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Authentication of misidentified *Pimenta* species in India using volatile compound profiling and multivariate analysis

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Abstract

Allspice (*Pimenta dioica*), a Caribbean tree spice introduced to the tropics, including India, has been found to share morphological and anatomical traits with the Bay Rum tree (*P. racemosa*), though only Allspice berries are suitable for culinary use. Thus, the present work was undertaken to reiterate the identity of *Pimenta* species grown in India using volatile compounds-based multivariate analysis. The essential oil (EO) was distilled from the samples, and its constituents were profiled using GC-MS. The results showed that the berry essential oil EO of *Pimenta* species from Kerala showed eugenol as the major constituent (39.9 %) followed by myrcene (22.2 %). Other major constituents were chavicol, limonene, linalool, and *trans*-β-caryophyllene. The EO distilled from samples in three different locations in the Western Ghats region of India also showed a similar composition, which matched the EO profile of *P. racemosa*. Moreover, of the five market samples tested, three indigenous samples showed a volatile profile of *P. racemosa*, and the remaining two exotic samples matched the profile of *P. dioica*. The findings indicate that the sampled Indian-grown and locally marketed material labelled as Allspice was chemically consistent with *P. racemosa* rather than *P. dioica*.

Key words: Allspice; bay rum; volatile constituents; chemotypes

Introduction

Allspice or Pimento, the spice known for its combined flavor and aroma of four important spices *i.e* . cloves, nutmeg, cinnamon and black pepper. The Genus *Pimenta* comprises 20 species of small to medium trees or shrubs native to the Caribbean and Central American regions (Landrum, 1986). Among these, Allspice (*Pimenta dioica* (L.) Merrill) and bay rum, (*Pimenta racemosa* (Miller) J. Moore) are the economically important species (Paula *et al*., 2010). Allspice berries are widely used for seasoning, flavouring soups, desserts, and juices in Caribbean cuisines. Unlike Allspice, leaves are the major economically important part of the Bay rum. The EO distilled from the leaves is used in the manufacture of cologne, ointment, aftershave and many products (Contreras-Moreno *et al*., 2014). The use of dried berries of bay rum as a spice is not reported in the literature, and some even state that their fruits and essential oils are toxic and not recommended for culinary use (Boning, 2010). Contreras-Moreno (2018) extensively reviewed the chemical composition of the essential oil (EO) of diverse Pimenta species from different parts of the world. He reported that in Jamaican Allspice, eugenol (66.38–79.24 %) is the major constituent, whereas Mexican Allspice contains methyl eugenol (48.3–62.7 %) and eugenol (8.3–17.3 %) as the major volatile compounds.

The main morphological differentiating characters between *P. dioica* and *P. racemosa* are the number of calyx lobes, which is 4 in *P. dioica* and 5 in *P. racemosa* (El-gizawy *et al*., 2019). Allspice seems to have been introduced in India during the first decades of the 19th century by British people, and there is evidence of Allspice trees growing at Burliar Estate, Tamil Nadu, India during the 1850’s (Mahindru, 1982). However, a recent study from our institute reports that Allspice has been widely misidentified in India, and the species grown and marketed as Allspice is not the true Allspice (*P. dioica*) but an allied species, *P. racemosa* (Muhammed Nissar *et al*., 2024). The determination of the species identity in the dried or ground form of marketed produce is difficult as the calyx portion in the berry is lost during the process of its postharvest curing. In this context, chemical authentication based on EO composition will help identify the correct species and its chemotypes. Since India is the major exporter of processed spices, the differentiation of species in the marketed produce is the need of the hour.

With this background, the present study aims to authenticate *Pimenta* species marketed as Allspice in India by analyzing their volatile profiles and applying multivariate statistical techniques for differentiation of species.

Materials and methods

Plant materials collection: Three different sets of plant materials were used in the present study. Since available information about the volatile constituents of *Pimenta* species from India is limited and to better understand the distribution of volatile compounds in different plant parts, leaf, berries and fruit stalk of *Pimenta* sample collected from Wayanad, Kerala (11°66’26 “N & 76°10’98”E) was used for the distillation of EO. The botanical identity of the collected species is established, and a voucher specimen is deposited in the herbarium of ICAR-IISR. Subsequently, to ascertain the identity of Pimenta species spread across the Western Ghats region, two more samples from

Kozhikode, Kerala (11°29'80"N & 76°10'98"E) and Kodagu, Karnataka (12°38'87"N & 75°71'70"E) were collected. These two samples, along with Wayanad samples, were analyzed to determine the variation in the volatile constituents of EO from different locations. These locations are selected because of the widespread distribution of the species under study and its commercial cultivation. From each site, samples were collected from different trees, pooled, and used for hydrodistillation of EO. Samples of leaves, matured unripe berries and fruit stalk were separated, air-dried and pulverized to obtain coarse powder for distillation. For third part of the study, samples marketed as Allspice were obtained from an e-commerce platform. The details of the samples are as follows:

Sample	Source/Sample description	Place
Sample 1	Imported (Berry)	Unknown
Sample 2	Local (Berry)	Kerala, India
Sample 3	Local (Berry)	Kerala, Wayanad, India
Sample 4	Local (Berry)	Kerala, Wayanad, India
Sample 5	Imported (Berry powder)	Mexico
Sample 6	Local (Allspice EO)	India
Sample 7 (Control)	Local (Berry)	Kerala, Wayanad India

Sample origin and trade identity were recorded from package labels; botanical identity was inferred from volatile profiles. The powdered samples are used as such, whereas the berry samples are pulverized as mentioned before and used in the distillation of EO.

Essential oil extraction: The EO was distilled using the hydrodistillation method in a Clevenger-type apparatus, as specified in AOAC method 962.17 (AOAC, 2005). Coarsely ground samples (40 g) were boiled with water and distilled for 4 h under normal pressure. The distilled oil was collected from the apparatus, residual water was removed using anhydrous sodium sulphate, and the oil was stored in an amber bottle at 4 ºC before Gas Chromatography – Mass Spectrometry (GC-MS) analysis. The distillation was performed three times, and the oil yield (v/w) was calculated as follows: % EO = (Volume of oil (mL) / Weight of sample (g)) x 100. All analyses were performed in triplicate.

Gas Chromatography–Mass Spectrometry (GC-MS) analysis: EO was analyzed qualitatively for its volatile constituents using a QP-2010 GC-MS system (Shimadzu, Japan). The separation was achieved in a column packed with 100 % Polyethene Glycol (PEG) with dimensions of 30 m x 0.25 mm x 0.25 µm (Restek, USA). The column temperature program was as follows: 65 °C for 2 min, then a gradient of 3 °C/min to 220 °C, and hold at 220 °C for 3 min. The ion source and interface temperatures were set to 200 °C and 240 °C, respectively. Other operational Parameters were as follows: column over temperature – 65 °C; Injection temperature – 240 °C; Helium Flow Rate - 1.0 mL/min; Injection Volume- 0.2 µL; Injection Mode - split (1:50 split ratio); electron ionization energy - 70 eV; acquisition Mode – scan; scan Range, 40-650 m/z with the scan speed of 1428. The peaks are integrated by scanning through each peak for its mass spectra to avoid the contamination of the peak with other compounds.

The mass spectra of the components were compared with the NIST/WILEY standard mass spectral library and identified by similarity search (Babushok *et al*., 2011). The linear retention indices (LRI) of volatile constituents are calculated with reference to a homologous series of n-alkanes standard (C8 – C40) (Sigma–Aldrich, USA) using the formula suggested by Van den Dool and Kratz (1963). For compound identification, a set of standard solutions is prepared and run under the same chromatographic conditions as the sample.

Statistical analysis: All the analyses were carried out three times. The normalized peak area from the GC-MS total ion chromatogram (TIC) was used to detect significant differences in volatile constituents across different geographical locations using Duncan's multiple range test (DMRT) at the 5% level of significance. The multivariate analysis of the market samples was performed in an R based platform, Metaboanalyst 5.0. The data were normalised by sum normalisation, log transformation, and auto-scaling using the features available on the platform to reduce sample-to-sample variation and regulate the variance of metabolites across samples. Partial Least Squares-Discriminant Analysis (PLS-DA) and Hierarchical clustering Heatmap (HCH) analysis was done to understand the relationship between different samples based on the volatile constituents (Hu *et al*., 2023).

Results and discussion

Variation in volatile constituents in different plant parts of *Pimenta* species collected from Wayanad region: The average yield of EO of berries, leaves and fruit stalks was 3.5 %, 3.0 % and 1.4 %, respectively. The harvested berries contained 5-10% fruit stalk, necessitating the inclusion of fruit stalk as separate samples in our study. There is no significant variation in EO yield among samples from different locations. This suggests that the Indian peninsular region, especially the Western Ghats region, has suitable climatic conditions for the growth and spread of this *Pimenta* species. In Allspice, both leaf and berry EO is used as economic produce and whole berries are used as spice in the preparation of seasonings, confectionery and juices. Unlike Allspice, leaf EO was given importance in bay rum, though tree produces a large number of fruits (Boning, 2010). In our study, unripe fruits were collected and processed, and the volatile composition of dried leaves, fruits, and fruit stalks was analysed.

Qualitative analysis of GC-MS data identified 39 major volatile compounds in the EO (Table 1). The major compound in in EO of different plant's parts was found to be eugenol (39.9 % in berries, 52.3 % in leaves and 57% in fruit stalk). The monoterpene compound myrcene was found to be highest in berries (22.2 %), followed by leaves (15.8 %) and fruit stalks (11.6 %). Chavicol, a phenylpropanoid, was found to be highest in leaves (13.5 %), followed by fruit stalk (8.9 %) and berries (8.2 %). Other major constituents are limonene (7.0 %, 5.3 % and 4.7 % in berries, leaves and fruit stalk respectively), *trans*-ocimene (2.6 %, 0.4 % and 3.9 % in berries, leaves and fruit stalk respectively), δ-cadinene (1.9 %, 1.4 %, 2.5 % in berries, leaves and fruit stalk respectively) and β-caryophyllene (1.6 %, 0.5 % and 0. 7 % in berries, leaves and fruit stalk respectively). The monoterpene sabinene (0.2%) was detected only in berries. The compounds, namely zingiberene (0.4%), β-bisabolene (0.11%) and β-sesquiphellendrene (0.14%), were detected only in leaves. The fruit stalk contained the following unique compounds compared to other parts: β-caryophyllene epoxide (0.07%), spathulenol (0.05%), and a phytol isomer (0.14%). Different EO constituents are grouped according to their chemical classification, and the phenolic group consists of eugenol and chavicol, which together

Table 1. Percentage composition of the berry, leaf and fruit stalk of EO of *Pimenta* species from the Wayanad region

Volatile components	RT	RI*	LRI**	Normalized Area % (Absolute peak area %)			Identification of the peak
				Berries	Leaves	Fruit Stalk	
α-pinene	3.08	1025	1026	0.71	0.58	0.21	RI, MS, STD
β-pinene	4.42	1110	1111	0.07	0.03	0.02	RI, MS, STD
Sabinene	4.71	1122	1122	0.16	ND	ND	RI, MS, TI
Myrcene	5.90	1160	1167	22.17	15.82	11.57	RI, MS, STD
carene isomer	6.22	1133	1179	0.23	0.13	0.10	RI, MS, TI
Limonene	6.78	1198	1200	6.98	5.28	4.73	RI, MS, STD
β-Phellandrene	6.99	1209	1207	1.17	0.74	0.71	RI, MS, TI
cis-ocimene	7.87	1234	1235	0.07	0.04	0.06	RI, MS, TI
Υ-terpinene	8.11	1245	1243	0.33	0.15	0.14	RI, MS, TI
trans-ocimene	8.38	1250	1252	2.61	0.42	3.87	RI, MS, TI
p-cymene	8.81	1270	1266	0.25	0.56	0.52	RI, MS, STD
Terpinolene	9.19	1282	1278	0.44	ND	0.17	RI, MS, TI
3-octanol	12.68	1391	1392	0.41	0.43	0.23	RI, MS, TI
1-octen-3-ol	14.22	1444	1445	0.72	1.18	0.71	RI, MS, TI
α-copaene	15.15	1491	1477	1.00	0.50	0.75	RI, MS, TI
n-decanal	15.40	1495	1486	0.15	0.14	0.09	RI, MS, TI
Linalool	16.88	1543	1535	1.21	1.23	0.85	RI, MS, STD
β-caryophyllene	18.03	1588	1574	1.55	0.48	0.74	RI, MS, STD
terpinen-4-ol	18.27	1601	1582	0.64	0.57	0.84	RI, MS, STD
α-humulene	20.12	1666	1640	0.67	0.27	0.49	RI, MS, STD
Υ-muurolene	20.78	1689	1661	0.49	0.49	0.75	RI, MS, TI
α-terpineol	21.14	1694	1672	0.12	0.14	0.21	RI, MS, STD
Germacrene D	21.34	1708	1679	4.03	0.04	0.11	RI, MS, TI
Zingiberene	21.90	1720	1696	ND	0.38	ND	RI, MS, TI
α-muurolene	21.91	1723	1696	0.24	0.05	0.38	RI, MS, TI
β-bisabolene	22.09	1727	1702	ND	0.11	ND	RI, MS, TI
δ -cadinene	22.9	1755	1727	1.91	1.37	2.45	RI, MS, TI
1-decanol	23.35	1754	1740	0.07	ND	0.14	RI, MS, TI
β-sesquiphellandrene	23.37	1771	1741	ND	0.14	ND	RI, MS, TI
Caryophyllene oxide	29.74	1986	1933	ND	ND	0.07	RI, MS, STD
Trans-cinnamaldehyde	31.28	2033	1979	ND	0.16	0.32	RI, MS, STD
Eugenol	35.62	2162	2113	39.87	52.27	57.02	RI, MS, STD
τ-cadinol	35.74	2176	2117	0.12	0.09	0.16	RI, MS, TI
α-cadinol	36.14	2180	2130	0.24	0.22	0.35	RI, MS, TI
δ -cadinol	36.53	-	2142	0.16	0.14	0.22	MS, TI
τ-muurolol	37.45	2224	2171	0.43	0.32	0.56	MS, TI
Chavicol	40.41	2337	2267	8.17	13.52	8.94	RI, MS
Phytol isomer	48.26	2613	2549	ND	ND	0.14	RI, MS
Phenolics				48.04	65.79	65.96	
Monoterpenes				35.16	23.91	22.08	
Sesquiterpenes				10.45	3.66	6.03	
Oxygenated Monoterpenes				2.12	2.16	2.06	
Aliphatic alcohol				1.12	1.61	0.94	
Sesquiterpene alcohols				0.95	0.76	1.32	

*-Retention Indices available in literatures; **- Linear Retention Index calculated by injecting homologous series of n-alkanes (C8-C40); ND – Not Detected; Values represent mean of three independent distillations/GC-MS runs.; MS-Mass spectrum; STD-identification through authentic standard; TI-Tentative Identification

account for the major proportion, followed by monoterpenes and sesquiterpenes. The combined chromatogram of berries, leaves and fruit stalk EO with identification of major peaks is provided as Fig. 1.

When the results were compared with the essential oil profiles of Jamaican and Mexican Allspice and Bay Rum reported in the literature (Table 2), our sample contained chavicol (8.17 %), whereas neither Jamaican nor Mexican Allspice contained this compound. On the other hand, Bay-rum was reported to have chavicol (10.49 %) (Kim *et al*., 2008). It clearly showed that the species grown in India, labelled Allspice, is not *Pimenta dioica* but *Pimenta racemosa*. Several studies reported that eugenol, methyl eugenol, 1,8-cineole, caryophyllene, and myrcene as the major volatile constituents in leaves and berries of Jamaican and Mexican Allspice (Green and Espinosa, 1988 and Morsy and Hammad, 2018; Padmakumari *et al*., 2011; Zabka *et al*., 2009; Jiang *et al*., 2013). Andrade *et al*. (2023) reported the presence of chavicol (5.12 %) in Allspice leaves from Brazil. Volatile analysis of Allspice samples from Sri Lanka showed the presence of eugenol (85.3 %) followed by β-caryophylene (4.4 %), 1,8-cineole (4.2 %) linalool (0.8 %) and α-humulene (0.8 %) as the major constituents. Myrcene (0.07 %) and chavicol (0.33 %) was found in very low amounts (Dharmadasa *et al*., 2015). In Egypt, volatile analysis of the leaves of *P. racemosa* EO showed high amounts of eugenol (37.95%), Myrecene (21.01%), Pinene (17.82%), linalool (6.15%) and limonene (5.93%) (El-gizway *et al*., 2018). Only a few reports from India have reported the presence of chavicol in the purported Allspice leaves (Sarathamabal *et al*., 2020; Ashokkumar *et al*., 2022) collected from the Western Ghats region. However, the correct identity of the species could have been overlooked in their analysis.

Tucker *et al*. (1991) reported the variation in leaf EO constituents of three varieties of *P. racemosa*. *P. racemosa* var. *girsea* contains geraniol, methyl eugenol and/ or trans-methyl isoeugneol as major constituents whereas *P. racemosa* var. *hispaniolensis* are dominated by 1, 8 cineole, methyl chavicol, methyl eugenol, γ-terpinene and terpinen-4-ol and thymol. *P. racemosa* var. *ozua* contains 1,8 cineole, limonene and/or α-terpineol as major compounds.

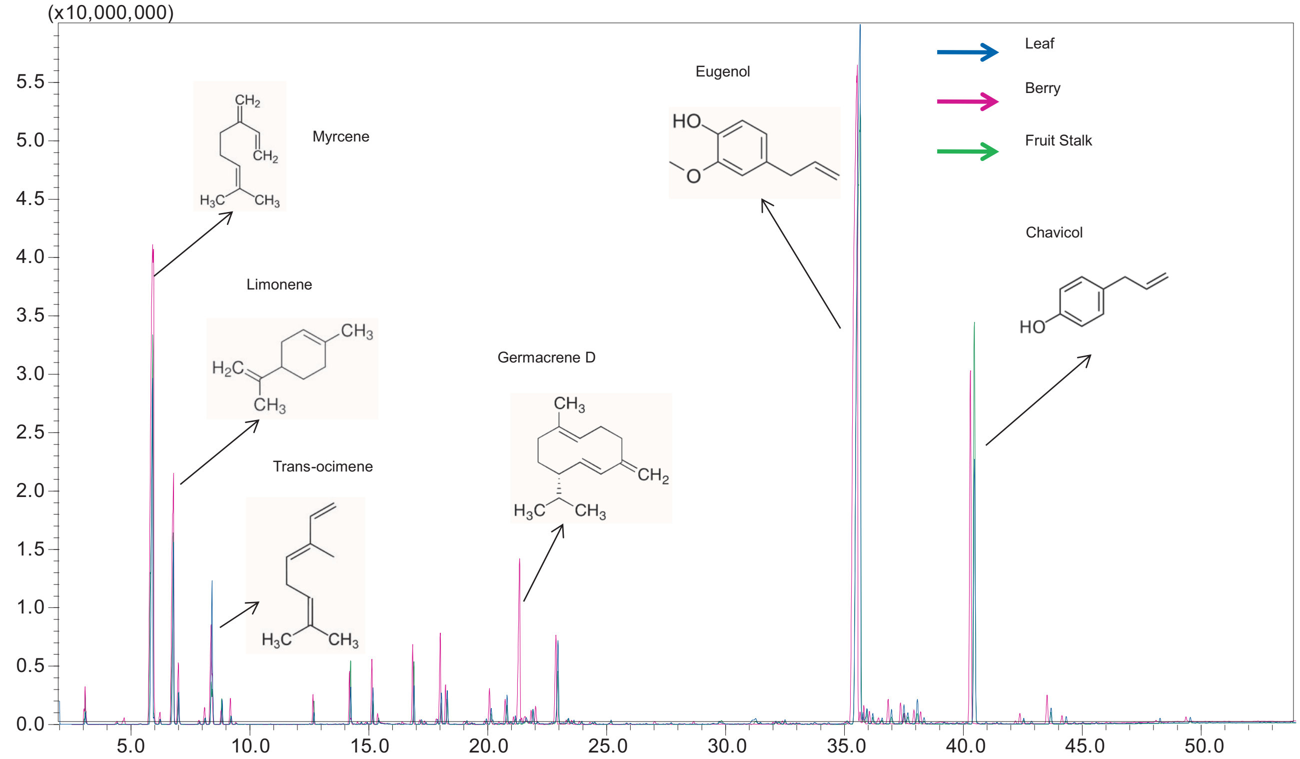

Fig. 1. Combined GC-MS chromatogram of volatile constituents of *P. racemosa* leaves, berries and fruit stalk

Table 2. Comparison of the relative area percentage of major constituents of essential oil of *Pimenta*

Volatile components	Present study$	Jamaican Allspice*	Mexican Allspice#	Bay-rum&
Myrcene	22.17	0.17-0.30	12.72	25.88
Limonene	6.98	0.14-0.22	0.56	3.10
ϒ-terpinene	0.33	0.33-0.73	0.2	0.1
trans-ocimene	2.61	NR	0.73	NR
β-caryophyllene	1.55	4.25-5.39	3.79	0.38
Eugenol	39.87	68.8-78.2	7.09	46.25
Methyl Eugenol	A	2.9-13.1	65.14	0.45
Chavicol	8.17	NR	NR	10.49

* - Green and Espinosa, 1988; # - Morsy and Hammad, 2018; $ - Present study, 2023; & - Kim *et al*., 2008; NR – Not Reported

Garcia *et al*. (2002) reported that *P. racemosa* var. *grisea* leaf EO contains 4-methoxy isoeugenol and 4-methoxy eugenol as major compounds and *P. racemosa* var. *terebinthina* has α-terpineol acetate, α-terpineol and 4-methoxy eugenol as major constituents.

By comparing the results with existing literature, the study conclusively demonstrated that the species is *P. racemosa* var. racemosa (Adjou *et al*., 2017; Garcia *et al*., 2002). The EO composition of var. *racemosa* from different parts of the Caribbean island countries and elsewhere has been found to vary in flavour. The botanical classification of the species into varietal form did not match with their volatile oil constituents. Therefore, *P. racemosa* var. *racemosa* has again divided into three chemotypes based on its flavor profile. First is "clove" type which contains eugenol, chavicol and myrcene as major compounds, the second is "anise" type wherein methyl eugenol, methyl chavicol and myrcene are reported as major compounds and third type is known as "lemon" because of higher percentage of geranial and neral compounds in its EO (Abaul *et al*., 1995). Chemotypes are distinct groups within a plant's subspecies that differ in chemical composition, though they morphologically appear similar. In our study, eugenol was the major volatile constituent in both leaves and berries followed by myrcene, chavicol, limonene and β-caryophyllene. Based on differences in the volatile constituents reported for these two species, the species analysed in the present study was *P. racemosa* var. racemosa "clove" chemotype, as no methyl eugenol or geranial was detected. There is no scientific literature available on the volatile composition of *P. racemosa* berry essential oil. By far, our knowledge extends our study is the first report of volatile constituent analysis of bay rum tree fruit and fruit stalk. Since the higher percentage of myrcene and lower amount of eugenol was detected in berries compared to leaves, berry EO has mellowed flavor than its leaf EO.

Variation in volatile constituents of leaves and berries: Once the chemical identity of the species at the collected location was established, three more samples were collected from different geographical locations varying in altitude within the Western Ghats region of India. Since the trees in the Kozhikode location did not bear fruit, only Wayanad and Kodagu samples were compared for their volatile constituents. Statistical analysis showed that among major constituents, only few compounds showed significant differences among the three locations. Eugenol

Table 3a. Percentage composition of the berry EO of *Pimenta* samples collected from different geographical location

Berry EO constituents	Normalized area % (Absolute peak area %)	
	Wayanad	Kodagu
Myrcene	23.17^{B}	26.58^{A}
Chavicol	8.15^{A}	6.64^{B}
Limonene	7.44	8.16
Linalool	1.31	1.25
trans-ocimene	2.78	3.09
Germacrene D	4.21^{A}	2.77^{B}
β-caryophyllene	1.72^{A}	1.24^{B}
Eugenol	39.32	40.18

The values represented are the average of three replications; Different superscripts (^{A,B}) in the column indicate a significant difference ($P < 0.05$) between locations

Table 3b. Percentage composition of the leaf EO of *Pimenta* samples collected from different geographical locations

Leaf EO constituents	Normalized area % (Absolute peak area %)		
	Wayanad	Kodagu	Kozhikode
Myrcene	16.18^{A}	14.40^{B}	15.98^{A}
Chavicol	13.77^{B}	15.64^{A}	12.36^{C}
Limonene	5.38^{A}	4.55^{B}	4.62^{B}
Linalool	1.25^{A}	1.11^{B}	1.0^{C}
trans-ocimene	0.42	0.72	0.10
1-octen-3-ol	1.20^{B}	0.99^{C}	2.68^{A}
δ -cadinene	1.39	1.58	1.51
Eugenol	53.23	54.48	54.61

The values represented are the average of three replications; Different superscripts (^{A,B}) in the column indicate a significant difference ($P < 0.05$) between locations

did not show significant differences in berry EO between Wayanad and Kodagu samples, whereas myrcene was higher in Kodagu (26.6 %) than in Wayanad (23.1 %). The contrary results were observed in the case of chavicol, germacrene D and β-caryophyllene, wherein the Wayanad sample had higher content (8.2 %, 4.2 % and 1.7 % respectively) than the Kodagu sample (6.6 %, 2.8 % and 1.2 % respectively) (Table 3a). In the case of leaf EO, no significant differences in eugenol content among the three locations. Myrcene was found to be higher in Wayanad and Kozhikode, whereas chavicol was higher in Kodagu. The leaf and berry EOs showed alteration in major constituent content across three locations (Table 3b). This showed that, like many spices and aromatic crops, Pimenta also exhibits climate-dependent variation in its volatile constituents. Despite these minor variations, the overall volatile profile of these three samples is found to be the same.

Thus, the study showed that the species present in this region is *P. racemosa*, not *P. dioica*. In India, Pragadeesh *et al*. (2013) studied the variation in leaf EO composition of *P. racemosa* during spring and autumn. They reported 72.9-92.9% eugenol content, whereas our study recorded only 53.2–54.6% eugenol in leaves; however, myrcene and chavicol content in our study were higher than their reported values. This variation could be attributed to climatic differences in the northern part of India and the Western Ghats region. They suggest that chavicol could serve as a marker compound to differentiate between *P. dioica* and *P. racemosa*. Our study also corroborates this finding, as chavicol is detected at higher percentages in both leaves, berries, and fruit stalks of the *P. racemosa* samples collected in Western Ghats regions. Apart from that our study also suggests the use of methyl eugenol and methyl chavicol to differentiate between these two speices.

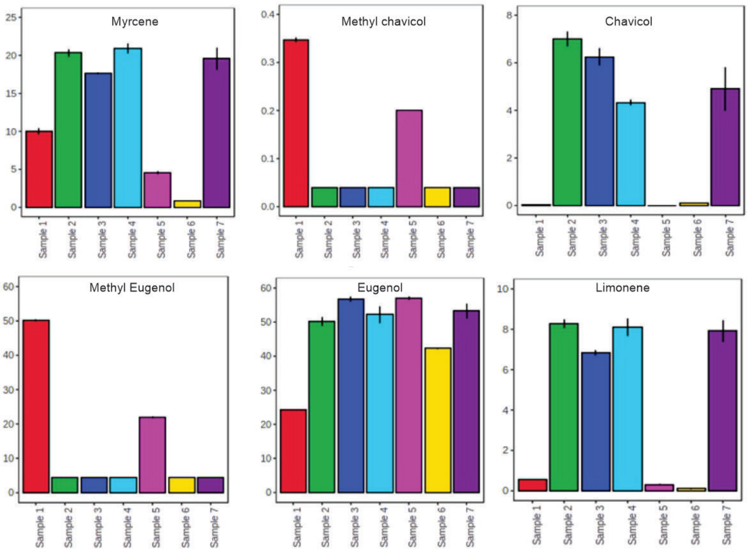

Fig. 2. Comparison of major volatile compounds of different market samples of Pimenta (Samples 1 & 5 – *P. dioica*; Samples 2, 3, 4 & 7 – *P. racemosa*; Sample 6 –*P. dioicia* EO)

Authenticating the samples marketed as Allspice using volatile profiles: For the next part of the study, six market samples (five berry samples and one commercial Allspice essential oil) and one local sample were used to authenticate the species available in the market. The analysis showed that sample 1 and sample 5 had methyl eugenol (50.16 % and 21.93 %, respectively) and it is not detected in other three samples. The eugenol content was found to be highest in Sample 5 (57.04 %), followed by Sample 3 (56.79 %), Sample 4 (52.29 %), Sample 2 (50.16 %), and Sample 6 (42.23 %), and lowest in Sample 1 (24.34 %). The compound methyl chavicol was detected only in Sample 1 (0.35 %) and Sample 5 (0.2 %) and in the remaining samples, it was not detected. The alternate trend was observed in case of limonene, wherein sample 2 (8.29 %), sample 3 (6.84 %) and sample 4 (8.10 %) and sample 7 (7.91 %) showed higher content, whereas sample 1 (0.56 %) and sample 5 (0.32 %) and sample 6 (0.11 %) showed lower content. Similar trend was observed in case of myrcene; Sample 1 (10.01 %) and sample 5 (4.57 %) showed lower content whereas sample 2 (20.36 %), Sample 3 (17.62 %), Sample 4 (20.91 %), sample 7 (19.57 %) showed higher content (Fig. 2). Sample 6, which is the commercial essential oil had more of the diluent named α-toluenol (32.36 %), eugenol (42.23 %) and β-caryophyllene (18.58%)

The GC-MS data were used to perform multivariate analysis to assess the correlation and interconnectedness of samples with respect to their volatile constituents. The 2D score plot created using partial least squares-discriminant analysis (PLS-DA) showed that samples 1, 5, and 6 were separated from the other samples. Though Sample 1 and

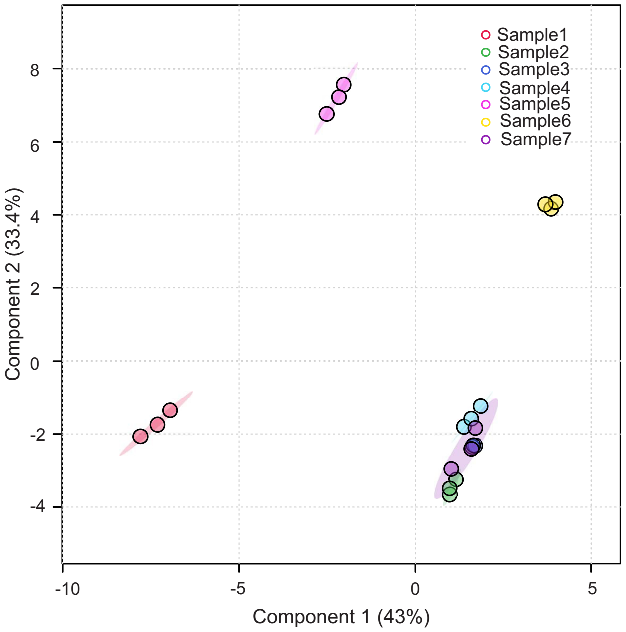

Fig. 3. 2D score plot of PLS-DA analysis of volatile constituents of *Pimenta* market samples (Samples 1 & 5 – *P. dioica*; Samples 2, 3, 4 & 7 – *P. racemosa*; Sample 6 –*P. dioicia* EO)

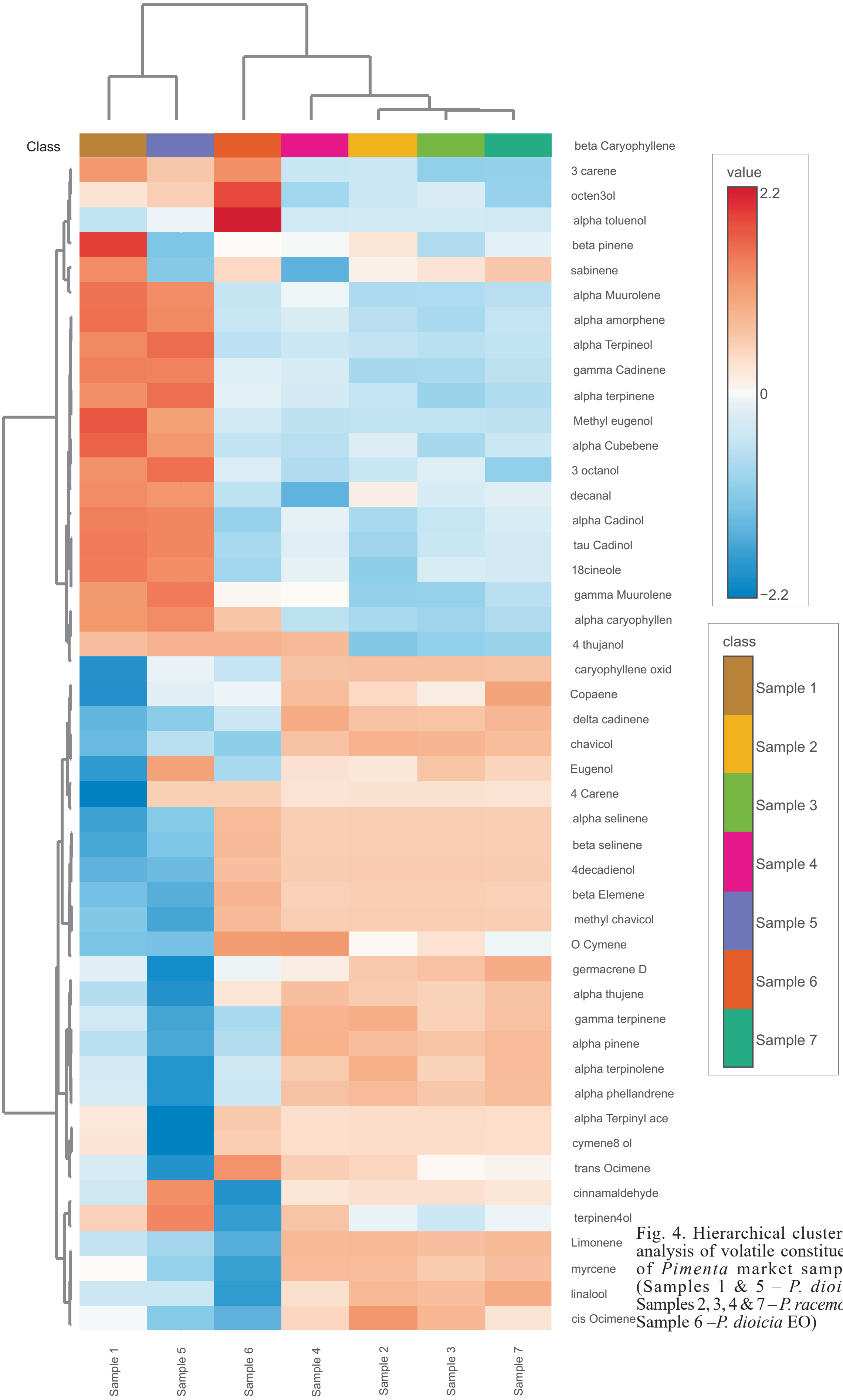

Fig. 4. Hierarchical clustering analysis of volatile constituents of *Pimenta* market samples (Samples 1 & 5 – *P. dioica*; Samples 2, 3, 4 & 7 – *P. racemosa*; Sample 6 –*P. dioicia* EO)

Sample 5 were positioned in the upper left quadrant, the distance between them was greater due to differences in their volatile constituents. Sample 2, sample 3 and 4 were clustered together with sample 7 (control samples) in lower right quadrangle. They had similar percentages of volatile constituents (Fig. 3). The hierarchical clustering heat map constructed from the datas*et al*.o confirms this (Fig. 4). Sample 6, a commercial essential oil, had more of the diluent α-toluenol. It is separated from the rest of the sample and located in the upper right quadrant. This analysis establishes that three samples of India (Kerala) origin marketed as Allspice are in fact *P. racemosa*, not *P. dioica*. Two exotic-origin samples (sample 1 and sample 5) showed a distinct volatile profile similar to that of *P. dioica* reported in the literature. Based on exploration in Western Ghats regions, *P. dioica*, *i.e* ., true Allspice, is found only in Bonacuad estate in Thiruvananthapuram district, Kerala, and the tree is very irregular in bearing, and the area is located in a remote forest (Muhammed Nissar *et al*., 2024). Due to these difficulties, in the present study, the volatile constituents of a sample collected from the market with the place of origin mentioned in the label is compared to information available in research literature to identify the species as *P. dioica*

Mass spectrometry-based species authentication was previously explored in several crop species, including saffron, rose, and honey (Dou *et al*., 2023). In our study, volatile compound analysis using GC-MS and subsequent statistical analysis revealed the identity of the species in samples marketed as Allspice. Identifying the correct species is important when the plant is used for culinary purposes. Our study focused solely on volatile constituents of *Pimenta* species. Youssef *et al*. (2021) analyzed the seasonal variation in volatile constituents of stems and leaves of both *P. dioicia* and *P. racemosa* and also studied the effect of essential oil against several cancer cell line and concluded that leaf essential oil from *P. dioicia* showed good cytotoxic effect against breast, hepatic and cervical cancer cell lines. Likewise, the compound named pimentol, an active phenolic glycoside present in (*P. diocia*) was found to inhibit the androgen receptor in prostate cancer cell lines and also involved in multiple processes of cancer cell development, leads to death of cancer cells (Shamaladevi *et al*., 2013). The same compound was not reported in *P. racemosa*. Therefore, authenticating the correct species also helps in utilization of the crop for its pharmacological properties.

The present study on the profiling of volatile constituents of Pimenta species further confirms the recently reported work on the misidentified Allspice in India. The results showed that the population of *Pimenta* widely grown in India is “clove” type of *P. recemosa* var. *racemosa*. Even though morphological features such as leaf shape and the number of calyx clearly distinguish between *P. dioica* and *P. racemosa*, these characters were overlooked because it is an introduced crop, and farmers and traders are completely ignorant of its botanical identity. The bay rum tree is still multiplied and sold through nurseries as Allspice, and cultivation is being expanded into nontraditional areas in East and North East India owing to favorable climatic conditions. The volatile constituents present in leaf and berry decide the culinary quality of this spice. This study will help various stakeholders, such as farmers, research organizations, and policymakers, to correct the incorrect identity of Allspice grown in India and to initiate appropriate actions to rectify the trade of the commodity under the wrong identity. The misidentification might have occurred due to the morphological similarity between Allspice and Bay-rum berries. This could have happened in other Asian countries too, as the species is non-native and has multiplied from its initial source of introduction. Although the present study included only a few market samples, it represented the major growing areas of *Pimenta* species in India. Based on the present findings, volatile compound-based authentication of Allspice grown and marketed in other Asian countries may be undertaken to prevent the willful spread of the wrong species for culinary and pharmaceutical applications.

Declaration of competing interest: The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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