KATHMANDU UNIVERSITY JOURNAL OF SCIENCE, ENGINEERING AND TECHNOLOGY
VOL. 5, No. II, SEPTEMBER, 2009, pp 51- 65.

VOLATILE ORGANIC COMPOUNDS OF MEDICINAL VALUES FROM NEPALESE *Acorus calamus* L.

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Received 13 May, 2009; Revised 26 July, 2009

ABSTRACT
The volatile organic compounds (VOC's) of rhizomes of Nepalese *Acorus calamus* L were isolated by simultaneous distillation–extraction (SDE) technique and then analyzed by gas chromatography–mass spectrometry (GC-MS). A total of fifty three VOC's so far belonging to chemical classes of alcohol (11), aldehyde (14), ester (3), furan (1), hydrocarbon (19), ketone (4), N-containing, miscellaneous (1) were identified. The yield of volatile compounds obtained from rhizomes of *A. calamus* was 7493.59 mg/kg. Several bioactive compounds; β-asarone 46.78%), [*E,Z*]-2,4-decadienal (14.15%), linalool (0.41), farnesol (11.09%), methyleugenol (6.10%), α- and β-pinene (both 0.06%), [E]-caryophyllene (0.11%), β-elemene (0.39%), ocimene(0.7%), aromadendrene (0.26%), camphor (0.03%), were detected and identified.

Key Words: *Acorus calamus* L*,* rhizome, volatile organic compounds, β-asarone

INTRODUCTION

Essential oils are hydrophobic liquid containing complex mixtures of volatile organic compounds (VOC's). These oils are the end product of secondary metabolism, and most of their components are terpenoids, generally monoterpenes and sesquiterpenes, as well as sometime diterpenes and aromatic compounds derivatives. Oils with standarized content of components have to contain certain chemicals which determine the therapeutic quality. The molecular structures of essential oils are extremely small allowing absorption into different parts of body. Due to the lipophilic nature of compounds, the essential oils are readily cross cell membranes and are therefore absorbed through the skin and the lung (Römmelt, 1988). Single oil possess wide variety of actions including antiseptic, diuretic, antimicrobial, sedative, antiphlogistic, analgesic, anti-inflammatory and anti-tumor properties (Singh, 2003; Alexander, 2001; Singh, 2003). Each component of the essential oils contributes to the beneficial or adverse effects of these oils because the component of each essential oil has different properties and bioavailabilities (Buchbauer, 2000). When diffused molecules of volatile oils

come in contact with sensory buds of nasal mucosa, energy transfer takes place, which in turn gives rise to electrical impulses and give odor of sensation to hypothalamus, from where they enter the bloodstream (Lawrence, 1994). Beside the pharmaceutical uses of essential oils, they are also used in air fresheners, candles, cosmetic, industrial cleaners, masking agents, soaps and detergents, confectioneries, sauces, beverages, and dairy products.

Rhizomes of *A. calamus* are the useful parts. Traditional use of rhizomes is for a number of medicinal reasons including: as an analgesic for the relief of toothache or headache, for oral hygiene to cleanse and disinfect the teeth, to relief the effects of exhaustion or fatigue, and to help cure/prevent a hangover. Other Native tribes used it to treat a cough, made a decoction as a carminative and as an infusion for colic (Baral and Kurmi, 2006). The ancient Chinese used it to lessen swelling and for constipation. Ayurvedic medicinal practice has used to cure fevers, for asthma and bronchitis, and as an all around sedative. The rhizome was also used by the ancient Greeks and included in the traditional remedies of many other European cultures. The unpeeled, dried rhizome was listed in the U.S. Pharmacopoeia until 1916 and in the National Formulary until 1950, for medicinal use on humans. In previous studies, the essential oils of *A. calamus* from different countries has been reported (Vashist and Handa, 1964; Keller and Stahl, 1983; Rsst and Bos, 1979, Mazza, 1984) but there is no any report on Nepalese *A calamus* found in literature. Therefore, this study aimed to evaluate the therapeutic quality of Nepalese *A. calamus* oil.

MATERIALS AND METHODS

Materials

Acorus calamus L

Sample of *Acorus calamus* L was collected from Godawari area (altitude of 2051 m.), Kathmandu, Nepal. Samples were dried at room temperature, packed on vacuum free condition by removing air from the package and stored at -18 ^{0}C before the experiment.

Reagents

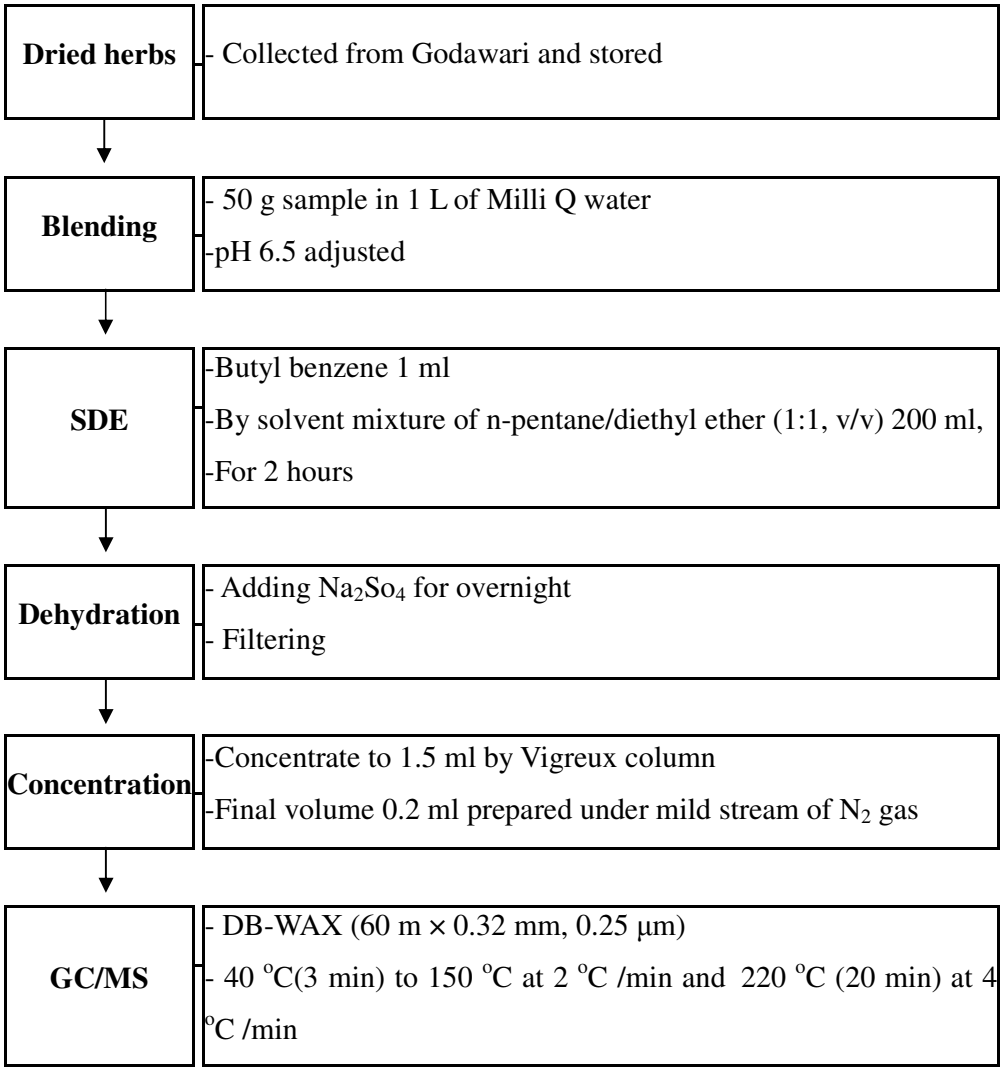
The regents used in experiments were purchased from Sigma Co. (USA) and Fisher Scientific (USA). HPLC grade organic solvents (*n*-pentane & diethylether), used for extraction and chromatography, were redistilled using a spiral packed double distilling apparatus (Normschliff Geratebau, Wertheim, Germany) and Milli-Q water that was generated with a water purification system (Millipore Corporation, Bedford, USA).

Methods

Extraction of volatile organic compounds

Fifty grams of sample were homogenized in a blender (MR 350CA, Braun, Spain) and mixed with 1L of distilled water. After adjusting the pH at 6.5 with 1% NaOH, 1 μl *n*-butylbenzene was added as an internal standard. The resultant slurry

was used for extraction of VOCs with 200 ml redistilled *n*-pentane:diethylether (1:1, v/v). The extraction of volatile organic compound was carried out for 2 h, using simultaneous distillation-extraction (SDE) apparatus of Nikerson and Likens (1966) type as modified under atmospheric pressure by Schultz *et al.* (1977). The solvent, containing compound extract, was dehydrated for 12 h using 10 g anhydrous Na_2SO_4 and then concentrated to approximately 1.5 ml using the vigreux column. This extract was further concentrated to 0.5 ml under gentle stream of N_2 gas and used for gas chromatography-mass spectrometry (GC-MS) analysis.



Scheme 1 . Scheme for analysis of volatile organic compounds of *A. calamus*

Chromatographic analysis

Chromatographic analysis was carried out using a Shimadzu GC-MS (Model QP-5000, Shimadzu Co., Kyoto, Japan) in EI (electron impact) mode. The ionization voltage was 70eV and temperatures of ion source and injector were 230^{o}C and 250^{o}C respectively. The capillary column used was a DB-WAX (60m × 0.2mm, i.d., and 0.25 μm, film thickness; J & W, USA). The oven temperature programmed at 40^{o}C (Isothermal for 3 minutes) was ramped to 150^{o}C at 2^{o}C/min and to 220^{o}C at 4^{o}C/min (Isothermal for 20 minutes) followed by 230^{o}C at 5^{o}C/min. Helium was used as the carrier gas at a flow rate of 1ml/min, with injector volume of 1μl using 1:20 split ratio. The standard value of retention index (RI) was determined by two different mixture of *n*-alkane, mixture I (C_7 ~ C_{17}) and mixture II (C_{13} ~ C_{23}) considering as standard (Fig. 1) . 1 µL mixture of alkane was analyzed to find out the retention time (RT) of standards by GC-FID. RI and RT of each peak of *n*-alkane confirmed at GC chromatogram.

Table 1. GC conditions for identification of VOCs of *A. calamus* L

GC	Hewlett-Packard 5890 series II Plus
Column	DB-Wax (60 m × 0.25 mm I.D., 0.25 µm film thickness, J&W, USA)
Detector	FID
Carrier gas	He (1.0 ml/min)
Make up gas	N_2 (20 ml/min)
Temp. program	40^{o}C (3 min), to 2^{o}C /min-150^{o}C, to 4^{o}C /min-220^{o}C (20 min)
Detector temp.	300^{o}C
Injector temp.	250^{o}C
Injection volume	1 μl

Table 2. GC/MS conditions for identification of VOCs of *A. calamus* L

GC/MS	Shimadzu GC/MS QP-5000
Column	DB-Wax (60 m × 0.25 mm id, 0.25 µm film thickness, J&W, USA)
Carrier gas	Helium (1.0 ml/min)
Tempterature program	40 ^{o}C (3 min), to 2 ^{o}C /min-150 ^{o}C, to 4 ^{o}C /min-220 ^{o}C (20 min)
Injector	250 ^{o}C
Ion source temp.	230 ^{o}C
Ionization	Electron Impact (EI)
Ionization voltage	70 eV
Mass range (m/z)	40~350
Injection volume	1 μl

Identification of volatile organic compounds

Qualitative analysis of volatile compounds was carried out by identification of compounds from mass spectra with the aid of mass spectral data book (Schultes, 1986; Arvigo and Balick, 1993). The spectrum of each analyzed volatile compound agreed with that present in the mass spectrum library of WILLY 139, NIST 12 and NIST 62. The content of the volatile flavor compounds was calculated on a dry weight basis by comparing with peak area percent of the internal standard. The mass spectrometer scanned was ranged from 41 to 450 *m/z*.

The following formula was used for quantitative analysis of volatile compounds.

$$\text{Compounds Content (mg/kg)} = \frac{C \times 1000}{A \times B}$$

A : Peak area of internal standard
B : Amount of sample (g)
C : Peak area of each compounds in sample

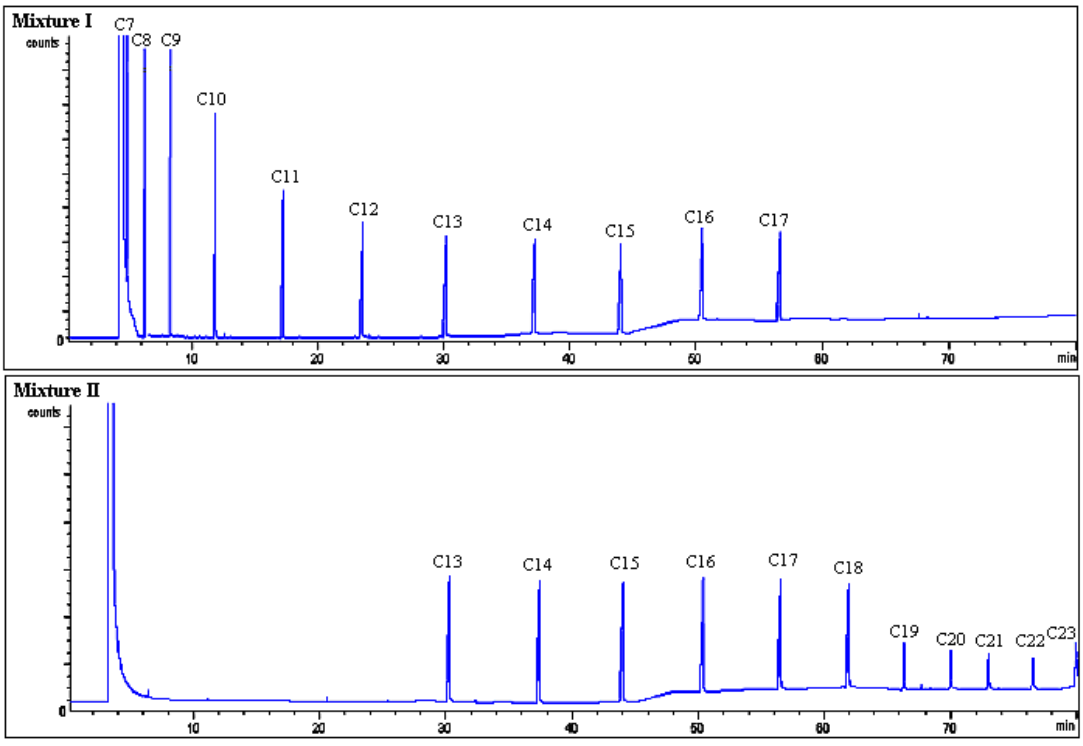


Fig. 1. GC chromatograms of *n*-alkane mixture I (C_7~C_{17}) and II (C_{13}~C_{23}).

RESULTS AND DISCUSSION

The essential oil of *A. calamus* was extracted by solvent extraction (P:E, 1:1) method for 2 h using SDE apparatus and analysed by GC/MS (Fig. 3). Investigation confirmed that the yield of essential oil obtained from Nepal originated *A. calams* rhizome was 7493.59 mg/kg. The identified VOC's are listed together according to their elution order on DB-WAX column with their amounts (Table 1). A total of fifty three VOC's so far belonging to chemical classes of alcohol (11), aldehyde (14), ester (3), furan (1), hydrocarbon (19), ketone (4), N-containing, miscellaneous (1) were tentatively identified and quantified (Table 2).

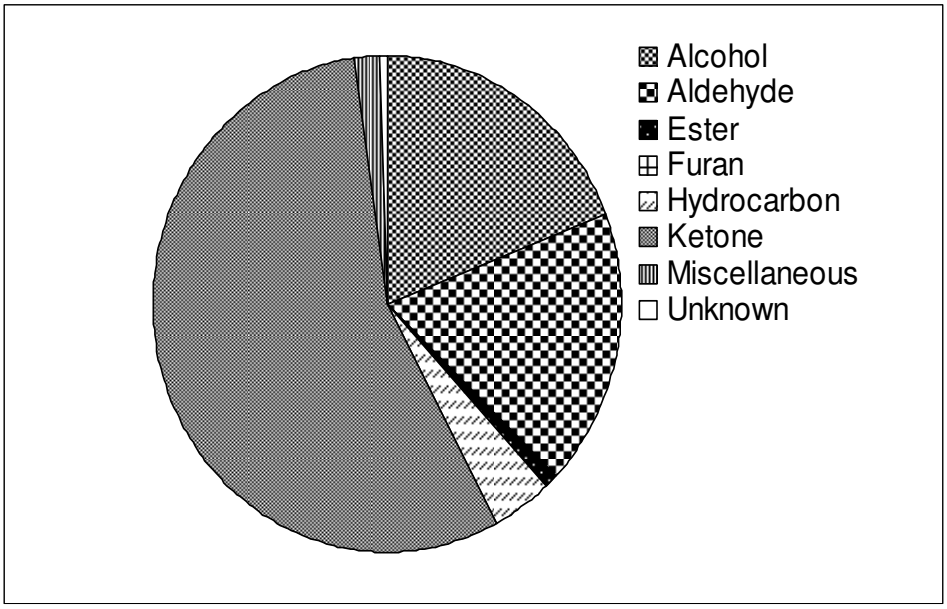


Fig. 2. Relative content of functional groups of volatile organic compounds in *A. calamus* L.

Ketones were dominant with highest proportion (55.40%). The major ketone compounds were α-asarone (8.71%) and β-asarone (46.78%). Alcohol (19.29%) group was also present in remarkable amount. Farnesol (11.09%) and methyleugenol (6.10%) were detected as the main components of alcohol group while remaining 9 alcohols were quantified at levels lower than 1%. Similarly, aldehyde was the third major group accounting 17.87%. Except myrtenal (3.07%) and [*E,Z*]-2,4-decadienal (14.15%) almost all aldehyde compounds were detected at levels lower than 0.2%. All of the compounds related to hydrocarbon group were terpene hydrocarbons. The major hydrocarbons were patchulane (0.81%), δ-cadinene (0.69%) and [*Z*]-ocimene (0.68%) while remaining 16 hydrocarbons were detected at levels lower than 0.5%. Beside these hydrocarbon terpenes some other terpenoids such as alcohol terpenoids and aldehyde terpenoids were also

detected. Oxygenated sesquiterpene, farnesol (11.09%) and α-bisabolol (0.96%) occupied the major position in terpenoids (12.05%). Similarly hydrocarbon monoterpenes accounted 0.94%, oxygenated monoterpenes accounted 3.51% and hydrocarbon sesquiterpene accounted 3.31%. This result indicates that β-asarone was the dominant compound and some major compounds ranged in content order as follows: [*E,Z*]-2,4-decadienal, farnesol, α-asarone and methyleugenol.

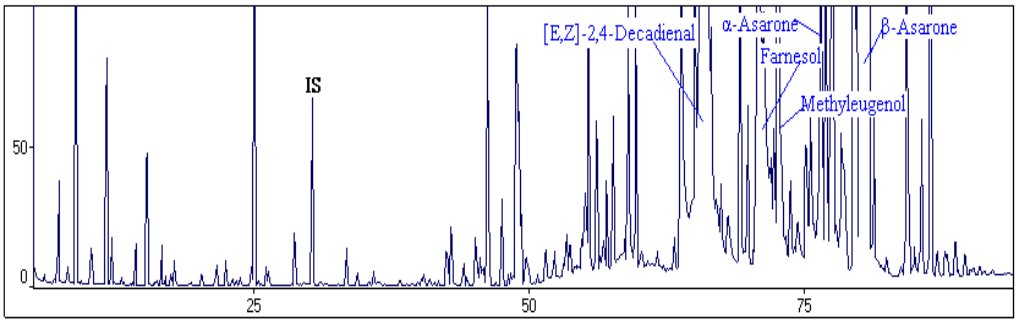


Fig. 3. GC/MS chromatogram of VOCs obtained from *A. calamus* L.

Qualitative studies of chemical constituents of essential oils provide an idea to evaluate the quality of such oils. The percentage composition of the essential oil provides probably the most important parameter for the characterization of that oil. Therefore our discussion is concentrated towards individual components and their pharmacological properties those based on previous studies. In previous investigation, Indian *A. calamus* yields oil containing 5~75% β-asarone (Vashist and Handa, 1964). Interestingly, in our sample β-asarone was detected in high amount. Keller and Stahl (1983) determined that β-asarone was absent in diploid varieties. According to Rsst and Bos (1979), β-asarone constituted 96 % in the oil of the triploid variety. European triploid type has been found to contain average 5% β-asarone (Mazza, 1984). β-Asarone is useful against insects, acting as repellent (Streloke et. al., 1989) and as sleeping time enhancer (Seto and Keup, 1969). This is also used in production of alcoholic beverages and foods at lower level. But FDA prohibited the utilization of this herb owing to the potential carcinogenic effects of its essential oil, with particular reference to β-asarone (FDA, 1974). Annex II of Directive 88/388/ ECC on flavorings fixed the maximum levels of β-asarone to 0.1 mg/kg in foodstuffs and beverages, with the exception of 1 mg/kg in alcoholic beverages and seasonings used in snack foods (ECC. 2002). Compound [*E,Z*]-2,4-decadienal, a major aldehyde compound of this oil, possess dioxygenase and fatty acid lyase activities (Andrianarison *et al.*, 1991). It strongly inhibits cell growth and affects cell viability (Nappez *et al.*, 1996).

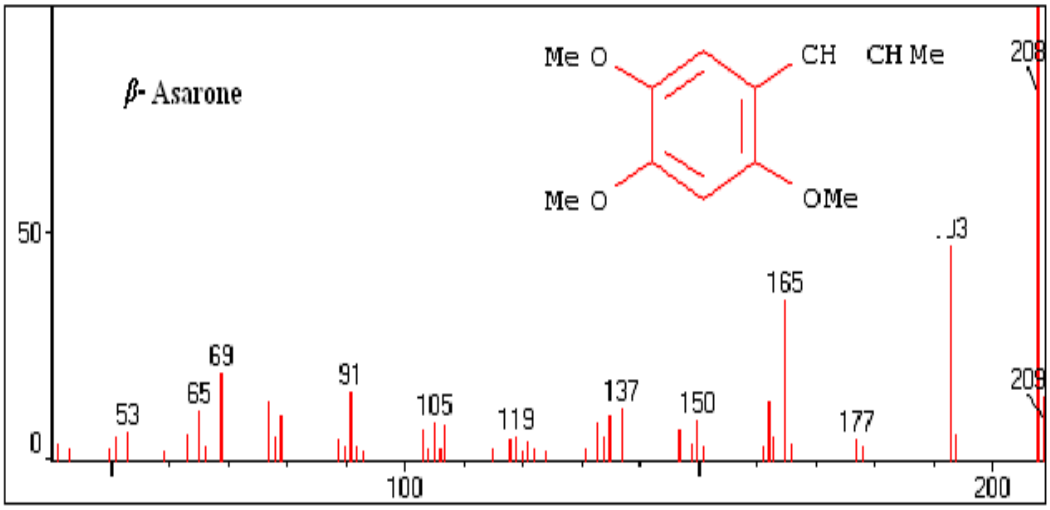


Fig. 4. Mass spectra of principal compound *β*-Asarone identified in *A. calamus* L.

Linalool is very important compound used in foodstuffs as food additives (JECFA, 1999) and pharmacology as sedative effect inducer (Sugawara *et al.*, 1998), glutamatergic neurons inhibitor (Elisabetsky et al, 1995) and also exhibits anti-inflammatory (Peana *et al.*, 2002) anticarcinogenic (Crowell *et al.*, 1992), antiseptic (Mazzanti *et al.*, 1998). However the concentration of linalool was very low in this oil. Compound farnesol was detected as a dominant alcohol compound among the 11 compounds of alcohol group. Anti-cancer effects of farnesol have been demonstrated in a number of studies that showed suppression of tumor cell proliferation (Adany *et al.*, 1994) and induction of tumor cell apoptosis in vitro (Burke *et al.*, 2002), anticarcinogenic (Rao *et al.*, 2002) and antibacterial activity (Brehm-Stecher and Johnson, 2003). Another major alcohol compound was methyleugenol, which is used as a fragrance in cosmetics, soaps and shampoos and as flavouring agent in jellies, baked goods, nonalcoholic beverages, chewing gum and icecream (Council of Europe, 2001). Many biological actions of methyleugenol have been previously reported to induce hypothermic, myorelaxant, antispasmodic, anticonvulsant and anesthetic effects (Sell and Carlini 1976; Dallmeier and Carlini, 1981; Sayyah *et al.*, 2002). Camphor is well-known chemical with its pronounced antimicrobial potentials (Pattnaik *et al.*, 1997). Similarly, *α*-pinene and its structural isomers have strong inhibition of AChE and prevent the audiogenic seizures in susceptible rats and antifungal properties (Miyazawa and Yamafuji, 2005;). Some of the important bioactive hydrocarbon compounds such as limonene, *β*-caryophyllene, β-elemene, [*E*]-*β*-ocimene, myrcene were also detected in this species. Caryophyllene have a woody spicy fragrance and exhibit an anti-cancer property (Opdyke, 1973) and also has been reported to have antiseptic (Verghese, 1994) activities. The smell of *β*-caryophyllene, is described as woody and spicy (Verghese, 1994). *β*-Elemene, has

been proved for anti-tumor activity including brain tumors (Tan *et al.*, 2001). It has also shown strong inhibition of AChE (Miyazawa and Yamafuji' 2005) and anti-inflammatory activities (Lorente *et al.*, 1989). [*E*]-β-Ocimene is a component of floral scents and has flavor and fragrance values (Knudsen *et al.*, 1993). β-Myrcene, has been used as flavoring additives in foods and beverages, as fragrances in cosmetics, and as scent in household products (Gomes-Carneiro *et al.*, 2005). The compounds [*E,Z*]-2,4-decadienal, farnesol, aromadendrene, α- and β-pinene, and [*E*]-farnesene have flavor characteristics as follows: seaweed, flower, wood, terpentine and sweet. It possesses a peculiar but pleasant, slightly sweetish and fatty odor reminiscent of stale milk.

Table 3. Relative content of functional groups of VOCs identified in *A. calamus* L

No.	Functional groups	Relative peak area (%)	Number of compounds
1	Alcohol	19.29	11
2	Aldehyde	17.87	14
3	Ester	0.77	3
4	Furan	0.15	1
5	Hydrocarbon	4.27	19
6	Ketone	55.40	4
7	Miscellaneous	1.75	1
8	Unknown	0.50	6
	Total	**100**	**59**

Table 4. Volatile organic compounds of *A. calamus* L

No.	RT[a)]	RI [b)]	Compound name	MW[c)]	Amount (mg/kg)	Content (%)
1	7.36	861	Ethyl acetate	88	8.98	0.12
2	8.17	895	3-Methylbutanal	86	1.55	0.02
3	8.89	923	Ethanol	46	42.79	0.57
4	10.29	969	2-Pentanone	86	5.58	0.08
5	11.67	1008	Methyl 2-methylbutyrate	116	19.89	0.27
6	12.15	1019	α-Pinene	136	4.62	0.06
7	14.34	1063	Camphene	136	3.61	0.05
8	15.33	1080	Hexanal	100	11.89	0.17
9	16.07	1093	Isobutanol	74	0.43	0.02
10	16.70	1104	β-Pinene	136	4.11	0.06
11	17.03	1110	3-Pentanol	88	0.89	0.02
12	17.55	1119	Sabinene	136	0.82	0.02
13	17.84	1124	2-Pentanol	88	2.31	0.03
14	20.32	1164	β-Myrcene	136	1.23	0.02
15	21.68	1184	Heptanal	114	1.90	0.03
16	22.51	1196	Limonene	136	2.35	0.03
17	23.12	1205	β-Phellandrene	136	0.48	0.02
18	23.50	1211	3-Methylbutanol	88	0.43	0.02
19	23.83	1216	2-Hexenal	98	0.84	0.02
20	24.82	1232	2-Pentylfuran	138	1.62	0.15
21	25.11	1236	[*Z*]-Ocimene	136	45.87	0.68
22	26.18	1252	[*E*]-Ocimene	136	1.58	0.02
23	26.41	1256	Pentanol	88	1.35	0.02
24	28.75	1288	Octanal	128	5.28	0.08
IS	**30.37**	**1312**	***Butylbenzene***	***134***	**-**	**0.00**
25	33.48	1359	Hexanol	102	3.87	0.05
26	35.94	1393	Nonanal	142	1.45	0.02
27	40.50	1464	Furfural	96	1.63	0.02
28	42.54	1494	α-Copaene	204	3.77	0.05
29	42.71	1497	Unknown	-	3.22	0.05
30	42.97	1500	Decanal	156	6.91	0.09
31	44.13	1519	Camphor	152	2.57	0.03
32	44.43	1524	Benzaldehyde	106	1.14	0.02
33	45.19	1536	[*Z*]-6-Nonenal	140	5.86	0.08

[a)]retention time, [b)]retention index, [c)]molecular weight

Table 4. Continued……..

No.	RT[a)]	RI [b)]	Compound name	MW[c)]	Amount (mg/kg)	Content (%)
34	45.61	1542	[*Z*]-4-Decenal	154	3.95	0.05
35	46.29	1553	Linalool	154	31.59	0.41
36	47.60	1573	Unknown	-	10.16	0.14
37	48.89	1592	*β*-Elemene	204	29.66	0.39
38	49.12	1595	Junipene	204	22.95	0.38
39	49.35	1598	[*E*]-Caryophyllene	204	8.29	0.11
40	49.78	1605	Unknown	-	4.55	0.06
41	52.36	1649	*α*-Humulene	204	3.69	0.05
42	53.47	1667	Unknown	-	4.21	0.06
43	54.82	1689	Dodecanal	184	3.40	0.05
44	55.15	1694	Unknown	-	10.01	0.14
45	55.43	1698	Geramerene B	204	31.37	0.42
46	56.20	1712	Aromadendrene	204	18.90	0.26
47	56.81	1724	Unknown	-	3.14	0.05
48	57.09	1729	[*E*]-Farnesene	204	10.92	0.15
49	57.68	1740	Geranyl acetate	196	28.09	0.38
50	59.07	1764	*δ*-Cadinene	204	52.04	0.69
51	63.92	1864	Myrtenal	152	230.92	3.07
52	65.09	1890	Patchulane	206	61.27	0.81
53	65.92	1910	[*E,Z*]-2,4-Decadienal	152	1063.93	14.15
54	70.88	2047	Farnesol	222	833.84	11.09
55	72.73	2099	Methyleugenol	178	458.33	6.10
56	76.52	2167	Elemicin	208	131.43	1.75
57	76.97	2175	*α*-Bisabolol	222	72.88	0.96
58	77.59	2186	*α*-Asarone	208	654.97	8.71
59	80.22	2252	*β*-Asarone	208	3508.28	46.78
				Total	**7493.59**	**100.00**

[a)]retention time, [b)]retention index, [c)]molecular weight

CONCLUSIONS

The gentle curative action of essential oil of *A. calamus* rhizome is due to its various constituents acting together synergistically. It would be advantageous to use one or two characteristic compounds instead of the whole oil. Moreover, though there are a number of bioactive components in the essential oil of *A. calamus*, it seems use of this oil could be riskable due to particular reference with *β*-asarone in high amount.

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