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Research Article

A comparative study of essential oil components of selected promising small cardamom (*Elettaria cardamomum* (L.) Maton) landraces in Cardamom Hill Reserve (CHR) of South India

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Abstract
Elettaria cardamomum (L.) Maton originated in the evergreen forests of the Western Ghats of South India. In order to compare the percentage of essential oil and its components of selected small cardamom landraces, the study was conducted at the Indian Cardamom Research Institute, Myladumpara, Idukki Dt. of Kerala. Essential oil components of nine farmer's varieties such as Njallani Green Gold, Thiruthali, Panikulangara Green Bold No.1, Wonder Cardamom, Elarajan, Arjun, Pappalu, PNS Vaigai, Pachaikkai; and ICRI-5 as control were compared to evaluate the quality of genotypes. Essential oil content was significantly higher in Najllani Green Gold (9.18%), followed by Wonder Cardamom (8.36%). GC-MS profiling identified twenty-five compounds contributing to 90-93% of essential oil. The higher percentage of 1,8-cineole was observed in PNS Vaigai (38.04%) and the control ICRI-5 (38.99%), while Panikulangara Green Bold No.1 had the lowest percentage (24.30%). α- terpinyl acetate was higher (43.39%) in Panikulangara Green Bold No.1 followed by Thiruthali (39.21%), and was lowest in ICRI-5 (35.97%). Panikulangara Green Bold 1 had a higher percentage of α-terpineol, Geranyl acetate, Geraniol and Linalyl acetate than other genotypes. The percentage of sabinene was higher in Njallani Green Gold (7.56%), and in the genotype, Elarajan (5.46%) was lowest. Due to the medicinal and flavour-contributing properties, the essential oil components can be used in medicinal and food industries.

Keywords: 1,8-cineole, α- terpinyl acetate, Cardamom, *Elettaria cardamomum* , Essential oil

INTRODUCTION

Elettaria cardamomum (L.) Maton is the small cardamom often referred as 'Queen of Spices' belongs to the monocotyledonous family Zingiberaceae. It is a pseophytic, perennial, rhizomatous plant believed to have originated in the moist evergreen forests of the Western Ghats of South India (Ravindran, 2002; 2005). It is grown extensively in the central part of Western Ghats, known as Cardamom Hills. It is located in 8' and 30' N latitudes and 75' and 78'30' E longitudes, where it exhibits extensive variability (Anisha *et al.,* 2020). The mean annual rainfall ranges from 1500 mm to 7000 mm and depends on the elevation and geographical location across Cardamom Hill Reserve (Murugan *et al.,* 2022). The ideal temperature for cardamom cultivation is 15 to 25^{o}C and it is grown in forest loamy acidic soil with a pH of 5.5-6.5 (Vijayan *et al.*, 2018). Small cardamoms are cultivated in Kerala, Karnataka and Tamil Nadu states of India. Earlier India was the leading producer, consumer and exporter of cardamom and at present second position with production of 23,340 tons during 2021 - 22 and the total area of small cardamom cultivation in India is 69,190 hectares (www. indianspices.com). *E. cardamomum* contains various compounds such as anthocyanins, alkaloids, phenols, starch, tannins, terpenoids, flavonoids, proteins, sterols (Rajan *et al*., 2017), and it also possesses various pharmacological properties, such as antimicrobial, antioxidant, anti-inflammatory and anticancer activities (Souissi *et al*., 2020). The use of cardamom in various industries, such as food, cosmetics and pharmaceuticals, is due to the diverse composition of various photochemical compounds present in cardamoms (Ivanović *et al*., 2021). Therefore, the present study aimed to evaluate the percentage of essential oil and its components of selected small cardamom landraces.

MATERIALS AND METHODS

Material

The present study was conducted during the period 2018 - 2021 at the Indian Cardamom Research Institute (ICRI), Spices Board, Myladumpara, Idukki District. of Kerala, India, to evaluate the quality characters of nine elite landraces of small cardamom. The genotypes selected for the study were the cardamoms which received the National Grassroots Innovation Awards from the National Innovation Foundation (NIF), Government of India. The nine landraces were Njallani Green Gold, Thiruthali, Panikulangara Green Bold No.1, Wonder Cardamom, Elarajan, Arjun and Pappalu were selected from Idukki District and PNS Vaigai, Pachaikkai from Theni District of Tamil Nadu and ICRI-5 was used as control. The planting materials were collected from the farmer's field and were planted in the experimental farm of ICRI, situated in Idukki District of Kerala in the Cardamom Hill Reserve (CHR) with a longitude of 9° 8'E and latitude of 77°2'N. This region is at an altitude of 1050 mean sea level with a well-distributed rainfall of 2790 mm per annum and the soil is forest loam with a pH of 5 - 6.5 (Vijayan *et al*., 2018).

Methodology

The field trial was laid out at the farm of the Indian Cardamom Research Institute, Spices Board, Myladumpara, in Randomized Block Design (RBD) with 3 replications. Each treatment with 12 plants in the plot was adopted with a spacing of 3 m x 3 m, as per the cultivation practices for small cardamom recommended by Spices Board (Anonymous, 2015). When the capsules attained physiological maturity, they were harvested and subjected to post-harvest operations such as washing, curing, cleaning, grading, packing and storage.

Extraction and estimation of essential oil

The extraction of cardamom essential oil was conducted using the hydro distillation method. Ten grams of powered small cardamom samples were taken to a 1000 ml round-bottomed flask with 500 ml deionized water. The oil trap with condenser was placed on the round-bottomed flask and was placed on the electric heating mantle and boiled. The refluxing rate was adjusted to drop per second by adjusting the regulator of the mantle. Reflux until two consecutive readings taken at one hour interval showed no change of oil volume in the trap. Then, the apparatus was cooled to room temperature and oil drops sticking to the sides of the condenser were pulled down to the trap using a steel rod. The total amount of oil collected in the trap in milliliter was recorded (AOAC, 2000).

Calculated the percentage of volatile oil as follows;

Volatile oil (% v/w) = Volume of oil (ml) / Weight of the sample (g) x 100 Eq. 1

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

To compare the essential oil content of the nine promising small cardamom landraces with ICRI-5 as control, GC-MS profiling of the essential oil from the small cardamom varieties were done. The chemical composition of selected small cardamom essential oil was analyzed by using GC-MS (Gas Chromatography-Mass Spectrometry) Agilent Technologies 5977. All samples were analyzed once without replication. Identification with name, chemical structure, and molecular weight of each small cardamom essential oil component was calculated based on the chromatogram's relative peak area (per cent area) and confirmed chemical components by comparing retention times with PubChem data and Willey 9 library database.

RESULTS AND DISCUSSION

The percentage of essential oil present in the selected small cardamom genotypes is presented in the Table 1. Among the selected genotypes, the highest essential oil content was observed in Najllani Green Gold (9.18%) followed by Wonder Cardamom (8.36%). The essential oil content in chosen genotypes varies between 7.01% and 9.18%. Several researchers have documented that the essential oil content of small cardamom falls within the range of 3.50% to 10.6%. Purseglove *et al.* (1981) reported that essential oils were at their maximum at earlier harvest stages. During the fruit maturation process, the fruits undergo physicochemical changes, which affect the quantity of essential oils they contain. Peter (2001) reported that small cardamom's essential content ranges from 6.6% to 10.6%. Ravindran *et al.* (2002) reported that the small carda-

Table 1. Percentage of essential oil present in cardamom genotypes

Genotype	Essential oil (%)
Njallani Green Gold	9.18
Wonder Cardamom	8.36
Elarajan	8.19
PNS Vaigai	7.12
Panikulangara Green Bold No.1	8.18
Thiruthali	8.01
Pachaikkai	7.69
Pappalu	7.75
Arjun	7.03
ICRI 5 (Control)	8.59

mom essential oil content was 3.50% volume by weight. Hymete *et al.* (2006) reported that the dried seeds of small cardamom contain 3.77% essential oil. According to Endashaw (2007), the essential oil content of small cardamom was reported as 3.50% by weight. On the other hand, the range of percentage of essential oil content of small cardamom was reported by Parthasarathy *et al*. (2008) as 5.5 – 10.0%. Ashokkumar *et al*. (2020) reported that the essential oil content of small cardamom exhibited variability across various extraction methods, plant varieties, and plant parts. Highest oil content was also reported in small cardamom genotypes such as HY-3 (9.6%) and SAM-5 (9.3%) at Pampadumpaara, Kerala, by Ashokkumar *et al*. (2021). They further reported that the cardamom essential oil was obtained through the hydrodistillation method, and the average yield from three distinct analyses ranged between 4.5% and 9.6%.

The comparative data on the components of essential oil from the selected varieties are given in Table 2. The essential chromatograms of all the 10 genotypes, including the control, are presented in Fig. 1-10. The GC-MS analysis of cardamom oils indicated 25 compounds contributing to 90-93% of oil. Among these, there were 11 major compounds, namely, 1,8-cineole, α-terpinyl acetate, α-pinene, sabinene, α-myrcene, linalool, 4-terpineol, α-terpineol, nerol, geranyl acetate and nerolidol (Table 2). The basic cardamom aroma is produced by a combination of 1,8-cineole and α-terpinyl acetate; these are the major components in the small cardamom volatile oil. PNS Vaigai (38.04%) and the control ICRI-5 (38.99%) had a higher percentage of 1,8-cineole (38.04%), while Panikulangara Green Bold No.1 had the lowest percentage (24.30%). It is reported that a harsh camphory note of small cardamom was due to the presence of 1,8-cineole in the volatile oil (Zachariah, 2002). Hymete *et al.* (2006) reported that the main constituent of small cardamom essential oil is 1,8-cineole. Nonetheless, certain studies have documented lower proportions of α-terpinyl acetate and linalool, accompanied by higher percentages of 1,8-cineole in the essential oil of cardamom originating from various countries, ranging from 25.6% to 26.71% of 1,8-cineole. (Soussi *et al.* 2015; Asghar *et al*. 2017). Ashokkumar *et al.* (2021) documented that the essential oil of all cardamom accessions contains 1,8-cineole as the second most significant monoterpene constituent, ranging from 15.2% to 49.4%.

The percentage of α- terpinyl acetate was higher (43.39 %) in Panikulangara Green Bold No.1 followed by Thiruthali (39.21%) and the lowest percentage was found in the control ICRI-5 (35.973%). Pillai *et al*. (1984) reported that the higher percentage of α- terpinyl acetate indicates the superior quality of small cardamom capsules. The essential oil of cardamom was abundant in monoterpene constituents such as α-terpinyl acetate (Yashin *et al.* 2017; Murugan *et al.* 2019; Ashokkumar *et al*. 2019). Wael *et al*. (2022) noted that α-terpinyl acetate constituted the primary component in cardamom essential oil.

In the present study, each of the ten genotypes exhibited a higher concentration of α-terpinyl acetate compared to 1,8-cineole. Ashokkumar *et al.* (2020) found that capsules of small cardamom are the storehouse of several bioactive metabolites like flavonoids, carotenoids, terpenes, etc. Ashokkumar *et al.,* 2021 further stated that capsules' characteristic strong aromatic aroma is due to the presence of primary bioactive metabolites in the cardamom essential oil. Using GC-MS analysis, Abdullah *et al.* (2022) discovered that the main bioactive components in small cardamom were 1,8-cineole and α-terpinyl acetate.

Content of α-terpineol, Geranyl acetate, Geraniol and Linalyl acetate were higher in Panikulangara Green Bold No. 1 compared to other genotypes. Zachariah (2002) reported that the sweet flavour of the volatile oil was due to the presence of α-terpinyl acetate, geranyl acetate and α-terpineol. The ruling variety, Njallani Green Gold had a higher percentage of sabinene (7.559%) and lowest percentage (5.495%) was found in the genotype Elarajan. According to Yamasaki *et al*. (2007), sabinene possesses anti-fungal and anti-inflammatory properties. The antimicrobial property of cardamom volatile was due to the presence of sabinene (Arunkumar *et al.,* 2014). The antioxidant activity of cardamom has been attributed to the existence of sabinene in cardamom oil, as noted by various researchers (Quiroga *et al.,* (2015), Zheljazkov *et al.,* (2015), Zheljazkov *et al.,* (2017).

This study revealed the composition percentages of essential constituents in cardamom, including α-terpinyl acetate (ranging from 35.97% to 43.39%), 1,8-cineole (24.30% to 38.99%), α-terpineol (2.56% to 7.20%), sabinene (5.5% to 7.6%), limonene (2.4% to 4.1%), terpinene 4-ol (1.84% to 2.99%), α-pinene (0.51% to 1.8%), linalyl acetate (0.57% to 3.48%), geraniol

Table 2. GC-MS analysis of the essential oil components of small cardamom landraces

Compound Name	RI	Njallani Green Gold	Wonder Cardamom	Elarajan	PNS Vaigai	Panikulangara Green Bold No.1	Thiruthali	Pachaikkai	Pappalu	Arjun	ICRI-5
α-Thujene	929	0.215	0.129	0.091	0.174	0.077	0.103	0.231	0.195	0.134	0.167
α-Pinene	937	1.575	0.991	0.572	1.346	0.506	0.751	1.803	1.484	1.007	1.225
Sabinene	974	7.559	6.148	5.495	6.421	6.023	5.612	6.765	6.864	6.226	5.746
β-Pinene	979	0.586	0.515	0.438	0.559	0.385	0.449	0.570	0.556	0.548	0.491
α-Terpinene	1017	0.492	0.271	0.315	0.324	0.316	0.325	0.200	0.253	0.282	0.299
p-Cimene	1025	-	0.422	0.477	0.291	0.497	0.297	0.264	0.364	0.595	0.274
D-(+)-Limonene	-	3.593	2.936	3.681	3.152	2.440	3.052	3.296	3.700	4.068	3.298
Eucalyptole (1,8-Cineole)	1032	33.884	36.996	35.157	38.042	24.303	32.637	37.331	34.487	34.52	38.994
γ-Terpinene	1060	0.837	0.494	0.568	0.622	0.514	0.628	0.370	0.491	0.572	0.487
4-Thujanol (Sabinene hydrate)	1075	0.452	0.269	0.298	0.293	0.275	0.299	0.853	0.340	0.210	0.521
Linalool	1099	0.070	0.057	0.027	0.048	0.008	0.021	0.040	0.051	0.018	0.041
(E)-4,8-Dimethylnona-1,3,7-triene	1116	0.255	0.140	0.205	0.217	0.073	0.101	0.077	0.192	0.049	0.111
trans-Sabinene hydrate	1070	0.328	0.252	0.261	0.233	0.248	0.279	0.583	0.276	0.171	0.406
Terpinen-4-ol	1177	2.322	2.997	2.459	2.326	2.935	2.933	1.837	2.411	2.522	2.392
α-Terpineol	1189	2.840	2.670	3.747	2.562	7.207	5.118	3.250	2.703	4.731	4.331
Linalyl acetate	1257	1.307	1.134	2.002	0.822	3.484	1.717	2.862	0.802	0.575	1.047
Geraniol	1255	1.693	2.092	1.922	1.545	2.435	1.940	0.402	1.367	0.963	0.799
α-Citral	1270	0.381	0.588	0.693	0.432	0.852	0.691	0.623	0.634	0.996	0.504
trans-Geranic acid methyl ester	1324	0.238	0.231	0.198	0.281	0.125	0.205	0.143	0.263	0.071	0.115
δ-Terpineol acetate	1315	0.254	0.290	0.265	0.350	0.100	0.265	0.317	0.317	0.199	0.391
α-Terpinyl acetate	1350	37.810	36.974	37.681	36.801	43.393	39.211	36.394	38.240	38.71 5	35.973
Geranyl acetate	1382	1.491	1.178	1.207	1.041	2.869	1.821	0.112	0.906	1.569	0.652
β-Selinene	1486	0.757	1.106	0.914	1.248	0.124	0.562	0.637	2.013	0.107	0.443
γ-Cadinene	1513	0.134	0.177	0.190	0.145	0.034	0.113	0.124	0.180	0.352	0.151
E-Nerolidol	1564	0.858	0.951	1.136	0.722	0.778	0.871	0.915	0.909	0.800	0.910

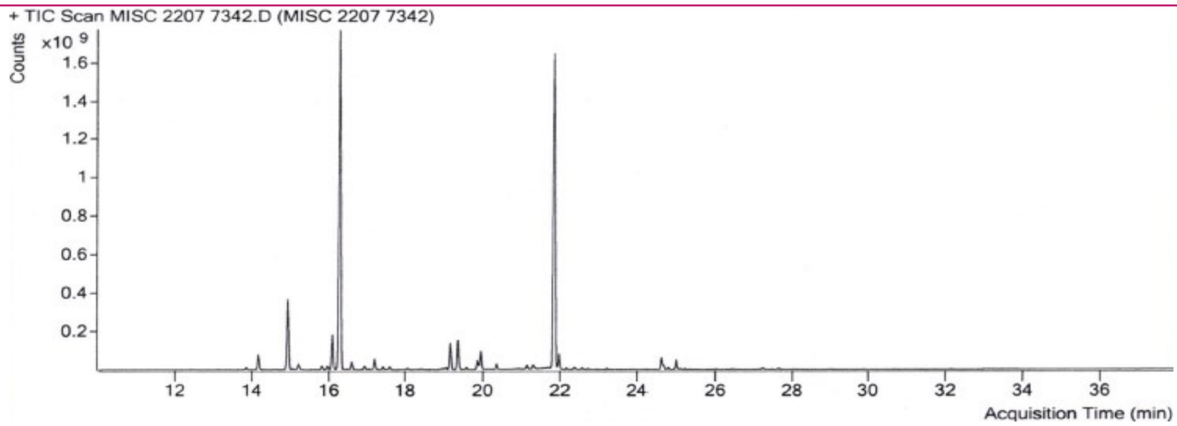

Fig. 1. *Essential oil chromatogram of small cardamom genotype PNS Vaigai*

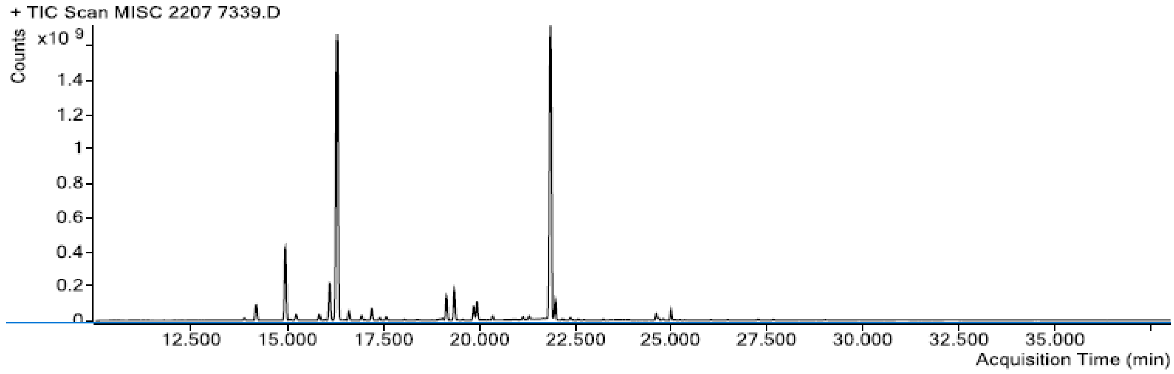

Fig. 2. *Essential oil chromatogram of small cardamom genotype Njallani Green Gold*

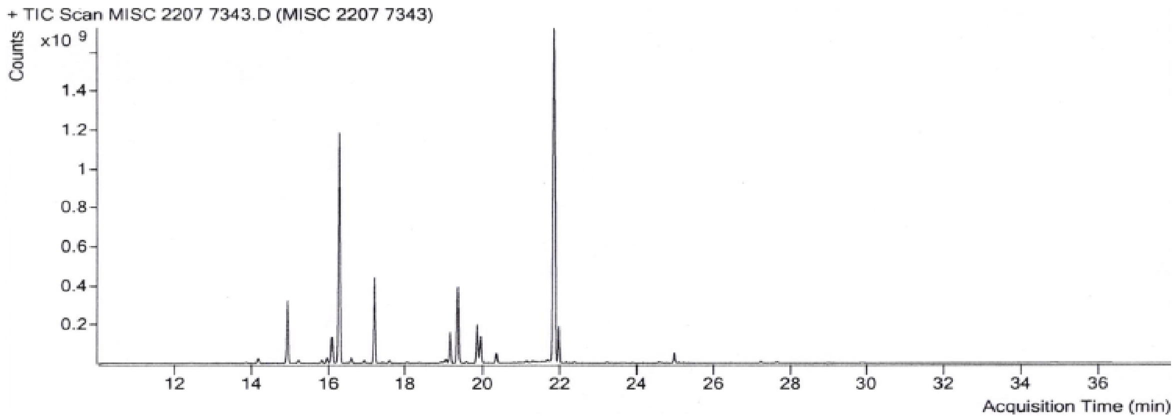

Fig. 3. *Essential oil chromatogram of small cardamom genotype Panikulangara Green Bold No. 1*

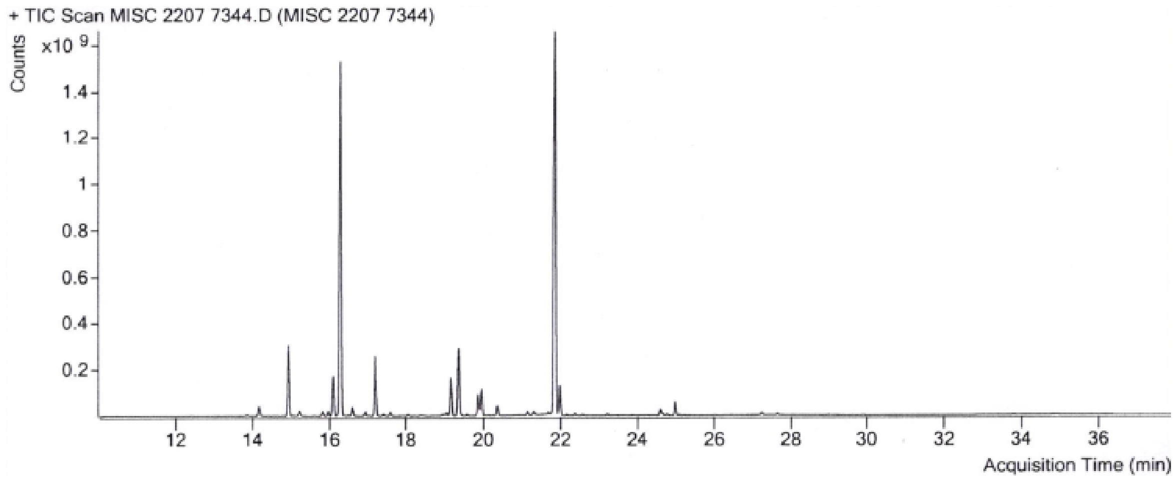

Fig. 4. *Essential oil chromatogram of small cardamom genotype Thiruthali*

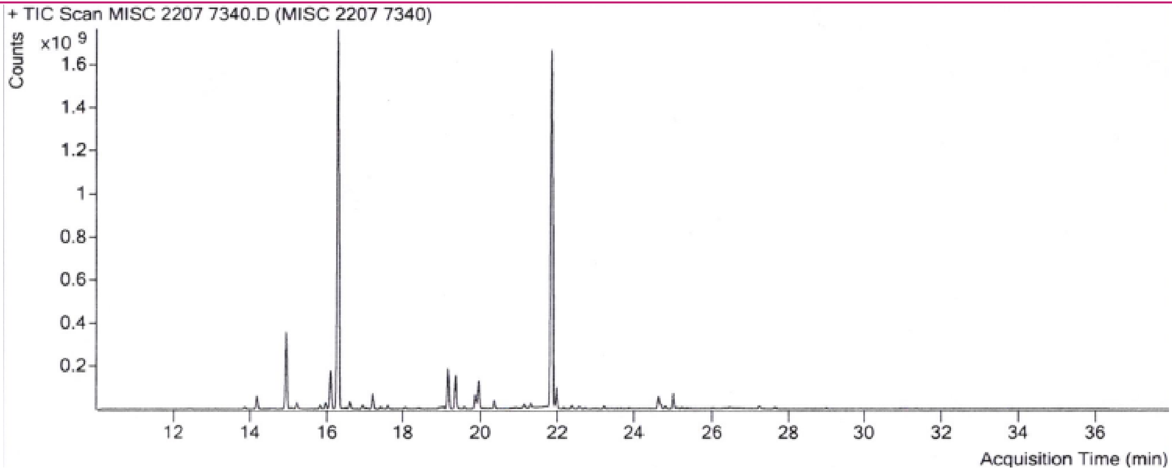

Fig. 5. *Essential oil chromatogram of small cardamom genotype Wonder Cardamom*

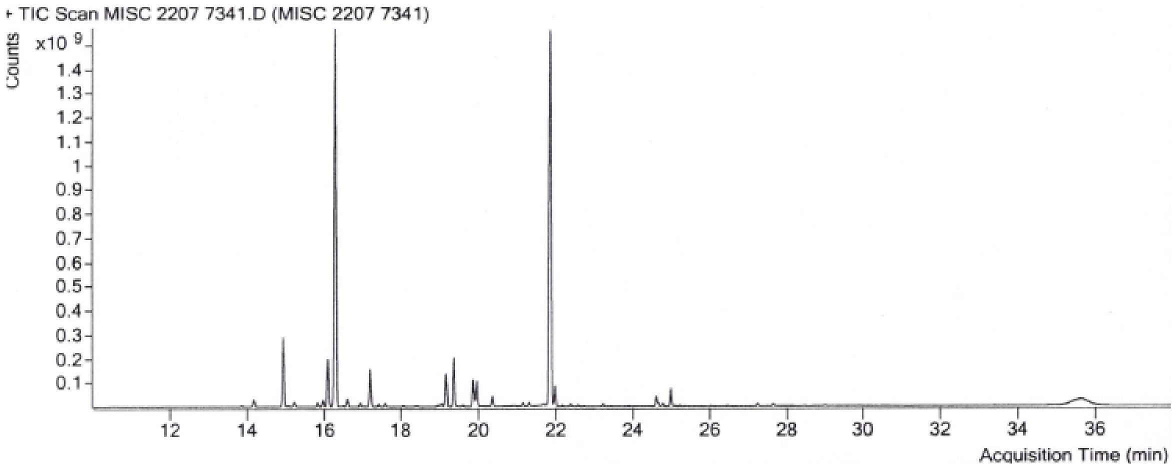

Fig. 6. *Essential oil chromatogram of small cardamom genotype Elarajan*

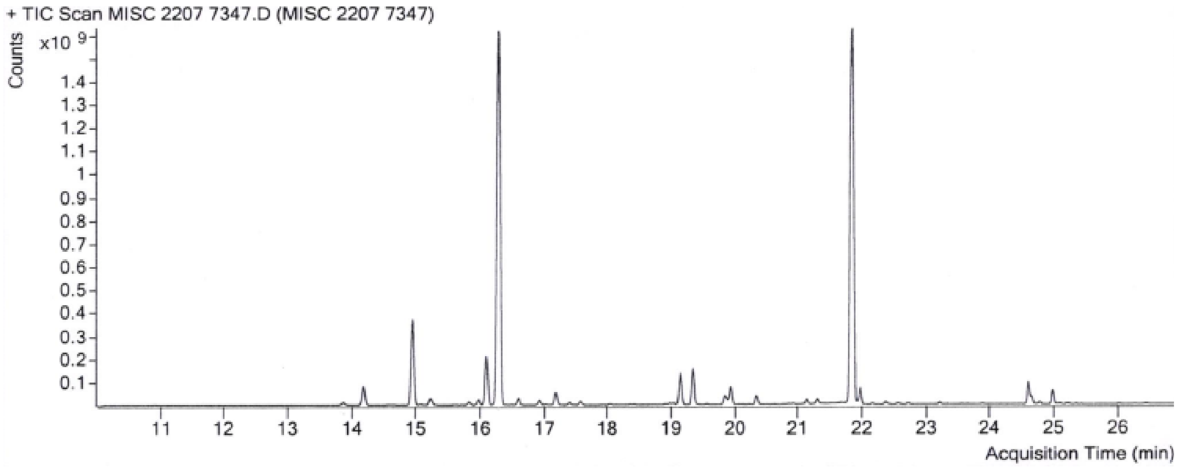

Fig. 7. *Essential oil chromatogram of small cardamom genotype Pappalu*

(0.40% to 2.43%), and geranyl acetate (0.11% to 2.86%). Murugan *et al*. (2019) found that the cardamom essential oil is rich in monoterpene constituents. Significant accumulations of various monoterpene compounds were observed, including sabinene (ranging from 1.9% to 4.9%), β-terpineol (from 0.3% to 2.7%), β-linalool (between 0.4% and 11.0%), terpinen-4-ol (ranging from 0.4% to 3.2%), α-terpineol (between 0.8% and 13.2%), geranyl acetate (from 0.1% to 2.3%), linalyl acetate (between 2.2% and 4.4%), D-limonene (from 0.9% to 9.4%), and nerol (between 0.2% and 1.1%). The concentrations of these compounds varied among different cardamom accessions, with distinct accessions exhibiting higher levels of sabinene (4.9%), β-linalool (11.0%), α-terpineol (13.2%), and nerol (1.1%) as reported by Ashokkumar *et al.* (2021).

In their study, Gochev *et al.* (2012) documented that essential oil primarily treats fevers, digestive concerns,

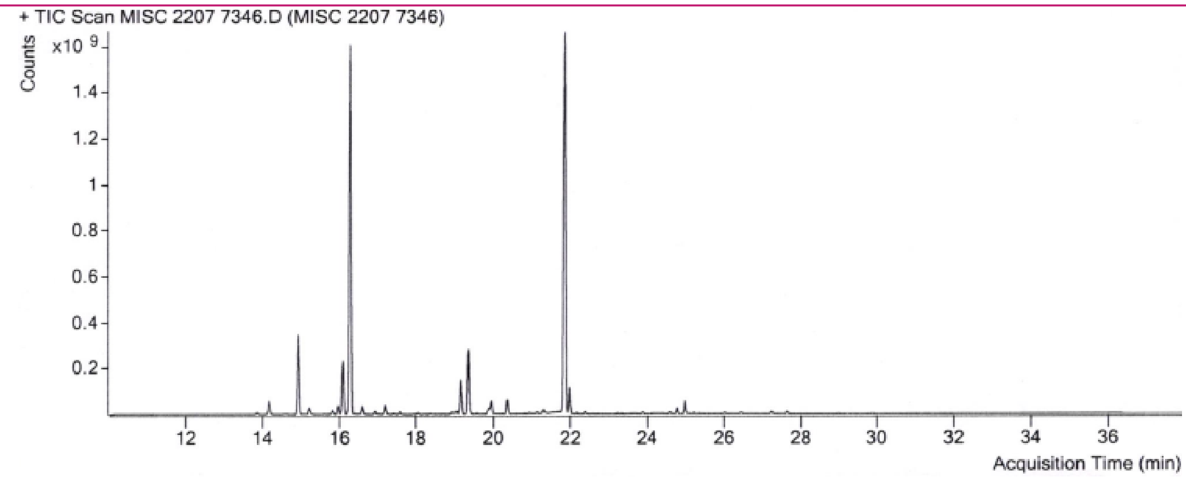

Fig. 8. *Essential oil chromatogram of small cardamom genotype Arjun*

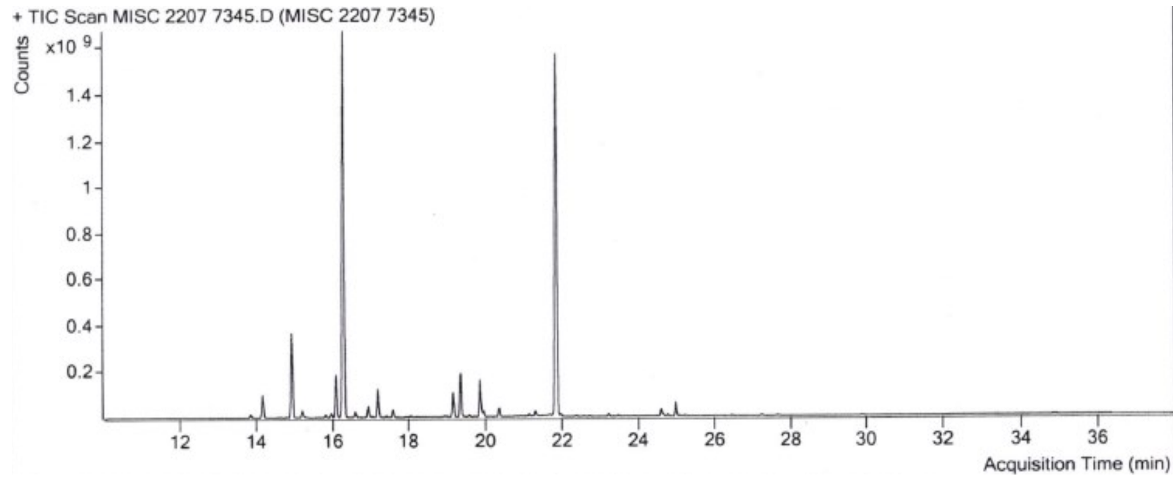

Fig. 9. *Essential oil chromatogram of small cardamom genotype Pachaikkai*

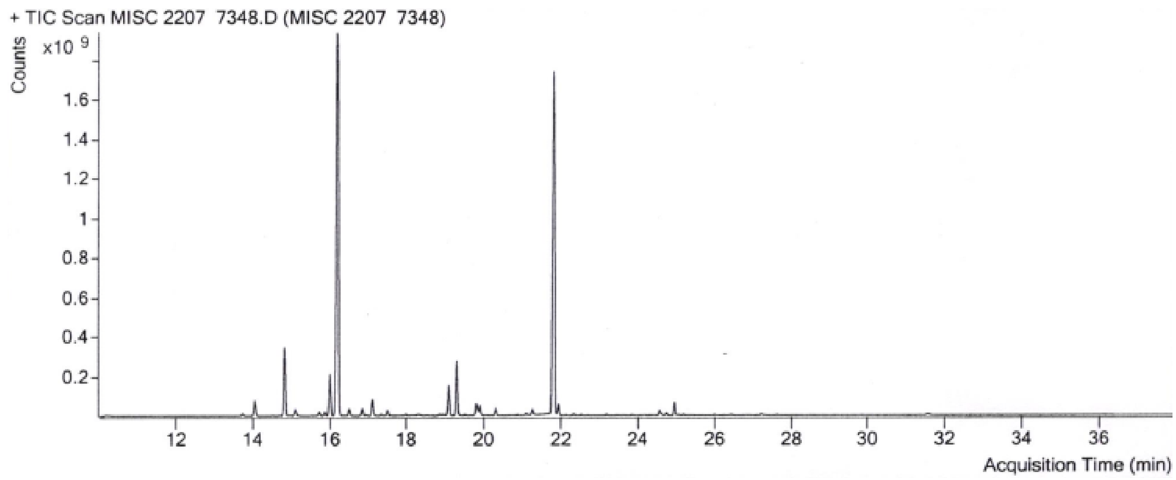

Fig. 10. *Essential oil chromatogram of small cardamom genotype ICRI 5*

and respiratory conditions. According to Asghar *et al.* (2017), aromatic compounds found in essential oils demonstrate stimulant, astringent, diuretic, carminative, anti-inflammatory, and antioxidant properties. Ashokkumar *et al.* (2021) and Jha *et al.* (2022) documented that the main components exhibit antioxidant, antidiabetic, anti-inflammatory, antimicrobial, antiviral, anticancerous, and gastroprotective activities. The distinctive fragrance and premium quality of cardamom essential oil arises from its elevated α-terpinyl acetate content in comparison to 1,8-cineol. Additionally, the sweet flavor of cardamom can be attributed to the presence of α-terpinyl acetate and other terpenoids like α-terpineol, linalool, linalyl acetate, and geraniol (Sayed *et al.*, 2023).

In this study, there was no significant difference in essential oil content between small cardamom genotypes (Table 1). Essential oils are a key class of phytochemicals and are a rich source of pharmaceutical agents and food additives, so the research on essential oils with biochemical, pharmacological and therapeutic aspects are important to nowadays (Cimino *et al.,* 2021; Angane *et al.,* 2022). The different volatile constituents, mainly the monoterpenes in the essential oil give the characteristic aroma of small cardamom. Zachariah (2002) reported that the camphorous note of essential oil is due to the presence of 1,8-cineole and the compound α-terpinyl acetate gives the sweet flavour. Therefore, the flavour quality of small cardamom essential oil is determined by the proportion of 1,8-cineole and α-terpinyl acetate. In their study, Oommen *et al.* (2018) noted fluctuations in the volatile constituents of cardamom essential oil cultivated in four distinct zones of Idukki hills. They observed that as the season advanced, there was a decline in α-terpinyl acetate and an increase in 1,8-cineole, showing variation across the different zones. Numerous studies have documented that the percentage of volatile constituents fluctuates due to factors such as climatic conditions, methods employed in essential oil extraction, storage conditions, soil characteristics, maturity of capsules, harvesting periods, and also exhibits variation among different genotypes (Gopalakrishnan and Cadavallur (1991), Sultana *et al.,* (2009), Oommen *et al.* (2018), Alagupalamuthirsolai *et al.* (2019). Ni *et al.* (2021) and Pirmohammadi *et al.* (2022) highlighted that various factors, including climate, seasonal and geographic conditions, harvest timing, and extraction methods, influence essential oils' chemical composition and subsequent biological activity. The present study assessment of essential oil and its constituents revealed that the quality of cardamom essential oil is significantly influenced by various factors such as climatic conditions, extraction methods, storage, soil characteristics, maturity, harvest timing, and genotypic factors.

Conclusion

The present investigation examined the essential oil content and constituents of nine promising landraces (Njallani Green Gold, Thiruthali, Panikulangara Green Bold No.1, Wonder Cardamom, Elarajan, Arjun, Pappalu, PNS Vaigai, and Pachaikkai) of small cardamom from South India, alongside the check variety ICRI-5, the first manmade hybrid in small cardamom. No significant difference was observed in essential oil content among small cardamom genotypes, with substantial variation only found in the percentage of essential oil constituents. Given that all genotypes were grown under identical environmental conditions, the variation in essential oil components was attributed to the genotypic influences of the selected accessions. PNS Vaigai and ICRI-5 exhibited higher essential oil content percentages. Notably, landraces Panikulangara Green Bold No.1 and Thiruthali, with elevated α-terpinyl acetate content, were identified as superior quality genotypes with a sweet flavor, suitable for utilization in the food industry. The dominant variety, Njallani Green Gold is recognized for its potential antimicrobial properties owing to a higher percentage of sabinene.

Conflict of interest

The authors declare that they have no conflict of interest.

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