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Composition of essential oils from four major cultivars of large cardamom (*Amomum subulatum* Roxb.) grown in Sikkim

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Abstracts

An experiment was conducted to study the essential constituents in four major cultivars of large cardamom (*Amomum subulatum* Roxb.). Each sample was analysed for volatile oil content using hydro-distillation in a Clevenger-type apparatus. The essential oil content varied from 2.70% to 1.90% and highest amount of essential oil content 2.70% was obtained from variety Golsey. The composition of the essential oil was determined by GC-MS which led to the identification of 47 compounds representing 93%-99.7% of the total oil. The major components identified in the oil were 1,8-Cineole, α-pinene, β-Pinene, α-Terpineol, α-thujene, **δ**-terpineol, limonene, δ-terpineol, δ- terpinene and myrcene. Out of 47 metabolites analysed it was found that 1,8-Cineole was the major component in the large cardamom oil. Among the cultivars tested, Golsey cultivar contained the highest numbers of major metabolites. Further, Ramsey cultivar was found with high percentage of 1,8-cineole.

Key words: Large cardamom, essential oil, GC-MS, *Amomum subulatum*, capsule, Zingiberaceae.

Introduction

Amomum subulatum Roxburg, commonly known as large cardamom, belongs to the plant family Zingiberaceae under the order Scitaminae, a major cash crop of Sikkim. It is a tall, evergreen, monoceious and monocot perennial herb. In India, it has been a well known spice since ancient time and it has been valued for its essential oil which possesses acceptable flavour as well as medicinal properties. It is native to Sikkim Himalayas and from there it spread to the sub- Himalayan areas like Darjeeling, Uttaranchal, Nagaland, Assam, Nepal and Bhutan (Vijayan *et al.*, 2017 and Kumar *et al.*, 2013). Its annual production in India is between 8000-8500 MT and with an area of 26,459 ha Sikkim produce 3863 MT which has been emerging as India's organic hub (Bisht *et al.*, 2011 and Gudade *et al.*, 2013).

Large cardamom capsules contain 2-3 % of essential oil rich in cineole having powerful flavouring and aromatic compounds, mainly used as spice and condiment in preparation of curries, soups, sausages and other meat preparations in India and around the globe (Vijayan *et al.*, 2017).There are mainly six cultivars of large cardamom *viz.,* Sawney, Golsey, Seremna, Ramsey, Ramla and Varlengey. Though all metabolite profiling was done in six large cardamom varieties by first author during 2013-15, the metabolite profiling was partial as it was carried out using High Performance Liquisd Chromatography (HPLC). Therefore, the present study was done targeting four major cultivars of Sikkim. Further, this work is done to know the unknown major constituents of the crop and may add some more economical and medicinal value to the crop.

Materials and methods

Cured and dried capsules were collected from four major cultivars of large cardamom found in Sikkim *i.e.,* Sawney, Golsey, Ramsey and Seremna from the farmers of Sikkim. The samples were ground by using Willey's mill. The powdered samples were subjected to hydro-distillation in a Clevenger apparatus for 4 hours at 40 ℃ and the oil yield was recorded. The essential oil from samples was stored in refrigerator until further analysis (Rout *et al.,* 2003). The essential oil percentage data of four cultivars under study was analysed using Completely Randomised Design. The number of treatments and number of replications was four.

Gas chromatography-Mass spectrometry analysis (GC-MS): The essential oil analysis was carried out on GC-MS (Shimadzu QP-2010 Ultra, Japan). The column oven temperature was maintained at 50°C. Helium gas was used as a carrier at a flow rate of 1.5 mL/minute and ionization energy was maintained at 70 eV. The injection temperature was 250°C, and the pressure was maintained at 69 kPa, total flow was 125.2 mL minute^{-1}, column flow was 1.21 mL minute^{-1}, linear flow was maintained at 39.9 cm second^{-1}, purge flow was 3 mL minute^{-1} The iron source temperature was 230°C, interface temperature was 270°C (Vijayan *et al.,* 2017).

Results and discussion

The four cultivars under study Sawney, Golsey, Ramsey, Seremna differed significantly concerning essential oil percentage. The range of oil percentage in four major cultivars of large cardamom varied from 1.90-2.70 mL/100g capsule (Table 2). The highest essential oil percentage was obtained in Golsey (2.70 mL/100g capsule) followed by Ramsey (2.11 mL/100g capsule), Seremna

Table 1. Essential oil constituents of the four major cultivars of large cardamom

SI. No.	Compounds	Sawney		Golsey		Ramsey		Saremna	
		Retention time	Peak area (%)	Retention time	Peak area (%)	Retention time	Peak area (%)	Retention time	Peak area (%)
1	α-Thujene	7.39	0.12	7.39	0.27	7.38	0.21	7.37	0.11
2	α- pinene	7.63	2.24	7.63	4.77	7.62	1.93	7.63	2.72
3	Camphene	8.22	0.05	8.22	0.13			8.22	0.07
4	Sabinene					9.18	0.34	9.19	0.79
5	β-phellandrene	9.2	0.39	9.21	0.37				
6	β-Pinene	9.35	5.91	9.36	7.63	9.32	4.43	0.35	5.37
7	Myrcene	9.9	0.45	9.91	0.73	9.9	0.19	9.89	0.44
8	α- Terpinene	11	0.12	11.01	0.19			10.99	0.06
9	p-cymene	11.35	0.07						
10	Limonene	11.55	1.82	11.56	1.89	11.51	1.44	11.56	2.99
11	1, 8-Cineole	11.93	54.78	11.97	60.1	11.71	86.09	12.09	59.38
12	ϒ-Terpinene					12.8	0.26		
13	δ-Terpinene	12.82	0.52	12.83	0.7	17.93	0.47	12.85	0.3
14	4-Thujanol							18.44	3.95
15	δ-Carene	14.02	0.07	14.03	0.12			14.02	0.06
16	trans-Sabinene hydrate							14.77	0.25
17	Linalool	14.78	0.08	14.79	0.86				
18	β-Fenchol			15.56	0.07				
19	cis-p-menth-2-en-1-ol	15.83	0.18	15.83	0.18			15.82	0.17
20	cis-pinocarveol	16.53	0.15	16.53	0.09	16.51	0.06		
21	trans-p-menth-2-en-ol	16.66	0.1	16.66	0.1			16.65	0.13
22	Pinocarvone	17.5	0.06	17.5	0.04			16.52	0.53
23	δ-terpineol	17.95	1.89	17.95	1.9	17.93	0.47	17.98	2.37
24	4-Terpineol							18.44	3.95
25	terpinnen-4-ol	18.42	4.39	18.42	4.2	18.37	1.79		
26	α-Terpineol	19.2	11.63	19.19	10.36	19.09	2.46	18.44	3.95
27	Isoascaridole	24.01	0.11						
28	α- terpinyal acetate	25.46	0.34	21.05	0.06			25.45	0.48
29	limonen oxide			25.46	0.25				
30	β-elemene	27.61	0.08						
31	(E)-caryophyllene	28.8	0.28	28.8	0.13	28.79	0.34	28.79	0.13
32	α- Caryophyllen	30.28	0.06						
33	allo- Aromadendendrene	30.46	0.1					30.45	0.05
34	ϒ-Muurolene							31.13	0.08
35	Germacrene D	31.36	1.32	31.35	0.45			31.35	0.37
36	Germacrene B	31.95	0.55						
37	α- Muurolene	32.11	0.09						
38	δ-Elemene			31.95	0.24				
39	ϒ-Elemene							31.94	0.21
40	ϒ-Cadinene	32.66	0.12						
41	δ-Cadinene	32.9	0.36	32.9	0.09			32.89	0.1
42	Nerolidol	34.74	5.63	34.71	3.25			34.76	4.89
43	Spathulenol	35.19	0.6	35.18	0.25			35.19	0.59
44	muurolol			38.19	0.18				
45	longifolene			38.8	0.14				
46	Globulol	35.53	0.2						
47	α-Cadinol	38.19	0.54						

(2.01mL/100g capsule), Sawney (1.90 mL/100g capsule). GC-MS profile of essential oil constituents of the four cultivars is shown in Table 1 and Fig. 1. The identification of the compounds was done by comparison of their mass spectra with the Wiley & NIST library and also with the published mass spectra (Adam *et al.,* 2001). Forty seven compounds were identified by GC-MS analysis which contributed 93%-99.7% of total oil. The major constituents of the essential oils from the cultivars are 1,8-cineole (54.7%-86%), α-terpineol (2.4%-11.6%), β-pinene (4.4%-7.6%), α-pinene (1.9%-4.7%), limonene (1.4%-2.9%),δ-terpineol (0.4%-2.3%), myrcene (0.1%-0.7%), δ-terpinene (0.3%-0.7%) and α-thujene (0.1%-0.2%).

Among the four cultivars, the major compounds were high in the essential oils of Golsey were β-pinene (7.6%), myrcene (0.7%), δ-terpinene (0.7%), α-pinene (4.7%), and α-thujene (0.2%). It was having thirty major compounds while Sawney had thirty five compounds in which α-terpineol (11.6%) was the major compound which showed antioxidant activity (Bisht *et al.,* 2011). In Seremna twenty eight metabolites were identified with high amounts of δ-terpineol (2.3%) and limonene (2.9%). 1,8-cineole (86%) was found high in essential oils of Ramsey cultivar where as it showed lowest no. of components (14). Ramsey showed the highest 1,8-cineole which has strong therapeutic properties, strong healing potential, anti-inflammatory, antibacterial, antioxidant, antispasmodic, antiviral and hypotensive properties (Vijayan *et al.,* 2017).

Bhandari *et al.,* (2013) analyzed essential oil of Sawney cultivar from Uttarakhand and found the amount of 1,8-cineole at 73.27%. Joshi *et al.,* (2012) also reported 50.55-60.46% of 1,8-cineole in the analyzed oil of large cardamom collected from Himachal

Table 2. Essential oil (mL/100g capsule)

Varieties	Mean
Sawney	1.90
Golsey	2.70
Ramsey	2.11
Saremna	2.01
C.D.	0.025
SE(m)	0.008
SE(d)	0.011
C.V.	0.742

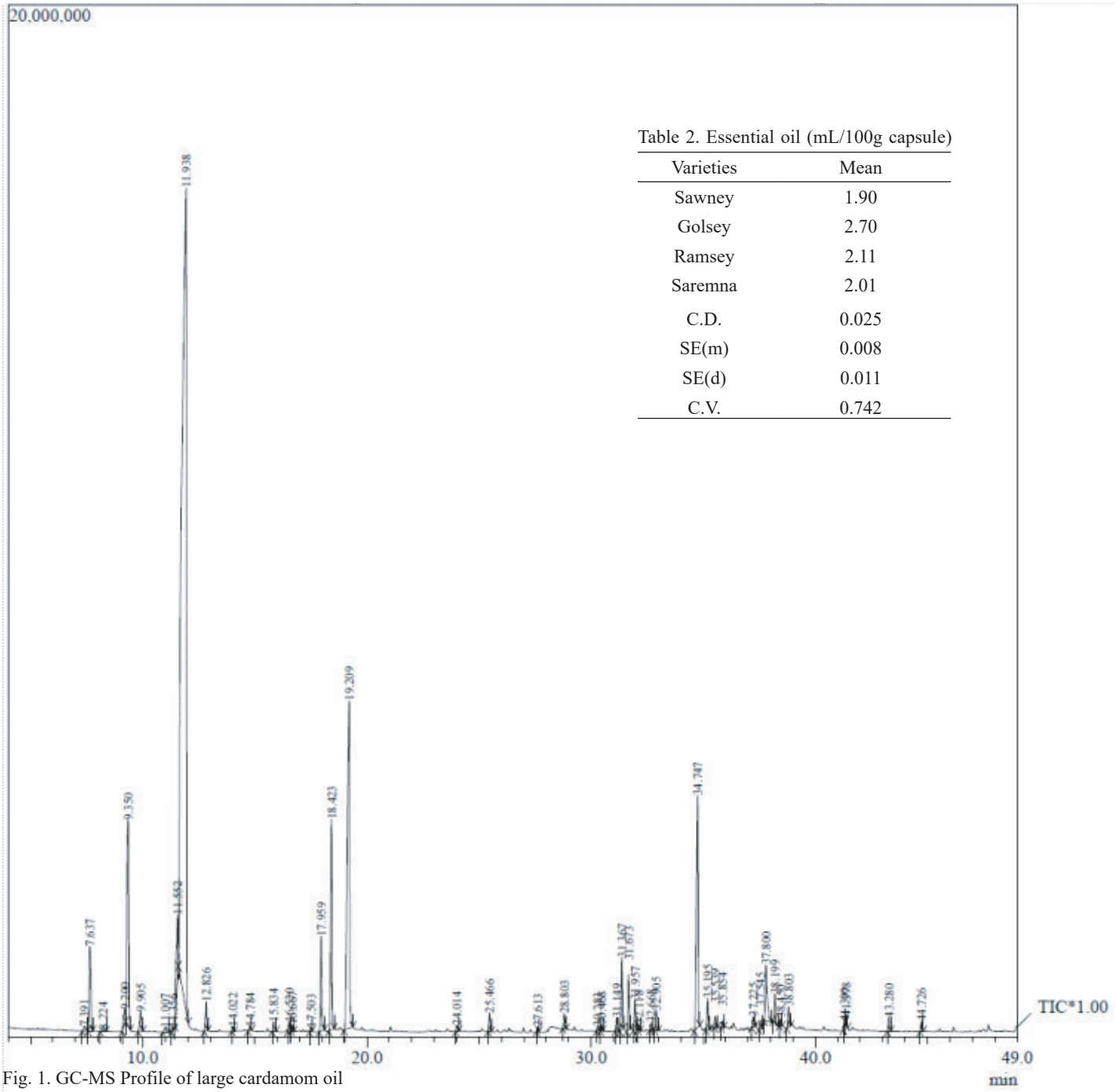

Fig. 1. GC-MS Profile of large cardamom oil

Pradesh. Kumar *et al.* (2012) collected a large cardamom sample from Sikkim subjected to GC-MS analysis and reported 65.39% of 1,8-cineole. Vijayan *et al.,* (2017) reported 68.8% of 1,8-cineole in Sawney collected from Pangthang, Sikkim, 72.2 % 1,8-cineole in Sawney cultivars from Nagaland and 70.1% 1,8-cineole in Sawney cultivars from Myanmar (Burma). In the present study, 1,8-cineole in Sawney cultivar was found to be 54.7%. Vijayan *et al.* (2017) reported 69.5% of 1,8-cineole in Ramsey cultivar oil collected from Upper Lingzey (East Sikkim) where as in the present study it was found to be 86% which was also highest among the other tested cultivars. These variations in the metabolite compounds may be attributed to different protocol used and geographical variations, *i.e.* climatic conditions, altitude, etc.

In the present study 1,8-cineole was found as the major component in all the four cultivars of large cardamom analysed through the GC-MS. The highest percentage of essential oil was obtained from Golsey cultivar. Among the cultivars tested Golsey was found to contain high percentage of major compounds namely β-pinene, myrcene, δ-terpinene, α-pinene, and α-thujene (0.2%). Whereas the number of components were highest in Sawney and the highest percentage of 1,8-cineole was found in Ramsey cultivar. The comparative study among different cultivars showed that Ramsey and Golsey cultivar can be recommended to farmers for commercial cultivation as 1,8-cineole, essential oil was highest which can be utilised for pharmaceuticals, flavouring agent and cosmetic industries. As Sikkim is largest producer of large cardamom and as essential oil has great medicinal uses, the study has great importance regarding its industrial value.

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