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Essential oil Composition of the Fruits of *Amomum subulatum* Roxb

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ABSTRACT

Amomum subulatum Roxb. (Zingiberaceae) is a perennial herb grown in Sikkim, Darjeeling, Assam, Bhutan and Nepal. Its fruits are used to treat in digestion, vomiting, biliousness, abdominal pain, rectal, throat and lung diseases, inflammation of the eyelids and liver complaints. Hydrodistilled essential oil obtained from the fruits of *A. subulatum* grown in northeast region of Sikkim, was analyzed by capillary GC and GC-MS techniques. The oil was consisted of 20 components including the major ones 1,8 cineole (65.39%), α-terpineol (10.15%), β-pinene (7.23%), α-pinene (4.06%), linalool oxide (3.23%), and limonene (2.53%).

Keywords: *Amomum subulatum* , Zingiberaceae, Fruits , essential oil composition.

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INTRODUCTION

Amomum subulatum Roxb (Zingiberaceae), commonly known as large cardamom, is a perennial herbaceous plant with subterranean rhizomes which produces several leafy shoots and panicles. It is a native to Sikkim and from there it is spread to neighboring areas like Darjeeling, Assam, Bhutan and Nepal between 700 to 800 m with annual rainfall of 3300 mm. India is the largest producer of large cardamom with an annual production of 4000 MT, followed by Nepal (2500 MT) and Bhutan (1000 MT)[6]. Sikkim state of India alone contributes 50% of the world's production of large cardamom[23]. The fruit is a trilocular many-seeded capsule. It contains 1.95 to 3.23% of essential oil having typical characteristic flavor and possesses stimulant, stomachic, alexipharmic and astringent effects[3,4,10]. The fruits are prescribed for the treatment of indigestion, vomiting, biliousness, abdominal pains, rectal diseases, throat troubles, congestion of the lungs, inflammation of the eyelids, digestive disorders, pulmonary tuberculosis, loss of appetite, and liver complaints [12, 16, 27]. Due to its pleasant aroma, it has been used as an essential ingredient in mixed spices for flavorings vegetables, many food preparations, confectionary, pickles, liquor and beverages. The seeds are official as a drug in The Ayurvedic Pharmacopoeia [26]. A preparation called 'Alul' is prepared by mixing cumin (Cuminium cyminum) and large cardamom and administered to treat renal area [25]. The seed decoction is useful as gargle to overcome teeth and gum affections; the fruit pericarp is beneficial to ameliorate headache and stomatitis [24]. The seeds are prescribed as antidote to cure scorpion sting and snake bite and to prevent Hyperlipidaemia[7,13]. Cardamom cola is prepared by blending caramel acid, large cardamom flavour and carbonating the mixture. One teaspoon of the fruit powder of *A.subulatum* is taken with honey twice a day to treat ischemic heart diseases [21]. The major constituent of large cardamom essential oil is 1, 8-cineole (65–80%) while the content of terpenyl acetate is low (traces to five per cent). The monoterpene hydrocarbon content is in the range of 5–17% of which limonene, sabinene, the terpinenes and the pinenes are significant components. The terpineols comprise approximately five to seven per cent of the oil. The high cineole and low terpenyl acetate probably account for the very harsh aroma of this spice in comparison with that of true cardamom [18]. The present paper describes the isolation of the essential oil of the fruits of *A.subulatum* of Sikkim region and characterization of the chemical constituents of the oil by analysis of GLC and GC-MS data.

MATERIAL AND METHODS

Plant Material

The fruits of *A. subulatum* were collected from the local market of Sikkim and identified by Prof. M. P. Sharma Department of Botany, Jamia Hamdard, New Delhi-110062. A specimen No. PRL/JH/11/02 is deposited in the herbarium of the Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi.

Isolation of volatile oil

The air dried fruits (250 g) were hydro distilled in an all-glass apparatus according to the method recommended by the British Pharmacopoeia, 2008, (Anonymous, 2008). The yellowish oil possessed an aromatic odour of eucalyptus oil. It was dried over anhydrous sodium sulphate and stored at 4 ºC in the dark. The yield was 2.9 % based on the dry weight of sample.

Gas chromatography

The major constituents of the extracted oil of the fruits were separated by GLC using Omega SPTm column width, film thickness: 0.25 um, column length: 30.0 m and inner diameter: 0.25 mm. The column maximum temperature was 260ºC. The flame ionization detector was used to detect the percentage area of the peaks. Nitrogen gas was used with a makeup flow of 30ml/minute. The sample was injected 2µl at a time.

Gas chromatography- Mass spectrometry:

The essential oil was analyzed by GC-MS Shimadzu QP-2010 Plus instrument. [GCMS-QP2010 Plus. The ion source temperature was 250°C, interface temperature was 280°C, and column temperature was maintained at 80º C, injection temperature was 270º C. The pressure was maintained at 86.6 kPa. Total flow was 100.9 mL/minute; column flow was 1.21 mL/minute, velocity was 40.5 cm/second; purge flow 3.0 mL/minute N_2 was used as a carrier gas. Flame ionization detector was coupled to detect the total peak area of the elements.

Identification

Most of the constituents were identified by GC comparing their Kovat's indices with those of authentic standards available in the author's laboratory or with Kovat's indices in the close agreement with reference. Further identification was achieved by analysis of GC-MS. The fragmentation patterns of the mass spectra were compared with those stored in the spectrometer data base using the NBS54 K.L. and Wiley L built-in libraries and with those published in the literature [8,9,15,17,20]. Further identification of the compounds was done by comparison of Kovats indices with those reported in the literature [1, 11].

Result and Discussion

The volatile component of *A.subulatum* fruits, relative peak area percentage composition of each component and KI values are listed in Table.1 in which the constituents are arranged in order of

GC and GLC elution on omega SPTm fused silica capillary columns. Analysis of the volatile oil by GC-MS resulted in the identification of 20 components comparing 100% of the total volatiles.

Table 1: Chemical composition of volatile oil of the fruits of *Amomum subulatum*

S. Num.	Components	KI	Area (%)
1.	α – Pinene	928	4.06
2.	Sabinene	960	1.22
3.	β - Pinene	965	7.23
4.	β - Myrcene	971	0.64
5.	Δ^3- Carene	988	0.33
6.	β - Phellandrene	1015	0.56
7.	1,8-Cineole	1019	65.39
8.	Limonene	1022	2.53
9.	ρ- Cymene	1027	0.37
10.	(E)-β-Ocimene	1029	0.28
11.	Cis-Sabinene hydrate	1045	0.89
12.	Linalool oxide	1077	3.23
13.	α – Terpineol	1160	10.15
14.	β – Selinene	1439	0.31
15.	2,3-Epoxygerenyl acetate	1510	0.23
16.	Nerolidol	1564	2.06
17.	Spathulenol	1577	0.18
18.	Aromadendrene epoxide	1602	0.08
19.	Globulol	1584	0.18
20.	Bisabolol oxide	1658	0.06

KI:- Kovat's indices

Quantitatively, the oil was characterized by high proportion of monoterpenes (97.11%), the predominant one being 1, 8-cineole (65.39%) followed by α-terpineol (10.15%), β-pinene (7.23%), and α-pinene (4.06%). Cineole contributes to pungency of the fruit. Among the 14 monoterpenes there were 9 hydrocarbons (17.22%), two alcohols (75.54%), one oxide (3.23%) and one ester (0.23%). Among six sesquiterpenes constituents, comprising 2.87 % of total volatile oil, there were one sesquiterpenes hydrocarbon (0.31%), three alcohols, and two oxides (0.24%). All the sesquiterpenes occurred in trace amounts. The volatile oil did not contain any aliphatic or aromatic constituent. Gurudutt et al. (1996) reported 12 sesquiterpenes and 26 monoterpenes from the volatile oil of *A.subulatum* of which allo-aromadendrene was the major constituent. The chemical composition of the oil from the Nigerian cardamom [*Aframomum daniellii* (Hook.f.) K.Schum.] was very similar to the oil of Indian *A. subulatum*, particularly with respect of monoterpenes and their oxygenated derivatives [2]. The large cardamom pericarp volatile oil also contained 1, 8-cineole (38.7%) and other components detected as β-pinene, α-terpineol, spathulenol, 4-terpineol, germacrene-D, α-pinene and β-selinene [17]. In the present study only 14 monoterpenes and six sesquiterpanes were detected. The earlier reported

monoterpenes i.e α-thujene, p-cymene, α-terpinene, linalool, δ-mycenol, campholene aldehyde, norbornyl acetate, dihydrolinalool, pinocarveol, β-terpineol, δ-terpineole terpene-n-ol, α-terpineol, myrtenol, verbenone, cis-carveol, cuminaldehyde, carvone, linalyl acetate and hydroxycitronellal, and the sesquiterpenes α-copaene, β-cedrene, allo-aromodandrene, aromodendrene, α- and β-bisabolenes and nerolidole, reported by Gurudutt et al. (1996) could not be detected in the present study of the oil. The monoterpenes including sabinene, β-phellandrene, linalool oxide and 2, 3-epoxygeranyl acetate and sesquiterpenes, aromadendrene epoxide, globulol and bisabolol oxide are reported for the first time in the essential oil of *A. subulatum*. GC analysis of the volatile oils showed that there was considerable variation among the cultivars with respect to α-pinene (3.2- 4.5 %), β-pinene (6.7 – 8.5 %), 1, 8-cineole (65.39 - 84.6 %) and α-terpineol (3.3 – 10.15 %). The physical and chemical quality of the large cardamom obtained from different regions of Sikkim, Bhutan and Nepal vary significantly. GC analysis of the volatile oil of these cultivars showed that there was considerable variation with respect to α-pinene (3.2- 4.5 %), β-pinene (6.7 – 8.5 %), 1,8-cineole (80.4 - 84.6 %) and 4-terpineol (0.6 – 1.3 %) and α -terpineol (3.3 – 4.3 %). The oil was similar with respect to specific gravity and refractive index but optical rotation values were different to some extent [19]. Liquid CO_2 extracted oil contained higher levels of high-boiling oxygenated compounds such as terpineol, linalyl acetate, geranyl acetate and geraniol in comparison to lower levels of these constituents in steam distilled oil [14]. The chemical constituents α-pinene, β-pinene, 1, 8-cineole, myrcene, γ-terpinene, limonene, ρ-cymene, terpinene-4-ol, terpineol and nerolidole were reported as major components of large cardamom oil [22].

CONCLUSION:

In the present study sabinene, β-phellandrene, linalool oxide, 2,3-epoxygerenyl acetate, aromadandrene epoxide, globulol and bisabolol oxide are reported for the first time in the volatile oil of *A. subulatum*.

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