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Assessment of Intra-Specific Variation in Essential Oil Composition in *Amomum subulatum* Roxb. Cultivated in Uttarakhand, India

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Abstract

Large cardamom *(Amomum subulatum)* is native to Sikkim Himalaya and is important cash crop grown under the forest canopy. It thrives well between altitudes of 600 to 2000 m having an annual rainfall of 2500 to 5000 mm. Due to similar climatic condition and economic potential of the species, its cultivation was also started in Uttarakhand. Present study was design to analysis the chemical composition of essential oil and major compounds present in essential oil of capsule of A. *subulatum* grown in different agro-climatic region of Uttarakhand. The essential oil of 07 plant sample collected from different agro-climatic zones of Uttarakhand was analyzed through Gas Chromatography Mass Spectrometry (GC-MS). The aroma analysis of chemicals present in essential oil was also carried out by two-fold, step wise dilution with dichloromethane. Oil yield was found between 2.5% to 3% for the sample of different agro-climatic regions. A total of 31 compounds were identified through Gas Chromatography Mass Spectrometry. 1-8 Cineol with 68% to 82.0% was the most dominant compound in all the samples. Other major compounds are 4-trimethyl (1.87% to 4.82%), β-pinene (1.88% to 3.56%), β-Terpineol (1.21% to 3.83%), Nerolidol (0.62% to 2.82%), DL limonene (1.01% to 2.51%) and Limonene (0.98% to 3.82%). Presence of higher concentration of essential oil (%) in all analyzed sample of Uttarakhand indicated that the species grown in Uttarakhand is may be of higher genotype. The GC-MS analysis of essential oil indicates quantitative variations within intra-specific and may attribute to the micro-climate under which the species grown.

Keywords: 1, 8 Cineole, Essential Oil, GC-MS, Genotype, Uttarakhand

1. Introduction

Amomum subulatum Roxb. (family, Zingiberaceae) is commonly known as large cardamom or Nepal cardamom. It is perennial spice indigenous to moist deciduous and semi-evergreen forests and cultivated mostly in north-eastern and recently introduced in the central Himalayan states of India[1]. The plant is well known for spice and medicinally useful as carminative, stomachic, diuretic, cardiac stimulant, thus acknowledged as boon to the Himalaya inhabitants[2,3]. It is cultivated commercially in India, Nepal, China and Bhutan. India produces the largest share with 54 percent of total world production followed by Nepal with 33.3 percent and Bhutan with 13.3 percent share[1]. In India, the states of Sikkim including the Darjeeling district of West Bengal are the leading areas for its production. Being a cash crop of the state of Sikkim, it is registered as a geographical indication (GI-376) for the state[4]. Flavonoids, steroids, triterpenoids, amino acids, tannins etc. are among the phyto-chemicals being reported in the essential oil and extract of fruit[5].

Realizing the economic importance of species and

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being preferable climatic and topographic conditions, its cultivation has also been started in the state of Uttarakhand, India[3,6]. But commercial cultivation of *A. subulatum* in Uttarakhand may inevitable leads to maintain the quality of essential oil in terms of chemical composition and presence major compounds in capsule. Moreover, even a minute change in climatic conditions may leads to the change in chemical composition of essential oil and capsule extract. Keeping the view of commercial cultivation, analysis of chemical composition and major compounds present in *A. subulatum* is prerequisite. Therefore, this study was carried out to assess the intra-specific variation in essential oil compositions and major molecules of *A. subulatum* grown in different agro-climatic regions of Uttarakhand, India.

2. Material and Methods

2.1 Collection of Plant Materials

The capsule of the *A. subulatum* were collected during the month of October-November from Lamgarha (Almora), Mandal and Guhad (Chamoli), Singot (Uttarakashi), Kwiti (Pithoragarh), Sema (Tehri), Parkandi (Rudraprayag) of Uttarakhand. The plant sample was identified through herbarium of Botanical Survey of India (accession number BSD-112741). The collected capsule was dried in modified curing unit designed by Spices Board, ICRI, Gangtok, Sikkim. The unit is composed of two parts viz. a lower bhatti and an upper curing chamber. Firewood is burnt inside the fireplace (drum; lower bhatti) to generate heat and smoke laden hot air passes through the flue pipes and escapes through the exhaust (upper chamber). This hot air moves upward through the cardamom capsules spread over the iron net. The cardamom put in to the net may stirred at regular interval for even drying. This system gives early drying and better quality. Under this system, cardamom is cured by indirect heating and takes 17-18 hours for 200 Kg of fresh capsule.

2.2 Oil Extraction

The dried whole capsules of different sites were then subjected to hydro-distillation for 6 h using Clevenger for oil extraction at a temperature of 90^{0}C. The collected concentrated oil was then subjected to anhydrous sodium sulphate to remove the moisture and then stored at 4°C for further analysis[7].

2.3 Aroma Dilution Analysis

The aroma analysis of chemicals present in essential oil was carried out by two-fold, step wise dilution with dichloromethane. The highest dilution at which an individual Component was detected was given as the Flavour Dilution (FD) factor for that odorant, and the FD factor was expressed as a power of 2.

2.4 Gas Chromatography-Mass Spectrometry Analysis (GC-MS)

Analysis of the essential oil extracted from fruits were carried out on a GCMS (Thermo, Focus-Polaris Q) equipped with ZB-5 capillary column (30 m × 0.25 mm, film thickness 0.25 mm). During analysis, the temperature of oven was fixed at 60°C (hold time 3 min), then after, it was fixed at 3°C min^{-1} to 220°C (hold for 10 min). Helium was the carrier gas has a flow rate of 1 ml/min. 0.5 µl of 10.0% oil prepared in hexane is used as injection volume with split flow of 20 ml/min while the injector temperature was 220°C. Ion Trap mass spectrometer in EI mode at 70 eV (m/z 40 to 350 at 1 scan/s) was used for the mass spectra recording. Ion source and transfer line temperatures were 200 and 230°C, respectively. The components presents in the mass spectra of essential oil were identified through matching up their spectra with mass spectra data already available in the NIST library and literature.

3. Results and Discussion

Light yellowish color essential oil was obtained with highest yield of 3.0 percent w/v in Guhad (Chamoli) sample followed by 2.9 percent in Singot (Uttarakashi), 2.7 each in Parkandi (Rudraprayag), Sema (Tehri) and Kwiti (Pithoragarh), 2.6 in Lamgarha (Almora) and 2.5 in Dasholi (Mandal) (Figure 1). The oil yield reported in present study was found higher than reported earlier[8]. Bhandari et al.[3] described that percentage of oil yield was affected by maturity of the capsule, climatic conditions, seed quality, curing and time of harvesting. On the basis of oil yield in the *A. subulatum* grown in Guhad (Chamoli) was found to be of higher quality. This may be attributed to the presence of better environmental conditions present in Uttarakhand for the growth of the species. On the basis of higher yield of essential oil, *A. subulatum* grown in Uttarakhand may considered of

superior genotype which may be used as mother plant for the production of superior quality suckers and seeds.

Among the total of 31 compound identified, 1-8 Cineol, 4-trimethyl, β-pinene, β-Terpineol, Nerolidol, DL limonene, Limonene are the predominant compounds in the essential oil of *A. subulatum* (Table 1). Among these, 1-8 cineol was the major compound (68.48% to 82.0%) followed by 4-trimethyl (1.87% to 4.82%), β-pinene (1.88% to 3.56%), β-Terpineol (1.21% to 3.83%), Nerolidol (0.62% to 2.82%), DL limonene (1.01% to 2.51%) and Limonene (0.98% to 3.82%). Minimum concentration was found in *trans*-sabinene hydrate (0.04-0.33 %), Linalool (0.08-0.19 %) and Palmitic acid (0.08- 0.31 %) (Table 1). The individual compound separated by gas chromatography were identified on the basis of comparison of retention indices and by comparing their MS with those of standard NIST (National Institute of Standards and Technology, Department of Commerce, USA) and Wiley (John Wiley and Sons Ltd.) libraries. Agnihotri et al.[9] reported that leucocyanidin-3-O-β-D-glucopyranoside, petunidin 3,5-diglucoside, cardamonin, cholcone, flavanone, subulin and alpinetin are among dominant compounds in *A. subulatum* essential oil. Earlier studies reported the presence of a total of 18 compounds[3] and 25 compounds in essential oil of different strains of *A. subulatum*[10]. Earlier studies carried out through gas chromatography and infrared spectra, also reported that 1,8 cineole is the major component in the essential oil[3,11,12]. Bhandari et al.[3] also found that 1-8 cineole is the dominant compound of essential oil of *A. subulatum* grown in Uttarakhand which is in comparable with the findings of present study. Likewise, Gupta et al.[13] also reported 77% to 89% of 1-8 cineole in essential oil of different strains of *A. subulatum* grown in different part of the Sikkim, Uttarakhand. Joshi et al.[7] also reported 1,8 cineole as the major component with maximum of 60.46% found in Palmpur and minimum of 50.55% for Bilaspur. The major and minor compounds found in the essential oil showed quantitative variations within intra-specific and may attribute to the micro-climatic conditions of the region. The variation in chemical composition of the essential oil is depends on stage of growth, microclimate, altitude, latitude and method of extraction[14].

On the basis of FD factors, DL-limonene had citrus, *α*-pinene has woody, *β*- myrcene has metallic, *α*-bisabolol has floral, *trans*-sabinene has hydrate sweet aromas (Table 1). The quality of aroma/flavour of the compound present in essential oil was found maximum for woody (6 compounds) and citrus (5 compounds). The variation in the aroma/flavour of the compounds was varied and found maximum for woody and citrus aroma. Since the characteristic aroma of a number of crops is often derived from only a very small number or even a single volatile compound[15], the correlation of volatile constituents with sensory analysis is essential to determine which odor-active compounds are important. Pinocarvone has spicy or turmeric aroma. A detailed study of the mechanism and the factors involved in the synthesis of these critical essences in the oil would be valuable.

4. Conclusion

The yield of essential oil (%) in all analyzed sample was found higher than the sample cultivated in Sikkim, indicating that the species grown in Uttarakhand may have better environmental conditions to thrive. Moreover, GC-MS analysis of essential oil indicates quantitative variations within intra-specific and may attribute to the micro-climate under which the species grown.

5. Conflict of Interest Statement

We declare that we have no conflict of interest.

6. Acknowledgement

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7. Competing Interests

The authors declare that they have no competing interests.

Table 1. Chemical composition of essential oil from capsules of *Amomum subulatum* from different locations of Uttarakhand, India

Compounds	Lamgarah (Almora)	Mandal (Chamoli)	Guhad (Chamoli)	Singot (Uttarkashi)	Kwiti (Pithoragarh)	Sema (Tehri)	Parkandi (Rudraprayag)	Aroma/ flavour quality
1,8-Cineole	69.28±1.95	72.27±2.03	82.0±2.013	78.0±2.10	70.63±2.03	71.32±2.11	68.48±1.98	Mint
1-Phellandrene	0.40±0.12	0.32±0.01	0.32±0.05	0.31±0.04	0.30±0.07	0.42±0.09	0.55±0.20	Citrus
2,3,3-Trimethy-le-1-hexene	0.31±0.05	0.17±0.02	0.09±0.03	0.09±0.03	0.26±0.03	0.09±0.02	0.16±0.03	Sweet fatty
2,6,6-trimeth-yl-acetate	0.27±0.01	0.09±0.02	0.04±0.01	0.05±0.1	0.05±0.01	0.09±0.01	0.19±0.02	-
2-Eth-yl-2-methyl-tri-decanol	0.27±0.09	0.26±0.02	0.11±0.02	0.38±0.08	0.11±0.03	0.38±0.09	0.31±0.06	Sweet-fatty
3-Cyclohexene	0.75±0.03	0.40±0.02	0.45±0.02	0.35±0.07	0.54±0.06	0.62±0.06	0.82±0.21	Sweet-fatty
4-Terpineol	1.86±0.17	2.68±0.09	1.1±0.04	2.42±0.26	1.66±0.08	3.34±0.58	3.52±0.32	Woody earthy
4-Trimethyl cyclohexene	2.36±0.24	1.87±0.06	2.06±0.09	3.36±0.23	4.22±0.02	4.43±1.02	4.82±0.62	Sweet
Benzyl acetate	0.57±0.06	0.35±0.04	0.25±0.02	0.55±0.13	0.37±0.04	0.54±0.08	0.77±0.08	-
Carvone	0.10±0.01	0.10±0.03	0.10±0.01	0.19±0.02	0.69±0.08	0.23±0.04	0.63±0.02	Caraway
Cis-Sabine Hydrate	1.50±0.09	0.27±0.02	0.5±0.02	0.5±0.01	1.06±0.04	0.60±0.10	0.70±0.09	Herbal
DL-Limonene	2.32±0.01	1.21±0.23	1.01±0.02	1.02±0.02	2.51±0.18	2.23±0.11	2.56±0.09	Citrus
Geraniol	0.23±0.02	0.06±0.02	0.07±0.01	0.04±0.01	0.21±0.05	0.13±0.02	0.10±0.02	Woody
Isogeraniol	1.23±0.16	0.16±0.05	0.05±0.01	0.09±0.01	0.13±0.06	0.12±0.02	0.12±0.04	-
Limonene	3.10±0.23	3.13±0.09	1.98±0.20	1.26±0.11	3.82±1.01	0.98±0.08	1.64±0.03	Citrus/Citric
Linalool	0.11±0.01	0.19±0.04	0.08±0.02	0.13±.03	0.13±0.06	0.15±0.03	0.12±0.06	Citrus
Nerolidol	2.82±0.21	0.62±0.08	1.06±0.01	2.06±0.09	1.48±0.11	2.20±0.13	1.95±0.05	-
Nerolidol B	0.18±0.03	0.19±0.01	0.18±0.05	0.18±0.02	0.18±0.01	0.22±0.07	0.32±0.06	Woody
Palmitic acid	0.12±0.05	0.31±0.07	0.08±0.02	0.18±0.04	0.19±0.02	0.24±0.06	0.24±0.08	Herbaceous
Pinocarvone	0.38±0.08	0.27±0.04	0.08±0.04	0.14±0.07	0.16±0.08	0.27±0.14	0.29±0.02	Spicy/ Turmeric
Sabinene	0.35±0.05	0.42±0.05	0.30±0.21	0.27±0.14	0.40±0.05	0.36±0.12	0.36±0.08	Piney
trans- Sabine Hydrate	0.36±0.12	0.17±0.01	0.04±0.01	0.04±0.01	0.13±0.05	0.09±0.03	0.15±0.02	Herbal
trans-Sabinene hydrate	0.11±0.03	0.13±0.02	0.04±0.01	0.07±0.01	0.04±0.02	0.13±0.02	0.33±0.02	Sweet
α-pinene	1.10±0.09	2.69±0.63	1.44±0.26	1.14±0.08	1.20±0.06	2.14±0.11	1.84±0.10	Woody
α-Terpenyl acetate	1.52±0.08	1.79±0.08	0.09±0.01	0.07±0.01	0.07±0.01	0.62±0.08	1.25±0.11	Woody
α-terpinene	0.10±0.01	1.28±0.06	0.23±0.019	0.29±0.14	0.38±0.02	0.42±0.05	0.49±0.09	Herbaceous
α-terpineol	1.35±0.02	3.73±0.13	0.11±0.01	0.21±0.08	0.11±0.03	0.19±0.01	0.17±0.5	Sweet like
α-terpinyl acetate	0.05±0.01	0.36±0.02	0.05±0.03	0.11±0.06	0.13±0.03	0.36±0.08	0.56±0.05	-
β-pinene	2.26±0.12	1.88±0.12	3.56±0.53	2.83±0.22	3.13±0.08	3.08±0.23	3.08±0.12	Woody
β-Terpineol	3.11±0.12	0.33±0.02	1.21±0.03	2.61±0.10	3.83±0.23	2.71±0.41	1.98±0.07	-
γ-terpineol	1.23±0.08	1.70±0.08	1.04±0.24	0.94±0.09	1.50±0.23	0.96±0.17	0.96±0.20	Citrus-pine

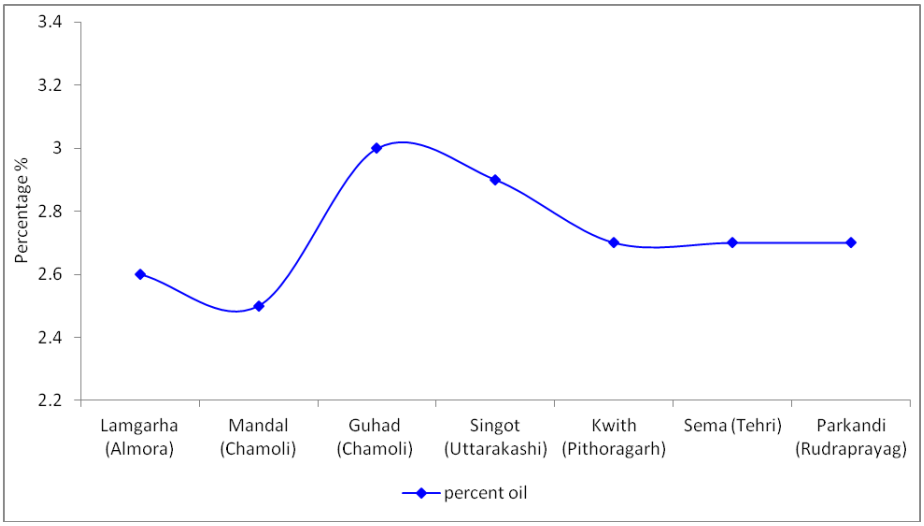

Figure 1. Variation in essential oil yield from capsules of Amomum subulatum from different locations of Uttarakhand, India.

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