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1, 8-Cineole: A predominant component in the essential oil of large cardamom (*Amomum subulatum* Roxb.)

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Oil obtained by hydro-distillation of the dried capsules of large cardamom (*Amomum subulatum* Roxb.) grown in Uttarakhand, India was analyzed by GC-MS. A total 18 components representing 99.21% of the total oil contents were identified by mass spectra and relative retention indices. The predominant constituents of the oil were found to be 1, 8-cineole (73.27%) followed by α-terpineol (4.23%), limonene (4.2%), α-terpinyl acetate (3.33%), α-pinene (2.9%), terpinen-4-ol (2.82%), β-pinene (2.12%), v-terpinene (1.8%) and α-bisabolene (1.4%).

Key words: *Amomum subulatum*, Clevenger, hydro-distillation, essential oil, gas chromatography-mass spectrometry (GC-MS)

INTRODUCTION

Since ancient times, all the aromatic and medicinal plants available worldwide have been used for their preservative and medicinal values. *Amomum subulatum* Roxb. (Family: Zingiberaceae) is native to the Eastern Himalayas, commonly known as large cardamom and locally known as *Bari Elaichi* in India and Nepal. It is well known in Ayurvedic system of medicine. Since ancient times, seeds of *A. subulatum* are valued for its aroma and flavor, thus used as spice and condiment. It is an important cash crop of Eastern Himalayas and typically cultivated in woodland areas between altitudes of 500 to 1,800M mean sea level (MSL) on slopes under chequered shades, preferably along the streams (Nandkarni, 2000). This crop is believed to be originated in Sikkim, a small state in the North Eastern region of India. Sikkim is the largest producer of large cardamom in India with 80 to 85% of total production (Bhandari et al., 2011; Bisht et al., 2012). Later, the cultivation of large cardamom spreads to other states including West Bengal, Arunachal Pradesh, Nagaland, Mizoram and Manipur in the north eastern region and Uttarakhand in the northern region of India. Simultaneously, it is also cultivated in neighboring countries of Nepal and Bhutan.

Large cardamom is a perennial herb with leafy stem up to 90 to 100 cm in height, large coarsely striated fruits of brown to dark red brown in color, measuring 2 to 3 cm in length and 1.5 cm in width. The plant consists of subterranean rhizomes and several leafy aerial shoots (tillers) that grow up to 1.5 to 2.5 m tall. Capsules are 20 to 25 mm in length, oval to globose and contain 30 to 55 seeds. The capsules possess certain medicinal properties such as carminative, stomachic, diuretic, cardiac stimulant, antiemetic and are also used in the treatment of throat and respiratory disorders (Singh et al., 1978; Bisht et al., 2011).

Qualitative chemical examinations of various extracts of *A. subulatum* revealed the presence of carbohydrates, flavonoids, amino acids, steroids, triterpenoids, glycol-sides and tannins (Bisht et al., 2010; Arora and Kapoor, 2013). There have been great efforts to find safe and

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potent natural drugs from various plant sources (Sundriyal et al., 1998; Negi et al., 2011, 2012; Bisht et al., 2013; Negi et al., 2013). Quantitative chromatographic analysis of essential oil of large cardamom was also carried out (Nigam and Purohit, 1960; Lawrence, 1970). Shankaracharya et al. (1990) reported that seeds of large cardamom contain moisture (8.5%), protein (6%), volatile oil (2.8%), crude fiber (22%), starch (43.2%), ether extract (5.3%) and alcohol extract (7%). It has been reported that 100 g of large cardamom seeds contains 666.6 mg calcium, 412.5 mg magnesium, 61 mg phosphorous and 14.4 ppm fluoride. Besides all these studies, cardamom grown in Uttarakhand is yet not analyzed for its oil percent and contents. Therefore, the present study was design to analyze the chemical constituents present in the cardamom oil grown in Uttarakhand, India. *A. subulatum* has been introduced for cultivation in the state of Uttarakhand, India, in recent years and successfully grown at farms field with excellent biomass and seeds production capacity. Therefore, the present study reports the volatile oil composition of *A. subulatum* together with a comparison with those from previous studies.

MATERIALS AND METHODS

Plant

Large cardamom capsules were harvested in the month of October, 2012 from the nursery of Herbal Research and Development Institute (HRDI), Mandal, Uttarakhand, India. It is bounded by North Latitude 30° 27' 13.40" and East Longitude 79° 16' 21.61" and 1545M. Capsules were collected from 5 to 6 years old Sawney cultivar raised from seedlings. Appropriate agro-technique developed by Spices Board, Indian Cardamom Research Institute was followed for cultivation.

Isolation of the essential oil

Freshly harvested capsule were hydro-distilled for 6 h using a Clevenger apparatus. Large cardamom capsules were collected from three places (250 g from each place) of the same population and used for hydro-distillation. Dark brown essential oil was obtained and stored at 0°C in air tight container after drying over anhydrous sodium sulphate.

Gas chromatography-mass spectrometry analysis (GC-MS)

Analysis of the fatty acid methyl esters were carried out on a GC-MS (Thermo, Focus-Polaris Q) equipped with ZB-5 capillary column (30 m × 0.25 mm, film thickness 0.25 mm). The oven temperature was held at 60°C (hold time 3 min) then programmed at 3°C min^{-1} to 220°C (hold for 10 min). The carrier gas was helium at a flow rate of 1 ml/min. The injection volume was 0.5 µl of 10.0% oil prepared in hexane (split flow 20 ml/min) and injector temperature was 220°C. Mass spectra were recorded using Ion Trap mass spectrometer in EI mode at 70 eV in the range m/z 40 to 350 at 1 scan/s. Ion source and transfer line temperatures were 200 and 230°C, respectively. The individual compounds were identified by comparing their mass spectra with data already available in the NIST library and literature (Adams, 2007).

Table 1. Chemical components of *A. subulatum* essential oil.

S/N	Compound	Component (%)
1	α-terpinene	1.2
2	α-pinene	2.9
3	β-pinene	2.12
4	Sabinene	0.6
5	Camphene	0.22
6	v-terpinene	1.8
7	Limonene	4.2
8	ρ-cymene	0.26
9	1, 8-Cineole	73.27
10	Linalool	0.21
11	Geraniol	0.06
12	α-terpineol	4.23
13	Terpinen-4-ol	2.82
14	Nerlidol	0.21
15	Nerlacetate	0.08
16	α-terpinyl acetate	3.33
17	α-bisabolene	1.4
18	β-terpineol	0.3

RESULTS AND DISCUSSION

In this study, capsules of *A. subulatum* yielded 3.5% w/v dark brown color oil. The oil percentage in the cardamom grown in Uttarakhand was found higher than earlier reports (Gupta, 1986; Shankaracharya et al., 1990). GC-MS analysis of *A. subulatum* essential oil led to the identification of 18 compounds representing 99.2% of the total oil contents (Table 1). The GC-MS chromatograms of standard and test oil are presented in Figure 1. The predominant constituents of the oil were found to be 1, 8-cineole (73.27%) followed by α-terpineol (4.23%), limonene (4.2%), α-terpinyl acetate (3.33%), α-pinene (2.9%), terpinen-4-ol (2.82%), β-pinene (2.12%), v-terpinene (1.8%) and α-bisabolene (1.4%). This is the first study on isolation and chemical composition of *A. subulatum* cultivated in Uttarakhand, India.

Lawrence (1970) separated the components of the oil and found 1, 8-cineole (74%) as major constituent using preparative gas chromatography and infrared (IR) spectra. Patra et al. (1982) also analyzed the oil using GC packed column and reported 1, 8-cineole as a major component (63.3%). In another study, Gupta et al. (1984) analyzed oils derived from different strains of *A. subulatum* wildly growing in Sikkim and found 1, 8-cineole as the major constituent varied from 77 to 89%. Gupta (1986) isolated 1.95 to 3.32% oil from *A. subulatum* capsules. Rout et al. (2003) also reported 84.5 and 86% 1, 8-cineole in fresh seed and laboratory dried seed oil, respectively. Gurudutt et al. (1996) isolated

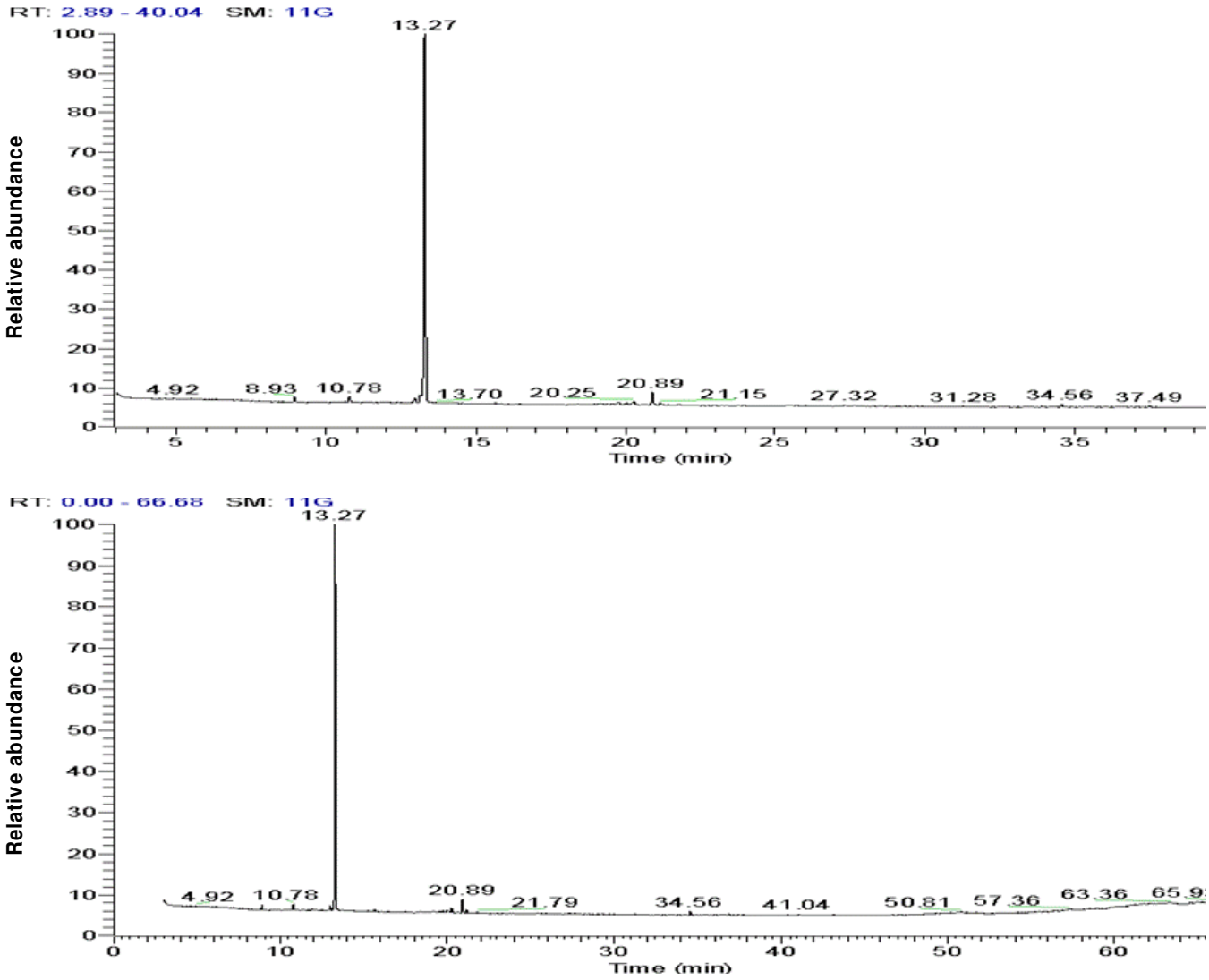

Figure 1. GC-MS spectra of *A. subulatum* essential oil and 1, 8-Cineole (RT 13.27).

volatile oil by steam distillation of large cardamom seeds grown in Sikkim, India, and identified 25 components by GC-MS, of which 16% were monoterpene hydrocarbons and 75.3% were oxygenated monoterpenes, with 1, 8-Cineole (61.3%) as major constituent. Present study reports that, the capsule contains 3.5% w/v essential oil which consist 18 constituents representing 99.21% of the total oil. Our results varied from previous studies on essential oil composition of *A. subulatum,* which might be due to different environmental conditions, genetic factors and agricultural practices. Agnihotri et al. (2012) described that *A. subulatum* mainly contain petunidin 3, 5-diglucoside, leucocyanidin-3-O-β-D-glucopyranoside, cholcone, cardamonin, flavanone, alpinetin and subulin. Terpenes, steroids and flavonoids are well known to have antimicrobial and curative properties against several bacterial pathogens (Nwaogu et al., 2007). Ravichandran et al. (2005) evaluated *A. subulatum* for facial skin wrinkles by prospective, open, phase III clinical trial and showed that protocatechualdehyde and protocatechuic acid were active constituents. Mihir et al. (2012) demonstrated the hepatoprotective effect of *A. subulatum* seeds which scientifically supports the traditional treatment of liver disorders. In conclusion, the percent of essential oil was found higher than that reported for Sikkim and other part of the country with 1, 8-cineole as major constituent extracted from *A. subulatum* capsules. Therefore, large scale cultivation shall be promoted in the state for improvement of economy of the growers.

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