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Essential oil composition of clove and nutmeg from Andaman and Nicobar Islands, India

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In spices, chemical variability is a known phenomenon. Here, we studied the clove and nutmeg grown in different islands both Andaman and Nicobar groups of islands situated in the Bay of Bengal, India. Essential oils were extracted and profiling was carried out which revealed considerable variations among the collections for essential oil content as well as composition. *P*-Eugenol was observed in island-grown clove samples in the range of 59.895 to 76.059%, whereas, β-Caryophyllene and Eugenyl acetate were other important constituents of oil. In case of nutmeg, myristicin, the hallucinogenic compound, was present in the range of 7.070 to 20.331%. Considering the quality of island-grown spices, these crops could be recommended as profitable intercrops in the existing plantations in the islands.

Keywords: β-Caryophyllene, Eugenyl acetate, Eugenol, Hallucinogenic, Myristicin, Nutmeg, Spices, *Syzygium aromaticum*

Clove of commerce is obtained from dried unopened buds of *Syzygium aromaticum* (Myrtaceae), a species native to Indonesia. It is commercially grown in Tanzania, Madagascar, Mauritius, Indonesia, Malaysia, Sri Lanka and India. It is one of the oldest known spices used in culinary preparations and for curing respiratory and digestive troubles[1]. It is an important constituent of various medicinal and aromatic preparations, flavoring agents, soaps, oral care products apart from its use in enhancing shelf life of fresh fruits[2,3]. Nutmeg (*Myristica fragrans*, Myristicaceae) is an important species yielding nutmeg and mace as two spice products. It is native to Indonesian Moluccas islands and is cultivated in Malaysia, Indonesia, Grenada, Sri Lanka, India, Tanzania, Mauritius, Reunion, Trinidad & Tobago, and China. Nutmeg is also valued for its diversified applications in food, confectionery, perfumery, and pharmaceutical industries[2].

Andaman and Nicobar Islands (ANI) are a group of 572 humid tropical islands, isles and rocks in the Bay of Bengal. Both clove and nutmeg are grown in ANI covering about 40 and 70 ha, respectively, (www.indianspices.com). Andaman group and Nicobar group of islands are geographically separated by ten-degree channel. The quality of spices produced in different regions of ANI is largely unknown. Except for reports by Raina *et al.*[4] and Pal *et al.*[5], which describe the composition of clove leaf and nutmeg, respectively, from Andaman islands, no other reports are available which provide information about the quality of spices grown in these islands. Determining the quality of spices grown in various regions could help in the identification of potential areas for their commercial-scale production. Hence, the present report concerned the determination of essential oil composition in clove and nutmeg grown in different parts of the Andaman and Nicobar Islands.

Materials and Methods

Collection of samples

Samples of nutmeg and clove were collected from various parts of Andaman and Nicobar groups of islands in the Bay of Bengal and a commercial market in Chennai, Tamil Nadu, India (Table 1). While collecting samples from the islands, the produce from available trees in each collection location was pooled to get bulk samples. Samples were packed in sample bags and transported to the authors' Institute for further processing.

Extraction of essential oils

Oils of nutmeg and clove were extracted using the hydro-distillation method. For extraction, ground

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Table 1 — Details of sample collection

Crop	Collection code	Site	Place of collection
Clove	SF	Institute farm	Sippighat, South Andaman Island
	LA	Farmer's field	Ramkrishnapur, Little Andaman Island
	KAT	Demonstration farm, Department of Agriculture	Katchal andKamorta islands (Nicobar group of islands)
	CHE	Market	Chennai, Tamil Nadu
Nutmeg	MT	Farmer's field	Mile Tilak, South Andaman Island
	KPM	Farmer's field	Ramkrishnapur, Little Andaman Island
	KAT	Demonstration farm, Department of Agriculture	Katchal and Kamorta islands (Nicobar groups of islands)
	CHE	Market	Chennai, Tamil Nadu

samples were accurately weighed and a known quantity of powder was extracted using deionized water in the Clevenger apparatus for oils lighter than water (Borosil, India). Extracted oils were dried using anhydrous sodium sulphate (HiMedia, Mumbai) and packed in airtight containers and stored at 4°C until further use.

Determination of volatile composition using GC MS analysis

The volatile composition of clove and nutmeg samples was determined using Gas Chromatograph (Varian-3800) coupled with Ion-Trap Mass Spectrometer (Varian-4000). For analysis, essential oils of spice samples were injected into the injector port. MS column *viz*. VF-5MS (Factor four; Varian, USA) fused-silica capillary column (30 m × 0.25 mm id and 0.25 mm film thickness) was used in the study. Injector temperature was set at 250°C and all injections were made initially in split mode (1:20) for 30 s followed by split-less. Detector temperature was maintained at 270°C and temperature program for a column was: 40°C for 3 min at an increment of 3°C/min to 190°C, hold for 1 min, then 5°C/min to 220°C and maintaining the constant temperature for 5 min.

Mass spectrometer details during operation were as followed- mode: external electron ionization; carrier gas: Helium (1 mL/min); injector temperature: 250°C, trap temperature: 180°C, for ion source-heating: 190°C, transfer line temperature: 260°C, EI-mode; 70 eV and full scan range: 50–350 amu. Total volatile content was estimated by summing all GC peak areas in each chromatogram and individual compounds were quantified as relative percent area. Identification of compounds was done by comparing the retention index, which was determined by using homologous series of n-alkanes (C5–C32) as standard[6] and also by comparing the spectra using spectral libraries: Wiley and NIST-2007.

Results and Discussion

The essential oil composition of a species is known to vary significantly with several factors. Area of cultivation is one of the important factors contributing to variations in the volatile constituents in spice crops[7,8] and hence, the present study was carried out to study the quality variations in oils of clove and nutmeg collected from the mainland and different islands of Indian Territory. Essential oil content and constituents in both clove and nutmeg samples collected from different locations varied considerably as revealed by the data.

Quality evaluation of clove oil

Unopened buds of clove are known to have essential oil which is being utilized for a variety of industrial and medicinal purposes. During the present study, the highest oil yield of 10.0% was obtained from samples collected from Katchal-Kamorta islands (Fig. 1), which was followed by a sample collected from Dundas Point in South Andaman (9.75%). Oil content was as low as 5.70% in a sample from Chennai. Interactions between genotype, growing conditions, and postharvest operations could have resulted in the observed variations. Earlier reports have suggested wide variations[9-11] for oil yield from various regions *e.g.* 4.58% (Manado), 4.99% (Java), 10.1% (China) and 0.70-0.92% (Gulf).

The GC-MS analysis of clove oil resulted in the identification of 96.995% (South Andaman) to 98.437% (Chennai) of the total constituents (Table 2). Irrespective of the site of sample collection, *P*-Eugenol and β-Caryophyllene were found to be the major volatile constituents in the essential oils of clove, contributing to about 80.968 to 92.256% of total volatiles identified. The proportion of *p*-Eugenol varied amongst the collections and was of the order:

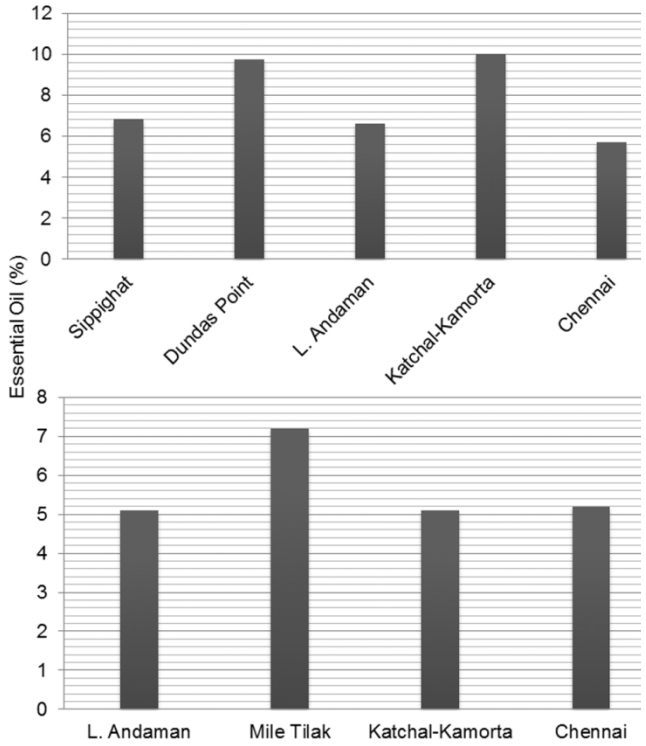

Fig. 1 — Essential oil content (%) in (A) Clove bud samples; and (B) Nutmeg samples collected from different locations in Andaman & Nicobar Islands and mainland India

South Andaman (59.895%) < Little Andaman (61.058%) < Chennai (74.801%) < Katchal- Kamorta islands (76.059%). β-Caryophyllene content in the oils was found to be of order: Katchal- Kamorta islands (13.185%) < Chennai (17.455%) < South Andaman (21.073%) < Little Andaman (24.856%). Interestingly, Eugenyl acetate was the third dominant constituent in samples of the islands (3.659 to 4.058%), whereas it was found in limited quantity (0.935%) in a sample from mainland India. Instead, the Chennai sample had *δ*-Cadinene as a third major constituent (2.164%), which was also present at above 1% quantity in samples from the islands.

Eugenol, a phenol, has been reported to be the major constituent in the essential oil of clove. This compound has commonly been employed in the preparation of synthetic vanillin[2]. Earlier studies have suggested that Eugenol content in Indian conditions varied between 44-55%[12]. During the present study, all the collections from the island as well as the mainland had a higher content of Eugenol. Eugenol content has been reported to vary with the growing environment mainly temperature, light and relative humidity and method of extraction[8,13]. A report from Indonesia suggested Eugenol as a major constituent in

Table 2 — Essential oil composition of clove bud oil from different locations in mainland India and Andaman & Nicobar Islands

Name of the compound	KI	Concentration (area %)			
		SF	LA	KAT	CHE
1-Ethyl-1H-pyrrole-2-carbaldehyde	1067	-	0.061	0.020	0.047
β-Ylangene	1216	0.331	0.254	0.122	0.108
Chavicol	1254	0.108	0.053	0.083	0.026
p-Eugenol	1355	59.895	61.058	76.059	74.801
β-Cubebene	1393	3.453	3.058	0.842	0.137
β-Clovene	1398	-	0.048	0.021	0.033
S-Neoclovene	1411	-	0.066	0.067	0.015
(+)-Aromadendrene	1441	-	0.010	0.003	-
β-Cadinene	1445	0.348	0.632	0.401	0.569
β-Caryophyllene	1455	21.073	24.856	13.185	17.455
α-Humulene	1459	2.316	2.575	1.665	2.076
Germacrene D	1464	-	0.016	0.007	-
β-Selinene	1483	0.046	0.022	0.029	0.033
Eugenyl acetate	1526	4.058	3.659	3.772	0.935
ĩ-Cadinene	1536	0.055	0.048	0.032	0.035
δ-Cadinene	1538	1.168	1.675	1.235	2.164
6-Isopropenyl-4,8a-dimethyldecahydro-1-naphthalenol	1651	-	0.014	0.021	0.003
Torreyol	1646	-	0.019	0.010	-
Octanoic acid		0.771	-	-	-
Octanoic acid, 2- methyl-		2.332	-	-	-
Nonanoic acid		0.842	-	-	-
Junipene		0.199	-	-	-
Total		96.995	98.124	97.574	98.437

clove leaf oil and the content ranged between 59.56 to 68.37% among different locations[8]. Comparison of bud oil constituents from India and Madagascar revealed variations for all the constituents[14]. As the samples were collected from different regions having dissimilarities in microclimates, variations observed in the present study could be justified.

Apart from the major constituents, β-Cubebene and *α*- Humulene were also present in a concentration above 1% in all the samples studied. In general, oils from Little Andaman, Nicobar group and Chennai had 18 identified constituents each, whereas oil of clove from the South Andaman had 15 constituents, of which four compounds *viz*. Octanoic acid (0.771%), Octanoic acid, 2-methyl- (2.332%), Nonanoic acid (0.842%) and Junipene (0.199%) were not found in other samples studied. Also, the other six minor constituents thatwere found in the other samples were not reported from this collection. This finding is in agreement with earlier reports by Amelia *et al*.[11], who

reported the presence of unique minor constituents in clove bud oil. Several earlier reports suggested variations in kind and concentrations of compounds in oils grown in different geographical regions[8,14].

Quality evaluation of nutmeg oil

Volatile oil recovery from nutmeg seeds varied between 5.1 to 7.2% (Fig. 1). Sample from the Mile Tilak region of South Andaman Island had the highest content of essential oil. Essential oil content is known to vary greatly as reported by previous studies. Report by Kapoor *et al.*[15] suggested the essential oil recovery of 3.40%, whereas, Muchtaridi *et al.*[16] found it to be 6.85%. Evaluation of nutmeg collections from South Indian region revealed essential oil content of 3.9-16.5%[17].

The GC-MS analysis of the oil samples revealed the identification of 31 compounds each from Little Andaman and Katchal- Kamorta islands, while 30 and 32 compounds were reported from samples from Chennai and Mile Tilak, respectively. Considerable variations were observed for various compounds among the tested samples (Table 3). Compounds such as *α*-Thujene, *α*-Pinene, Sabinene, *α*-Terpinene, (-)-4-Terpineol, Myristicin, and Elemicin were found to be in concentration above 10% in one or more collections.

Though the complete mechanism of action of various compounds present in nutmeg oil is largely unknown[18], compounds namely Myristicin and Elemicin are known to possess hallucination/ intoxication properties, whereas, safrole is reported to be a carcinogen[19,20]. Myristicin has also been regarded to have hepatoprotective and chemoprotective activity against cancer[21,22]. Elemicin is known to have anti-proliferative, tumoricidal, antibacterial and anti-allergic activities[23-25].

Content of Myristicin in the present study was of in the order: 7.070% (Mile Tilak) < 8.957% (Chennai) < 19.729% (Little Andaman) < 20.331% (Katchal-Kamorta islands), whereas Elimicin was ranked as: 1.129% (Little Andaman) < 3.609% (Mile Tilak) < 3.958% (Katchal- Kamorta islands) < 15.154% (Chennai). Earlier reports suggested varying levels of Myristicin in essential oils of nutmeg grown in different regions *e.g.* 3.28% to 3.80% in India[15,26], 16.2 % in Austria[19], 12.93% to 13.57% in Indonesia[16,27]. A study by Dupuy *et al.*[7] with samples from Indonesia suggested wider variations and the values varied between 1.74% and 12.92%. As per the Indonesian National Standard, the presence of minimum 5% Myristicin is one of the criteria for determining the quality of nutmeg essential oil[28]. All the collections in the present study met this requirement. Elemicin content has been reported to be inversely proportional to Myristicin content in

Table 3 — Essential oil composition of nutmeg oil from different locations in mainland India and Andaman & Nicobar Islands

Name of the Compound	KI	Concentration (area %)			
		KPM	CHE	KAT	MT
Cyclofenchene	890	0.234	0.241	0.388	0.469
Tricyclene	920	1.813	1.162	2.222	4.052
α-Thujene	923	4.549	7.544	12.050	16.874
α-Pinene	931	4.613	8.378	9.553	10.135
Camphene	942	1.214	1.114	1.342	1.226
Sabinene	974	15.292	26.428	19.248	0.172
α-Myrcene	992	3.011	1.774	1.235	2.509
α-Terpinene	1015	3.475	2.051	2.774	11.165
5-Isopropyl-2-methylbicyclo[3.1.0] hexan-2-ol	1029	0.769	0.797	0.392	0.176
Limonene	1032	3.827	4.978	5.893	5.224
√-Terpinene	1053	4.980	2.752	4.170	9.617
Dehydro-p-cymene	1097	0.933	0.429	0.698	4.970
Cis-p-2-menthen-1-ol	1112	0.699	0.244	0.285	0.240
Methyl octanoate	1118	1.116	0.000	0.031	0.025
cis-α-Terpineol,	1123	0.242	0.246	0.277	0.163
1-Terpinenol	1136	0.124	0.343	0.331	0.238
(-)-4-Terpineol	1181	14.161	6.491	7.113	13.611
Piperitone	1192	-	-	-	0.050
trans-p-Menth-1-en-3-ol	1209	0.310	0.060	0.079	0.059
Ethyl octanoate	1245	0.801	-	-	-
Linalyl acetate	1248	-	-	0.048	-
Isobornyl acetate	1275	1.454	0.732	0.863	1.299
Borneol, acetate	1281	0.475	0.312	0.296	0.204
4-Terpineol acetate	1286	0.000	0.080	0.115	0.070
Myrcenyl acetate	1311	0.794	0.098	0.753	0.117
β-Isosafrole	1312	3.587	1.650	1.457	1.644
Citronellyl acetate	1348	-	0.093	-	0.076
Eugenol	1355	0.720	-	-	-
Copaene	1365	1.093	1.228	0.835	0.650
2-Allyl-6-methoxyphenol	1385	0.434	0.247	0.228	0.144
δ-Neoclovene	1390	5.819	2.795	1.374	1.588
β-Cubebene	1391	0.513	0.349	0.301	0.087
á-Ylangene	1395	0.682	0.864	0.314	0.424
Myristicin	1522	19.729	8.957	20.331	7.070
Elemicin	1542	1.129	15.154	3.958	3.609
Total		98.590	97.590	98.956	97.956

collections of nutmeg from India[17]. The present study supports this report as it was found that collections with a relatively high content of Myristicin had lower levels of Elemicin and vice versa.

Place of collection and genotype have been considered as two sources of variations in the composition of nutmeg. Among the ecotypes of nutmeg from Indonesia, Myristicin content varied between 5.57% and 13.76%, whereas, Elemicin content was 0.67 to 2.05%[29]. Maya *et al.*[17] found that genotype had a profound influence on essential oil composition, wherein Myristicin content amongst 65 collections from India varied between 1.1 and 45.6%, while Elimicin ranged from 1.0 to 29.7%. Higher levels of hallucinogenic compounds found in the island samples could be exploited for pharmaceutical purposes.

Sabinene, a compound known to impart sweetness to nutmeg, was found to be the highest in a sample collected from Chennai (26.428%), followed by that in Katchal-Kamorta islands (19.248%) and Little Andaman island (15.292%). Interestingly, a sample from Mile Tilak had the lowest levels of Sabinene (0.172%). Earlier reports suggested the presence of Sabinene between 6.00 to 36.08 % in Indonesia[7] and between 6.3 to 50.2% in India[17].

Farming in the Nicobar group of islands is of subsistence in nature[30], while organic farming is being promoted in the Andaman group of Islands by the island administration. As a result, these spices are produced with the use of limited or no chemical inputs, thereby reducing the chances of residues in the final product. Clove and nutmeg are recommended crops for commercial cultivation in the islands to increase the profitability without causing ecological degradation[31]. About half of the total cultivated area in the Andaman and Nicobar islands is under coconut and areca nut plantations. However, most of these plantations are under monocropping and intercropping is rarely being practiced. The findings of the present study indicate acceptable qualities of spices grown in the islands and hence, would provide the scientific backup in promoting their cultivation in the islands. Further, industries on spice essential oil extraction could be established in the islands to increase the profitability of the system.

Conclusion

The present study is possibly the first attempt in comparing the quality of clove and nutmeg produced in different islands of Andaman and Nicobar groups of islands. Considering the content of major compounds in the essential oil samples, the cultivation of clove and nutmeg could be successfully promoted in the islands including those located in the Nicobar group of islands. As the spice gardens in the islands are of seedling origin, detailed surveys could be taken up to identify desirable genotypes.

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Conflict of Interest

All authors declare no conflict of interest.

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