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GC-MS based chemical fingerprinting large cardamom cultivars of Sikkim

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ABSTRACT

Amomum subulatum **Roxb, also known as large cardamom, is an herbaceous plant cultivated in Sikkim, India for its aromatic seeds with diverse applications in food, beverages, perfumery, and medicine. The study aimed to GC-MS based chemical fingerprinting through analyzing volatile compounds in seeds and assess the relatedness among seven large cardamom cultivars grown in the Sikkim hill region. The results revealed significant differences in volatile compounds among the cultivars. The major compounds identified were 1,8-cineole (76.37-90.63%), α-terpineol (1.73-13.40%), α-terpinolene (0.23-11.51%), β-pinene (0.54-4.13%), γ-terpinolene (0.77-4.47%), and α-pinene (0.35-1.86%). These compounds possess notable antioxidant, anti-inflammatory and antibacterial properties. Through PCA and dendrogram analyses, it was observed that ICRI SKM-1 showed similarity to ICRI SKM-2 and Seremna, while Ramsey, Ramla, and Sawney exhibited close relatedness based on their phytochemical composition. These findings have important implications for large cardamom breeding programs. These findings also suggest that large cardamom seed extracts could have potential commercial applications in various industries, including medicine, food, and cosmetics.**

Key words : *Amomum subulatum* Roxb., Aroma, Large Cardamom, 1,8-Cineole, Terpenoid.

INTRODUCTION

Large cardamom (*Amomum subulatum* Roxb) is an important perennial tropical herb belonging to the Zingibaraceae family and native to the eastern Himalayan region, particularly in Darjeeling, Sikkim, and Assam hills in India (Mishra *et al.*, 10). It has been widely used as an important herb in traditional medicinal practices for centuries. Additionally, large cardamom has been found to contain several bioactive metabolites like flavonoids, carotenoids, terpenes, etc. (Ashok Kumar *et al.*, 2). Its seeds and oil have been utilized for various purposes, such as aiding digestion, treating urinary problems, and reducing symptoms of the common cold, cough, and headaches. More than 50% of the global production of large cardamom comes solely from the Indian state of Sikkim (Noumi *et al.*, 11). It was also found that Saudi Arabia was the largest cardamom importer in 2017, constituting 19.3% of the worldwide market (Ashok Kumar *et al.*, 2). Its seeds and oil have been utilized for various purposes such as aiding digestion, treating urinary problems, and reducing symptoms of the common cold, cough, and headaches. Another study reported that the essential oil of black cardamom contains around 40 components, including 1,8-cineole, α and β-pinene, α-terpineol, and also-aromadendrene (Gurudutt *et al.,* 5). The essential oils found in large cardamom are volatile and play a critical role in determining the quality of the spice (Debabhuti *et al.,* 4). The chemical makeup of large cardamom essential oil is characterized by a high concentration of oxygenated monoterpene and a low number of monoterpenes hydrocarbons. This is valued for its unique taste, pleasant flavour, and rich aroma. Major constituents of large cardamom oil include 1,8-cineole, α-pinene, β-pinene, γ-terpinene, terpinene 4-ol, myrcene, terpineol, limonene, p-cymene, and nerolidol (Shankaracharya *et al.,* 12). Further research by Jabbar and Ghorbaniparvar (6) revealed the presence of 82 volatile chemical components in black cardamom essential oil using GC/MS analysis.

However, our literature review indicates the little information is available on variety specific GC-MS based chemical fingerprinting and diversity study in large cardamom cultivars from sourced from Northeastern India. Therefore, this study aims to analyze the volatile components in different cultivars of large cardamom and underrtand the variation and relatedness among the culivars.

MATERIALS AND METHODS

We collected capsules of seven distinct cultivars of large cardamom, namely Sawnney (S-1), Varlangey

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(S-2), Ramla (S-3), ICRI SKM-2 (S-4), Seremna (S-5), Ramsey (S-6), and ICRI SKM-1 (S-7), from the Indian Cardamom Research Institute (ICRI), Spices Board, Gangtok, Sikkim, India (27º9'-27º25'N, 88º27'-88º56'E, 300-5000 m above MSL) (Fig. 1). To extract the volatile compounds, fresh large cardamom capsules were air-dried at 40–45°C using a hot air oven. Separation of the pericarp and seeds was performed very carefully, and the seeds were crushed in methanol (1:5 w/v) for subsequent GC/MS analysis.

To analyze the volatile compound present in large cardamon seeds, GC-MS 7890A GC (Agilent Technologies) equipped with an HP-5MS column (30 m × 0.25 mm;/0.25 µm, Agilent Co., USA) was utilized, which was directly linked to a triple axis HED-EM 5975C mass spectrometer (Agilent Co., USA). The sample (1 µl) was injected in split mode with helium as the carrier gas at 1 ml min^{-1} flow rate and 10 psi head pressure. The GC-MS analysis had a total run time of 46 min, with the initial oven temperature held at 90°C for 1 min, followed by a gradual increase to the final temperature of 290°C with a gradient of 5°C min^{-1} and held for 5 min. Additionally, the interface temperature was set at 250°C and the ion source temperature at 200°C with EI mode operated at 70 eV. The metabolites were identified by comparing the fragmentation patterns of the mass spectra and retention times (RT) with the NIST standard reference database.

RESULTS AND DISCUSSION

The results revealed a broad spectrum of compounds in large cardamom, including monoterpenes, aldehydes, terpene alcohols, ketones,

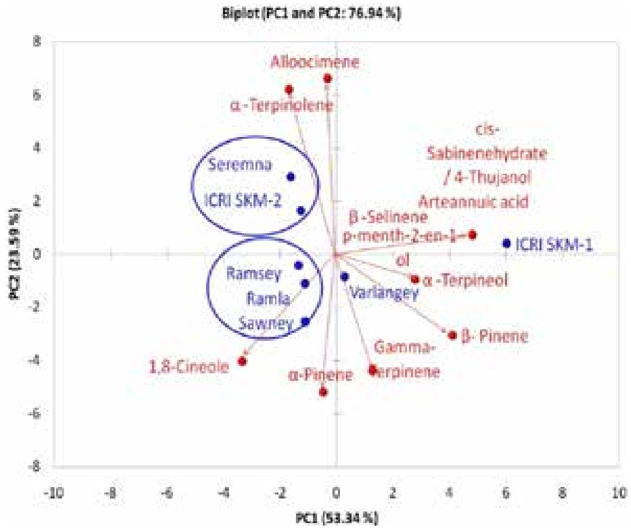

Fig. 1. PCA analysis of seven large cardamom cultivars based on the volatile compounds identified by GC/MS analysis.

fatty acids, alkanes, alkenes, and sesquiterpenes (Table 1). It was observed that the primary constituents identified were β-pinene, 1,8-cineole, and γ-terpinene in the large cardamon cultivars. A study by Noumi *et al*. (11) reported the predominant class of compounds in cardamom such as oxygenated monoterpenes, comprising 71.4%, 63.0%, and 51.0% of the total compounds. Among these, 1,8-cineole was the most abundant compound, with the highest percentage in cardamom seeds. Moreover, the collected essential oils exhibited variations in both qualitative and quantitative compositions, with presence of oxygenated monoterpenes (ranging from 65.31%

Table 1. The percentage composition of volatile chemicals in different varieties of large cardamom seeds.

Sl. No.	Compounds	Different varieties of large cardamom seeds (% Area)						
		Sawney (S-1)	Varlangey (S-2)	Ramla (S-3)	ICRI SKM-2 (S-4)	Seremna (S-5)	Ramsey (S-6)	ICRI SKM-1 (S-7)
1.	α- pinene	1.86	-	1.28	-	0.35	0.73	0.65
2.	β- pinene	2.25	2.63	2.36	0.83	0.67	0.54	4.13
3.	1, 8- cineole	90.63	79.89	90.36	80.97	82.90	89.10	76.37
4.	γ- terpinene	4.47	4.08	1.36	3.11	0.77	2.25	3.14
5.	α- terpineol	-	13.40	3.53	1.73	1.56	5.59	9.35
6.	Alloocimene	-	-	0.50	1.09	3.37	0.83	1.20
7.	α-terpinolene	-	-	-	11.51	9.54	-	0.23
8.	p-menth-2-en-1-ol	-	-	-	-	-	-	0.53
9.	Arteannuic acid	-	-	-	-	-	-	0.53
10.	Cis-sabinenehydrate/ 4-thujanol	-	-	-	-	-	-	0.96
11.	β- selinene	-	-	-	-	-	-	0.06

to 75.54%), followed by monoterpene hydrocarbons (10.53% to 17.12%), terpene alcohols (15.32% to 18.80%), and sesquiterpene hydrocarbons (5.02% to 9.19%) (Joshi *et al.,* 7). These compounds are known to be involved in mediating the interactions between plants and their surroundings, including inhibiting the nitrogen cycle.

The oil content and the presence of volatile compounds varied among the large cardamom cultivars, with α-terpineol being detected in all except S-1 and alloocimene present except S-1 and S-2. The oil content varied from 1.2 to 2.5 %. The S-7 (ICRI SKM-1) was the only large cardamom variety to contain eucalyptol, p-menth-2-en-1-ol, arteannuic acid, cis-sabinenehydrate/4-thujanol, and β-selinene. This variety has been developed by the Indian Cardamom Research Institute specifically for growth in hill climates. Interestingly, the GC-MS profiling showed that 1,8-cineole was the predominant compound in large cardamom, and it varied in abundance among different regions. This compound is commercially valuable and is used as a raw material in various industries. The percentage composition of 1,8-cineole varies with different regions, as seen in *E. Globules* leaves (Boukhatem *et al*., 3). The compounds present in large cardamom contribute to its medicinal properties, as summarized in Table 2. Previous studies have demonstrated that α-pinene and β-pinene, which are organic terpenes, possess antimicrobial and anti-inflammatory properties, and α-Pinene aids in memory (Lee *et al*., 8). β-pinene has been shown to have antimicrobial activity against various organisms, including *C. albicans, C. neoformans, R. oryzae*, and MRSA (Silva *et al*., 13). γ-terpinene is a plant metabolite, volatile oil ingredient, and human xenobiotic metabolite with free radical scavenging properties. Eucalyptol or 1,8-Cineole is vital in controlling airway mucus hypersecretion and asthma by inhibiting anti-inflammatory cytokines (Silveira *et al*., 14).

Moreover, PCA analysis was performed to determine the relationship between the volatile compounds found in large cardamom. PC1, which accounted for 53.34% of the variance, and PC2, which recorded 23.59%, indicated that ICRI SKM-2 and Seremna are rich in alloocimene and α-terpinolene (Fig. 1). Ramsey, Ramla, and Sawnney formed a distinct cluster that was different from the cluster formed by Seremna and ICRI SKM-2. ICRI SKM-1 exhibited unique properties with eucalyptol, p-menth-2-en-1-ol, arteannuic acid, cis-sabinenehydrate/4-thujanol, and β-selinene. Dendrogram analysis revealed similar trends (Fig. 2), with Varlangey and ICAR SKM-1 sharing a common axis and ICAR SKM-2 and Seremna being closely related. This might be due to genetic variations in cultivars as edaphic and climatic condition of cultivated area is remain same for all the cultivars. Our study provides insights into the relatedness of various large cardamom varieties based on GC-MS based chemical fingerprinting.

Table 2. Medicinal values of different volatile chemical components present in large cardamom seeds.

Sl. No	Name of Metabolites	Chemical formula	Molar mass (g/mol)	Function in the cellular system
1.	α- pinene	$C_{10}H_{16}$	136.24	Anti-inflammatory; antimicrobial; aiding memory (Silva *et al.,* 13; Lee *et al.,* 8).
2.	β- pinene	$C_{10}H_{16}$	136.24	Antimicrobial activity against *C. albicans*, *C. neoformans*, *R. oryzae,* and MRSA (Silva *et al.,* 13).
3.	1, 8- cineole/eucalyptol	$C_{10}H_{18}O$	154.25	Antibacterial activity and food additive (Boukhatem *et al.,* 3); Anti-inflammatory cytokine inhibitor (Silveira *et al.,* 14).
4.	γ- terpinene	$C_{10}H_{16}$	136.24	Antioxidant.
5.	α- terpineol	$C_{10}H_{18}O$	154.25	It has a role as a plant metabolite
6.	Alloocimene	$C_{10}H_{16}$	136.24	Semiochemicals
7.	α- terpinolene	$C_{10}H_{16}$	136.23	Used as flavor ingredients
9.	p-menth-2-en-1-ol	$C_{10}H_{18}O$	154.25	Acts as a flavouring agent, membrane stabilizer, energy source, and energy storage
10.	Arteannuic acid	$C_{15}H_{22}O_2$	234.34	Highly effective against multidrug-resistant strains of malaria (*Plasmodium spp*.) (Li *et al.,* 9)
11.	Cis-sabinenehydrate/4-thujanol	$C_{10}H_{18}O$	154.25	Used as a food additive and flavoring agent (Pubchem database)
12.	β- selinene	$C_{15}H_{24}$	204.36	Antioxidant and antimicrobial activity (Ali *et al.,* 1)

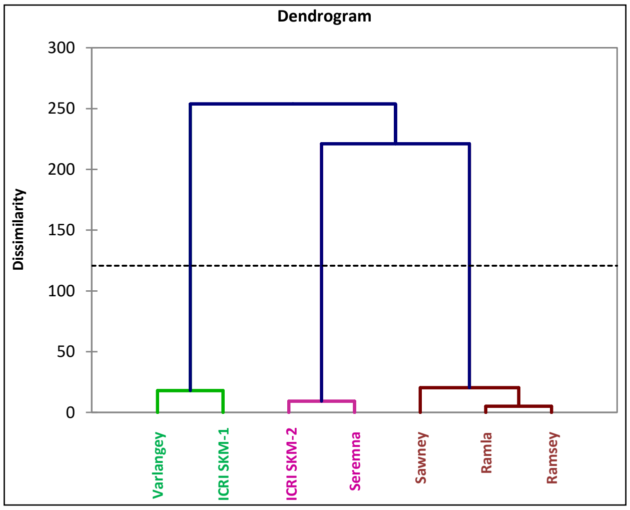

Fig. 2. Dendrogram analysis of large cardamom cultivars based on dissimilarity between the presence of volatile compounds.

In conclusion, large cardamom seeds contained diverse volatile compounds, with 1,8-cineole being the most abundant. These compounds contribute to its medicinal properties, including anti-inflammatory and antimicrobial effects. GC-MS based chemical fingerprinting differentiate and classify large cardamom cultivars and also showed their relatedness.

AUTHORS' CONTRIBUTION:

Conceptualization of research and chemical fingerprinting of large cardamom (DN); Field survey and collection of experimental materials (BG and KS); GC-MS based chemical fingerprinting in Laboratory (SS); Data collection and interpretation (SS, SJ and BP); Preparation of the manuscript (DN, SJ, PB, SS, TC and AM).

DECLARATION

The authors declare that they have no conflict of interest.

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