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Ameeta Tiwari
M.B (Govt) P.G College, Haldwani, Kumaun University Nainital, Nainital, Uttarakhand, India

Sushma Kholiya
M.B (Govt) P.G College, Haldwani, Kumaun University Nainital, Nainital, Uttarakhand, India

Rakesh K Joshi
Department of Education, Government of Uttarakhand, India

Corresponding Author:
Ameeta Tiwari
M.B (Govt) P.G College, Haldwani, Kumaun University Nainital, Nainital, Uttarakhand, India

Variation in volatile constituents of *Acorus calamus* L.; A potential medicinal plant of Uttarakhand

Ameeta Tiwari, Sushma Kholiya and Rakesh K Joshi

Abstract
The investigation of essential oil yield (v/w) of *Acorus calamus* L. from eight natural sites in northern India, were studied and compared using gas chromatography- flame ionization detector and gas chromatography-mass spectrometry. The essential oil yield (v/w) varied from 1.0% to 1.3%. The major constituents identified in rhizome oil were β-asarone (75.2% -92.1%), α-asarone (0.5%-5.8%), α-selinene (<0.1%-3.9%), β-caryophyllene (<0.1%-2.5%), γ-asarone (<0.1%-2.3%), (E)-methyl isoeugenol (<0.1%-2.1%) and (Z)-methyl isoeugenol (<0.1%-1.2%). The oils differed in their qualitative and quantitative make up. Asarone was the major volatile constituents of rhizome oils, beside differences in content of other volatile constituents.

Keywords: gas-chromatography (GC) and gas chromatography mass spectrometry (GC-MS), β-asarone, α-asarone, β-caryophyllene, (E)-methyl isoeugenol and (Z)-methyl isoeugenol

1. Introduction
Acorus calamus Linn. Belongs to Araceae family. This family involves few temperate species and herbaceous plant [1]. *Acorus* is an aromatic herb. The genus *Acorus* consists of 40 species. It is a tall perennial wetland monocot plant. It has branched aromatic rhizome, thickness of cylindrical up to 2.5cm and can grow up to 6 feet tall. The colour of rhizome is internally white and purplish brown to light brown from externally. Central Asia and Eastern Europe is repository of *Acorus* species [2, 3, 4]. It covers wide range in India whether it is in terms of cultivation or in plains or in wild state or in lower elevation or even in Himalayan range up to altitude of 2200m [5].
According to geographical distribution and ploidy status, *A. calamus* has been categorise into four cytotypes, they are- diploid (North America), triploid (Europe), tetraploid (East Asia, India and Japan) and hexaploid (Kashmir, India) [6, 7]. Studies have revealed that tetraploids contain highest content of β-asarone with around 70-96%, triploids (5-19%) and diploids (0-2%). The varieties with very low percentage of β-asarone were permitted to be used [8].
Acorus calamus Linn. Is commercially and commonly known as "vacha" and its rhizomes are termed as "sweet flag". In Sanskrit "vacha" means one which improves induces vomiting when taken with lukewarm salt water [9]. Sweet flag is a valuable herb used for curing various diseases of nervous system, digestive ailments such as loss of appetite, abdominal pain and worms. Ayurveda had special place for "vacha" as it is main medhya drug, which improves memory power and intellect. It has to be said that rhizomes of *Acorus* are used in almost all civilizations of world while in Asia it has been used from at; least past 2000 years for various medicinal purposes [10]. In India to improve speech development and intellect, mixture of vacha powder and ghee is given virtually. This plant is credited with various medicinal purposes. It is used as holy incense by Egyptians and Sumerians. Scented leaves of this plant are used as air-fresheners and insecticides [11]. The dried and powdered rhizome has spicy flavour and is used as substitute for nutmeg, cinnamon and ginger for its odour. It eases cough and asthma [12].
The various study on essential oil or extract of *Acorus calamus* reported that it posses various biological activities which are of medicinal use, some of them are- antihelminthic [13], antibacterial [14], antioxidant [15], anti-seizures [16], antifungal [17], antimicrobial, insecticidal [18], antidiabetic [19], immunosuppressive [20], analgesic effect [21,22] and anti-inflammatory [23].
Literature survey revealed that composition of volatile oil of rhizomes of *Acorus calamus* have been explored extensively by many researchers throughout the world. Keeping in view of the medicinal use of *A. calamus* and enormous variations in essential oil constituents, the present study was performed to study the chemical variability in the rhizome volatile constituents from eight sites.

A. calamus is collected from different locations of foot and mid hills of Kumaun region of Uttarakhand, India. The chemical variability is observed by GC-FID and GC-MS.

2. Materials and Methods

2.1 Materials: Samples of *Acorus calamus* were collected from eight natural sites- Ranikhet, Bhowali, Pantnagar, Bageshwar, Haldwani, Jageshwar, Almora and Pithoragarh of Uttarakhand, India. The plant material was authenticated at Taxonomy Department of CSIR- Central Institute of Medicinal and Aromatic Plants, Research Centre, Pantnagar.

2.2 Isolation of essential oil: Fresh rhizomes of the plant were subjected to hydro distillation in a Clevenger apparatus for 3hrs, for isolation of essential oil. Essential oil was measured directly in the extraction burette and contents (%) were calculated as volume (ml) of essential oil per 100g of fresh weight of rhizomes of plant. The crude oil was dehydrated over anhydrous sodium sulphate and kept in a cool and dark place until further analysis.

2.3 GC and GC-MS Analysis: The analysis of oil was carried out on a Nucon gas chromatograph model 5765 equipped with flame ionization detector (FID) and DB-5 (30m x 0.32mm; 0.25µm film coating) fused silica capillary column. Hydrogen was used as a carrier gas at 0.1ml min^{-1}. Temperature programming was from 60 to 230ºC at 3ºCmin^{-1} with final hold time of 10min. Injector and detector temperatures were 220ºC and 230ºC, respectively. Injection volumes of the oils were 0.02µL neat with a split ratio of 1:40. The percentages of the individual constituents were calculated by electronic integration of the FID peak areas without response factor correction. GC-MS analyses were carried on PerkinElmer turbomass quadrupole mass spectrometer fitted with Equity-5 fused silica capillary column (60m x 0.32mm i.d., film thickness 0.25µm). The oven column temperature ranged from 70 to 250ºC; programmed at 3ºCmin^{-1} with initial and final hold time 2min, using He as carrier gas at 1.0mL min^{-1}. The injection volume was 0.02µL near with split ratio 1:30. The injector source and transfer line temperatures were 250ºC; mode of ionization was electron impact ionization (EI), with ionization energy 70eV and mass scan range of 40-400 amu.

2.4 Identification of Essential oil constituents: Identification of essential oil constituents was accomplished on the basis of retention index (RI), reference mass spectral library search (NIST version 2.1) and Wiley registry of mass spectral data and by comparing the mass spectral data with given literature (Adams, 2007). β-asarone is found as major constituent of essential oil of rhizome of *A. calamus.*

3. Results and Discussions

The essential oil of the fresh rhizomes of *Acorus calamus*, isolated by hydro distillation method, was analyzed by gas chromatography (GC-FID) and GC-mass spectrometry (GC/MS) techniques. The identified constituents with their respective percentage in the volatile oil from *Acorus calamus* have been listed in *table-1* as per increasing order of altitudes. The essential oil yield (v/w) varied from 1.0% to 1.3%. The major constituents identified in rhizome oil were β-asarone (75.2%-92.1%), α-asarone (0.5%-5.8%), α-selinene (<0.1%-3.9%), β-caryophyllene (<0.1%-2.5%), γ-asarone (<0.1%-2.3%), (E)-methyl isoeugenol (<0.1%-2.1%) and (Z)-methyl isoeugenol (<0.1%-1.2%). The oils differed in their qualitative and quantitative make up. Asarone was the major volatile constituents of rhizome oils beside differences in content of other volatile constituents.

Among the volatile constituent of rhizome of essential of *A. calamus*, the most interesting constituents is β-asarone, it is reported to be toxic due to its carcinogenic effect. The previous study reported that major constituents of essential oil of rhizome of *A. calamus* are β-asarone, α-asarone, elemicine, cis-isoelemicine, cis & trans isoeugenol & their methyl ethers, camphene, p-cymene, β-gurjunene, α-selinene, β-cadinene, camphor, terpinen-4-ol, α-terpineol, α-culacorene, acorne, acorenone, acoragermacrone, 2-deca-4,7-dienol, shyobunones, isoshyobunones, calamusenone, linalool and pre-isocalamendiol. {Kumar A & Vandan}

Some other compound identified in *A. calamus* are (-)-4-Terpineol, 2-Allyl-5-ethoxy-4-methoxyphenol, Epieudesmin, Lysidine, (-)-Spathulenol, Borneol, Furylethyl ketone, Nonanoic Acid, 2,2,5,5-Tetramethyl-3-hexanol, Bornyl acetate, Galgravin, Retsuin, (9E,12E,15E)-9,12,15-Octadecatrien-1-ol, ButylButanoate, Geranylacetate, Sakuranin, Acetic Acid, Camphor, Isoelemicin, α-Ursolic acid, Acetophenone, Dehydroabietic acid, Isoeugenol Methylether, Apigenin 4',7-dimethyl ether, Dehydrodiisoeugenol, Linalool, Elemicin, Linolenic acid. {Balakhumban R *et al.*}

The structure of major constituents is shown in figure 1.

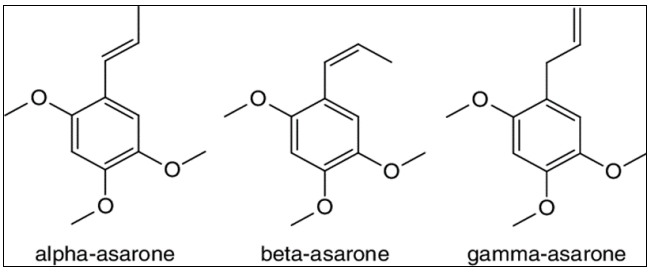

Fig 1: Chemical structure of asarone isomers

Table 1: Variability in content of volatile constituents of *Acorus calamus* L. from different locations of Kumaun region of Uttarakhand, India

Compounds	Content (%)							
	I	II	III	IV	V	VI	VII	VIII
α-Pinene	t	t	-	t	t	0.2	t	0.1
Camphene	0.2	t	t	t	0.3	t	t	t
β- Pinene	0.3	-	t	-	0.1	-	0.1	t
p- Cymene	0.7	1.3	0.7	t	0.5	t	1.0	0.4
β- Phellandrene	0.8	-	t	t	0.2	-	t	t
1,8-Cineole	1.0	-	-	t	t	t	0.1	t
Linalool	0.5	t	0.4	0.1	-	0.1	-	-
Geraniol	t	t	t	t	t	0.5	t	0.2
Bornyl acetate	0.8	0.3	0.1	0.8	0.1	-	0.5	-
α-Camphenol	t	t	t	t	t	t	t	t

α-Copaene	0.9	-	-	t	t	t	0.9	0.3
Geranyl acetate	t	t	t	-	-	0.2	0.2	t
Methyl eugenol	0.1	0.6	-	-	0.2	t	t	-
β-caryophyllene	0.1	2.5	0.1	0.3	1.0	t	0.3	0.2
(Z)-Methyl isoeugenol	0.8	1.0	0.9	0.6	0.1	1.2	0.3	t
Dehydroaromadendrane	-	0.6	t	t	0.5	t	t	-
(E)-Methyl isoeugenol	0.8	0.2	1.3	0.5	t	0.5	2.1	t
α- Selinene	1.3	2.5	t	0.6	3.9	1.2	0.8	t
Germacrene D-4-ol	0.1	0.5	t	t	2.3	t	t	t
Spathulenol	0.1	0.6	0.1	t	0.5	-	t	t
Caryophyllene oxide	t	1.5	t	t	3.0	0.5	0.1	t
γ-asarone	0.9	0.4	0.3	0.9	1.0	2.3	1.0	t
β-asarone	80.9	78.9	90.1	92.3	75.2	88.8	88.6	89.6
α-asarone	2.8	5.8	2.3	0.5	5.3	2.9	0.9	1.2
Total	93.1	96.7	96.3	96.7	94.4	98.4	96.9	92.1

*t = trace (<0.1%); (-) = absent.
I- Pantnagar, II- Haldwani, III- Bageshwar, IV- Almora, V- Pithoragarh, VI- Bhowali, VII- Ranikhet and VIII- Jageshwar.

The altitudinal variation wise bar graph representation of major component β-asarone along with its two isomer α-asarone and γ-asarone is represented in figure2. This bar graph clearly visualises that the variation in % composition of major composition does not vary linearly with altitude.

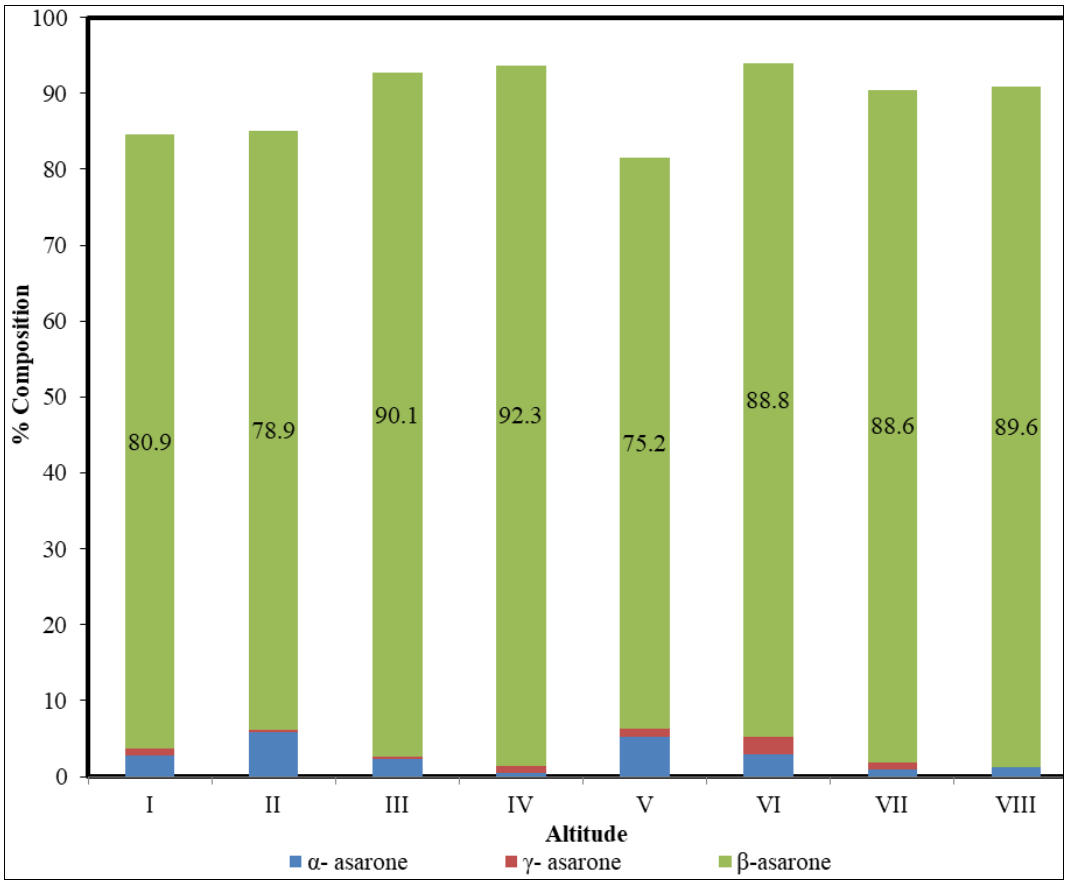

Fig 2: Altitudinal variation of major components of rhizome oil of *Acorus Calamus* L.

4. Conclusions

This report involves the altitudinal variation of essential oil constituents of rhizome of *Acorus calamus* L. from eight different sites of Kumaun and Garhwal region of Uttarakhand. It can be concluded from the bar graph that lowest and highest altitude may not vary gradually along major % composition. The highest % (92.3%) of major compound β- asarone was found in Almora and lowest % (75.2%) in Pithoragarh. Therefore it may be concluded that rhizome oil of *Acorus Calamus* from Pithoragarh shows least toxicity as it contains lowest % of β-asarone. Hence rhizome oil of *Acorus Calamus* from Pithoragarh can be used for industrial purpose.

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