, developmental stage and geographic location. Analysis by GC–FID and GC–MS enabled us to identify and quantify a total of 39 constituents accounting for 97.1–98.0% of the oils. The three essential oil samples, all obtained by hydrodistillation, showed a high percentage of the aromatic compound thymohydroquinone dimethyl ether (89.9–92.8%). All other minor components remained more or less unchanged both qualitatively and quantitatively with respect to the stage of growth. On the contrary, variations were observed with geographic distribution. The geographical variation of the chemical composition of the volatile oil of *A. triplinervis* from several sites in the world is also briefly discussed.

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1. Introduction

Ayapana triplinervis (Vahl) R.M. King & H. Robinson (syn.: *Eupatorium ayapana* Vent; *Eupatorium triplinerve* Vahl) belongs to the Asteraceae family and is commonly known as Ayapana in Hindi. It is also known by several other names, such as Ayapana tea in England, Ayapan, Bishallakarani in Bangladesh, Aiapana, Iapana, Japana, Japana-branca, Japana-roxa, Erva-de-cobra, Erva-santa in Brazil, Aiapana, Diapana, Thé de l'Amazone in French Guiana, Sekrepatoewiwiri in Surinam and Herbe à Thé, Herbe vulnéraire in French West Indies. This plant growing up to 1 m high is an ornamental erect perennial herb and semi-woody at base. The leaves (4.5–10.5 cm long and 0.8–1.7 cm wide) are aromatic, smooth, simple, opposite, sub-sessile, 3-nerved, acuminate, glabrous and lanceolate. The stems are reddish brown. The many flowering heads are each 6–13 mm long and bear about 40 pink flowers. *A. triplinervis* is native to South America and can be found in the Amazon region of Brazil, Ecuador, Peru, the three Guianas, Puerto Rico, Hawaii but is also well represented in other countries such as India, Vietnam and the Mascarene Islands (Reunion, Mauritius, Rodrigues). Since it is widely used in folk medicine (e.g. Agapito and Sung, 2003; Beckstrom-Sternberg et al., 1994; Bose and Roy, 1936; Brack Egg, 1999; Grenand et al., 1987; Gurib-Fakim and Guého,

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1995; Manfred, 1947; Mors et al., 2000; Trang et al., 1993a; Van den Berg, 1984), the plant has been extensively investigated for its biological and pharmacological properties (e.g. Bose et al., 2007; Chaurasia and Kher, 1978; Facknath, 1999; Garg and Nakhare, 1993; Gupta et al., 2002; Jelager et al., 1998; Kokate et al., 1971; Verpoorte and Dihal, 1987; Yadava and Saini, 1990). However, till now the information about its chemical composition has remained poor. According to previous phytochemical studies (Bose and Roy, 1936; Chaturvedi and Mulchandani, 1989; Natarajan and Natarajan, 1979; Späth et al., 1937; Trang et al., 1992, 1993a,b), the most characteristic secondary metabolites are coumarins (Fig. 1). A total of seven coumarins known under the trivial name ayapanin (or herniarin) (**1**), ayapin (**2**), daphnetin (**3**), daphnetin dimethyl ether (**4**), daphnetin-7-methyl ether (**5**), hydrangetin (**6**) and umbelliferone (**7**) have been characterized in the plant. Coumarins are considered to be components of the general defense response of plants to abiotic and biotic stresses and it has been proved that various substituted coumarins exhibit antimicrobial or anti-inflammatory activity and act as inhibitors of numerous enzyme systems (Murray et al., 1982). This may explain why *A. triplinervis* rich in coumarins, is used in herbal medicine. Concerning more specifically volatile compounds from *A. triplinervis*, few investigations (four references, all using the synonym *Eupatorium triplinerve* to designate the plant) exclusively devoted to essential oil samples of Brazilian, Indian and Vietnamese origins have been carried out (Table 1). According to this literature survey, it also appears that up to now the chemical composition of the volatile oils of *A. triplinervis* from Reunion Island has not been investigated. Yet this plant is locally really appreciated for its healing virtues in particular its digestive properties (Gurib-Fakim and Guého, 1995). Therefore, the present work concerns detailed GC and GC–MS examination of the leaf essential oil of three *A. triplinervis* specimens from Reunion Island, growing in two distinct locations (North of the island; samples 1 and 2, South of the island; sample 3), at two developmental stages (flowering; samples 1 and 3, vegetative; sample 2). The objective of our study was to (1) observe the homogeneity of the composition or, conversely, to evidence a chemical variability among *A. triplinervis* specimens on Reunion Island, and (2) compare the oil composition of *A. triplinervis* from Reunion Island with those of oils produced by the same species grown in different geographical regions of the world. This study is a part of the ongoing APLAMEDOM (Association for Medicinal and Aromatic plants of Reunion Island) program which aims at conserving biodiversity on Reunion Island through the valorisation of aromatic and medicinal plants. By reinforcing links between scientific information and ethnobotanical knowledge and by enabling systematic cultivation and mass propagation of aromatic and medicinal plants, the objective of this program is to develop a market of natural products on the island.

2. Materials and methods

2.1. Plant material

Three samples of *A. triplinervis* (Vahl) R.M. King & H. Rob were gathered at two different stage of development (flowering and vegetative) in two different localities of Reunion Island (Table 2). Voucher specimens were identified and deposited at the Herbarium of the University of Reunion Island (REU).

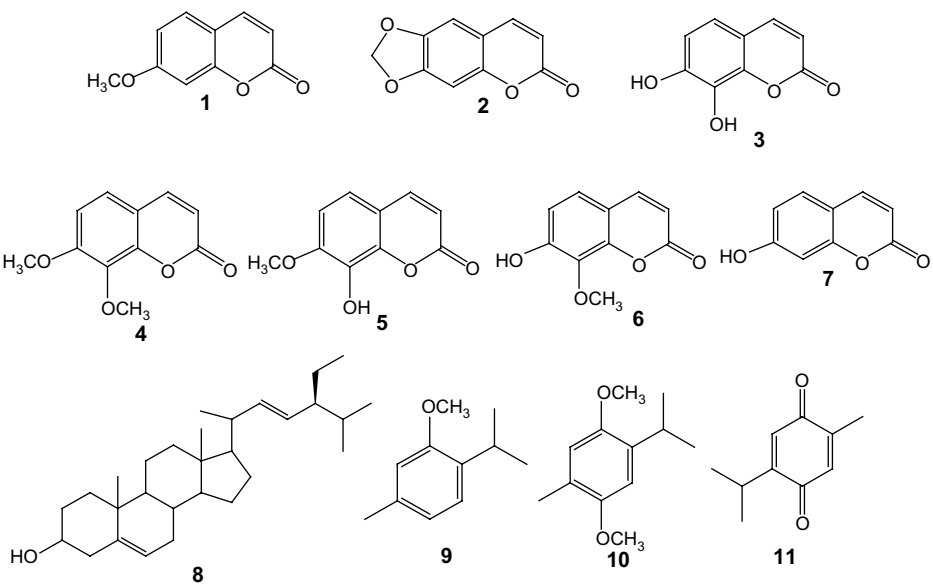

Fig. 1. Chemical structure of secondary metabolites isolated from *A. triplinervis*: ayapanin (**1**), ayapin (**2**), daphnetin (**3**), daphnetin dimethyl ether (**4**), daphnetin-7-methyl ether (**5**), hydrangetin (**6**), umbelliferone (**7**), stigmasterol (**8**), thymol methyl ether (**9**), thymoquinol dimethyl ether (**10**), and thymoquinone (**11**).

Table 1
Main compounds of essential oil of *Ayapana triplinervis* in different geographical sites in the world.

Countries	Sites	Plant part	Yield (%)	Main compounds (≥ 4%)	%	Ref.
Brazil	Lago Grande, Macapá	Leaves and stems	0.4	Thymohydroquinone dimethyl ether	69.7	Maia et al. (1999)
				β-Caryophyllene	19.7	
India	Saugar (Botanical garden)	Flowers	0.9	Thymohydroquinone dimethyl ether	50.3	Garg and Nakhare (1993)
				Borneol	5.8	
				α-Terpineol	4.6	
				Isoeugenol	4.0	
	Saugar (Botanical garden)	Leaves	0.6	Thymohydroquinone dimethyl ether	49.3	Garg and Nigam (1970)
				α-Phellandrene	8.9	
				Borneol	8.0	
				Dipentene	7.3	
				Linalool	6.5	
				Sabinene	5.3	
				α-Terpineol	5.2	
				Bornyl acetate	4.6	
	Lucknow (Campus of Central Institute of Medicinal and Aromatic Plants)	Leaves	0.4	Selina-4(15),7(11)-dien-8-one	36.6	Gupta et al. (2004)
				β-Caryophyllene	14.7	
				δ-Elemene	5.9	
				β-Sesquiphellandrene	4.7	

2.2. Essential oil isolation

Fresh leaves (300 g) of the three samples were hydrodistilled in a Clevenger-type apparatus for 3 h. The oils were taken up in dichloromethane, dried over anhydrous sodium sulphate and kept at 4 °C. The extraction yields estimated on the basis of the fresh weight of plant material are reported in Table 2.

2.3. Gas chromatography analysis

GC analyses were carried out using a Varian Gas chromatograph (Model CP-3800 – Star chromatography work station software) equipped with a flame ionization detection (FID) system and a non-polar SPB-5 capillary column (60 m × 0.32 mm i.d., film thickness 0.25 μm). The oven temperature was programmed from 60 °C to 230 °C at 4 °C/min and then held isothermally at 230 °C for 40 min. Injector and detector temperatures were maintained at 250 °C and 300 °C, respectively. Nitrogen was used as the carrier gas at a flow rate of 0.7 mL/min. Samples were injected in the splitless mode (injection volume, 0.1 μL of pure oil).

2.4. Gas chromatography–mass spectrometry analysis

GC–MS analyses using a non-polar phase were conducted on a Hewlett–Packard 6890 series-5972 GC–MS system equipped with a SPB-5 column (60 m × 0.32 mm i.d., film thickness 0.25 μm). GC–MS analyses using a polar phase were performed on a Hewlett–Packard 6890-5973 GC–MS system equipped with a Supelcowax column (60 m × 0.32 mm i.d., film thickness 0.25 μm). Both GC–MS instruments were operated at 70 eV in the EI mode over the *m*/*z* range 30–550. Helium was used as the carrier gas at a flow rate of 0.7 mL/min (SPB-5 column) and 0.8 mL/min (Supelcowax column). The oven temperature was programmed from 60 °C to 230 °C at a rate of 4 °C/min, held for 40 min. The injector and the transfer line were both programmed to 250 °C. All analyses were done using a 20:1 split ratio (injection volume, 0.1 μL of pure oil).

2.5. Identification and quantification

Retention indices of all the constituents were determined on both phases by Kovats method using *n*-alkanes as standards. The mixture of *n*-alkanes (C_8–C_{28}) was prepared from pure chemicals at 5% concentration in pentane. Constituents of the

Table 2
Collection places, voucher numbers, dates, life-cycle stages, and extraction yields of *Ayapana triplinervis*.

Sample no.	Collection places (altitudes)	Voucher numbers	Dates	Life-cycle stages	Oil yields (%, W/W)
1	Saint-Denis *Bellepierre* (440 m)	REU04807	December 2006	Flowering	0.28
2	Saint-Denis *Saint-François* (420 m)	REU05801	April 2007	Vegetative	0.28
3	Tampon (580 m)	REU05772	December 2007	Flowering	0.29

volatile oil were identified by comparison of their retention indices and their mass spectral fragmentation pattern with those reported in the literature (Adams, 2001) and stored on MS Libraries (Wiley 7, NIST 02). The quantification of the components was performed on the basis of their GC peak areas on the SPB-5 column without FID response factor correction.

3. Results and discussion

3.1. Oil yield

Several studies on aromatic plants have shown that oil yield may be affected by environmental conditions such as moisture level and may fluctuate daily or seasonally as well (Hay and Waterman, 1993). However, in this study, the three oil yields (0.28–0.29%, calculated from fresh material) were quite similar. Thus, by this first approach, it seems that oil yields of *A. triplinervis* from Reunion Island may neither be affected by the site of development (North of the island; sample 1, South of the island; sample 3) and the stage of growth (flowering; sample 1, vegetative; sample 2) nor may be apparently determined genetically.

3.2. Oil composition

The chromatographic analyses of the three yellowish essential oil samples allowed the detection and quantification of 47 compounds, among which 39 covering more than 97% of the total oils were identified. The detected components, their retention indices and their percentage composition are listed in Table 3. All the constituents grouped by chemical classes were arranged according to their elution order on the SPB-5 column. Mass spectra of the eight unidentified components are reported in Table 4. In the three *A. triplinervis* essential oils studied, the aromatic derivative thymohydroquinone dimethyl ether was found to be the predominant component accounting for 89.9–92.8% of the total oils. It was followed by the sesquiterpene hydrocarbon β-selinene in the range of 2.1–3.4%. Because only small differences were observed between the three essential oil samples for their first two principal components, the concentration of the minor constituents was used to analyse the relationship between essential oil composition, developmental stage and geographic locations.

When comparing results for samples 1 and 2, no major differences in both qualitative and quantitative were observed among the minor compounds. This enables us to conclude that there is no growth phase influence on the chemical composition of *A. triplinervis* essential oil from Reunion Island.

When comparing results for samples 1 and 3, some differences in the minor compounds composition were evident. Sample 1 was found to contain 45 minor compounds separated into the five categories of monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes and aromatic derivatives; whereas sample 3 was marked by the presence of only 17 minor components (all present in sample 1) with no trace of oxygenated monoterpenes and much smaller amounts of monoterpene hydrocarbons, sesquiterpene hydrocarbons, oxygenated sesquiterpenes and aromatic derivatives. Due to this difference in minor components' composition, we suggest that the oil composition of *A. triplinervis* is dependent of the geographic location on Reunion Island. This leads us to assume a possible connection with particular climatic conditions which need to be studied more thoroughly. Sample 3 was indeed collected from an area with a higher humidity level than samples 1 and 2.

The composition of the oils isolated from the three *A. triplinervis* specimens investigated here by us was then compared to that obtained from the same species collected in different geographical sites in the world (Brazil and India). For comparison purposes, only the main components were considered (Table 1). It appears then a clear geographical diversity in *A. triplinervis* with respect to the chemical composition and the main components of the essential oils. Two chemotaxonomic groups may be established.

Group 1: species that mainly synthesize the aromatic ether thymohydroquinone dimethyl ether. This was described for species from Brazil and India (Saugar).

Group 2: species producing principally the oxygenated sesquiterpene selina-4(15),7(11)-dien-8-one. This is the case of *A. triplinervis* from India (Lucknow).

On this basis, our *A. triplinervis* samples from Reunion Island considerably dominated by thymohydroquinone dimethyl ether (average: 91.2%) may be included in the group 1. However, in this group, by looking at the other main components, three chemotypes may tentatively be considered: chemotype thymohydroquinone dimethyl ether/α-phellandrene/borneol (India/Saugar); chemotype thymohydroquinone dimethyl ether/β-caryophyllene (Brazil); chemotype thymohydroquinone dimethyl ether (Reunion Island).

As chemical variation related to phenological and environmental factors is frequently encountered in aromatic plants, further studies are needed to see if the changes of chemical composition in the studied oils of *A. triplinervis* are on the account of different environmental conditions or the chemotypes are genetically fixed. Experimentation with plants of different origins being grown in consistent environments would be the most effective way to distinguish between the effects of genotype and environment on *A. triplinervis* chemotype. Moreover, if the chemotype thymohydroquinone dimethyl ether of *A. triplinervis* from Reunion Island is confirmed, the plant may be a good natural source of this aromatic compound.

Table 3
Chemical composition of the essential oil of three *Ayapana triplinervis* samples.

Compounds	LRI_1[a]	LRI_2[a]	Composition (%)[b]		
			Sample 1	Sample 2	Sample 3
Monoterpene hydrocarbons					
α-Thujene	930	–	tr	tr	–
α-Pinene	939	1022	tr	tr	tr
β-Pinene	984	1102	tr	0.1	tr
β-Myrcene	990	1156	tr	tr	–
α-Phellandrene	1009	1166	0.3	0.1	tr
Limonene	1033	1206	tr	tr	tr
β-Phellandrene	1035	1215	0.1	tr	tr
β-Ocimene	1047	1250	tr	tr	–
γ-Terpinene	1062	–	tr	–	-
Terpinolene	1094	1284	tr	tr	–
4,8-Dimethylnona-1,3,7-triene[c]	1116	1304	tr	tr	–
Oxygenated monoterpenes					
Linalol	1100	–	tr	tr	–
cis-*p*-Menth-2-en-1-ol	1128	1565	tr	tr	–
trans-*p*-Menth-2-en-1-ol	1145	1631	tr	tr	–
α-Terpineol	1197	1701	tr	tr	–
trans-Piperitol	1213	1749	tr	tr	–
trans-Pinocarvyl acetate	1305	1657	tr	tr	–
Neryl acetate	1363	–	tr	tr	–
Sesquiterpene hydrocarbons					
Cyperadiene	1379	1536	0.1	0.6	–
Isopatchoula-3,5-diene	1392	1553	tr	0.3	–
cis-β-Elemene	1396	1585	tr	tr	–
β-Elemene	1403	1596	1.2	0.8	0.8
Cyperene	1420	–	tr	0.2	tr
Caryophyllene	1439	1607	1.7	1.1	0.7
α-Humulene	1474	1680	tr	tr	–
Drima-7,9(11)-diene	1488	–	0.1	0.1	–
β-Selinene	1506	1732	3.4	2.6	2.1
α-Selinene	1514	–	tr	tr	–
Oxygenated sesquiterpenes					
(*E*)-Nerolidol	1568	2039	tr	tr	–
Selin-11-en-4-α-ol	1676	2262	tr	tr	–
Aromatic derivatives					
Benzaldehyde	963	1536	tr	tr	tr
p-Cymene	1028	1273	0.1	tr	tr
p-Cymen-8-ol	1189	–	tr	–	–
Methyl salicylate	1202	1789	tr	tr	–
Thymol, methyl ether	1237	1597	1.0	0.2	0.3
Carvacrol, methyl ether	1247	1609	0.1	0.3	0.2
Thymoquinone	1254	–	tr	–	0.2
Thymol	1293	2187	tr	tr	–
Thymohydroquinone dimethyl ether	1430	1885	89.9	90.9	92.8
Total identified			>98.0	>97.3	>97.1
Unidentified compounds (molecular weight)					
Unknown 1 (MW = 164)	1231	1582	tr	–	–
Unknown 2 (MW = 194)	1386	1821	tr	tr	–
Unknown 3 (MW = 204)	1492	–	tr	0.1	–
Unknown 4 (MW = 204)	1537	–	tr	tr	–
Unknown 5 (MW = 222)	1557	2452[d]	tr	tr	–
Unknown 6 (MW = 222)	1571	2487[d]	tr	–	–
Unknown 7 (MW = 220)	1638	2187[e]	0.4	0.4	0.5
Unknown 8 (MW = 220)	1642	2230[e]	tr	tr	tr
Total unknown			>0.4	>0.5	>0.5

[a] LRI_1: linear retention indices relative to C_8–C_{22} *n*-alkanes on the apolar SPB-5 column; LRI_2: linear retention indices relative to C_8–C_{28} *n*-alkanes on the polar Supelcowax column.
[b] tr: Trace (<0.1%).
[c] Correct isomer non-identified.
[d] Values may be reversed.
[e] Values may be reversed.

Table 4
Mass spectra data of major unidentified compounds of *Ayapana triplinervis*.

Compounds	LRI_1[a]	LRI_2[a]	Molecular weight	*m*/*z* (rel. int., 70 eV)
Unknown 1	1231	1582	164	41(8), 51(8), 65(6), 77(10), 91(18), 105(8), 119(8), 134(22), 149(100), 164(44)
Unknown 2	1386	1821	194	39(4), 51(4), 65(5), 77(11), 91(18), 105(4), 119(24), 136(7), 147(11), 164(70), 179(90), 194(100)
Unknown 3	1492	–	204	41(21), 55(17), 67(19), 79(39), 91(37), 93(36), 95(40), 105(46), 119(32), 133(44), 147(36), 161(37), 175(14), 189(100), 204(78)
Unknown 4	1537	–	204	41(21), 55(17), 67(14), 79(33), 91(36), 93(37), 105(58), 107(53), 119(46), 133(32), 147(21), 161(100), 179(10), 189(61), 204(33)
Unknown 5	1557	2452	222	43(8),77(7), 91(7), 165(100), 180(87), 222(16)
Unknown 6	1571	2487	222	43(6),77(6), 91(10), 107(9), 150(9), 165(100), 180(72), 222(14)
Unknown 7	1638	2187	220	41(19), 55(13), 67(10), 77(16), 91(33), 105(33), 119(34), 131(36), 145(46), 159(100), 177(15), 187(64), 202(40), 205(78), 220(51)
Unknown 8	1642	2230	220	41(20), 55(13), 67(11), 77(18), 91(38), 105(35), 119(34), 131(38), 145(50), 159(100), 177(15), 187(68), 202(43), 205(71), 220(47)

[a] LRI_1: linear retention indices relative to C_8–C_{22} *n*-alkanes on the apolar SPB-5 column; LRI_2: linear retention indices relative to C_8–C_{28} *n*-alkanes on the polar Supelcowax column.

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