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Chemical composition and antioxidant activity of *Acorus calamus* L. accessions from different altitudes of Uttarakhand Himalayas

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ABSTRACT

Background & Aim: *Acorus calamus* L. (Araceae) is an herb traditionally used in Indian and Chinees system of medicine. It is a perennial herb with long, cylindrical scented rhizomes which are creeping and extensively branched with the thickness up to 2.5 cm. The rhizomes are whitish internally and brownish externally in appearance. It is vernacularly known as Bach in India. The present investigation reveals the phytochemical screening and antioxidant activity of the rhizome and leaves methanolic extracts of *A calamus* from three different altitudes of Uttarakhand.

Experimental: The extracts compositions of *Acorus calamus* rhizomes and leaves collected from three different altitudes of Uttarakhand Himalayas, India. The yield of extracts ranged from 0.3- 4.8% w/v, were analyzed by GC/MS and in-vitro antioxidant assay were done by different methods.

Results: Over 65 contributing 80.90-90.55 % in different extracts among all the accessions were identified. The major compounds identified were β-asarone (44.9-51.9%), shyobunone (1.1%- 5.3%), Z-methyl isoeugenol (0.1%-2.4%), leinoleic acid (6.4%-18.9%), α-asarone(0.1%-4.6%) and Z-isoelemicin (2.2%-15.8%). The amount of phenolics in rhizome extracts, ranged from 4.10 mg-4.80 mg GAE/g respectively, whereas in leaf extracts the amounts were 2.40-3.26mg GAE/g respectively. All the extracts exhibited good in vitro antioxidant activity with the IC50 values ranging from 0.3- 4.8 % w/v determined by different methods compared to standard antioxidant.

Recommended applications/industries: Based on above observations it can be inferred that the herb may be a good source of bioactive compounds and can work as an antioxidant to prevent the oxidative deteriorative activity of food materials beside generation of database for its scientific and judicious in-situ exploitation.

1. Introduction

Sweet flag, *Acorus calamus* L., vernacularly known as Batch in India is, a semi-aquatic herbaceous plant growing in temperate to sub temperate regions. It is a perennial herb with long, cylindrical rhizomes which are creeping and extensively branched with the thickness up to 2.5 cm. The rhizomes are whitish internally and brownish externally in appearance. The rhizomes and leaves of this plant have been used in the Indian systems of traditional medicine for hundreds of years. The leaves and rhizomes essential oils of this herb have been reported to possess biological activities

like antispasmodic, carminative and also used for treatment of epilepsy, mental ailments, chronic diarrhea, dysentery, bronchial catarrh, intermittent and tumors (Avadhani *et al*., 2016). It has been reported that the extracts are used in the traditional *Chinese* prescriptions and its beneficial effects on memory disorders and on learning performance. Lipid peroxidase content and anti- aging effect in senescence have also been reported (Mehrotra *et al*., 2003). The plant has also been reported to possess insecticidal, antifungal, antimicrobial, moth, mosquitos, lice repellent, and as a toxicant for flying insects, ectoparasites and insects in stored products (Lewis and Elvin-Lewis, 2003; Phongpaichit *et al*., 2005). In view of its traditional uses as medicinal herb in indigenous system, the present communication deals with chemical composition and antioxidant assay. The chemical composition along with biological activities of essential oil of this herb has already been reported by our group (Joshi *et al*., 2012; Kumar *et al*., 2009).

2. Materials and Methods

2.1. Plant material

The samples were selected from different altitudes of Uttarakhand Himalayas. The plants were collected from Pithoragarh, District- Pithoragarh (altitude 1514m), Bhimtal, District- Nainital (altitude 1370m) and Pantnagar District Udhamsingh Nagar (altitude 344m) from their natural habitat. The rhizomes and leaves were shade dried and were powdered. The powdered material (100gm) was subjected for extraction in methanol by cold percolation method for seven days with constant shaking in between. The yield of extracts ranged from 0.3- 4.8% w/v.

2.2. GC-MS analysis

The GC-MS data were analysed by using GCMS-QP2010 Plus with following experimental conditions: Initial temperature 60°C with RAMP of 3°C/ min, final temperature 210°C, final hold time 10 min, carrier gas He, flow rate 1mL/ min. Column, silica DB-5, capillary(30m× 0.25mm,0.25 µm). MS were recorded under electron ionization (EI) condition (70 eV) with split mode of 40:1. The compounds were identified by matching their mass spectra with those recorded in NISTMS Wiley Library and comparing the data in literature (Adams, 2007).

2.3. Antioxidant activity

2.3.1. DPPH radical scavenging activity

It is a quick method to study the scavenging ability of the antioxidants. A standard protocol was followed for the same (Burits and Bucar, 2000). In brief different amounts of the tested sample (20-100 µg/mL) were added to 5 mL of a 0.004% methanol solution of DPPH. Finally the absorbance was read against a blank at 517 nm after 30 min of incubation at room temperature. All the observations were taken as triplicate. BHT (butylated hydroxyl toluene) and catechin were used as the standard antioxidant. Percent DPPH free radical Inhibition (IC%) was calculated by using the equation, IC % = $(A_0 – At/A_0) \times 100$, where A_0 and At are the absorbance values of the control and test sample respectively.

2.3.2. Reducing power activity

The reducing power of extracts was determined by following reported methods (Jeena *et al*., 2016). Varying concentrations of tested sample (20-100 µg/mL) were mixed with 2.5 mL of phosphate buffer (200 mM, pH= 6.6) and 2.5 mL of 1% potassium ferricyanide, $K_3[FeCN_6]$. The mixtures were incubated for 20 min. at 50°C. After incubation, 2.5 mL of CCl_3COOH was added to the mixtures, followed by centrifugation at 650 rpm for 10 min. The supernatant (1 mL) was mixed with 5mL distilled water and 1 mL of 0.1% $FeCl_3$. The absorbance of the resultant solutions was measured at 700 nm. BHT was taken as the standard. The reducing power of samples was calculated by the following formula, Reducing power % = $(A_0 – A_t/A_0) \times 100$, where A_0 and A_t are the absorbance values of the control and test sample respectively.

2.3.3. Metal chelating activity

The chelation of Fe^{2+} by extracts were evaluated by using the method developed earlier (Kunwar *et al*., 2013). In brief 0.1 mL of 2mM $FeCl_2.4H_2O$, 0.2mL of 5mM ferrozine and 4.7 mL of methanol was added to different concentrations of tested sample (20-100 µg/mL). The solutions were mixed and allowed to react for 10 min. The absorbance at 562 nm was measured. EDTA and citric acid was taken as the standards.

2.3.4. Total phenolics assay

In order to correlate the antioxidant activity of extracts the total phenolic content were estimated quantitatively by using the reported method (Shetty *et al*., 1995). Briefly, 1 mL of the sample extract was transferred into a test tube and mixed with 1 mL of 80% methanol and 8 mL of distilled water. To each sample 0.5 mL of 1 N Folin-Ciocalteu reagent(FCR) was added and mixed. After 5 min., 1 mL of saturated Na_2CO_3 solution was added to the reaction mixture and allowed to stand for 60 min. The absorbance was read at 650 nm using a Thermo Scientific UV spectrophotometer. The calibration curve was established using various concentrations of catechol and results were expressed as mg of catechol per gram of sample in dried weight (dw).

2.4. Statistical analysis

The experimental data were obtained by performing the experiments in triplicates. The data were 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. Phytochemical analysis of extracts

The constituents of leaves and rhizomes methanolic extracts from three populations viz., ACPGLME = *A. calamus* pithoragarh leaves methanolic extract, ACPNLME = *A. calamus* Pantnagar leaves methanolic extract, ACBTLME = *A. calamus* Bhimtal leaves methanolic extract, ACPGRME = *A. calamus* Pithoragarh rhizomes methanolic extract, ACPNRME = *A. calamus* Pantnagar rhizomes methanolic extract, ACBTRME = *A. calamus* Bhimtal rhizomes methanolic extract, were analyzed and compared using GC-MS. The analysis led to the identification of over 62 constituents comprising 80.70-90.07 % of total compositions. Persual of table 1 shows the relative percentage of the identified components in the methanolic extracts**.** Phenyl propanoids contributed the major part of the total compositions, being dominated by β-asarone (44.9-51.9%). The other constituents identified within this class were α-asarone (0.1-4.6 %), Z- isoelemicin(2.2- 15.8%), stigmast-4-en-3 one (0.3-1.3 %), *trans* methyl isoeugenol (0.1-2.4 %), *cis* methyl isoeugenol (0.-2.0 %) and γ-asarone (0.5-0.7%) Besides phenyl propanoids; β- gurjunene (0.3-0.9%, cuparene(2.4-3%), shyobunone(1.1- 5.3%), phytol(2.3-10.6%), leinoleic acid (6.4- 18.9%), 6-epishyobunone(0.8- 2.0%) were the major sesquiterpenoids identified.

β-asarone (70.1%), α-asarone (6.11%), γ-asarone (1.0%), methyl linolelaidate (0.73%), isocalamendiol (0.50%), methyl palmitate (0.39%), and methyl isoeugenol (0.26%) from hexane extracts of rhizomes have been reported in previous study (Adfa, 2015). 2,4,5-trimethoxy benzaldehyde with its activity against insects has been reported from Bangladesh (Hossain *et al*., 2008). Saponins, resins, alkaloids, steroids, tannins, phenolics, flavonoids, proanthocyanidins, glycosides, diterpenes and triterpenes have also been reported from *A. calamus* (Barua, *et al*., 2014; Nanda, 2014; Pandy *et al*., 2009). The sesquiterpenes, 1β,7α(H)-cadinane-4α,6α,10α-triol, 1α,5β-guaiane-10α-O-ethyl-4β,6β-diol and 6β,7β(H)-cadinane-1α,4α,10α-triol in ethanolic extracts from China have also been reported (Dong *et al*., 2010). Shyobunone, with its insecticidal activity and linoleic acid's anti-inflammatory, acne reductive, and moisture retentive properties have also been reported (Chen *et al*., 2015; Diezel *et al*., 1993; Letawe *et al*., 1998; Darmstadt *et al*., 2002).

The constituents in various extracts are different both qualitatively and quantitatively from the results reported in the literature. The chemical diversity was also found among the extracts both from leaves and rhizomes which might be possibly because of climatic, altitudinal or edaphic factors. To the best of our knowedge and search of literature it is the first report on GC – MS analysis of methanolic extracts from rhizome and leaves of *A. calamus.* In continuation to our research on *A. calamus* we have reported the essential oil composition of *A. calamus* revealing the presence of major compounds like β-asarone, α-asarone, Z-methyl isoeugenol, β-caryophyllene, methyl eugenol, spathulenol, α-calacorene and caryophyllene oxide etc. along with biological activities of essential oil as a whole (Joshi *et al*., 2012; Kumar *et al*., 2009).

Table 1: Chemical constituents in methanolic extracts from leaves and rhizomes of *A. calamus*

S.N.	Compound name	% Cntribution in extracts						Method of identification
		ACPGR	ACPGL	ACPNR	ACPNLM	ACBTRM	ACBTL	
1	Syringol	-	-	-	0.5	-	-	**M**$^+$ 154, **M/z**: 154,139,93
2	Linalool	0.2	1.1	-	-	0.3	2.6	**M**$^+$ 154, **M/z:** 71,93,55
3	caryophyllene	-	-	-	-	-	0.2	**M**$^+$ 204, **M/z:** 93,669,133
4	β gurjunene	0.9	-	0.4	-	0.3	-	**M**$^+$ 204, **M/z:** 161,105, 41
5	*trans*-methyl isoeugenol	0.7	0.6	0.1	0.1	2.4	1.1	**M**$^+$ 178, **M/z:** 178,107,163
6	γ- muurolene	-	-	0.1	-	0.1	-	**M**$^+$ 204, **M/z:** 161,105,119
7	δ cadinene	-	-	-	-	0.2	-	**M**$^+$ 204, **M/z:** 161,119,105
8	β asarone	51.9	45.9	47.0	44.9	46.2	51.2	**M**$^+$ 208, **M/z:** 208,193,165
9	Cuparene	2.6	-	3.0	-	2.4	-	**M**$^+$ 202, **M/z:** 132,145,107
10	methyl hexadecanoate	-	-	-	-	0.8	-	**M**$^+$ 270, **M/z:** 74, 87, 143
11	asaronic acid	-	1.6	-	0.8	0.3	-	**M**$^+$ 212, **M/z:** 197, 137, 169
12	hexadecanoic acid	4.8	-	5.1	-	7.9	2.0	**M**$^+$ 256, **M/z:** 73,60,43
13	linoleic acid, methyl ester	1.0	-	0.6	1.2	1.5	-	**M**$^+$ 294, **M/z:** 67, 81, 41
14	α- copaene	0.1	-	.02	-	t	-	**M**$^+$ 204, **M/z:** 161, 105, 119
15	decanoic acid	-	-	-	-	t	-	**M**$^+$ 172, **M/z:** 60, 73, 129
16	bicyclogermacrene	t	-	-	-	t	-	**M**$^+$ 204, **M/z:** 121, 93, 41
17	β- elemene	0.3	-	0.2	-	t	-	**M**$^+$ 204, **M/z:** 81,93,68
18	β –farnesene	-	-	-	-	0.1	-	**M**$^+$ 204, **M/z:** 41, 69, 93
19	Shyobunone	5.3	-	3.0	-	1.4	1.1	**M**$^+$ 220, **M/z:** 150,83,81
20	γ asarone	-	0.5	-	0.7	-	0.6	**M**$^+$ 208, **M/z:** 208, 193, 69
21	γ gurjunene	-	-	-	-	-	1.5	**M**$^+$ 204, **M/z:** 81, 107, 161
22	Elemol	-	-	-	-	-	0.3	**M**$^+$ 222, **M/z:** 59,93,161
23	hydroxycineol	-	-	-	-	0.2	-	**M**$^+$ 170, **M/z:** 43, 108, 126
24	z- isoelemicin	-	15.8	-	2.2		9.0	**M**$^+$ 208, **M/z:** 208,193,124
25	α –calacorene	-	-	-	-	0.07	-	**M**$^+$ 200, **M/z:** 157,142,200
26	4-propylbiphenyl-4-carboxylic acid	-	-	-	-	0.4	-	**M**$^+$ 240, **M/z:** 211, 240, 165
27	Spathulenol	0.4	-	0.5	-	0.5	-	**M**$^+$ 220, **M/z:** 43, 41, 91
28	isocalamendiol	0.8	-	0.8	-	0.7	-	**M**$^+$ 238, **M/z:** 43, 111
29	9 octadecenoic acid (z)	-	-	-	-	0.1	-	**M**$^+$ 282, **M/z:** 43, 73, 57
30	2,4,6 trimethoxy acetophenone	-	0.8	-	0.4	.07	-	**M**$^+$ 210, **M/z:** 195, 210, 180
31	asaronaldehyde	-	0.5	-	0.8	-	-	**M**$^+$ 208, **M/z:** 96,181,150
32	3,4,5-trimethoxy phenyl-2-	-	6.3	-	0.3	-	1.9	**M**$^+$ 224, **M/z:** 181, 224, 148
33	heptadecanol	-	-	-	1.5	-	1.2	**M**$^+$ 256, **M/z:** 55, 83, 97
34	Citronellol	-	0.9	-	-	-	-	**M**$^+$ 156, **M/z:** 71, 68, 41
35	methyl palmitate	-	-	-	1.8	-	-	**M**$^+$ 270, **M/z:** 74, 87, 143
36	heptadecanol-1	-	1.5	-	1.2	-	-	**M**$^+$ 208, **M/z:** 83, 57
37	phytol	-	-	-	10.6	-	2.3	**M**$^+$ 296, **M/z:** 71,57,123
38	2,13-octadecadien-1-ol	-	-	-	5.0	-	-	**M**$^+$ 266, **M/z:** 55, 41, 67
39	9,12,15-octadecatrienoic acid,	-	-	-	1.3	-	-	**M**$^+$ 292, **M/z:** 79, 67, 41
40	2,4,6 trimethoxy benzonitrile	-	-	-	0.2	-	-	**M**$^+$ 193, **M/z:** m/e: 193, 164, 71
41	leinoleic acid	14.6	-	6.4	-	18.9	-	**M**$^+$ 280, **M/z:** 67, 81, 95
42	Carinol	-	-	-	1.2	-	-	**M**$^+$ 378, **M/z:** 137, 240, 175
43	Pentacosane	0.03	-	-	-	0.2	-	**M**$^+$ 352, **M/z:** 57, 71, 43
44	cyclohexyl palmitate	-	-	-	-	0.2	-	**M**$^+$ 338, **M/z:** 82, 257, 55
45	1,3,12-nonadeca triene	-	-	-	-	1.2	-	**M**$^+$ 262, **M/z:** 67, 41, 82
46	ergost-5-en-3-ol	0.5	-	-	-	0.5	-	**M**$^+$ 400, **M/z:** 107, 96, 145
47	stigmast-4-en-3 one	0.3	0.9	1.0	0.7	1.3	0.5	**M**$^+$ 412, **M/z:** 124, 43, 229
48	vitamin e	0.2	-	0.1	-	0.2	-	**M**$^+$ 403, **M/z:** 165, 430, 164

49	stigmast-5-en-3 ol (3 beta)	3.4	-	3.7	1.3	2.0	-	**M^+** 414, **M/z:** 43, 55, 107
50	4z- decenal	0.1	-	-	-	-	-	**M^+** 154, **M/z:** m/e: 84, 55, 41
51	6-dodecenol	T	-	-	-	-	-	**M^+** 184, **M/z:** 55, 67, 41
52	α- curcumene		0.6	-	0.4	-	-	**M^+** 202, **M/z:** 119, 132, 105
53	Aristolene	0.4	-	-	-	-	-	**M^+** 204, **M/z:** 161, 91
54	β copaene	0.1	-	0.1	-	-	-	**M^+** 204, **M/z:** 161, 105, 91
55	6-epishyobunone	1.2	0.8	-	-	-	2.0	**M^+** 220, **M/z:** 150, 41, 69
56	α- cadinene	-	-	0.2	-	-	-	**M^+** 204, **M/z:** 105,161,204
57	*cis* methyl isoeugenol	-	-	2	-	-	-	**M^+** 178, **M/z:** 178,107,163
58	α- calacorene	-	-	0.1	-	-	-	**M^+** 172, **M/z:** 157,142,200
59	α- asarone	0.1	4.2	4	3.6	0.2	4.6	**M^+** 208, **M/z:** 208,193,165
60	pentadecanoic acid	-	-	0.2	-	-	-	**M^+** 242, **M/z:** 74, 87, 143
61	methyl linolenate	-	-	0.1	-	-	-	**M^+** 292, **M/z:** 79, 67, 95
62	Tricosane	-	-	0.4	-	-	-	**M^+** 324, **M/z:** 57, 43, 71
63	Stigmasterol	-	-	2.0	-	-	-	**M^+** 412, **M/z:** 55, 83, 69
	Total	90.07	82.00	81.12	80.70	90.75	82.10	-

ACPGLME = *Acorus calamus* Pithoragaeh leaves methanolic extract, ACPNLME = *Acorus calamus* Pantnagar leaves methanolic extract, ACBTLME = *Acorus calamus* Bhimtal leaves methanolic extract, ACPGRME = *Acorus calamus* Pithoragarh rhizomes methanolic extract, ACPNRME = *Acorus calamus* Pantnagar rhizomes methanolic extract, ACBTRME = *Acorus calamus* Bhimtal rhizomes methanolic extract, M^+= molecular mass, M/z= major fragments, t= trace <.05.

3.2. Antioxidant activity

All the extracts exhibited significant DPPH radical scavenging activity as a function of concentration. The radical scavenging potential of the extracts in terms of their IC_{50} values revealed the order ACPNRME (108.99 µg/mL)> ACBTRME (206.78 µg/mL) > ACPGLME (298.30µg/mL)> ACBTLME (521.40 µg/mL)> ACPNLME (799.39 µg/mL)> ACPGRME (919.48 µg/mL) compared to the standards catechin (IC_{50}= 64.07µg/mL)> BHT (IC_{50}= 101.88µg/mL). In the radical form DPPH is purplish/pink in colour and absorb at 517 nm. It has been observed that the radical scavenger in the DPPH solution donates electrons and reduce the colour of solution and convert it to a stable diamagnetic molecule (Matthaus, 2002). In present investigation the observations were with the total agreement of the above fact. Thus it can be inferred that the phytochemicals in the extracts stabilized the DPPH molecule by donating electrons. It has also been reported that the phenols posses radical scavenging activity and proportionally correlate (Devi and Ganjewala, 2011). In our study the phenols positive correlated between antioxidant activity of the extracts (Table 2 and 3).

Table 2: Antioxidant potentials in terms of IC_{50} values for different methanolic extracts from *A. calamus* along with their total phenolic content.

Sample/ standard	DPPH scavenging activity/ IC_{50} (µg/mL)	Reducing power ability /RP_{50}(µg/mL)	Metal chelating ability/ IC_{50}(µg/mL)	Total phenols (mg/g GAE)
ACPGLME	298.30±1.25^{d}	177.44±4.80^{c}	270.62±3.52^{b}	3.26±.052^{c}
ACPNLME	799.39±5.75^{f}	105.45±1.34^{a}	202.75±0.19^{a}	2.40±.058^{a}
ACBTLME	521.40±0.82^{e}	267.57±0.34^{e}	314.62±0.06^{bc}	2.45±.051^{b}
ACPGRME	919.48±6.89^{g}	190.50±2.28^{d}	331.30±1.27^{c}	4.10±.055^{e}
ACPNRME	108.99±0.70^{b}	148.91±1.42^{b}	387.78±8.21^{d}	3.41±.052^{d}
ACBTRME	206.78±0.23^{c}	263.21±0.35^{e}	776.23±0.81^{e}	4.80±.054^{f}
BHT	101.88±0.70^{b}	102.23±1.06^{a}	-	-
Catechin	64.077±0.40^{a}	-	-	-
EDTA	-	-	194.06±0.11^{a}	-
Citric acid	-	-	207.56±0.46^{a}	-

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Not applicable, 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 ($p<0.05$), GAE= gallic acid equivalent

It has been reported that antioxidant potential of certain compounds is related to their reducing power and this serves as an important indicator of antioxidant activity. In this assay, ferric ions (Fe^{3+}) were reduced to ferrous ions (Fe^{2+}) with change in colour from yellow to bluish green. The intensity of colour depends on the reducing potential of the compounds present in the reaction medium, consequently, related to the antioxidant activity (Siddhuraju *et al*., 2002). In present study the Fe^{3+} to Fe^{2+} reducing activity was determined in terms of their RP_{50} values which exhibited the order of ACPNLME(105.45 µg/mL) > ACPNRME (148.91 µg/mL)> ACPGLME (177.44 µg/mL)> ACPGRME (190.50 µg/mL)> ACBTRME (263.21 µg/mL)> ACBTLME (267.57 µg/mL) compared to standard BHT (RP_{50}= 102.23µg/mL).

Similarly in Fe^{2+} metal chelating activity the equilibrium between Fe^{2+} ion and ferrozine complex get disturbed in presence of competitor complexing agent and indicated by decrease in colour intensity thus shows its antioxidant activity (Sethi *et al*, 2016). In present study the various extracts under investigation revealed varying degree of binding capacity for Fe^{2+} ion which expressed their ability as a per oxidation inhibitor. ACPNLME and ACPGLME were found to have significantly higher Fe^{2+} chelating abilities followed by other extracts. The IC_{50} values for antioxidant potentiality of extracts was obtained as ACPNLME (202.75µg/mL)> ACPGLME (270.62 µg/mL)> ACBTLME (314.62µg/mL)> ACPGRME (331.30µg/mL)> ACPNRME (387.78 µg/mL)> ACBTRME (776.23µg/mL) compared to standards EDTA and citric acid which exhibited the IC_{50} values 194.06 µg/mL and 207.56 µg/mL respectively. The IC_{50} values of all the extracts, assayed for their *in-vitro* antioxidant activity by various methods along with their phenolic contents have been reported in table 2, while the correlation of phenols with IC_{50} has been recorded in table 3.

Table 3: Correlation of total phenols with IC_{50} values.

Phenols	Total phenols	DPPH Scavenging activity		Reducing power ability		Metal chelating ability	
		rhizomes	leaves	rhizomes	leaves	rhizomes	leaves
Total phenols	1.0	-0.901	-0.859	-0.998	-0.014	-0.317	0.172

The quantitative estimation of total phenols in all the extracts were observed in the range of 2.40 to 4.80 µg/mL. The highest phenolic content was observed in ACBTRME followed by ACPGRME, ACPNRME, ACPGLME, ACBTLME and ACPNLME. It has already been reported that the phenols in various medicinal herbs play important role as antioxidants (Skerget *et al*., 2005). From present study it can be inferred that the phenols might be possibly responsible for antioxidant activity in extracts. The total phenols were correlated with IC_{50}/RP_{50} values of different methods used for the determination of antioxidant assay. The results obtained showed negative correlation with DPPH radical scavenging, metal chelating ability and reducing power ability at α=0.01 or 0.05 (level of significance) except the metal chelating ability of methanolic extract of *A.calamus* leaves (Table 3).

Various biological properties, such as anti-inflammatory, antimicrobial, enzyme inhibition, anti-allergic, and antioxidant activities have been reported to be associated with phenolics and flavanoids (Raina *et al*., 2003; Shahidi *et al*., 1992). It has been reported that the hydroxyl group (–OH) in aromatic ring, mediates redox reaction, and is capable of scavenging of free radicals (Raina *et al*., 2003). Based on the results obtained in present study and traditional use of *A calamus* as herbal folk medicine it can be concluded that this plant may be a good source of phytochemicals like shybunone, leinoleic acid, phytol and phenyl propanoids etc. The potential of antioxidant activity in all the extracts and their phenolic content supports its use as safe natural antioxidants and food preservative over the harmful synthetic ones like BHT. However further clinical trials will be needed for its

commercialization and value addition. The study will be also helpful to generate the scientific data base for the researchers so that the herb can be exploited from nature judiciously.

4. Conclusion

Based on above observations observed in present investigation it can be inferred that the herb may be a good source for natural food preservative to prevent the oxidative deteriorative activity of food materials beside generation of database for its scientific and judicious *in-situ* exploitation.

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