Enantioenriched (3S)-(+)-Linalool in the Leaf Oil of *Cinnamomum tamala* Nees et Eberm. from Kumaon

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Abstract

Variations and genuineness in constituents of *Cinnamomum tamala* leaf essential oil are characterized by enantio-GC-FID, capillary GC-FID and GC/MS. The oil samples were analyzed for two consecutive years. (E)-Cinnamaldehyde, which was the principal component, was higher in the first year oil samples but lower in the second year samples. Similarly, (Z)-cinnamaldehyde was 5.8–7.1% in the first year oils and 1–1.1% of the second year oils. Linalool content varied (6.4–8.5% and 5.7–16.2%, respectively) in first and second year oil samples, while (E)-cinnamyl acetate was higher (10.0–22.7%) in oil samples of the second year collection. Using a 10% heptakis(2,3-di-O-methyl-6-O-tert-butyldimethylsilyl)-β-cyclodextrin as the chiral stationary phase, optically pure (3S)-(+)-linalool was found in both the oil samples leading to complete enantiomeric excess for (3S)-(+)-enantiomer.

Key Word Index

Cinnamomum tamala, Lauraceae, essential oil composition, (E)-cinnamaldehyde, (3S)-(+)-linalool, (Z)-cinnamaldehyde.

Introduction

In India, *Cinnamomum* (family Lauraceae) is represented by twenty species (1-2). *Cinnamomum tamala* Nees et Eberm. is a medium sized tree, up to 8 m high, and frequently occurs in the northwestern Himalaya, Sikkim, Assam, Mizoram and Meghalaya regions (2). Apart from this, *C. tamala* is the sole species cultivated for its *Tejpat* leaves in the whole region of Kumaon for the production of spice and related products (3). The essential oil isolated from the leaves, known as *Tejpat* oil, is medicinally used as carminative, antiflatulent and diuretic. The genus *Cinnamomum* is known for its antioxidant and antimicrobial properties. In particular, the antioxidant properties of the oil (4) and antibacterial activity against (E)-cinnamaldehyde were reported (5). Various chemical races of *C. tamala* exist viz., eugenol-type, cinnamaldehyde-type, or cinnamaldehyde/linalool-type, *trans*-sabinene hydrate-type named after the marker constituents present in oils from various regions (6-12).

To best of the authors' knowledge, no work has been reported so far on enantiomeric characterization of leaf oil of *C. tamala* from natural habitats of Kumaon Himalaya. However, a few reports have described trends in leaf oil (7-13). Earlier, cinnamaldehyde (41.2% and 55.19%) and linalool (15.67% and 15.28%) in two samples (each from natural and market collection) from Kumaon were reported (8). However, the authors did not identify the correct isomer of cinnamaldehyde. Therefore, in the present communication, we report the complete oil composition and enantioselective study of *C. tamala* leaf oil from the natural habitat of Kumaon.

Experimental

***Plant material*:** Fresh leaves of cultivated *C. tamala* Nees et Eberm. from two plants once in a year in February 2007 and February 2008 just at the beginning of spring at complete leaf maturity were collected from one of the author's (CSC) kitchen garden in the village of Jones Estate near the Nainital district (1,450 m) in Uttarakhand, India. The species authentication was very well established and identification was done in the Botany Department of Kumaun University and compared with herbarium records. The voucher specimens Chem./DST/CT1 and Chem./DST/CT2 have been deposited in the Herbarium of the Botany and Chemistry Department of Kumaun University, Nainital India.

***Isolation of oils*:** The fresh leaves (0.1 kg each) were subjected to hydrodistillation in a Clevenger-type apparatus for 3 h. The distillate was saturated with NaCl and the oil was extracted with hexane and methylene dichloride. The organic phase was then dried over anhydrous Na_2SO_4 and the solvent distilled off using a thin film rotary vacuum evaporator at 30°C. The oil yields (calculated as oil wt/wt of fresh leaves) were 0.1%, 0.11% and 0.1%, 0.09%, respectively.

***Enantio-GC-FID*:** All the oils were analyzed on a Varian CP-3800 GC apparatus using a BETA DEX 110 fused-silica capillary column (30 m × 0.25 mm, 0.25 µm film thickness, Supelco) equipped with a flame-ionization detector (FID). For enantio-GC/MS studies, a PerkinElmer AutoSystem XL GC apparatus (same column as above) coupled with a Turbomass Quadrupole mass spectrometer was used. The tempurature program was 60°C (isothermal for 2 min) to 180°C (isothermal

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for 2 min) at 3°C/min, then 180–220°C at 3.5°C/min with a final hold time of 5 min, with H_2 as carrier gas at 1.8 mL/min constant flow, and the injector and detector tempuratures were 220°C and 250°C, respectively. Injection volume was 0.04 µL prepared in diethyl ether with split ratio of 1:200. Mass spectra were recorded in the EI mode at 70 eV in the range m/z 40–450 in split mode (1:40), the injection volume being 0.06 µl. Both the enantiomers were identified based on their elution order in BETA DEX 110 capillary column, by co-injection with authentic enantiomer samples and enantiomeric linalool mixture (Sigma).

GC-FID and GC/MS: A PerkinElmer AutoSystem XL gas chromatograph fitted with Equity–5 column (60 m x 0.32 mm, film thickness 0.25 µm, Supelco), column temperature 70–250°C was programmed at 3°C/ min with initial and final hold time of 2 min. each using H_2 as carrier gas at 10 psi constant pressure, split ratio 1:30, injection size 0.03 µL neat, injector and detector (FID) temperature were 250°C and 280°C, respectively. GC/MS was done on PerkinElmer AutoSystem XL GC interfaced with Turbomass Quadrupole Mass spectrometer fitted with Equity–5 column fused silica capillary column (60 m x 0.32 mm; 0.25 µm film coating). The oven temperature program was the same in as in GC while the injector temperature was 250°C, transfer line and ion source temperatures were 250°C, injection size 0.03 µL neat, split ratio 1:30 using He as carrier gas at 10 psi constant pressure. MS were taken at 70 eV with

Table I. Percentage composition of cultivated *Cinnamomum tamala* essential oils

Compound	RI	RRI	First Year Samples %		Second Year Samples %		Mode of dentification
			I	II	I	II	
α-pinene	934	1022	1.4	3.1	-	t	RI, MS, STD
camphene	949	1066	1.8	4.0	-	-	RI, MS, STD
benzaldehyde	963	1525	2.3	2.5	4.1	2.8	RI, MS
p-cymene	1027	1262	-	-	t	0.2	RI, MS
limonene	1029	1195	-	-	-	0.3	RI, MS, STD
1,8-cineole	1035	1207	-	0.1	-	0.1	RI, MS
salicylaldehyde	1047	1680	1.6	2.1	3.2	2.5	RI, MS
γ-terpinene	1058		-	-	-	t	RI, MS
acetophenone	1070		-	t	0.1	t	RI, MS
cis-sabinene hydrate	1076	1476	0.4	0.1	0.2	0.2	RI, MS
trans-sabinene hydrate	1093		0.4	0.5	0.2	0.2	RI, MS
linalool	1105	1565	6.4	8.5	5.7	16.2	RI, MS, STD
3-phenyl propanala	1165		1.4	1.3	2.5	1.3	RI, MS
pinocarvone	1168		-	0.1	0.1	0.1	RI, MS
borneol	1171	1707	0.2	0.2	0.3	0.3	RI, MS, STD
2-methylbenzofuranb	1180	1748	-	t	0.2	2.0	RI, MS
tepinen-4-ol	1182	1609	-	t	0.1	0.1	RI, MS, STD
p-cymen-8-ol	1188	1840	0.1	0.1	0.1	0.1	RI, MS
α-terpineol	1195	1698	0.2	0.3	0.1	0.3	RI, MS
(Z)-cinnamaldehyde	1223	1886	5.8	7.1	1.0	1.1	RI, MS
carvone	1249		-	0.1	t	0.1	RI, MS, STD
(E)-cinnamaldehyde	1282	2034	62.3	53.7	52.8	35.8	RI, MS
bornyl acetate	1292	1577	0.4	0.1	0.7	1.3	RI, MS, STD
(E)-cinnamyl alcohol	1311	2284	0.1	-	0.3	0.3	RI, MS
eugenol	1362	2144	-	-	-	0.1	RI, MS, STD
hydrocinnamyl acetate	1374		-	0.1	-	0.4	RI, MS
(Z)-cinnamyl acetate	1393		0.9	0.6	1.0	0.7	RI, MS
β-caryophyllene	1429	1579	-	-	0.2	0.6	RI, MS
(Z)-cinnamic acid	1430		t	t	-	-	RI, MS
coumarin	1446		3.6	3.5	2.0	1.0	RI, MS
(E)-cinnamyl acetate	1453	2155	5.2	4.7	10.0	22.7	RI, MS
α-humulene	1463	1654	-	-	-	0.1	RI, MS
(E)-nerolidol	1567	2054	-	-	0.1	1.0	RI, MS
spathulenol	1589		0.3	0.3	0.4	0.2	RI, MS
β-copaen-4α -ol	1596		1.4	0.6	2.5	2.0	RI, MS
Total Identified			96.2	93.7	87.9	94.1	
Monoterpenoid Hydrocarbons			3.2	7.1	-	0.6	
Oxygenated Monoterpenes			8.1	10.1	7.5	18.9	
Sesquiterpens Hydrocarbons			-	-	0.2	0.7	
Oxygenated Sesquiterpens			1.7	0.9	3.0	3.2	
Phenylpropanoids			75.7	67.5	67.6	62.4	
Others			7.5	8.1	9.6	8.3	

RI on Equity-5; RRI on CP-Wax 52 CB capillary columns using a homologous series of n-alkanes (C_9-C_{28} hydrocarbons, Polyscience Corp. Niles IL), MS=mass spectrum, STD=Sigma Standard, t=trace <0.1%, * tentatively identified. MS Pattern for compound [a] m/z: 91(base peak), 92, 78, 134 (M^+), 77, 105, 51, 79, 65, 103, 115; compound [b] m/z: 131 (base peak), 132 (M^+), 51, 77, 103, 78, 63 etc.

mass range of m/z 40-450 with scan time of 1 s and 0.5 s inter-scan delay. Characterization was done on the basis of retention indices using index calculating software, Relative Retention Indices using a homologous series of n-alkanes or alkane pattern (C_9-C_{28} hydrocarbons, Polyscience Corp., Niles IL), coinjection with standards (Sigma), MS Library Search (NIST & Wiley) and by comparing with the mass spectral literature data (14-15). The relative amounts of individual components were calculated based on GC-FID peak areas without using correction factors.

Results and Discussion

The naturalness of the hydrodistilled oil from fresh leaves of the *C. tamala* Nees et Eberm. has been established by means of enantio-GC, capillary GC and GC/MS (Tables I and II). Both years' oil samples were marked by a high percentage of phenylpropanoids corresponding to 75.7%, 67.6% in samples I, and 67.5%, 62.4% in samples II, respectively. The marker phenylpropanoids were (E)-cinnamaldehyde, (Z)-cinnamaldehyde, (E)-cinnamyl alcohol, eugenol, hydrocinnamyl acetate, (Z)-cinnamyl acetate, (Z)-cinnamic acid and (E)-cinnamyl acetate.

The oil isolated from sample I in both years collection contained benzaldehyde (2.3–4.1%), salicyladehyde (1.6–3.2%), linalool (6.4–5.7%), 3-phenylpropanal (1.4–2.5%), (Z)-cinnamaldehyde (5.8–1%), (E)-cinnamaldehyde (62.3–52.8%), (Z)-cinnamyl acetate (0.9–1%), coumarin (3.6–2%) and (E)-cinnamyl acetate (5.2–10%). Similarly, oil sample II in both years' collection possessed benzaldehyde (2.5–2.8%), salicyladehyde (2.1–2.5%), linalool (8.5–16.2%), (Z)-cinnamaldehyde (7.1–1.1%), (E)-cinnamaldehyde (53.7–35.8%), bornyl acetate (0.1–1.3%), (Z)-cinnamyl acetate (0.6–0.7%), coumarin (3.5–1%) and (E)-cinnamyl acetate (4.7–22.7%). Hydrocinnamyl acetate was characteristic feature of sample II.

As far as the biogenesis of metabolites is concerned, cinnamaldehyde and eugenol are formed in shikimic acid pathway while linalool is formed from mevalonic acid. Similarly, cinnamaldehyde is reported to be directly formed by the reduction of cinnamic acid, which is a part of phenylalanine pathway. (Z)-Cinnamic acid was only found in the oils of the first year samples of *C. tamala*. The absence of (Z)-cinnamic acid in the successive year oil samples might be due to its reduction to the respective aldehyde. Apart from this, coumarin, which was solely unique in each year sample, differs from the previous reports (7-12). Thus, this could be an important marker for origin and authenticity establishment of *C. tamala*. Besides, the present investigation is comparable in terms of major compound cinnamaldehyde reported in *C. tamala* (8, 11-12) and *C. pauciflorum* from India (16).

In recent years, chirality evaluation of essential oil components has been introduced as a versatile indicator of genuineness and origin. In order to provide an alternative method for authenticity control, the authors analyzed the enantiomeric composition of linalool in *C. tamala* of Kumaon origin (Table II).

Analysis of linalool enantiomers using permethylated β-cyclodextrin capillary column revealed specific enantiomeric excess in *C. tamala*. Optically pure (3S)-(+)-linalool with a complete excess was observed in both years' samples.

This is the first enantiomeric separation report on *C. tamala* from Kumaon region. Moreover, enantiomeric excess could be useful in authenticity determination of this species because other similar species *C. zeylanicum* contained (3R)-(-)-linalool in with an excess of 90%, whereas (3S)-(+)-linalool was present in 5% optical purity level (17). Meanwhile, a sharp decrease in the relative percentage for three major entities (Z)- and (E)-cinnamaldehyde and coumarin have also been observed in both years' samples possibly due to the variations in climatic conditions viz., temperature and total rainfall in each year that had an impact on the secondary metabolite productions.

Thus, variations in terms of enantiomeric ratios and excess could be treated as clear adulteration. Furthermore, combination of GC-FID analysis and enantioselective analysis of its optically active ingredients could be considered a powerful tool in terms of quality control of essential oils.

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Table II. Enantiomeric distribution of linalool in cultivated *Cinnamomum tamala* leaf oil

Compound*		First Year		Second Year		Enantiomeric excess
		Enantiomeric ratio in each Sample (Enantio-GC-FID %)				
		I	II	I	II	
	(3*R*)-(-)-linalool	-	-	-	-	
	(3*S*)-(+)-linalool	100	100	100	100	100 (3S)
STD 1	(3*R*)-(-)-linalool	-	-	-	-	100 (3*R*)
STD 2	(±)-linalool	-	-	-	-	racemic mixture

* as per the elution order in Beta DEX 110 capillary column; STD1 & STD2 Sigma.

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Chemical Composition and Cytotoxic Activity of Leaves Essential Oil from *Mangifera indica* var. *coquinho* (Anacardiaceae)

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Abstract

The volatile composition of *Mangifera indica* var. *coquinho* has been studied. The essential oils were obtained by hydrodistillation in a modified Clevenger-type apparatus, and their analyses were performed by GC-FID and GC/MS. A total of 19 different compounds were identified. Sesquiterpene hydrocarbons were shown to be the main group of constituents of all taxa. The main constituents of the oil were α-gurjunene (24.0%), β-selinene (24.0%), β-caryophyllene (11.2%), α-humulene (7.2%), caryophyllene oxide (5.5%) and humulene epoxide (2.4%). The cytotoxic activity of the oil was more pronounced than antioxidant and antimicrobial activities.

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