SUPPLEMENTARY MATERIAL

Variation in volatile constituents of *Cinnamomum keralaense*, endemic to the Western Ghats, India

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Abstract: Leaf essential oils (EOs) of *Cinnamomum keralaense*, an endemic species to southern Western Ghats, India, were investigated. Leaves were collected from three distinct populations and hydro-distilled to obtain EOs, which were analysed by GC-FID and GC-MS. A total of 68 constituents were identified from three EOs. Wide variation was found in the composition of EOs. Ten major compounds were identified from three EOs, however, none of the compound was present in all three EOs as major constituent. High content of methyl eugenol (82.5%) in one of the EOs is significant as it could be evaluated for commercial utilization. This is the first report on the chemical compositions of leaf EOs of *C. keralaense*.

Keywords: Western Ghats; *Cinnamomum keralaense*; essential oil; methyl eugenol.

Experimental part

1. Plant material:

The leaves of *C. keralaense* were collected from three different locations in the Southern Western Ghats (WG), Kerala state, India for extraction of essential oils (EOs). Since it is a rare species, collection of leaf samples for the study was restricted to only three different populations. The sample CK¹ (accession number–68) was collected from Devikulam; CK² (123) from Kuttikkanam and CK³ (124) from Vagamon, Idukki, Kerala, India. Herbarium specimens were prepared for each accession (*Murugan 68, 123, 124*) and deposited at SASTRA University Herbarium, Thanjavur, India. The specimens were identified by one of us (RM) using the revision on the genus *Cinnamomum* of South India (Kostermans, 1983). The identity of species was confirmed by matching the herbarium specimens with the Type specimens (*Kostermans 26104*) deposited at the National Herbarium (L), Leiden, Netherland.

2. Essential oil extraction:

The leaves were kept under shade for a week before extraction of EOs. The shade dried leaves were hydro-distilled (leaves: water ratio was 1:8 w/v) for about 3 hours in Clevenger apparatus. After extraction, EOs were collected and dried over anhydrous Sodium sulphate. The EOs were stored under refrigeration at 4 °C for further study (Sriramavaratharajan et al. 2016).

3. GC-FID analysis of EOs:

EOs were quantitatively and qualitatively analyzed by GC-FID and GC-MS respectively. GC-FID analysis was performed on Agilent 7890B Gas Chromatograph with a Flame Ionization Detector (FID). A non-polar HP-5 capillary column (30 m × 0.32 mm × 0.25 µm coated with 5% phenyl – 95% methyl polysiloxane) was used. The running conditions were: Oven temperature

60 °C – 240 °C increased at 3 °C/min., final hold time 10 min.; injector and detector temperatures – 250 °C; carrier gas – helium, at a linear velocity of 30 cm/sec., pressure – 93.6 kPa, flow rate – 1 ml/min.; sample injection – 1 µl EO dissolved in hexane, split ratio – 70:1.

4. GC-MS analysis of EOs:

GC-MS analysis was performed on PerkinElmer Clarus 500 Gas Chromatograph with Turbo Mass Gold Quadrupole Mass Spectrometer. A non-polar, Elite-5MS capillary column (30 m × 0.25 mm × 0.25 µm coated with 5% phenyl – 95% dimethyl polysiloxane) was used. The running conditions were: Oven temperature 60 °C – 240 °C, increased at 3 °C/min., final hold time 5 min.; injector temperature – 270 °C; carrier gas – helium, flow rate – 1 ml/min.; sample injection – 1 µl EO dissolved in hexane, split ratio – 1:20. Mass spectra were recorded in electron ionization mode, scan range – 40-460 amu, ionization energy – 70 eV; transfer line temperature – 200 °C; ion source temperature – 180 °C.

5. Identification of EO constituents:

A standard *n*-alkanes solution with a mixture of carbons ranging from C₈ to C₂₀ was co-injected with EO sample to obtain the linear retention index (LRI) value of individual constituents. Chemical constituents were identified by comparing the LRI of the GC peaks with those reported in literature (Adams, 2007). The mass spectra of the peaks obtained from GC-MS were matched with spectra reported in the literature (Adams, 2007) and NIST 2005 MS library.

References

- Adams RP. 2007. Identification of essential oil components by Gas Chromatography / Quadrupole Mass Spectroscopy, 4th Ed. Allured Publishing Corporation, Carol Stream, Illinois, USA.
- Kostermans AJGH. 1983. The south Indian species of *Cinnamomum* Schaeffer (Lauraceae). Bull. Bot. Surv. India 25(1-4): 90-133.
- Sriramavaratharajan V, Stephan J, Sudha V, Murugan R. 2016. Leaf essential oil of *Cinnamomum agasthyamalayanum* from the Western Ghats, India – A new source of camphor. Ind. Crops Prod. 86: 259–261.

Table S1 Comparison of the compositions of leaf essential oils of *Cinnamomum keralaense*

Constituents	LRI ^{cal}	LRI ^{lit}	Relative amount %		
			CK ¹	CK ²	CK ³
α -Thujene	930	930	1.8	0.1	0.3
α -Pinene	936	939	0.3	0.3	3.1
Camphene	952	954	-	-	0.9
Sabinene	978	975	2.9	-	-
β -Pinene	981	979	-	-	1.3
Myrcene	993	990	1.1	0.1	1.1
α - Phellandrene	1002	1002	-	0.4	0.5
δ -3-Carene	1008	1011	2.3	-	0.2
α -Terpinene	1018	1017	13.0	1.5	-
ρ -Cymene	1024	1024	-	0.1	2.9
Limonene	1032	1029	-	-	4.7
β -Phellandrene	1033	1029	30.9	2.6	0.7
(<i>E</i>)- β -ocimene	1053	1050	0.2	-	-
γ -Terpinene	1062	1059	0.9	-	-
<i>cis</i> -Sabinene hydrate	1072	1070	0.1	-	-
Terpinolene	1091	1088	0.2	-	0.1
Linalool	1100	1096	1.2	0.4	1.7
<i>trans</i> -Sabinene hydrate	1104	1098	0.3	-	-
<i>cis</i> - ρ -mentha-2-en-1-ol	1118	1121	0.2	-	-
Citronellal	1155	1153	0.4	-	12.7
Borneol	1172	1169	-	-	0.1
Terpinen-4-ol	1181	1177	0.9	0.1	0.3
Cryptone	1190	1185	0.2	-	-
α -Terpineol	1195	1188	0.8	0.1	0.3
Citronellol	1227	1225	-	-	2.9
Piperitone	1255	1252	1.6	0.1	-
Safrole	1290	1287	-	0.1	-
<i>n</i> -Tridecane	1303	1300	-	-	0.1
δ -Elemene	1340	1338	-	0.1	0.3
α -Cubebene	1349	1348	0.1	0.1	0.1
Eugenol	1362	1359	2.2	-	-
α -Ylangene	1375	1375	-	0.1	0.4
α -Copaene	1380	1376	1.9	-	-
β -Cubebene	1388	1388	0.3	0.1	-
β -Elemene	1394	1390	-	0.1	0.4
Methyl eugenol	1403	1403	-	82.5	2.7
Dodecanal	1411	1408	0.2	-	-
(<i>E</i>)-Caryophyllene	1419	1419	10.1	-	3.7
α - <i>trans</i> -Bergamotene	1441	1434	0.1	-	0.4
α -Humulene	1454	1454	1.5	-	0.5
<i>allo</i> -Aromadendrene	1460	1460	-	-	0.7

γ -Muurolene	1481	1479	0.7	-	-
α -Amorphene	1484	1484	-	-	3.1
Germacrene D	1485	1485	1.9	1.6	-
Bicyclogermacrene	1503	1500	2.5	0.5	-
α -Muurolene	1503	1500	-	-	0.6
δ -Amorphene	1514	1512	-	0.4	2.5
γ -Cadinene	1516	1513	0.8	-	-
δ -Cadinene	1527	1523	2.7	1.1	6.1
α -Cadinene	1540	1538	0.2	0.1	0.3
Hedycaryol	1551	1548	-	-	0.3
Elemol	1552	1549	0.6	-	-
(<i>E</i>)-Nerolidol	1566	1563	-	0.7	-
Germacrene D-4-ol	1578	1575	1.0	0.1	1.4
Spathulenol	1580	1578	0.4	-	-
Viridiflorol	1594	1592	-	0.2	7.4
Humulene epoxide II	1608	1608	-	-	0.3
Tetradecanal	1615	1612	-	0.1	-
<i>cis</i> -Cadin-4-en-7-ol	1638	1636	-	-	2.0
Caryophyllene (II)*			-	-	3.7
<i>epi</i> - α -Muurolol	1644	1642	-	-	6.0
α -Muurolol	1645	1646	2.2	-	-
Cubenol	1647	1646	-	1.2	-
α -Eudesmol	1656	1653	9.9	-	-
α -Cadinol	1658	1654	-	3.2	16.1
<i>trans</i> -Calamenen-10-ol	1671	1669	-	-	0.4
Shyobunol	1691	1689	0.1	-	4.0
Benzyl benzoate	1763	1760	-	0.1	-
Monoterpenes			54.0	5.1	15.8
Oxygenated monoterpenes			5.3	0.8	18.0
Sesquiterpenes			22.8	4.2	22.8
Oxygenated sesquiterpenes			14.2	5.5	37.9
Miscellaneous compounds			2.4	82.6	2.8
Total			98.7	98.2	97.3

CK¹ – *Cinnamomum keralaense* collected from Devikulam; CK² – *Cinnamomum keralaense* collected from Kuttikkanam; CK³ – *Cinnamomum keralaense* collected from Vagamon, Idukki district, Kerala, India; LRI^{cal} – Linear Retention index calculated; LRI^{lit} – Linear Retention index reported in the literature (Adams, 2007); * – Identified using only mass spectrum.