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ESSENTIAL OIL COMPOSITION OF *ACORUS CALAMUS* FROM DISTRICT-PITHORAGARH, UTTARAKHAD, INDIA

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ABSTRACT
Many ethnomedicinal and ethnobotanical uses of the plant *A. calamus* has been used as traditional Chinese and Indian prescriptions for its beneficial effects on memory disorder, learning performance, lipid peroxide content, and anti-aging and anticholinergic activity. The essential oil of leaves of *Acorus calamus*, were extracted by steam distillation. The quantitative and qualitative analysis of volatile essential oil constituents of the plant was done by Gas Chromatography (GC) and GC -Mass Spectrometry. A total of 61 components of the essential oil of *Acorus calamus*, were identified accounting for 71.08 % of the total oil. The main compounds found were α-Asarone 16.54%, (E)- Methylisoeugenol 5.06 %, γ-Cadinene 3.00 %, α-pinene 2.96 % and Citronellal 2.82 %.

KEYWORDS: Essential oil, GCMS, α-Asarone and (E)- Methyl isoeugenol, Mass Spectrometry.

INRTODUCTION
Acorus calamus Linn. commonly known as Sweet Flag, belongs to the family Araceae (Adoraceae). It is also called as *Acorus odoratus.* The genus *Acorus* derived from *Acoron* (coreon = the pupil of the eye) and the species *calamus* is derived from the Greek word Calamos (a reed). The family Araceae comprises about 110 genera and more than 1,800 species. The members of the family are rhizomatous or tuberous herbs. *Acorus calamus* Linn. commercially occurs in both peeled and unpeeled forms.This perennial herb is common on the banks of streams and in damp marshy places.In Ayurvedic medicine *Calamus* is an

important herb, and is valued as a "rejuvenator" for the brain and nervous system, and as a remedy for digestive disorders.The plant is known by different names indifferent names in India and abroad (Divya G et al.,2011) viz;

Vernacular names

Bengali: Bach;

Gujarat: Vekhand

Ayurvedic: Vacha

Unani: Bacch

Hindi: Bajai

Gora-bach, Vasa bach

Marathi: Vekhand

Tamil: Vasambu

Telugu: Vadaja, Vasa

Kannada: Baje

Malayalam: Vayambu

Sanskrit: Bhutanashini, Ugragandh, Jatila

Italy: Plant of Venus.

A. calamus (Sweet flag) is a wetland perennial monocot plant, whose scented leaves and rhizomes have been traditionally used medicinally against different ailments like, fever, asthma, bronchitis, cough and mainly for digestive problems such as gas, bloating, colic, and poor digestive function, and also used as a sedative, nerve tonic, antimicrobial agent, and expectorant (Dong el. al. 2010). The ethanolic extracts of *A. calamus* leaves have wound-healing activity (Jain el. al. 2010).

Acorus calamus L. has had a long and rich history of ethnobotanical application by many different cultures in many countries (Motley, 1994). *A. clamaus* essential oil from different parts extensively investigated for its chemical compositions by various workers (Namba, 1993; Wang, 1998; Raina *et. al.* 2003 and Venskutonis & Dagityte, 2003).

A. calamus has been used medicinally for a wide variety of ailments, and its aroma makes calamus essential oil valued in the perfume industry. The essence from the rhizome is used as a flavor for pipe tobacco. *Acorus calamus* has a very long history of medicinal use in Chinese and Indian herbal traditions. The leaves, stems, and roots are used in various Siddha and Ayurvedic medicines. It is widely employed in modern herbal medicine as its sedative, laxative, diuretic, and carminative properties (Mukherjee el. al. 2007). In uttarkhand *Acorus calamus* used in the formulation of Aruvedic dugs by many pharmaceutical companies so this work is beneficial for researcher and pharmaceutical company.

EXPERIMENTAL

Plant Material

The plant *Acorus calamus* was collected in the month of September, 2013 from Bishar (Pithoragarh) 10 km away from Pithoragarh Discrict, Uttarakhand, India. The plant was authenticated by Botanical Survey of India (BSI), Dehradun. A voucher specimen (No.114852) was deposited in the Herbarium Section at BSI, Dehradun, India.

ESSENTIAL OIL EXTRACTION

The fresh aerial parts of *Acorus calamus* (5 kg) were chopped and steam- distilled using copper still fitted with spiral glass condensers. The distillate was saturated with NaCl and extracted with n-hexane. Anhydrous Na_2SO_4 was then added to dry the organic phase which was separate dusing separating funnel and finally the solvent was evaporated under reduced pressure. The percentage content of the oil was calculated on the basis of dry weight of plant material. The oil was then stored in screw-capped vials,under refrigeration until needed.

GAS CHROMATOGRAPHIC ANALYSIS (GC)

The oil was analysed by using a Shimadzu 2010 auto system GC. The column temperature was programmed at 80°C (holding time for 2 minute) to 210°C (holding time 5 minute) at 3°C min^{-1} and then 210°C - 300°C at 20°C min^{-1} with final hold time of 15 minute, using N_2 at 30.0 mL/min column head pressure as carrier gas, the injector temperature was 270°C and detector (FID, Flame ionization detector) temperature 280°C

GC- M S Analysis and Identification

The GC-MS used was Autosystem 2010 GC (Rtx- 5, 30m x 0.25mm, i.d. FID 0.25µm) coupled with Shimadzu QP 2010 plus with thermal desorption system TD 20 with (Rtx-5) fused silica capillary column (30 m x 0.25mm with film thickness 0.25µm). The column temperature was 80°C (holding time for 2 minute) to 210 °C (holding time 5 minute) at 3°C min^{-1} and then 210°C - 300°C at 20°C min^{-1} with final hold time of 21 minute, using helium as carrier gas. The injector temperature was 230°C and 0.2 µL in n-hexane, with split ratio of 1:30 MS were taken at 70 eV with a mass range of 40- 650 amu.

Identification of constituents were done on the basis of Retention Index (RI, determined with reference to homologous series of n-alkanes C8-C28, under identical experimental condition), MS library search (NIST and WILEY), and by comparison with MS literature data (Adams R.P. 2007). The relative amounts of individual components were calculated based on GC peak area (FID response) without using correction factor. Retention indices (RI) were determined with reference to a homologous series of normal alkanes, by using the following formula (Kovats, 1958).

$$KI = 100\left[n + (N-n) \times \frac{\log t^1_R(\text{unknown}) - \log t^1_R(C_n)}{\log t^1_R(C_N) - \log t^1_R(C_n)}\right]$$

t^1_R – the net retention time ($t_R - t_0$)

t_0 – the retention time of solvent (dead time)

t_R – the retention time of the compound.

C_N – number of carbons in longer chain of alkane

C_n– number of carbons in shorter chain of alkane

n - is the number of carbon atoms in the smaller alkane

N - is the number of carbon atoms in the larger alkane

RESULTS AND DISCUSSION

The yield of *Acorus calamus* leaves essential oil was 0.14 % (V/W). The GC and GC-MS analysis of leaf oil of *Acorus calamus* resulted in the identification of 61 constituents. The identified constituents of the oil are listed in Table in order of their elution in Rtx-5 column. The major compounds found were α-Asarone 16.54%, (E)- Methylisoeugenol 5.06 %, γ-Cadinene 3.00 %, α-pinene 2.96 % and Citronellal 2.82 %. The miner compounds were Acoradiene 0.10 %, Tridecanol 0.10 %, 2-(acetylmethyl)- (+)-3-Carene 0.12 %, 7-Hexadecyne 0.13 % and Rosoxide 0.14 %.

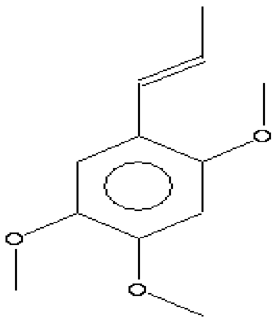

Fig. 1. α-Asarone

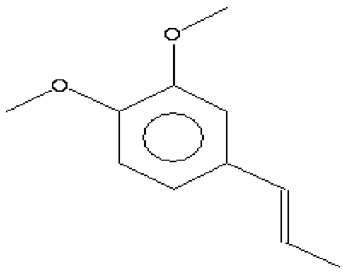

Fig. 2. Methylisoeugenol

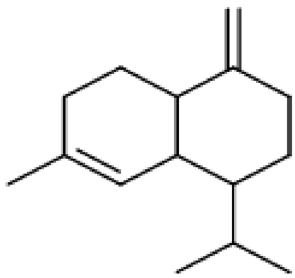

Fig. 3. γ-Cadinene

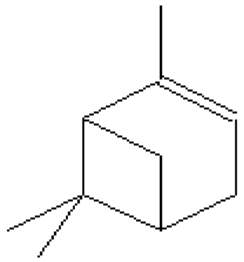

Fig. 4. α-pinene

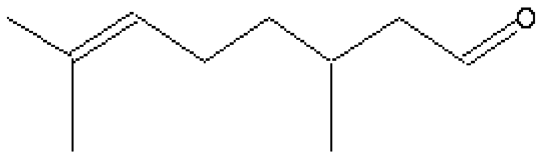

Fig. 5. Citronellal

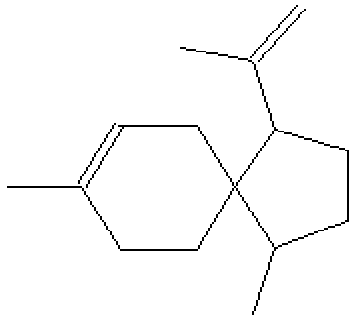

Fig. 6. Acoradiene

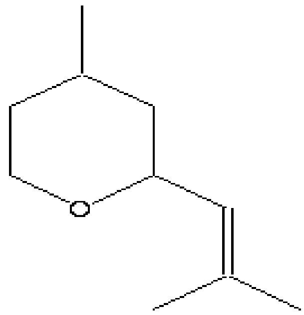

Fig. 7. Rosoxide

Table- 1 Main constituent compounds isolated from the essential oil of *A. calamus* leaves.

S. No.	Compound	(%)	Mol. formula	Mol. wt.	RI	Mode of I
1.	α-pinene	2.96	$C_{10}H_{16}$	136	937	a,b
2.	Camphene	0.71	$C_{10}H_{16}$	114	957	a,b
3.	Myrcene	0.43	$C_{10}H_{16}$	136	991	a,b
4.	α-Limonene	0.44	$C_{10}H_{16}$	136	1018	a,b
5.	o-Cymene	0.76	$C_{10}H_{14}$	134	1026	a,b
6.	Limonene	0.31	$C_{10}\ H_{16}$	136	1030	a,b
7.	1,8 -Cineol	0.16	$C_{22}H_{42}O_2$	338	1032	a,b
8.	Linalool	1.27	$C_{10}H_{18}O$	154	1097	a,b
9.	Rosoxide	0.14	$C_{10}H_{18}O$	154	1114	a,b
10.	Nona-(2E,6E)-dienal	0.96	$C_9\ H_{14}O$	138	1152	a,b
11.	Citronellal	2.82	$C_9H_{18}O$	142	1153	a,b
12.	α-Terpineol	1.16	$C_{10}H_{18}O$	154	1155	a,b
13.	(iso)-Borneol	0.42	$C_{10}H_{18}O$	154	1169	a,b
14.	beta.-Citronellol	0.21	$C_{10}H_{20}O$	156	1179	a,b
15.	(2Z)-2-Decenal	0.51	$C_{10}H_{18}O$	154	1212	a,b
16.	Neral	2.57	$C_{10}H_{16}O$	154	1238	a,b
17.	Pulegone	0.66	$C_{10}H_{16}O$	152	1241	a,b
18.	4-acetyl 2-Carene	0.03	$C_{12}H_{18}O$	178	1244	a,b

19.	Nerol	0.69	$C_{10}H_{18}O$	154	1254	a,b
20.	Geraniol	1.18	$C_{10}H_{18}O$	166	1256	a,b
21.	Dec-(2E)-enal	0.29	$C_{10}H_{18}O$	154	1265	a,b
22.	2-Decen-1-ol	0.52	$C_{10}H_{20}O$	156	1266	a,b
23.	Linalool, formate	2.28	$C_{11}H_{18}O_2$	182	1270	a,b
24.	(2E)-2-Methyl-2-octenedia	0.52	$C_9H_{14}O_2$	154	1278	a,b
25.	Bornyl acetate	0.15	$C_{12}H_{20}O_2$	172	1288	a,b
26.	2,4-Undecadienal	1.02	$C_{11}H_{18}O$	166	1319	a,b
27.	Farnesan	0.35	$C_{15}H_{32}$	212	1320	a,b
28.	2-(acetylmethyl)- (+)-3-Carene	0.12	$C_{13}H_{20}O$	192	1344	a,b
29.	α-Duprezianene	0.51	$C_{15}H_{24}$	204	1385	a,b
30.	E-Longipinane	1.28	$C_{15}H_{26}$	206	1393	a,b
31.	γ-Cadinene	3.0	$C_{15}H_{24}$	204	1418	a,b
32.	Trans-Farnesene	1.97	$C_{15}H_{24}$	204	1457	a,b
33.	α.-Farnesene	0.34	$C_{15}H_{24}$	204	1458	a,b
34.	α.-Farnesene	0.17	$C_{15}H_{24}$	204	1458	a,b
35.	Acoradiene	0.10	$C_{15}H_{24}$	202	1462	a,b
36.	Germacrene-D	0.49	$C_{15}H_{24}$	226	1479	a,b
37.	(E)- Methylisoeugenol	5.06	$C_{11}H_{14}O_2$	178	1500	a,b
38.	Tridecanal	1.66	$C_{13}H_{26}O$	198	1502	a,b
39.	Cuparene	1.21	$C_{15}H_{22}$	202	1504	a,b
40.	Pentadecane	0.64	$C_{15}H_{32}$	212	1512	a,b
41.	Humulene epoxide II	1.64	$C_{15}H_{24}O$	206	1522	a,b
42.	Ledol	0.37	$C_{15}H_{26}O$	222	1530	a,b
43.	3,7,11-trimethyl-1-Dodecanol	0.17	$C_{15}H_{32}O$	228	1563	a,b
44.	Tridecanol	0.10	$C_{13}H_{28}O$	200	1580	a,b
45.	Caryophyllene oxide	0.40	$C_{15}H_{24}O$	220	1587	a,b
46.	13-Tetradecenal	1.41	$C_{14}H_{26}O$	210	1591	a,b
47.	β-Asarone	2.22	$C_{12}H_{16}O_3$	208	1628	a,b
48.	7-Hexadecyne	0.13	$C_{16}H_{30}$	222	1629	a,b
49.	α-Asarone	16.54	$C_{12}H_{16}O_3$	208	1678	a,b
50.	Heptadecane	1.29	$C_{17}H_{36}$	240	1700	a,b
51.	cis Lanceol,	1.76	$C_{15}H_{24}0$	220	1737	a,b
52.	Acorone	0.54	$C_{15}H_{24}O_2$	236	1775	a,b
53.	Palmitaldehyde	0.27	$C_{16}H_{32}O$	240	1800	a,b
54.	Pentadecanoic acid	0.23	$C_{15}H_{30}O_2$	242	1869	a,b
55.	Methyl hexadecanoate	0.24	$C_{17}H_{34}O_2$	270	1878	a,b
56.	Farnesyl acetone	0.46	$C_{18}H_{30}O$	262	1902	a,b
57.	Palmitic acid	2.29	$C_{16}H_{32}O_2$	256	1968	a,b
58.	Eicosane	0.69	$C_{20}H_{42}$	282	2009	a,b
59.	2-methyl-Eicosane	0.66	$C_{21}H_{44}$	296	2045	a,b
60.	Tetracosane	1.19	$C_{24}H_{50}$	338	2400	a,b
61.	Butyl octyl phthalate	0.41	$C_{20}H_{30}O_4$	334	2434	a,b
	Total Identified	71.08				

a=Retention Index (RI), b=MS (GC-MS)

Essential oils are valuable natural products, which are used as raw materials in many fields including perfumes, cosmetics, aromatherapy, phytotherapy, spices and nutrition (Buchbauer, 2000). This has recently attracted the attention of many scientists and encouraged them to screen plants to study the biological activities of their oils from chemical and pharmacological investigations to therapeutic aspects. Hopefully, this will lead to new information on plant applications and new perspective on the potential use of these natural products.

The plant has a characteristic essential oil called as the asarone oil. The composition of each chemical compound in the oil varies according to the ploidy level of the plant. It is believed that tetraploid plant has the highest beta asarone content [90-96%], triploid contains a small portion and diploid lacks it (Bertea *et. al.* 2005). Individual plants also show variation in the percentage of chemical components depending on the part of the plant from which the oil was extracted (Van Lier *et. al.* 1986). The results are quite different from the previous reports. For example, preisocalamenediol (18.0%), acorenone (14.2%), shyobunone (13.3%) and cryptoacorone (7.5%) were major compounds of the essential oil of *A. calamus* collected from Quebec, Canada (Garneau *el. al.* 2008). Preisocalamendiol (17.3%), isoshyobunone (13.0%), 1,4-(*trans*)-1,7- (*trans*)-acorenone (10.5%), camphor (5.9%) 2,6-diepishyobunone (2.6%) and β-gurjunene (2.5%) were the main components of the essential oil of *A. calamus* roots obtained from Turkey (Ozcan et. al. 2002). However, the essential oil of *A. calamus* rhizomes collected from Italy contained acorenone (21.6%), (*Z*)-sesquilavandulol (13.0%), shyobunone (7.0%), α-asarone (5.1%), and dehydroxyisocalamendiol (3.5%) (Marongiu *et. al.* 2005). However, these differences of chemical composition of the essential oils might have been due to harvest time and local, climatic and seasonal factors as well as storage duration of medicinal herbs. For practical use, it is necessary to standardize the essential oil of the Uttarakand *A. calamus*.

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