Leaf Essential Oil of Cultivated *Pimenta Racemosa* (Mill.) J.W. Moore from North India: Distribution of Phenylpropanoids and Chiral Terpenoids

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Abstract

In this work, cultivated *Pimenta racemosa* from North Indian plains was studied for its leaf volatile composition twice in year at two distinct seasons (spring and autumn) using GC-FID, enantio-GC-FID, GC/MS and ^{1}H-, ^{13}C-NMR and DEPT experiments. Phenylpropanoid was the abundant class of compounds represented by eugenol (72.9%) followed by chavicol (7.7%) whereas β-myrcene (9.6%) and limonene (3.8%) were identified as the most exclusive terpene constituents in spring collection. The autumn sample was recorded with highest eugenol (92.9%) proportion. Notably, presence of chavicol differentiates this oil from *Pimenta dioica*. An enantiomeric excess for (*S*)-(-)-limonene (41.1-45.3%) and (*R*)-(-)-linalool (86.7-89.9%) was recorded over (*R*)-(+)-limonene and (*S*)-(+)-linalool, respectively when separated on 6-*tert*-butyldimethylsilyl-2,3-diethyl-β-cyclodextrin chiral phase. Moreover, both the monoterpene derivatives occur as enantiomeric mixture in *P. racemosa* leaf essential oil.

Keywords: *Pimenta racemosa;* 6-*tert*-butyldimethylsilyl-2, 3-diethyl-β-cyclodextrin; Essential oil composition; Phenylpropanoid; Eugenol; Chavicol; (*S*)-(-)-limonene; (*R*)-(-)-linalool

Introduction

The genus, *Pimenta* (family Myrtaceae), is comprised of about 2 to 5 species of aromatic trees [1]. *Pimenta racemosa* (Mill). JW Moore (syn. *Myrtus caryophyllata*, Lacq. Not L, *P. acris* Kostel) commonly known as Bay or Bay-rum-tree, up to 25 ft high, leaves leathery, obovate or elliptic, finely reticulate veined; flowers white with 5 lobed calyx, is indigenous to West Indies, Venezuela and Guiana and is used in preparation of bay-rum [2]. The plant is similar to allspice (*Pimenta dioica*) and can be differentiated by the presence of elliptic leaves with fine venation, slightly larger fruit and 5 lobed calyx whereas allspice is 4 lobed [1,3]. Welsh reported the plant as 4-12 m tall tree; 3-10 mm long petioles; (1.5) 4-10 (12.5) cm long leaf blades, 2.5-6 cm wide, obovate to oblanceolate or elliptic, coriaceous, obtuse, acute basally, entire, finely reticulately veined, with 5-7 pairs of rather obscure, main lateral veins, shiny above, dull and pale beneath; pedunculate cymes; white flowers; 5-lobed calyx; ovoid fruit, black at maturity [4]. In a Pacific Island Ecosystems (PIER) report, *Pimenta* has been identified under high risk category, which indicates that the species poses a high risk of becoming a serious pest or may be threat to ecosystems of the Pacific islands [5].

To date, there has been good number of publications on oil compositions from *P. racemosa* [6-9] and *P. dioca* [10-12] are available. However, gas chromatography using substituted cyclodextrin for stereochemical/enantiomeric characterization of the *P. racemosa* terpenoid compounds has not been undertaken for study till date. The present communication reports composition of cultivated *P. racemosa* oil using GC-FID, enantio-GC-FID, GC/MS and NMR techniques.

Materials and Methods

Plant material and isolation of essential oils: *P. racemosa* was collected from CSIR-CIMAP campus, Lucknow during spring and autumn seasons. The essential oil from leaf was extracted by hydro distillation using a Clevenger-type apparatus for 4 h. All oil samples were stored at 4ºC prior to analysis.

GC analysis

A PerkinElmer Auto System XL GC, fitted with an Equity-5 column (60 m×0.32 mm i.d., film thickness 0.25 µm), was used for GC analysis. The column oven was programmed from 70ºC to 250ºC at a rate of 3ºC/min, with initial and final hold time of 2 min, and programmed to 290ºC at 6ºC/min, with a final hold time of 5 min, using H_2 as carrier gas at a constant pressure of 10 psi, a split ratio of 1:35, and injector and detector (FID) temperatures of 290 and 280ºC, respectively. GC-MS utilized a Perkin Elmer Auto System XL GC interfaced with a Turbomass Quadrupole mass spectrometer based on the above oven temperature program. Injector, transfer line and source temperatures were 250ºC; ionization energy 70 eV; and mass scan range 40-450 amu. Characterization was achieved on the basis of retention time, elution order, relative retention index using a homologous series of n-alkanes (C_6-C_{28} hydrocarbons, Polyscience Corp. Niles IL), coinjection with standards in the GC-FID capillary column (Aldrich and Fluka), mass spectral library search (NIST/EPA/NIH version 2.1 and Wiley registry of mass spectral data 7th edition) and by comparing with the mass spectral literature data [13]. The relative amounts of individual components were calculated based on GC peak areas without using correction factors.

Chiral analysis

For chiral GC analysis, a TBDE-β-CD (RESTEK Rt™-β-DEXse fused silica capillary column (30 m×0.25 mm id, 0.25 µm) was used

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in a Varian CP-3800 gas chromatograph. The oven temperature was programmed from 70°C (hold 3 min) to 120°C at a rate of 3°C/min and 230°C at a rate of 5°C/min. Hydrogen was used as carrier gas at 1.8 mL/min constant flow. Injector and detector temperatures were 220°C and 230°C, while the elution order was confirmed as per the previous report [14].

NMR experiment

For NMR experiment, Bruker Avance-300 (300MHz) was utilized for ^{1}H- and ^{13}C-NMR experiments with tetramethylsilane (TMS) as internal standard. About 40 mg of the essential oil was dissolved in CDCl$_3$ and spectral data are reported. Chemical shifts are reported in ppm units relative to CDCl$_3$ set to 7.26 (^{1}H-NMR) and 77.0 (^{13}C-NMR) (multiplicity: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad). The identity of compounds was established by comparison of spectral data [15-17].

Results

The oil yield of *Pimenta racemosa* leaves was 0.02% (w/w). In total, 18 compounds were identified, accounting for 97.2-97.8% of volatile constituents (Table 1; Figure1). Phenylpropanoid contributes major percentage to the oil with eugenol as principal component (72.9-92.9 %; 2). Other minor constituents identified were β−myrcene (0.3-9.6; 1) limonene (0.2-3.8; 5) and chavicol (1.3-7.7; 3) suggesting a high molecular diversity in the essential oil. The total terpenoid proportion recorded was less than 3.9%. A comparison on various published reports on major constituents from genus *Pimenta* are listed (Table 2). The *P. racemosa* leaf oil may be distinguished from *P. dioica* by the presence of high β-myrcene proportion followed by chavicol. Moreover, chavicol may be regarded as marker constituent

(A) Terpenes and phenylpropanoids. (B) Chiral Terpenoids
Figure 1: Major volatile constituents identified in *Pimenta racemosa* essential oil.
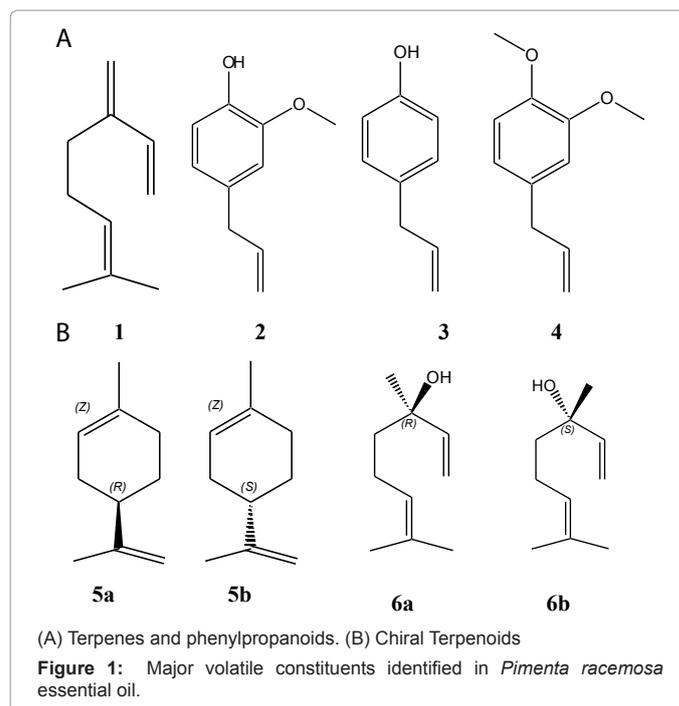
of *P. racemosa* leaf oil, which was not reported from natural *P. dioica* except in one market sample, possibly due to adulteration [12]. On contrary, methyleugenol (4), which contributes good proportion to *P. dioica* was completely absent in *P. racemosa* under present study. The characterization of eugenol and chavicol in essential oil was done using ^{1}H-, ^{13}C-NMR and DEPT experiments. In ^{1}H-NMR, sharp signals for aromatic protons were observed (δ ppm 6.69-6.88, 3H, m) followed by resonances for exocylic double bond (5.06-5.12, 2H, m) and (5.91-6.04, 1H, m), respectively. The presence of methoxy group (δ 3.88, 3H, s) in the arene ring system was also significant. The methylene protons in C$_6$-C$_3$ side chain was marked by a sharp doublet at δ 3.33-3.35 (d, J=6.3 Hz). In ^{13}C-NMR, a total of ten carbon resonances attributed to one methyl, two methylene, four methine and three quaternary carbons were identified for eugenol. The exocyclic double bond (δ 137.78, 1H, δ 115.46, 2H) and one methoxy (δ 55.79) groups were also present (Table 3; Figure S1-S3). Further, the NMR spectral data of eugenol were comparable to the published reports [15-16]. For chavicol, the characteristic aromatic ring proton signals were observed at δ 6.77 (2H, d, J=8.4 Hz); δ 115.20 and δ 7.05 (2H, d, J=8.1 Hz); δ 129.60 in ^{1}H and ^{13}C, respectively. The methylene protons in C$_6$-C$_3$ side chain was marked by a sharp doublet at δ 3.33-3.35 (d, J=6.3 Hz). In ^{13}C-NMR, a total of nine carbon resonances attributed to two methylene, five methine and two quaternary carbons were identified. The carbon directly attached to –OH was observed downfield at δ 154 ppm. The NMR spectral data of chavicol were comparable to the published reports [17]. The ^{1}H and ^{13}C chemical shift values for eugenol and chavicol were also calculated and verified using the expressions δ=7.27 + Σ S and δ=128.5 + Σ S, respectively where S represents substitution at *ortho-, meta-* or *para-*position. The agreement between the calculated and observed ^{1}H and ^{13}C values was good. However, the deviations from observed ^{1}H value were greater in eugenol as compared to chavicol because the former possessed *ortho* substitution. Hence, we conclude that, in addition to the GC-FID, enantio-GC-FID and GC/MS experiments, NMR could be utilized successfully to characterize *P. racemosa* leaf essential oil.

Compound	RI$_1$*	RI$_2$*	(FID response in %)		Mode
			Spring	Autumn	
α-Pinene†	937	1036	0.1	nd	RI, MS
1-Octen-3-ol†	977	1446	0.9	nd	RI, MS
3-Octanone	985	1245	0.7	nd	RI, MS
β –Myrcene	991	1153	9.6	0.3	RI, MS
3-Octanol†	996	1390	0.4	nd	RI, MS
α-Phellandrene	1009	1163	0.7	nd	RI, MS
α-terpinene	1016	1168	0.1	nd	RI, MS
p-Cymene†	1022	1254	0.3	nd	RI, MS
Limonene†	1025	1183	3.8	0.2	RI, MS
(Z)-β-ocimene	1142	1240	0.2	nd	RI, MS
γ-Terpinene†	1056	1245	0.1	nd	RI, MS
Linalool†	1101	1543	0.6	0.1	RI, MS
Chavicol	1256	2331	7.7	1.3	RI, MS, ^{1}H, [13]C
Eugenol†	1365	2148	72.9	92.9	RI, MS, ^{1}H, [13]C
β-Caryophyllene	1427	1564	0.1	0.7	RI, MS
α-Humulene	1461	1657	0.1	0.3	RI, MS
Germacrene D	1482	1684	0.1	0.5	RI, MS
δ-Cadinene	1528		nd	1.5	RI, MS
Phenylpropanoids			93.3	94.2	
Terpenoids			3.9	3.6	
Total identified			97.2	97.8	

Notes: *Retention Index (RI$_1$) on Equity-5 and (RI$_2$) Supelcowax 10 capillary columns using a homologous series of *n*-alkanes (C$_6$-C$_{28}$ hydrocarbons, Polyscience Corp. Niles IL), t=trace <0.1%, †= Sigma Standard, elution order and mass spectrum were used to confirm the identity of each constituent, nd: not detected.
Table 1: Relative percentage of constituents in *Pimenta racemosa* (Mill.) JW Moore essential oil.

	Pimenta racemosa (%)				*Pimenta dioica* (%)			
Constituents	I	II	III	IV	V	VI	VII	VIII
Phenylpropanoid								
eugenol	45.2-52.7	55.7-61.9	45.60	52.7	44.41-68.93	73.35-74.71	76.02	54.26
chavicol	7.1-9.3	8.0-15.3	9.31	6.3	0.01-15.57	-	0.12	-
methyleugenol	nd	nd	1.02	nd	0-11.88	4.08- 9.54	7.14	-
Terpene								
myrcene	25.1-29.4	12.5-22.3	24.97	26.6	0.1-16.17	nd	0.11	-
limonene	3.0- 4.0	-	2.92	-	-	-	0.12	-
β-caryophyllene	-	-	0.47	-	-	3.3- 4.9	6.47	-

I: Int. J Biosciences, 2012, 2(9), 1-12; II: J de la Societe Ouest- Africaine de Chimie, 2008 13(26), 11-19; III: Ernaehrung, 2007, 31 (7/8) 293-300; IV: J Essent Oil Res. 1996, 8(2), 207-209; V: J Essent Oil Res. 1991, 3(5), 323-329; VI: Nat Prod Res. 2011, 25(2), 152-160; VII: Ernaehrung, 2007, 31 (2) 55-62; VIII: : J Essent Oil Res. 1996, 8(3), 331-332.

Table 2: Comparison of compositions (%) of major constituents in *Pimenta* essential oil.

	Eugenol			Chavicol		
C/group	^{1}H	^{13}C	C/group	^{1}H	^{13}C
1	-	146.40[141.0]		-	154.00[155.4]
2	-	143.81[147.1]	2/6	6.77 (2H, *d*, J=8.4Hz) [6.77]	115.20[115.7]
3	6.69(1H, *d*) [6.70]	114.23[115.5]	3/5	7.05 (2H, *d*, J=8.1Hz) [7.13]	129.60[129.9]
4	-	131.88[131.3]		-	131.88[130.3]
5	6.71 (1H, *d*) [6.70]	111.07[122.2]		-	129.60[129.9]
6	6.86-6.88 (1H, *d*) [6.68]	121.12[116.7]		-	115.20[115.7]
1'	3.33-3.35 (*d*, J=6.3 Hz)	39.83	1'	3.33-3.35 (*d*, J=6.3 Hz)	39.28
2'	5.91-6.04 (1H, *m*)	137.78	2'	5.91-6.04 (1H, *m*)	137.85
3' Hα/ Hβ	5.06-5.12 (2H, *m*)	115.46	3' Hα/ Hβ	5.06-5.12 (2H, *m*)	115.34
-OH	5.59 (1H, br *s*)	-	-OH	5.59 (1H, br *s*)	-
-OCH$_3$	3.88 (3H, *s*)	55.79		-	-

^{1}H and ^{13}C chemical shift values for eugenol and chavicol were calculated and verified using the expressions δ = 7.27 + Σ S and δ = 128.5 + Σ S, respectively where S represents substituent position in benzene ring as shown in brackets.

Table 3: NMR (δ ppm, 300 MHz) data of *Pimenta racemosa* essential oil for eugenol and chavicol.

Enantiomers	cRI*	Optical Purity (in TBDE-β-CD chiral column)			
		Spring	ee(%)	Autumn	ee(%)
(*S*)-(-)-Limonene	1060	70.09	41.1	72.63	45.3
(*R*)-(+)-Limonene	1075	29.01		27.37	
(*R*)-(-)-Linalool	1193	93.35	86.7	94.95	89.9
(*S*)-(+)-Linalool	1203	06.65		05.05	

*Retention Index (RI) on TBDE-β-CD (6-*tert*-butyldimethylsilyl-2,3-diethyl-β-cyclodextrin) capillary column using a homologous series of n-alkanes (C$_6$-C$_{28}$ hydrocarbons, Polyscience Corp. Niles IL), ec: optical purity; ee: Enantiomeric excess.

Table 4: Enantiomeric composition (%) of chiral terpenoids in leaf essential oils of *Pimenta racemosa*.

Chiral analysis

Two marker chiral pairs such as limonene and linalool were studied for their enantiomer discrimination (Table 4). Chiral phase coated with 6-*tert*-butyldimethylsilyl-2,3-diethyl-β-cyclodextrin revealed enantiomeric excess for (*S*)-(-)-limonene (41.1-45.3%; Figure 5b) over (*R*)-(+)-limonene (Figure 5a). Similarly, (*R*)-(-)-linalool (86.7-89.9%; (Figure 6a) was recorded in high excess as compared to (*S*)-(+)-linalool (Figure 6b). Besides above, there has been no chiral differentiation observed for other constituents in the leaf oil. Since, phenylpropanoids lack chirality. Therefore, high (*R*)-(-)-linalool excess may be one of the authenticating tool for *P. racemosa* essential oil. In conclusion, the systematic chiral investigations have revealed that the presence of both enantiomers is common for monoterpenes such as limonene and linalool in *P. racemosa* essential oil and the similar signatures had been observed in many earlier reports [18,19].

Acknowledgements

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