



## Evaluation of Comparative metabolomic profile in *Cardamom elettaria* and *Amomum subulatum* fruits

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### ABSTRACT

Background: Cardamom is Queen of Spices belonging to Zingiberaceae family. The present study to reveals the comparative metabolomic profile of the two different types of cardamom such as *Cardamom elettaria* and *Amomum subulatum*. Methods: Physical parameters and chemical parameters were screened in both species. The essential oil was extracted by using Clevenger apparatus and analysed the volatile profile by GC-MS studies. Results: Capsule of *C. elettaria* was observed to dominant in bulk density and volatile oil content whereas *A. subulatum* is greater in size and shape. Oil of *C. elettaria* showed the good ascent of Optical rotation (+29.1), Specific gravity (1.4620), refractive index (0.927), aroma and taste. In *C. elettaria* and *A. subulatum* the terpinyl acetate, geranyl acetate, and myrcene were present in high level. As well, 1,8-cineole, pinene, 3-carene, and terpineol are the primary differentiators in both seeds with diverse therapeutic effects. Conclusion: The physico-chemical characteristics of *C. elettaria* and *A. subulatum* were contrasted in this work.  $\alpha$ -terpinyl acetate is a lead molecule in *C. elettaria*. 1,8-cineole are rich in both essential oils. Future researchers and flavourists can use this study to improve the scent and therapeutic elements of drug repurposing.

**Keywords:** Zingiberaceae, *Cardamom elettaria*, *Amomum subulatum*, Phytochemical fingerprinting, Physico-chemical properties.

### INTRODUCTION

Traditional medicine plays important role in curing several diseases. According to WHO, 88% of the world population depends on the plant-derived medications as home remedies and curation for the several ailments<sup>1,2</sup>. Essential oil (EOs) are extracted from leaves, fruits, stem and barks which have been natural aromatic, volatile

liquids possessing the antibacterial, antiviral and insecticidal activities<sup>3</sup>. Many foods, including meat, peaches, sweet peppers, lemon, apple juice, and milk, use EOs as a natural preservative because they kill harmful bacteria like salmonella, *E. coli*, and *Listeria monocytogenes* and boost antioxidant activity and active shelf life by up to 5-6 days. It is now used as natural food preservatives from ancient times<sup>4</sup>. Cardamom essential oils are most



effective for treating asthma, oral mouth infections, gastrointestinal disorders, cardiovascular issues, nausea, diarrhoea, hepatotoxicity, gastric and ulcerogenic diseases<sup>5</sup>. Cardamom is the best curative medicine for severe cough, cold, wheezing and asthma, bronchitis, tuberculosis, antibacterial and sore throat<sup>6,7</sup>.

*C. elelettaria* and *A. subulatum* are major types and its capable to slow down the spoiling process and stop the spread of food-borne infections and it used as antimicrobials in the food industries<sup>8</sup>. After saffron and vanilla, little cardamom is considered the priciest spice in the world. Essential oil of *C. elelettaria* (L.) Maton used in perfume production, and it ranks third in cost, behind only vanilla and saffron, among spices. Large-scale production occurs mostly in the tropical countries of Guatemala, India, and Sri Lanka<sup>8,9</sup> and it having higher economic values<sup>13</sup>. In this study, we have compared the phytoconstituents profile of both *C. elelettaria* and *A. subulatum* and their physico-chemical appearance that will be useful in further research.



Fig. 1(a). *Cardamom elelettaria* and (b) *Amomum subulatum*

## MATERIALS AND METHODS

### Sample Collection, oil extraction and physical characteristics observation

Fresh and healthy capsules of *C. elelettaria* and *A. subulatum* has been procured from the local shop of Cochin, Kerala. Physical parameters such as size and shape, bulk density and volatile content were observed manually. Seeds with capsules were then made steam distillation in Clevenger apparatus for extraction of essential oils from both the seeds

(*C. elelettaria* and *A. subulatum*) separately and the physical parameters of both oils was observed manually.

### Identification of Volatile phytoconstituent profile in *C. elelettaria* and *A. subulatum* Oil by GC-MS analysis

Gas chromatography coupled to a mass spectrometer detector (MSD) from an Agilent HP 6890/5977 was used to examine the oils from *C. elelettaria* and *A. subulatum*. Specifically, the machine condition was Injector at 270°C; Detector 300°C; oven 40°C @ 1 min hold to 110 °C @ 1 min hold up to 300 °C (5 mins hold), and 325 °C (10 min hold) at a rate of 10°C per minute. The steady flow of helium was set at 3 mL/minute. The scan mass/charge range was from 50 m/z to 550 m/z. NIST 05 and Wiley 275 intrinsic mass spectral libraries were used to determine the essential oil by comparing retention indices (RIs) to n-alkanes homologous series<sup>14</sup>.

## RESULTS

### Collection of Sample and Morphological appearance of Capsules

15 g of both fruits were collected (*A. subulatum* and *C. Elelettaria*) then observed the morphological characteristics and extracted oils for further studies. Capsules of *C. elelettaria* and *A. subulatum* shown greater difference in their physical morphological appearance. Where *C. elelettaria* has green colour and having slighter wider area of 5-7 mm and *A. subulatum* has daker brown in colour and having the wider area of 9-11mm which is more comparable to the green cardamom. The length of *A. subulatum* largely differs from the *C. elelettaria* having 22-30 mm larger were *C. elelettaria* has 16-20 mm length which measured by the vernier calliper. The Bulk density of the *A. subulatum* has the larger difference as 410gm/L whereas *C. elelettaria* has only 360 g/L. This shows the wild type of cardamom has larger volume and denser in quantity grade. The volatile content of *C. elelettaria* has greater 7.5% then *A. subulatum* having 5.5% shown in Table. 1. 15 g of separate samples of both species yielded 3.4  $\mu\text{L} \pm 0.02 \mu\text{L}$  (*Cardamom elelettaria*) and 2.5  $\mu\text{L} \pm 0.31 \mu\text{L}$  (*Amomum subulatum*) respectively.

Table 1: Raw material analysis of *C. elelettaria* and *A. subulatum*

S. No	Parameters	<i>Cardamom elelettaria</i>	<i>Amomum subulatum</i>
1	Size & Shape	(16-20 mm) X (5-7 mm) More parabolic or curved	(22-30 mm) X (9-11 mm) More Spherical
2	Bulk Density	360 g/lit Approx	410 g/lit Approx
3	Volatile Oil Content	7.5% Approx	5.5% Approx

### Physical parameters of oils

As mentioned before the respective oils were obtained and checked for the tentative values of Optical rotation, Refractive index, Specific gravity, Solubility, Odour and taste. Optical rotation of *C. eleteria* has +29.11/(25 to 35) which is higher and the *A. subulatum* has the negative scale of rotation such as -9.59/(-5 to -15). The Refractive index has the similar range of both of the species with difference of 0.0003 and with similar indexing

of 1.460 to 1.464. Specific gravity of the oil of *A. subulatum* (0.915) has the slighter difference while *C. eleteria* has 0.927 with the standard deviated range of *C. eleteria* 0.917 to 0.937 and *A. subulatum* 0.905 to 0.925 respectively. Oil texture and quality of *C. eleteria* has been identified as Spicy, Sweet, Pleasant, Oily but not that rancid, Slight minty and *A. subulatum* has the Waxier, Camphoraceous, Oily Rancid, more minty texture.

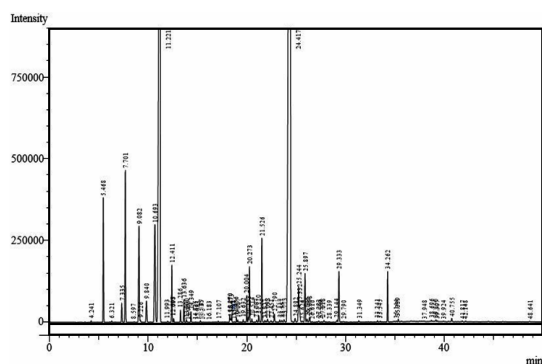
**Table 2: Physical parameter analysis of *C. eleteria* oil and *A. subulatum* oil**

S. No	Parameters	<i>Cardamom eleteria</i> oil	<i>Amomum subulatum</i> oil
1	Optical Rotation (Value/Range)	29.1/(25 to 35)	-9.59/(-5 to -15)
2	Refractive Index (Value/Range)	1.4620/(1.460 to 1.464)	1.4623 (1.460 to 1.464)
3	Specific Gravity (Value/Range)	0.927 (0.917 to 0.937)	0.915 (0.905 to 0.925)
4	Solubility	Partially soluble in ethanol	Partially soluble ethanol
5	Odor, Aroma & Taste	Spicy, Sweet, Pleasant, Oily but not	Waxier, Camphoraceous, Oily Rancid, more minty

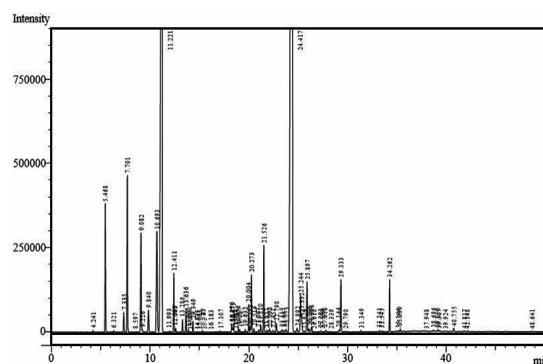
### Volatile phytoconstituents of *C. eleteria* and *A. subulatum*

Hydro distilled oils of *C. eleteria* and *A. subulatum* has been carried out in the phytoconstituent analysis using the GC-MS analysis and compared (Table 3). Within the detected Ester type phytoconstituents *C. eleteria* possess the greatest percentage area such as  $\alpha$ -terpinyl acetate (46.316),  $\delta$ -terpinyl acetate (0.24), Linalyl acetate (0.932), Geranyl acetate (1.111), Bornyl acetate (0.062). Where the major presence of Borneol acetate plays the major role comprising the volatile ester group in *C. eleteria*. 1,8 Cineole is plays key role in the aromaticity of the cardamom species as already reported in

many research. *A. subulatum* has the larger area percentile (70.061) while comparing to *C. eleteria* (30.148). Monoterpenes such as Sabinene (2.091) and  $\beta$ -myrcene (1.406) are greater in *C. eleteria* where in *A. subulatum* has the greater percentile area such as  $\beta$ -pinene (6.348), Limonene (d) (5.562) and  $\delta$ -3-carene (1.159) of monoterpenes. The monoterpene,  $\alpha$ -terpineol (5.205) is found abundant in *A. subulatum* showing they are strongest phytoconstituent associated to the monoterpene shown in figure 2b. The aldehyde, Geranial is found major in the oil source of *C. eleteria* (0.69) having the greater difference while comparing to the amomum species shown in Figure 2a.



**Fig. 2a. Chromatogram of *Cardamomum eleteria* Oil showing the major phytoconstituents**



**Fig. 2b. Chromatogram of *Amomum subulatum* Oil showing the major phytoconstituents.**

**Table 3: Comparison of oil phytoconstituents between the *C. eletheria* and *A. subulatum***

S.No	Compound ESTER	CAS No.	Molecular Formula	<i>Cardamom Eletheria</i> (%)	<i>Amomumsubulatum</i> (%)
1	$\alpha$ -terpinyl acetate	80-26-2	C <sub>12</sub> H <sub>20</sub> O <sub>2</sub>	46.316	4.078
2	$\delta$ -terpinyl acetate	93836-50-1	C <sub>12</sub> H <sub>20</sub> O <sub>2</sub>	0.24	0.0231
3	Linalyl acetate	115-95-7	C <sub>12</sub> H <sub>20</sub> O <sub>2</sub>	0.932	0.022
4	Geranyl acetate	105-87-3	C <sub>12</sub> H <sub>20</sub> O <sub>2</sub>	1.111	0.15
5	Bornyl acetate	76-49-3	C <sub>12</sub> H <sub>20</sub> O <sub>2</sub>	0.062	Not detected
	OXIDE				
6	1.8-cinoole	470-82-6	C <sub>10</sub> H <sub>18</sub> O	30.148	70.061
	MONOTERPENE				
7	Sabinene	3387-41-5	C <sub>10</sub> H <sub>16</sub>	2.091	1.31
8	$\beta$ -myrcene	123-35-3	C <sub>10</sub> H <sub>16</sub>	1.406	0.736
9	$\beta$ -pinene	127-91-3	C <sub>10</sub> H <sub>16</sub>	0.283	6.348
10	$\alpha$ -thujene	02-05-2867	C <sub>10</sub> H <sub>16</sub>	0.279	0.286
11	$\alpha$ -terpinene	99-86-5	C <sub>10</sub> H <sub>16</sub>	0.404	0.301
12	(E)- $\beta$ -ocimene	13877-91-3	C <sub>10</sub> H <sub>16</sub>	0.056	0.052
13	Limonene (d)	5989-27-5	C <sub>10</sub> H <sub>16</sub>	3.095	5.562
14	$\alpha$ -pinene	80-56-8	C <sub>10</sub> H <sub>16</sub>	1.17	3.08
15	$\gamma$ -terpinene	99-85-4	C <sub>10</sub> H <sub>16</sub>	0.749	0.493
16	p-cymene	99-87-6	C <sub>10</sub> H <sub>14</sub>	0.731	0.812
17	Terpinolene	586-62-9	C <sub>10</sub> H <sub>16</sub>	0.409	0.162
18	Camphene	79-92-5	C <sub>10</sub> H <sub>16</sub>	0.022	0.053
19	$\delta$ -3-carene	13466-78-9	C <sub>10</sub> H <sub>16</sub>	0.018	1.159
	MONOTERPENOL				
20	Linalool	78-70-6	C <sub>10</sub> H <sub>18</sub> O	0.438	0.158
21	Terpinen-4-ol	562-74-3	C <sub>10</sub> H <sub>18</sub> O	1.689	1.741
22	$\alpha$ -terpineol	98-55-5	C <sub>10</sub> H <sub>18</sub> O	1.809	5.205
23	$\beta$ -terpinol	7299-42-5	C <sub>10</sub> H <sub>18</sub> O	0.106	0.73
24	p-cymen-8-ol	1197-01-9	C <sub>10</sub> H <sub>18</sub> O	0.368	0.032
	ALDEHYDE				
25	Geranial	5392-40-5	C <sub>10</sub> H <sub>16</sub> O	0.69	0.0891
26	Neral	106-26-3	C <sub>10</sub> H <sub>16</sub> O	0.069	0.032
27	Decanal	112-31-2	C <sub>10</sub> H <sub>16</sub> O	0.072	0.046
	SEQUITERPENOL				
28	(E)-nerolidol	40716-66-3	C <sub>15</sub> H <sub>26</sub> O	1.084	1.533
	SEQUITERPENE				
29	$\beta$ -selinene	17066-67-0	C <sub>15</sub> H <sub>4</sub>	1.199	0.743
30	$\beta$ -caryophyllene	87-44-5	C <sub>15</sub> H <sub>4</sub>	0.0216	0.097

## DISCUSSION

*C. eletheria* grown in the regions of Kerala state and *A. subulatum* found in the regions of Karnataka (Mysore) which were adjoining states in the Western Ghats. Due to the increased export quality *C. eletheria* is preferred after the bleaching which is found in green elongated shape. The practice of employing Sulphur dioxide or compounds containing chlorine to brighten the hue was abolished by this system. In some cases, metanyl yellow solution was reportedly used to get a consistent, attractive bleached colour. Among the *E. cardamomum* species recognised by ISO 676:1995 are Thwaites Variety Major and Minuscule Thwaites Variety Burkill<sup>15</sup>. Rich sandy loams soil is

ideal for cardamom plants by enjoying the shade and need some rain. Capsules of cardamom are dried in artificial furnaces. At high temperatures and high humidity, the chlorophyll on the outside of the capsule can be degraded. Steam distillation was originally practiced in Western consumer countries. About 65% of the capsule is made up of the seeds when they are dry. The husk makes about 35% of the total. Grade level and geographical location can both cause for difference. *C. eletheria* Originally from South India, but now widespread around the world, wild maton is a long, perennial reed-like herb. The plant has been called "the queen of spices" because of its reputation for rarity and high price. The husk is valuable since it is employed in solvent extraction and its has many flavoring molecules. Essential oils

have varying chemical compositions depending on the plant's genus, species, chemotype, harvesting place, and period. The biological effects of *C. eleteria* essential oils are consistent with those predicted by their chemical composition. Monoterpenes such as 1,8-cineole, terpineol, linalool, and pinene, as well as the ester -terpinyl acetate, fall within this category. On a dry weight basis, cardamom capsules provide B vitamins, ascorbic acids, and minerals, along with 10% crude protein, 42% carbohydrates, and 20% crude fibre. Essential oil, found at concentrations of 5–11% depending on area and grade, is the most important component due to its primary usefulness as a flavouring substance. 1,8-Cineole is the primary component in eucalyptus oil. The component has a camphoraceous and refreshing aroma. Free and acetate forms of alcohols such linalool and terpineol are found. Thus, the volatile phytoconstituents of the *C. eleteria* and *A. subulatum* has the key differentiator and rich source that provide many medicinal properties such as anticancer, antidiabetic, antiasthmatic and anti-rheumatics.

## CONCLUSION

Morphological characteristics of *C. eleteria* showed predominant than *A. subulatum* except the size and length. The principal phytoconstituents of *A. subulatum* oil contains 1,8-cineole, pinene, limonene (d), and 3-carene, while the phytoconstituents of *C. eleteria* oil are rich in terpinyl acetate, geranyl acetate, sabinene, myrcene, and selinene. Further, the volatile profile of these two essentials oils was revealed and showed differences. These findings will help to the natural flavors in food industries.

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## Conflict of interest

The authors do not have any competing interests, either financially or otherwise.

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