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Comparative Study of Chemical Composition and Antioxidant Activity of Essential Oil Extracted from *Acorus calamus* L. Leaves

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ABSTRACT

Background & Aim: *Acorus calamus* L. is a native herb in India. The herb belongs to family Acoraceae. It is perennial and grows in marshy land with scented rhizomes and tapered reed like leaves with 80-100 cm in height. The leaves generally arise from the rhizomes. It is also known as sweet flag in English and vernacularly as Bach. The present investigation reveals the chemical diversity and antioxidant activity of *Acorus calamus* leaf essential oils from different altitudinal regions of Uttarakhand.

Experimental: The essential oil composition of *Acorus calamus* (Araceae) leaves, collected from twenty different ecological niches of Uttarakhand, in India with oil yield ranged between 0.2-1.4 percent, were analyzed by GC/MS. DPPH assay were performed for determination of antioxidant activity of each oil.

Results: The major component ranging 43.4-60.7% of the total oil was identified as β- asarone. The other predominant constituents were α-asarone (2.6-7.9%), shyobunone (3.4-6.3%) and Z- isoelemicin (3.2-5.4%). The antioxidant activity of essential oil was studied by their ability to scavenge free radicals with different IC_{50} values (10.79-106.44 µg/ml) in comparison to standard antioxidant.

Recommended applications/industries: The vast chemical diversity of the herb essential oil and its antioxidant potential can be good natural source for herbal neutraceuticals and phenyl propanoids, the biologically important class of terpenoids.

1. Introduction

Acorus calamus L. (Araceae) is perennial, semi aquatic and aromatic medicinal herb exist in both temperate and sub temperate region with sharp pointed, long erect leaves. *Acorus calamus* is a well known herb with useful bioactive properties in indigenous medicine for centuries in India (Mehrotra *et al.*, 2003).

This plant is an evergreen herb up to 6 feet tall and is cosmopolitan in distribution ranging from Europe, East Asia, and North America. It has been reported that in America and Indonesia *Acorus calamus* is used as herbal medicines to treat gastrointestinal disorders like colic pain, diarrhea and diabetes (Gilani *et al.*, 2006; Si *et al.*, 2006).

Its uses for the treatment of fever, cough, bronchitis, inflammation, depression, tumors, hemorrhoids, numbness, skin diseases, general debility and as antidotes for several poisoning have been reported (Vaidyaratnam, 1994). Terpenoids, flavonoid and

quinines as major constituents have been reported in *A. calamus* (Patra and Mitra, 1979).

High content of β-asarone (77.7%) accompanying α-asarone (6.8%) has been reported from India, however acorenone (8.1%), β-gurjunene (6.7%), isoshyobunone (6.3%), calamendiol (5.2%) and β-asarone (5.2%) have been reported as major constituents in European collection of *Acorus calamus* (Mazza, 1985). The compounds like (Z)-asarone, (Z)-methyl isoeugenol, (E)-caryophyllene, a-humulene, germacrene, linalool, camphor and isoborneol have also been reported from in leaf essential oil of *Acorus calamus* from Lithuania (Radusiene *et al.,* 2007). The aim of this study was to ascertain the chemical diversity and antioxidant activity of essential oils of *Acorus calamus* leaves collected from different ecological niches of Uttarakhand and to look for strains for the development of good herbal antioxidant to enrich the scientific knowledge for traditional folk herbal drugs.

2. Materials and Methods

2.1. Plant material

The plants were collected from the twenty different ecological niches of Uttarakhand in India (Table 1). The herb was taxonomically authenticated by Dr. D. S. Rawat, Department of Biological Science, College of Basic Science and Humanities.

Table 1. Collection sites of plant.

S.N.	Plant Part	Natural Habitat	District	Altitude (Mtr.)
1	Leaves	Chhoi (1L)	Nainital	369
2	Leaves	Kotsari (2L)	Almora	1750
3	Leaves	Devlat(3L)	Paudi Garhwal	1570
4	Leaves	Saraikhet (4L)	Almora	1850
5	Leaves	Jaitpur (5L)	U.S. Nagar	235
6	Leaves	Paithani (6L)	Almora	1100
7	Leaves	Chaukhutiya (7L)	Almora	1040
8	Leaves	Rikherikhal (8L)	Paudi Garhwal	1460
9	Leaves	Jadaukhan (9L)	Paudi Garhwal	1740
10	Leaves	Bageshwar (10L)	Bageshwar	950
11	Leaves	Someshwar (11L)	Almora	1400
12	Leaves	Palpur (12L)	Almora	1500
13	Leaves	Naulakot (13L)	Almora	1540
14	Leaves	Walka (14L)	Champawat	1660
15	Leaves	Gumti (15L)	Almora	1550
16	Leaves	Champawat (16L)	Champawat	1670
17	Leaves	Donprewa (17L)	Nainital	345
18	Leaves	Gairshen (18L)	Chamoli	1620
19	Leaves	Deghat (19L)	Almora	1650
20	Leaves	Khedagao (20L)	Almora	1356

2.2. Isolation of essential oil

The essential oils from fresh crushed and chopped leaves were isolated by hydrodistillation (Clevenger, 1928). The hydrodistillation was continued for about 7-8 hr. The oils were extracted with DCM and desiccated over anhydrous Na_2SO_4.

2.3. GC-MS Analysis

The GC-MS analysis was carried out using GCMS-QP 2010 with mass selective detector having capillary column (DB-5, 30m × 0.25mm, 0.25µm). The column initial oven temperature was 600C and then programmed at 30C /min to final oven temperature 240oC, isothemal for 30 min .Injection temperature was 260°C. Helium was carrier gas with flow rate of 3.0 ml/min and split ratio 40:1. For mass detection electron ionisation (70 eV) technique was used. The constituents ofessential oils were identified on the basis of their mass spectra obtained then matching them with those in NIST-MS, FFNSC Wiley Library and authenticating by comparing with literature reports and GC retention indices (Adams, 2007).

2.4. Antioxidant activity

In present investigation, the in vitro antioxidant property of leaf essential oils from *Acorus calamus* was studied by DPPH radical scavenging method compared to standard antioxidant. DPPH scavenging activity was evaluated according to the method developed earlier and recently being used by (Prakash *et al.,*2011). The

assay mixture contained 5 ml of 0.004% methanol solution of DPPH and different amount of test sample solution of different concentrations. The percent DPPH free radical inhibition (IC_{50}%) was calculated by using the equation. IC_{50}%=[(control-sample)/control]×100. Standard curve was drawn by plotting percent inhibition against concentrations. Finally using this curve IC50 values for standard and essential oil were calculated. The lower IC_{50} value indicated more radical scavenging activity.

2.5. Statistical analysis

The experimental data were obtained by executing the experiments in triplicates. The data was analysed by Tukey's test in conjunction with an ANOVA (post-hoc) analysis with the help of SPSS 16 version.

3. Results and discussion

3.1. Essential oil analysis

Depending upon environmental factors, seasonal factors, growth stage, climatic conditions and locality development the yield of essential oils ranged from 0.2-1.4% (v/w) among the accessions. A total of 37 constituents, contributing 80-86.8% of the essential oils were identified. Persual of Table 2 indicates the names and respective percentage of identified constituents while figure 1 represents the major compounds.

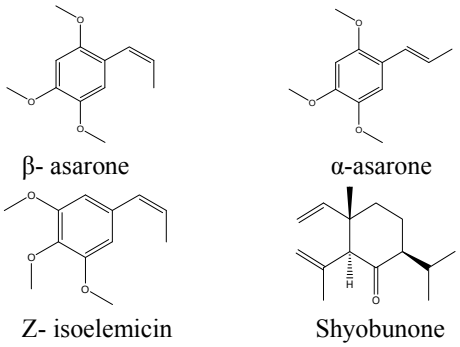

Figure 1. Structure of major chemical constituents of *Acorus calamus* leaf essential oils.

Table 2. Comparative GC/MS analysis of *A. Calamus* leaves essential oil from 20 different altitudes of Uttarakhand.

Compounds	Percent contribution in leaves																				
	MF (m/e)	1L	2L	3L	4L	5L	6L	7L	8L	9L	10L	11L	12L	13L	14L	15L	16L	17L	18L	19L	20L
limonene	68,67,93	-	-	-	-	-	-	0.4	-	-	-	0.5	-	-	-	-	-	-	0.5	-	-
linalool	71,93,55	-	-	0.4	4.4	3.2	4.8	7.1	6.6	3.0	3.4	4.3	3.7	2.6	4.9	5.0	3.0	4.8	3.2	5.8	1.2
camphor	95,81,41	-	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-
viridiflorene	107,93,119	-	1.5	-	-	-	0.7	-	0.7	-	1.3	-	-	0.3	0.3	-	0.5	0.5	-	-	0.6
bornyl acetate	95,93,43	-	-	-	-	-	-	-	-	-	-	0.2	0.1	-	-	0.7	-	-	-	0.1	-
β elemene	81,93,68	-	-	0.3	0.3	-	-	-	-	-	-	0.8	-	0.7	-	-	-	-	-	-	-
caryophyllene	93,69,133	-	-	1.1	2.8	1.8	3.2	2.1	2.6	2.4	2.1	1.8	2.2	1.8	2.0	1.7	2.0	2.5	2.6	2.7	2.7
calarene	161,105,119	-	0.2	-	-	-	-	-	-	0.1		0.2	0.2	-	-	-	-	-	0.6	0.1	-
Z-methyl isoeugenol	178,107,163	1.5		2.1	2.4	1.7	2.7	2.8	1.2	1.7	1.8	2.7	2.9	2.0	1.7	1.5	2.0	2.4	2.3	3.1	0.8
α-humulene	93,80,121	0.3	1.5	0.8	1.5	0.8	1.4	1.1	1.0	1.1	1.0	1.2	1.2	1.0	1.2	1.1	0.9	1.0	1.6	1.4	1.1
germacrene D	161,105,91	-	-	-	0.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
α-muurolene	105,161,93	-	0.5	0.3	-	0.6	0.6	-	0.3	0.3	0.3	0.4	0.4	0.3	0.3	0.2	-	0.3	-	0.5	0.3

(+)-cuparene	132,145,107	-	-	-	-	-	-	-	-	-	-	0.8	-	-	-	-	0.4	0.6	-	-	-
δ-cadinene	161,119,105	0.3	1.3	-	-	1.5	1.3	0.3	0.8	0.7	0.5	0.8	0.8	0.5	0.3	0.4	0.8	-	0.6	1.1	0.8
kessane	108,126,43	0.6	1.6	3.2	-	0.3	0.7	1.0	0.3	0.7	2.5	3.1	2.9	2.0	2.7	2.9	0.8	0.4	1.1	1.8	0.7
α-cadinene	105,161,204	-	-	-	0.7	-	0.1	-	-	-	-	0.1	0.1	-	-	-	-	-	-	-	-
α-calacorene	157,142,200	-	1.1	0.4	-	0.4	0.2	-	0.2	0.1	0.2	0.5	0.4	0.3	-	-	0.1	-	-	0.4	-
α elemol	59,93,161	-	-	0.7	0.5	0.6	1.0	0.8	0.7	0.9	0.8	0.8	0.9	0.9	0.8	0.7	0.7	0.6	0.5	0.8	0.6
β calacorene	157,142,200	-	0.2	-	-	-	-	-	-	-	-	0.1	0.1	-	0.1	-	-	-	-	-	-
Z isoelemicin	208,193,124	4.8	3.2	4.3	5.1	5.2	-	5.1	5.4	5.0	4.7	3.3	4.3	4.9	4.6	4.9	4.9	5.0	4.9	4.4	5.0
germacrene D-4-ol	81, 43, 41	-	-	-	-	-	4.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
β – asarone	208,193,165	60.7	56.4	53.6	60.6	54.4	46.2	52.3	48.5	51.2	49.7	43.4	47.6	50.7	47.8	49.3	53.7	52.0	57.5	48.0	54.0
α-asarone	208,193,165	7.4	7.9	3.7	2.6	4.8	3.5	2.9	5.2	4.2	6.0	2.9	4.2	3.7	3.0	3.4	3.4	3.5	3.4	2.6	4.2
shyobunone	150,83,81	6.3	3.8	5.6	3.4	4.4	5.0	5.5	4.0	3.9	5.1	5.9	4.3	4.5	5.7	5.7	4.8	4.4	5.4	5.2	5.9
cadin-4-en-10-ol	95,121,43	-	2.1	0.7	0.4	1.5	1.4	-	-	-	0.4	0.3	-	-	-	-	-	-	-	-	0.9
phytol	71,57,123	-	-	-	0.7	0.5	1.5	0.4	2.0	2.0	1.1	0.5	0.9	1.6	1.2	1.0	1.2	2.2	0.2	0.5	-
aspidinol	181,182,224	-	-	-	-	-	-	-	-	-	-	1.7	-	-	1.3	-	-	-	-	-	-
asaronaldehyde	196,181,150	2.1	2.5	3.7	1.0	-	-	-	-	-	-	2.5	1.4	-	1.1	0.6	-	-	-	0.8	-
palmitic acid	73,60,43	-	-	-	-	-	-	-	-	-	-	-	-	3.6	-	-	-	-	-	-	-
E- nerolidol	69,93,41	-	-	-	-	-	0.9	-	0.7	0.7	-	-	0.7	-	-	0.6	-	0.4	-	-	0.7
cis ocimene	93,91,92	-	-	-	-	-	-	0.4	-	-	-	0.8	-	-	-	-	-	-	0.4	0.1	-
pentadecanol	83,97,69	-	-	-	-	-	0.4	-	-	1.7	-	-	-	-	0.9	-	-	-	-	-	-
γ- muurolene	161,105,119	-	-	-	-	-	0.2	-	-	-	-	0.1	0.2	-	-	-	0.1	-	-	0.2	-
δ- elemene	121,93,136	-	-	-	-	-	0.1	0.1	-	-	-	0.1	0.1	-	-	-	0.1	-	-	-	-
γ- cadinene	161,105,91	-	-	-	-	0.4	0.7	0.5	-	0.5	-	-	0.4	0.4	0.5	0.6	0.6	-	-	0.5	0.5
α-guaiene	105,93,119	-	-	-	-	-	0.3	-	-	-	0.3	-	-	0.3	-	0.3	-	-	-	-	-
α -muurolol	161,119,204	-	-	-	-	-	-	-	-	-	-	0.4	-	-	-	-	-	-	-	-	-
Total		84.0	83.8	80.9	86.8	82.1	81.6	82.8	80.2	80.2	81.2	80.3	80	82.1	80.4	80.6	80.0	80.6	84.8	80.1	80.0

Whereas, MF= Mass Fragment, 1= Chhoi(Ramnagar), 2= Kotsari, 3= Devlat, 4=Saraikhet, 5= Jaitpur, 6=Paithani(Dwarahat), 7=Bagdigaon(Chaukhutiya), 8=Rikherikhal, 9=Jadaukhan, 10=Thapla(Bageshwar), 11= Someshwar, 12=Palpur, 13=Naulakot, 14=Walka, 15=Gumti, 16=Champawat, 17=Patkot, 18=Gairsen, 19=Fatehpur(Deghat), 20=Khedagaon, L= Leaf

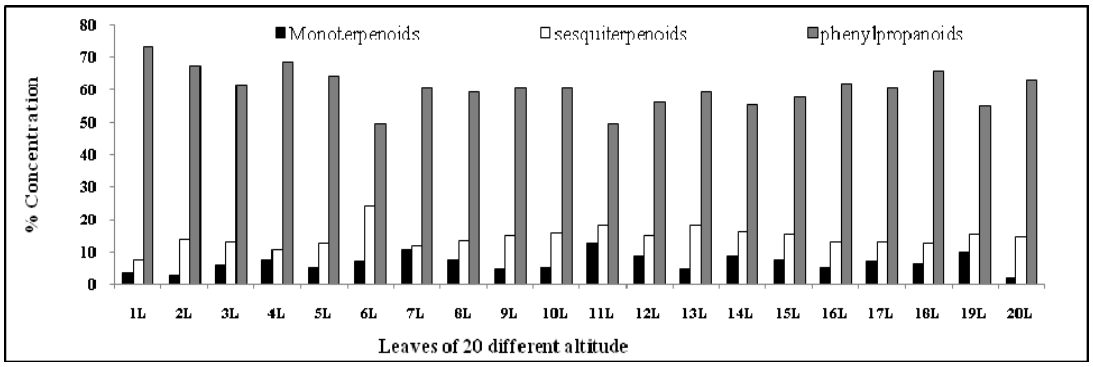

Figure 2.Comparative class composition of terpenoids of *A. calamus* leaves from 20 different altitudes.

Table 3. Comparative class composition of terpenoids of ACLEO from 20 different altitudes.

S.N	Compounds	1L	2L	3L	4L	5L	6L	7L	8L	9L	10L	11L	12L	13L	14L	15L	16L	17L	18L	19L	20L
1	MT	3.6	2.5	6.2	7.8	4.9	7.5	10.7	7.8	4.7	5.2	12.8	9.0	4.6	9.0	7.8	5.0	7.2	6.4	9.9	2.0
2	ST	7.8	13.8	13.1	10.7	12.8	24.4	11.8	13.3	15.1	15.6	17.9	14.9	18.2	16	15.2	13	12.9	12.6	15.2	14.8
3	PT	72.9	67.5	61.6	68.3	64.4	49.7	60.3	59.1	60.4	60.4	49.6	56.1	59.3	55.4	57.6	62	60.5	65.8	55	63.2

MT=monoterpenoids, ST=sesquiterpenoids, PT=Phenylpropanoids

All the oils were different in their qualitative and quantitative make-up of major and minor constituents and showing vast molecular diversity In term of class composition the components of essential oil were mainly composed of phenylpropanoids (49.7-72.9%), monoterpenoids (2.0-12.8%) and sesquiterpenoids (7.8-24.4%) (Figure 2 and Table 3). A positive correlation between major constituents and essential oil was observed (Table 4). The results vary from the previous reports. A report by Raal *et al.* (2016) on *Acoruscalamus* essential oil revealed the dominating presence of oxygenated sesquiterpenes in his report. In present communication the dominating constituents in the leaf essential oils from *Acorus calamus* were β-asarone (43.4-60.7%), α-asarone (2.6-7.9%), shyobunone (3.4-6.3%), and α- humulene (0.3- 1.6%) in twenty different accessions. The constituents linalool (0.4-7.1%), caryophyllene (1.1-3.2%) and α-elemol (0.5-1.0%), were identified in all the accession except 1L and 2L. It was also observed that germacrene D-4-ol (4.7%) was identified in 6L while palmitic acid (3.6%) was in 13L whereas in other accession these compounds were totally missing. Similarly Z-methyl isoeugenol (0.8-3.1%) and Z- isoelemicin (3.2-5.4%) showed variable concentrations which were totally absent in samples collected from 2L and 6L respectively.

In our previous study by Kumar *et al.* (2009) and Joshi *et al.* (2012) we have published a report on antibacterial and anthelmentic activity in rhizome essential oils of *Acorus calamus* from different locations.

The presence of major (27.4-45.5%) constituent β-asarone in *Acorus calamus* leaves essential oil by Venskutonis and Dagilyte (2003) has supported our findings. However, Keller and Stahl (1983) revealed the absence of β-asarone in diploid varieties. Rost and Bos (1979) have reported 31-44% of β-asarone in leaf essential oils of *Acorus calamus* collected from 24 different regions of India, Europe and North America. As per the report by Mazza (1985) acorenone (8.1%), isoshyobunone (6.3%), β-asarone (6.7%), calamendiol (5.2%) and β-asarone (5.2%) were the major constituents in triploid *Acorus calamus* oil, whereas Raina *et al.* (2002) have reported 10-40% β-asarone in tetraploid species of *Acorus calamus* from Far East Russia. Li and Jiang (1993) have reported (Z)-methylisoeugenol (36.4% and 17.7%),

acoragermacrone (4.1% and 7.4%) and δ-cadinene (4.1%–3.7%) in leaf and rhizome essential oils of *Acorus calamus*. This variability in the leaf essential oils of *Acorus calamus* might be possibly due to their ploidy natures, seasonal factors or geographical conditions.

Table 4. Correlation between the total EOs content and main constituents of essential oil of *A. calamus* Leaves.

	Total	β-asarone	α-asarone	Shyobunone	*Z*-isoelemicin	*Z*-methyl isoeugenol
Total	1					
β-asarone	0.752**	1				
α-asarone	0.186	0.359	1			
Shyobunone	-0.215	-0.140	-0.101	1		
Z-isoelemicin	0.028	0.375	-0.019	-0.071	1	
Z-methyl isoeugenol	-0.065	-0.350	-0.704**	0.108	-0.163	1

**Significance at 0.01 level, EOs= essential oils

3.2. Antioxidant activity

Natural antioxidants are responsible for preventing the oxidative stress and much safer to use due to its less toxicity and side effects. Antioxidants play a major role for prevention of tissue damage that stimulates the wound healing process (Barku *et al*., 2013). DPPH antioxidant activity is based on the capability of DPPH a stable free radical which is discolored in the presence of antioxidants (Subathraa and Poonguzhali, 2012). The results of the antioxidant activity are demonstrated in Figure 3 and Table 5.

Strong to good DPPH radical scavenging property in *Acorus calamus* leaf essential oils was observed as a function of concentrations at selected dose levels. The maximum scavenging activity (IC_{50} 10.79±0.042b µg/ml) was observed in the sample 2L. The essential oil of this collection contained highest amount (7.9%) of α-asarone. The maximum radical scavenging activity of this sample might be possibly due to the presence of α-asarone. The results are in total agreements with the previous reports by Manikandan and Devi (2005); Devi *et al.* (2014) which revealed that the antioxidant property of *Acorus calamus* extract is partly expected due to the active constituents, α- asarone.

Eessential oil of *Acorus calamus* is known for its medicinal and insecticidal properties. β-asarone the major chemical constituents reported in Phytochemical investigation has been reported to possess many biological activities along with a potential therapeutic agents for Alzheimer's disease (Geng *et al.,* 2010), However, the other isomeric phenylpropanoid α-asarone have been reported to posses neuroprotective, anticonvulsive, anti-oxidative, cognitive enhancing action and to improve various central nervous system disorders along with prevention of the noise stress induced memory impairment (Manikandan and Devi 2005; Shin *et al*., 2014; Kim *et al*., 2015).

The compounds shyobunone and isoshyobunone isolated from essential oil have been reported to possess insecticidal and repellant activity against Lasioderma serricorne and Tribolium castaneum (Chen *et al.,* 2015).

The essential oil of Pakistanian *Acorus calamus* have been reported to exhibit potential pesticidal activity (Tariq *et al.,* 2010). It has been reported that the essential oils containing linalool and the corresponding acetate play a major role in the anti-inflammatory activity (Peana *et al.,* 2002).

Acoruscalamus essential oils and its constituent like methyleugenol, methylisoeugenol and α-asarone possessed insecticidal activity against booklouse (Liu *et al.*, 2013). Some of the important bioactive hydrocarbons such as limonene, β-caryophyllene and β-elemene, have also been identified in this species. Caryophyllene reported with a woody spicy fragrance, exhibits anticancer property (Opdyke, 1973) and antiseptic activities (Verghese, 1994).

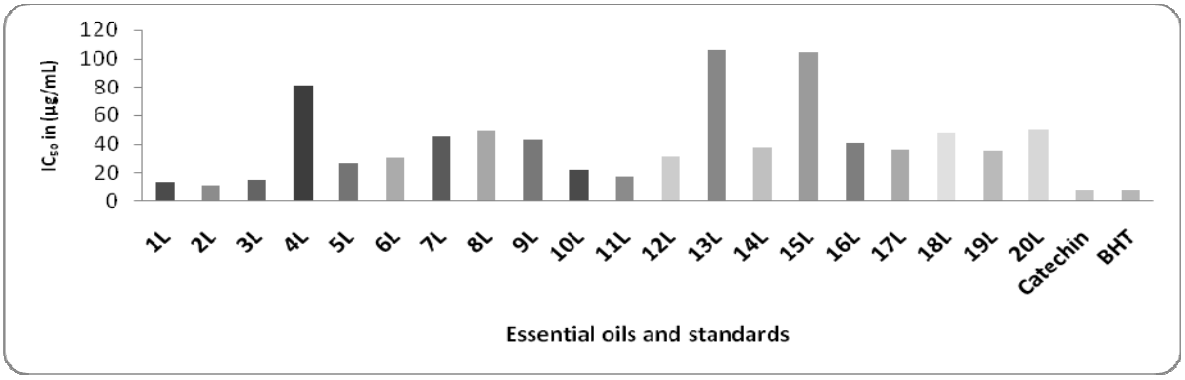

Figure 3. DPPH radical scavenging activity of leaf essential oils from *Acorus calamus*

Table 5. IC_{50} of *A. calamus* leaf essential oil of different altitudes.

SN.	Sample Name	IC_{50} values (µg/ml) in triplicate			Mean IC50 value (µg/ml)±SD
		I	II	III	
1	1L	12.78	12.88	12.91	12.86 ± 0.068^{bc}
2	2L	10.75	10.78	10.83	10.79 ± 0.042^{b}
3	3L	14.20	14.23	14.23	14.22 ± 0.016^{c}
4	4L	83.49	79.00	81.31	81.26 ± 2.245^{o}
5	5L	26.38	26.43	26.54	26.45 ± 0.080^{f}
6	6L	59.35	59.96	55.16	58.16 ± 2.614^{n}
7	7L	45.29	45.17	45.00	45.15 ± 0.146^{ik}
8	8L	49.49	49.57	49.26	49.44 ± 0.161^{lm}
9	9L	42.90	42.85	42.62	42.79 ± 0.152^{ij}
10	10L	21.90	21.76	21.96	21.87 ± 0.103^{e}
11	11L	17.44	17.43	17.43	17.43 ± 0.007^{d}
12	12L	31.06	30.99	31.15	31.06 ± 0.080^{g}
13	13L	105.70	107.60	106.00	106.44 ± 1.022^{p}
14	14L	37.90	37.87	37.87	37.88 ± 0.019^{h}
15	15L	105.10	104.15	104.15	104.47 ± 0.553^{p}
16	16L	40.82	41.27	41.19	41.09 ± 0.239^{i}
17	17L	36.23	36.15	36.15	36.18 ± 0.045^{h}
18	18L	47.29	47.61	47.80	47.57 ± 0.259^{kl}
19	19L	35.52	35.27	35.39	35.39 ± 0.125^{h}
20	20L	50.57	50.60	49.43	50.20 ± 0.666^{m}
21	Catechin*	7.70	7.40	7.49	7.53 ± 0.153^{a}
22	BHT*	7.22	7.25	7.23	7.23 ± 0.018^{a}

*= Standard antioxidants, Values are means of three replicates ±SD. Within a column, mean values followed by the same letter are not significantly different according to Tukey's test ($p<0.05$).

4. Conclusion

Fromthe above result it is infered that the herb *Acorus calamus* is a rich source of phenylpropanoids with major compounds like β-asarone, α-asarone and Z-isoelemicin. The present study revealed that β-asarone is positively correlated with the total amount of essential oil. These results confirmed that essential oil

composition of herb can be different in quality and quantities in different geographical and environmental condition. Hence, the chemical diversity of the herb *Acorus calamus* essential oil can be a good source for herbal, neutraceuticals and phenylpropanoids, the biologically important class of terpenoids.

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