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Diversity study of leaf volatile oil constituent of *Piper* species based on GC/ MS and spatial distribution

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Abstract

Ten wild *Piper* species of Western Ghats were studied for leaf volatile oil constituents using GC/MS. The total number of major components (≥ 1%) in different species varied from 5 to 10. The leaf oil was rich in sesquiterpenoids. The most abundant compounds in *Piper* leaf oil of Western Ghats were β - Caryophyllene, Nerolidol and β - Elemene. Different accessions of *P. nigrum* collected from different parts of Western Ghats were used to study the spatial diversity of aroma with the help of DIVA GIS. The results indicated that the latitudinal influence was strong in aroma diversity. Total number of components varied from 17 to 73, while the major components found were 10 in *P. nigrum* leaves. The diversity index of the total component was 2.87.

Keywords: gas chromatography mass spectrum, geographic information system, *Piper* species, volatile oil

Introduction

Piper is a model genus for research in ecology and evolutionary biology. The large number of species and ecological diversity of this genus makes it an obvious candidate for biodiversity studies. Essential oils, odorous and volatile products of plant secondary metabolism, have wide application in folk medicine, food flavouring and preservation as well as in fragrance industries (Kalemba & Kunicka 2003). Ibrahim *et al.* (1994) made a comparative study of essential oil of five *Piper* species of Malaysia with GC/MS protocol. Orav *et al.* (2004) studied the qualitative and quantitative composition of the essential oil from black, green, and white pepper and found that the most abundant compounds in pepper oil was β-caryophyllene, limonene, β-pinene, sabinene and eugenol. Enormous level of chemical diversity was observed in *Piper* species collected from the same location in Brazil (Potzernheim *et al*. 2006). According to Mundina *et al.* (1998) the main components in the leaf essential oil were sesquiterpenes in three Panamanian *Piper* species. Francois *et al.* (2009) reported that among essential oils obtained from different tissues of *Piper* species from Cameroon, *P. nigrum* leaf oil was found to have high caryophyllene content.

Any database that deals with diversity information has to be geo-referenced. A geographic information system (GIS) is an important tool for this purpose. The GIS software can accommodate large amount of spatial and non-spatial (attribute) data for interpretation and analysis. Utpala *et al.* (2008) found variation in the volatile oil component as well as in percentage of occurrence of the component with eco-geographic variation in *Piper* species. Potzernheim *et al* (2006) observed differences in the chemical constituents of same species harvested from different biome. Combination of species occurrence data with climatic data delimits the potential distribution of many morphological and biochemical characters of the species. The aim of the present study was to record the important leaf volatile oil components of 10 *Piper* species of Western Ghats. The study also focused on the spatial influence on volatile oil component of the leaves of *P. nigrum* and the most common *Piper* species collected from different places of Western Ghats.

Materials and methods

Essential volatile oil was extracted by hydrodistillation procedure using Clevenger apparatus from fresh leaves (160-240 g) of 10 wild *Piper* species collected from the germplasm of IISR Experimental Farm, Peruvannamuzhi. GC/MS analyses were performed using Shimadzu GC-2010 gas chromatograph equipped with QP 2010 mass spectrometer. The column used was DB-5. The oven temperature was programmed as follows; 70°C for 5 minutes and then increased to 110°C at the rate of 5°C min^{-1}, then up to 200°C at the rate of 3°C min^{-1} and again upto 220°C at the rate of 5°C min^{-1}, at which the column was maintained for 5 minutes; injector temperature of 250°C. Helium was used as carrier gas at a flow rate of 1 mL min^{-1}; Identification of the constituents was done on the basis of comparison of their retention indices and mass spectra with those given in the NIST and Wileys libraries and by co-injection with authentic standards.

Volatile oil component of 19 accessions of *P. nigrum* collected from different parts of Western Ghats were also studied. This data was analyzed for spatial variation with the help of DIVA GIS software, and compared with similarity cluster prepared with SPSS software. The samples were named according to the place of collection. Shannon's Diversity index was calculated from the species domain option of DIVA GIS. This index is the measure of species diversity in a given community.

Results and discussion

The GC-MS profile of leaf volatile oil of 10 wild *Piper* species is given in Table 1. About 26 volatile components were identified as major components (≥ 1%). *P. sugandhi* had 10 components, *P. thomsoni* and *P. colubrinum* -9, *P. chaba* and *P. hymenophyllum* -8, *P. arboreum* -7, *P. betle, P. longum, P. nigrum* -5 and *P. attenuatum* -4. β-Caryophyllene and Nerolidol were the most common compounds. Percentage of β- Caryophyllene varied from 1-45%, while that of Nerolidol varied from 1-75%. β-Elemene and α-Humelene were another two generalized compounds but the variations were (0.2% to 12.4%) and (0.1% to 19.9%) respectively.

The total number of components detected in the accessions of *P. nigrum* collected from different forest regions of Western Ghats varied from 16 to 68 (Table 2) and β-Caryophyllene, Nerolidol and β-Elemene were the most generalized compounds, while 4 accessions collected from Karwar did not have β-Caryophyllene. Myristicin was detected in some of the Karwar collections and Nerolidol was absent in these samples (Table 2), whereas, Dakshin Karnataka samples did not possess both Nerolidol and Myristicin. Kerala samples had β- Caryophyllene and Nerolidol but not Myristicin. α- Pinene, a common oil of *Piper* fruits was present only in some Karwar and Dakshin Karnataka samples, but not in Kerala samples. A cluster diagram prepared with the volatile oil components showed a clear separation of higher latitudinal samples from the lower latitudinal samples (Figure 1). All the Karwar, Dapoli and Goa samples clustered together while Kerala samples formed another cluster. Rainfall and altitude of the collection sites were studied with the help of GIS software. There was no clear demarcation, though there was a tendency among the samples (Table 3).

Essential oil from 10 *Piper* species revealed around 4 and 10 major (≥ 1%) components, but total number of components varied from 28 to 84 (Table 1). This type of GC-MS profile

Table 1. GC/MS profile of ten wild *Piper* species

Constituents	*P. sugandhi*	*P. thomsoni*	*P. chaba*	*P. hymenophyllum*	*P. colubrinum*	*P.attenuatum*	*P. arboreum*	*P. betle*	*P. longum*	*P. nigrum*
β- Caryophyllene	1.10	4.32	21.21	5.14	19.02	8.20	5.16	45.30	21.69	4.1
β-Cubebene	-	8.13	-	-	-	-	-			
γ-Elemene	-	13.65	-	-	21.15	-	-			
α-Farnesene	-	6.54	-	-	-	6.01	2.01	5.69		
Nerolidol	1.08	39.24	2.33	1.57	1.36	75.28	27.27		37.74	43.2
Sapthulenol	-	12.16	-	-	-	-	-			
α- Pinene	4.8	-	-	-	-	-	-		3.39	
β-Phellandrene	2.80	-	-	-	1.61	-	-			
γ-Muurolene	3.57	-	-	-	-	-	-			
Cadinene	6.15	-	-	-	-	-	4.32			
Sabinene	28.19	-	-	-	-	-	-			
Linalool	-	-	5.98	-	-	-	-			3.72
β-Elemene	2.77	1.85	5.55	12.46	1.15	1.23	6.38		5.59	
α-Humulene	1.18	1.03	19.97	5.59	1.56	-	2.92	6.11		4.3
β-Cubebene	-	-	5.04	2.08	-	-	-			
β-Selinene	1.64	1.20	3.85	16.91	-	-	7.34			
β-Citronellal	-	-	-	1.18	-	-	-			
Gurjunene	-	-	-	23.80	-	-	-			
Aromadendrene	-	-	-	-	3.91	-	-			
Safrole			3.5							
Eugenol					5.2					
Eugenyl acetate					1.3					
Germacrene B								14.30	8.83	3.58
Germacrene D								7.44		
Total compounds	84	60	83	67	74	70	61	28	63	68

Table 2. GC/MS profile of *Piper nigrum* accessions of the Western Ghats

Collection No.	Latitude	Longitude	β-Caryophyllene	Nerolidol	β-Elemene	α-Humulene	Germacrene-D	Myristicin	α-Pinene	β-Selinene	Sabinene	safrole	Others	Total No. of components
KRW 1	14°12'	74°52'	7.49	4.2	0	5.3	0	0	10.25	0	43.68		0	38
KRW 2	14°11'	74°59'	0	36.40	0	0	0	0	3.9	0	11.7		3.6	35
KRW 3	14°29	74°61'	4.65	0	0	0	0	7.29	28.21	0	0		0	45
KRW 4	14°54'	74°63'	0	0	3.91	0	0	30.99	0	0	0		0	34
KRW 5	14°30'	74°75'	0	0	0	0	0	66.7	0	0	0		0	16
KRW 6	14°34'	74°12'	4.58	0	0	0	16.12	59.4	0	0	0		0	27
KRW 7	14°33'	74°67'	0	0	0	0	14.7	60.8	0	0	0		0	32
KRW8	14°54'	74°76'	0.99	0	5.36	0	0	54.38	0	0	0		0	28
DK 1	12°52'	75°23'	5.43	2.13	1.46	2.07	2.89	0	0	7.86	0		0	53
DK 2	13°24'	75°26'	3.86	0	9.57	0	0	0	0	0	22.31		22.31	39
DK 3	12°38'	75°35'	2.25	0	1.19	0	0	0	0	0	26.95		26.95	46
DK 4	13°02'	75°23'	1.1	0	0	0	0	0	29.6	0	2.1		26.3	73
DK 5	13°15'	75°32'	1.19	0	0	0	0	0	50.71	11.23	11.23		0	46
KER 1	10°60'	76°35'	3.73	1.15	1.51	4.03	0	0	0	8.26	0		0	49
KER 2	09°39'	77°04'	5.18	1.12	0	5.99	3.58	0	0	15.49	0		0	68
KER 3	10°58'	76°35'	2.88	1.52	2.3	0	28.03	0	0	0	0		0	40
KER 4	08°54'	77°10'	4.57	43.2	0	0	0	0	0	0	0		3.72	29
GOA	15°60'	73°95'	5.99	0	2.84	1.62	0	55.06	0	4.8	0	3.38	0	62
DP	17°76'	73°18'	1.67	0	3.68	1.14	2.6	20.25	26.81	0	0	3.61	3.81	52
Shannon's Diversity index			2.66	1.37	1.71	1.18		1.18	1.18					2.87

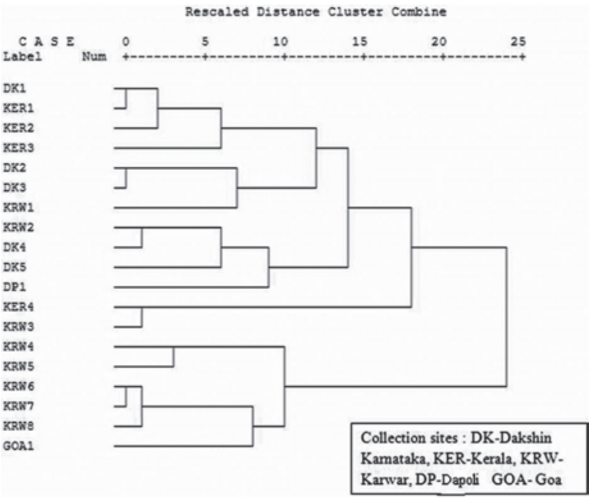

Fig. 1. Dendrogram of volatile oil components of different accessions of *P. nigrum* leaves using Average Linkage (Between Groups) method

Table 3. Altitude and rainfall of collection sites in the Western Ghats

Collection No.	Altitude (M)	Rain fall (mm)
KRW1	450	3000-4000
KRW2	450	2500-3000
KRW3	200	4000-4500
KRW4	200	3500-4000
KRW5	200	4000-4500
KRW6	350	3000-3500
KRW7	650	3500-4000
KRW8	450	3000-3500
DK1	250	4000-4500
DK2	350	4000-4500
DK3	350	4000-4500
DK4	350	4000-4500
DK5	350	4000-4500
KER1	250	4000-4500
KER2	350	2000-2500
KER3	450	3000-3500
KER4	150	1500-2000
GOA1	50	2500-3000
DP1	250	3500-4000

correlation has not yet been reported for Western Ghats *Piper* species. It was noted that β-Caryophyllene and Nerolidol were present in all the wild samples under study except in *P. betle*. Nerolidol is a natural pesticide and gives irritating sensation to the skin (Jayalekshmy *et al.* 2003). The percentage of β-Caryophyllene was very high (45.3%) in *P. betle*. β-Caryophyllene is known as anti inflammatory sesquiterpene which is also having antibacterial activity (Utpala *et al.* 2008). Rimando *et al.* (1996) from Philippines reported that *P. betle* had 15.5% of β-Caryophyllene and high concentration of chavibetol (53.0%) which was absent in Western Ghats species. *P. betle* had only 5 major components out of which Germacrene B and Germacrene D were the two components which are known for their antimicrobial properties (Himeyima *et al.* 1992). Germacrene B was also present in the leaf of *P. longum* and in *P. nigrum*. Germacrene D is a common volatile component in *Laurus nobilis* leaf oil (Kilic *et al.* 2004). According to Adewale (2009), germacrenes are typically produced in a number of plant species for their antimicrobial and insecticidal properties, though they also play a role as insect pheromones.

P. sugandhi was reported to have highest number of components. β-seline was a compound which was present in *Piper* (*P. capense*, *P. guineense*) leaf oil of Cameroon, (Francois *et al.* 2009). Western Ghats species like *P. thomsoni*, *P. chaba*, *P. hymenophyllum* and *P. arboreum* also had β-seline in their leaf oil. α- Pinene is an important component of *Piper* berry oil which was present only in *P. sugandhi* and *P. longum*. Sumathykutty *et al.* (1999) reported β-cubebene as the major constituent of *P. attenuatum* berry oil, which was present in *P. chaba* and *P. hymenophyllum* leaf oil. *P. sugandhi* and *P. hymenophyllum* were the two wild species of high altitude (≥ 1000MSL) of Western Ghats. The species are of not much use economically. About 4.8% α-Pinene was present in *P. sugandhi* but in *P. hymenophyllum,* α-Pinene was absent. Both the species had very high number of volatile components (10 and 8, respectively). *P. attenuatum* is a low altitude wild species very common in the forest areas of Western Ghats, but not cultivated. *P. attenuatum* differed from the high altitudinal wild species like *P. sugandhi* and *P. hymenophyllum,* in that it had low number of volatile components (4).

The geographic distribution of diversity is an important consideration at all stages of genetic resource exploration and conservation, yet it is a difficult parameter to visualize and analyze. Heaton *et al*. (1999) explained the correlation between the morphological distance matrix and GIS - derived matrices. A study by Utpala *et al.* (2006) revealed a strong relation between morphological characters and environmental factors.

Leaves from different accessions of *P. nigrum* collected from different forest regions of Western Ghats were studied for aroma diversity. The results were plotted in SPSS cluster to find the similarity among the accessions. Most of the accessions collected from similar geographic locations were the first ones which grouped together indicating clear location influence. Two Karwar samples of *P. nigrum* were segregated while all other Karwar samples (6) and Goa and Dopoli samples from latitude 14°12' to 17°76' were clustered together. Southern Karnataka samples (4) and Karwar samples (2), did not have both Nerolidol and Myristicin while in all other cases, one of them was present. Santos *et al.* (2001) reported the differences in chemical constituents of the same species when harvested from different biomes. Dakshin Karnataka and Kerala samples did not have Myristicin at all, while higher latitudinal samples had Myristicin (Table 2). Myristicin was not found as a common compound in Western Ghats samples while it is a generalized compound in Nigerian samples (Oyedeji *et al.* 2005). Though β-Caryophyllene is a generalized compound in *P. nigrum,* it was absent in four Karwar samples. β-Caryophyllene is one of the chemical compounds that contribute to the spiciness of black pepper. Two Karwar samples having Nerolidol did not have Myristicin but had high concentration of Sabinine (46.685% & 11.73%), whereas, Dakshin Karnataka samples did not have Nerolidol and Myristicin, but it had Sabinine. This profile was similar to the pepper berry oil, where sabinene and pinene were present.

The study showed that intra species aroma diversity could very easily be interpolated with the help of GIS software. Important component diversity of *P. nigrum* varied from 16 to 62 (Table 2). The GIS interpretation showed that the high latitudinal samples namely the samples of Karwar of Maharashtra had lower number of components. The diversity index of total number of components was 2.87. β-*Caryophyllene*, the most generalized component of *Piper* leaves had diversity index of 2.66, while Nerolidol which is also a generalized compound of Southern part of Western Ghats (Utpala *et al.* 2008) had a diversity index of 1.37 and was available in the latitude between 8°4' to 10°6'. Myristicin was present in higher latitude from 14° 29' to 17° 76′ namely in Goa, Dapoli and Karwar samples and had a diversity index of 1.18. Altitude and rainfall study of the collection sites revealed that there was no specific relation of altitude for the presence or absence of Myristicin, but high rainfall from 3000 mm to 4000 mm showed a favorable relationship. As this is the first report of GC/MS profile and GIS relation for Western Ghats *Piper* species, an extensive survey of the higher latitudes of Western Ghats may give a better idea about this finding. In this study it was found that *P. nigrum* from 13° 9' to 17° 8' latitudes had different types of volatile oil components. In this study, Myristicin recorded an average of 46.5% in leaf oil of *Piper nigrum* collections. Martins *et al.* (1998) has reported that Myristicin was the main constituent of *P. guinense*. Two accessions from Karwar (KRW1 & KRW2) showed a profile which is similar to pepper berry oil, where sabinene and pinene were present. It was also interesting to note that in these two accessions, Nerolidol was present and Myristicin was absent. Two accessions from Dapoli and Goa showed the presence of Safrole but in other samples from Western Ghats it was not detectable. Sabinine was present mainly in between Dakshin Kanada and lower part of Karwar (between 13°5' to 14°25' latitude). Nerolidol, was found in very high concentration in lower latitudes 9° to 11°, but in higher latitudes it was not present except in two samples from Karwar. Utpala *et al.* (2008) recorded the presence of Nerolidol in lower latitudes of Western Ghats and Ibrahim *et al.* (1994) reported Nerolidol as a major constituent of *P. penangense* of Malaysia. The study clearly indicated that the volatile oil components are influenced by environmental factors.

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