Indian Journal of Natural Products and Resources
Vol. 14(4), December 2023, pp. 581-590
DOI: 10.56042/ijnpr.v14i4.5912

Chemical composition and antioxidant activity of *Acorus calamus* L. accessions from different ecological niches

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Received 06 September 2022; revised received 13 October 2023; accepted 01 November 2023

The essential oil composition of *Acorus calamus* (Acoraceae) rhizomes, collected from twenty different ecological niches in Uttarakhand, India, with an oil yield ranging between 0.7–5.4% (v/w), was examined by GC/MS. Among the identified components, *β*-asarone (62.3–75.9%), *α*-asarone (2.2–6.1%), Z-isoelemicin (2.4–6.2%), *Z*-methyl isoeugenol (2.3–6.4%) and shyobunone (1.5–5.3%) were found to be the major ones. The antioxidant activity of different essential oils was compared to that of standard antioxidants to assess their free radical scavenging potential, metal chelating ability, and reducing power. The essential oils exhibited significant *in vitro* antioxidant activity. The IC_{50} values for DPPH radical scavenging, metal chelating, and reducing ability exhibited by the rhizomes essential oils were observed between 22.28–61.96 µg/mL, 29.55–159.26 µg/mL, and 21.41–61.19 µg/mL, respectively. Based on the above observations, the chemical diversity of *A. calamus* essential oil can be a good source of herbal nutraceuticals and phenylpropanoids. The possible mode of action and structure-activity relationship between major compounds of essential oils and proteins of antioxidant activity were studied using *in-silico* molecular docking and were found to support the *in-vitro* results.

Keywords: *Acorus calamus*, Antioxidant, Essential oil, Molecular docking, *β*-asarone

IPC code; Int. cl. (2021.01)– A61K 36/00, A61K 125/00, A61P 39/00

Introduction

Essential oils, often referred to as essences, volatile oils, or etheric oils, are complex natural concoctions of volatile, lipophilic, and odoriferous chemicals that are commonly found in aromatic plants. Essential oils have been reported as food-preserving agents mainly due to the presence of phenolic compounds as major constituents, which might be due to antioxidant properties[1]. *Acorus* is a genus monocot flowering plants found in wetlands, especially marshes, which are native to Northern Asia, Eastern and Southern Asia, North America, as well as Europe. The traditional indigenous plant *Acorus calamus* (sweet flag) is typically used to cure haemorrhoids, cough, gout, bronchitis, tumours, numbness, skin problems, and general weakness[2]. The primary plant antioxidants are polyphenols, which have a variety of biological properties as well as structural and functional qualities. Plant-based antioxidants are mainly phenolic compounds, carotenoids, and vitamins[3]. In case of essential oils, the antioxidant activity has been associated with compounds like terpenoids and phenolic, which diffuse and damage cell membrane structures[4]. *β*-asarone has been reported to be the major bioactive phytoconstituent of the volatile oil. *α*- and *β*-asarone are mainly responsible for the biological activity of *A. calamus*. The herb has also been reported to be an integral part of Ayurveda and Unani medicines. The concentration of asarone in *A. calamus* essential oils depends on the parts of the plant used to extract the oil and the ploidy[5-8].

The phenolic content of rhizomes has been reported in treating dyspepsia, dysentery, intestinal worms, cough and fever[9]. The sesquiterpenes, monoterpenes, lignans, phenyl propanoids, flavones, steroids, and xanthone glycosides from *A. calamus* have been reported to show insecticidal, antimicrobial, mutagenic, cytotoxic, anticonvulsant, neuroleptic, hepatoprotective, smooth muscle stimulant and relaxant activity[10]. *A. calamus* has also been reported to exhibit significant antioxidant activity. *A. calamus* essential oils from Pakistan, has been reported to exhibit potential pesticidal activity and also found to be effective in wound healing[11].

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The phytochemical investigation of *A. calamus* revealed the presence of *β*-asarone as a major chemical constituent and was reported to possess most of the biological activities[12]. *β*-asarone has been reported as a potential therapeutic agent to manage diseases like Alzheimer's, whereas *α*-asarone has been used as a, anticonvulsive, neuroprotective and cognitive enhancer to improve various central nervous system disorders[13-14]. The insecticidal and repellant activity of shyobunone and isoshyobunone has been shown previously against *Tribolium castaneum* and *Lasioderma serricorne*[15]. The chemical analysis and biological activities from essential oils of *A. calamus* like antioxidant, antibacterial and anthelmintic activity have also been reported from our laboratory[16-19].

The study using molecular docking (Autodock 4.2) showed the necessity for a structure-based drug-designing approach to develop new drugs that counteract the inhibition of potential therapeutic targets[20]. The comparative chemical compositions of essential oils among twenty accessions of *A. calamus* along with their *in-vitro* and *in silico* antioxidant activity have not been thoroughly studied because of the non-availability of literature.

Material and Methods

Plant material

The fresh plant materials were collected from twenty different ecological locations of Uttarakhand in India (Table 1) from June to December 2015. The plant material was identified and authenticated by Dr. D.S. Rawat, Plant taxonomist, Department of Biological Science, College of Basic Science and Humanities, vide voucher specimen number GBPUH-756.

Table 1 — Collection sites of *Acorus calamus* L.

S. No.	Natural Habitat	District	Altitude (m)
1	Chhoi	Nainital	369
2	Kotsari	Almora	1750
3	Devlat	Paudi Garhwal	1570
4	Saraikhet	Almora	1850
5	Jaitpur	U.S. Nagar	235
6	Paithani	Almora	1100
7	Chaukhutiya	Almora	1040
8	Rikherikhal	PaudiGarhwal	1460
9	Jadaukhan	PaudiGarhwal	1740
10	Bageshwar	Bageshwar	950
11	Someshwar	Almora	1400
12	Palpur	Almora	1500
13	Naulakot	Almora	1540
14	Walka	Champawat	1660
15	Gumti	Almora	1550
12	Palpur	Almora	1500
13	Naulakot	Almora	1540
16	Champawat	Champawat	1670
17	Patkot	Nainital	345
18	Gairshen	Chamoli	1620
19	Deghat	Almora	1650
20	Khedagao	Almora	1356

Isolation of essential oil

Essential oils were isolated from fresh rhizomes of the *A. calamus* by hydrodistillation method in a Clevenger-type apparatus[21]. Rhizomes were crushed and hydro-distilled for 7-8 hours. The essential oils were collected as pure and desiccated over anhydrous sodium sulphate to eliminate traces of water, if any. The process of hydrodistillation of oil was repeated three times to get a reproducible yield.

GC-MS analysis

The essential oils were analysed using GCMS-QP 2010 Plus equipment with the following conditions: initial temperature: 60.0°C for 3 min, final temperature: 210.0°C, and final hold time: 10 min., detector used: FID, Column oven temperature: 60.0°C, injection temperature: 260.00°C, pressure: 76.7 kPa, total flow: 64.7 mL/min, column flow: 1.0 mL/min, linear velocity: 40.1 cm/sec, purge flow: 3.0 mL/min, and split ratio: 50.0. The constituents of essential oils were identified by matching their mass spectra with those in NIST-MS, FFNSC, and Wiley Library by comparing them with literature reports and GC retention indices[22].

Antioxidant activity

The antioxidant activity of the essential oil of *A. calamus* rhizome was determined by DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay, metal chelating, and reducing power activity compared to standard antioxidants.

DPPH radical scavenging activity

Free radical scavenging activity by DPPH assay was evaluated according to the method developed earlier with slight modifications[23-24]. Different concentrations of essential oils were mixed with a 0.004% methanol solution of DPPH. Per cent radical inhibition was plotted against different concentrations, and the standard curve was drawn using standard antioxidants to calculate the IC_{50} values for standard and different concentrations (5, 10, 15, 20 and 25 µL) of essential oils.

Metal chelating activity

The metal chelating activity was carried out by following standard protocols and being practiced with some modification[25]. Briefly, 2mM $FeCl_2.4H_2O$, 5mM

ferrozine and 4.7 mL of methanol were added to different concentrations (5–25 µL) of essential oils obtained from *the A. calamus*. After incubation, the absorbance was measured at 562 nm.

Reducing power assay

The reducing power assay of essential oils was taken to measure their antioxidant properties. The reductive ability is due to the presence of reductones[26]. A volume of 2.5 mL of phosphate buffer (200 mM, pH = 6.6) and 2.5 mL of 1% $K_4[Fe(CN)_6]$ were mixed with different concentrations (5-25 µL) of essential oils obtained from the *A. calamus*. After incubation, 2.5 mL of trichloroacetic acid was added to the mixtures, followed by centrifugation at 650 rpm for 10 min. The upper layer was mixed with 5 mL of distilled water and 1 mL of 0.1% $FeCl_3$. The absorbance of the resultant solutions was measured at 700 nm using a Thermo Scientific UV spectrophotometer.

***In-silico* molecular docking**

In order to comprehend the host-guest connection at the lowest possible energy level, molecular docking is a virtual method for predicting the activity and affinity of small molecules with macromolecules like proteins in various orientations. Based on the Lamarckian genetic algorithm with local search, AutoDock 4.2.6 is the most reliable automated programme used as a research engine to understand protein-ligand interactions and protein-protein interactions (The Scripps Research Institute, USA)[27]. Using Autodock Tools (ADT ver. 1.5.7), Gasteiger charges were calculated and registered as a pdbqt file. The 3D complex structures for antioxidant protein xanthine oxidase (PDB ID: 3NRZ)[28] were gained from the protein data bank (www.rscb.org/pdb).

The main identified compounds like *β*-asarone, α-asarone, Z-methyl isoeugenol, shyobunone and elemicin, which were common in the essential oils of different accessions of *A. calamus*, were used as ligands obtained from PubChem (3D structures). To examine the chemical interactions of complicated structures, 3D structures of ligands and proteins were created using BIOVIA Discovery Studio 2019. By introducing Kollmann charges and polar hydrogens into proteins, all of the water molecules were eliminated. Using the graphical user interface program (AutoDock Tools), the pdbqt files were prepared for protein and ligands, and a grid map was created using a grid box called AutoGrid (grid space of 0.375 Å and the grid size was set to 40 x 40 x 40 points).

Cluster analysis was used to assess the docked results with an RMS (root mean square) tolerance of 2.0. The dock score used to express the binding affinity of the test ligands (compounds) with the receptors (protein) indicates the estimated binding free energy ΔG (kcal/mol) in negative terms, which is reported as the enzyme inhibition constant (Ki) value. Higher calculated binding free energy values in negative terms or lower enzyme inhibition constant values suggest higher ligand–receptor binding affinities[29].

Statistical analysis

All the experiments were performed in triplicate, and the results were expressed as mean±SD. The recorded data were analysed by Tukey's test in conjunction with ANOVA analysis with the help of SPSS software (Statistical Package for the Social Sciences). Differences were considered significant at $P < 0.05$.

Results and Discussion

The chemical components of twenty populations of *A. calamus* with their per cent contribution for each essential oil have been depicted in Table 2, whereas structures have been shown in Fig. 1. Altogether, 17 constituents were identified, representing 86.0–94.3% of the essential oil composition. The constituents in the essential oils were mainly phenylpropanoids (72.0-84.7%), monoterpenoids (2.3–6.4%) and sesquiterpenoids (4.3–11.3%). The yield of *A. calamus* rhizome essential oil was found to be 0.7-5.4% (v/w) based on fresh weight of plant material. It was examined that oxygenated sesquiterpenes were the major fraction of *A. calamus* essential oil. The presence of monoterpenes (1.0–6.0%) and sesquiterpenes (0.4–7.1%) was also observed. The major constituents in the rhizome essential oil of most of the population were *β*-asarone (62.3–75.9%), *α*-asarone (2.2–6.1%), *Z*-isoelemicin (2.4–6.2%), *Z*-methyl isoeugenol (2.3–6.4%), shyobunone (1.5–5.3%), elemicin (0.7–1.7%) and calarene (0.2–3.4%). Other predominant constituents, *viz*. α-cadinene (0.5–1.7%) and *δ*-cadinene (0.4–1%), showed variable concentration. Asaronaldehyde (0.6–2.4%) and kessane (0.2–1.1%) were also detected in some collection sites.

The variation in the qualitative and quantitative difference of the constituents in the present

Table 2 — Comparative GC/MS analysis of ACREO from 20 different altitudes of Uttarakhand

S. No.	Compounds	KI	ACREO																				MF (m/e)
			1R	2R	3R	4R	5R	6R	7R	8R	9R	10R	11R	12R	13R	14R	15R	16R	17R	18R	19R	20R	
1	linalool	1095	-	-	-	-	-	-	0.4	-	-	0.2	-	-	-	-	-	-	-	-	-	-	M^+ 154; M/z 71,93,55
2	bornyl acetate	1288	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	M^+196; M/z 95,93,43
3	calarene	1433	2.5	0.9	1.7	0.2	1.3	2.4	0.8	2.4	2.8	0.4	0.9	0.7	2.7	1.1	2.3	3.4	2.0	1.3	2.9	1.4	M^+ 204; M/z161,105,119
4	*Z*-methyl isoeugenol	1453	2.6	3.0	3.5	6.4	4.4	3.4	4.3	3.8	2.5	3.2	4.2	4.5	5.6	3.0	2.3	3.9	3.3	4.2	3.7	2.9	M^+ 178; M/z178,107,163
5	δ-cadinene	1522	0.7	0.6	0.6	0.5	0.5	0.9	0.4	0.7	0.8	0.5	0.7	0.5	0.9	-	0.6	1.0	-	0.6	0.4	-	M^+ 204; M/z161,119,105
6	kessane	1529	-	0.3	0.2	-	0.5	0.3	-	-	0.4	-	0.3	-	1.1	0.5	0.4	0.5	-	0.4	-	-	M^+222; M/z 108,126,43
7	α-cadinene	1538	-	-	1.0	-	0.8	1.6	0.5	1.4	1.7	-	0.5	-	1.6	0.5	1.3	-	-	0.7	-	-	M^+ 204; M/z105,161,204
8	α-calacorene	1544	0.5	1.0	0.7	1.8	1.7	1.4	0.8	1.3	1.2	0.9	1.4	1.5	1.5	1.0	0.4	1.1	0.5	0.6	-	-	M^+ 200; M/z157,142,200
9	α- elemol	1549	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	M^+ 222; M/z 59,93,161
10	elemicin	1555	1.0	0.8	1.0	1.3	1.2	1.0	0.9	1.7	1.0	1.0	0.8	1.2	1.1	0.9	1.1	0.8	0.9	0.9	1.1	0.7	M^+ 208; M/z208,193,133
11	β- calacorene	1564	-	0.2	0.1	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	M^+ 200; M/z157,142,200
12	Z- isoelemicin	1568	4.7	4.7	4.9	6.2	5.0	4.7	7.3	5.2	2.4	4.5	4.6	5.4	5.1	4.7	4.7	2.6	5.1	4.8	5.3	4.7	M^+ 208; M/z208,193,124
13	β – asarone	1616	63.8	70.9	71.0	68.7	65.6	62.3	73.1	65.2	70.4	72.9	68.9	69.1	65.2	72.5	73.4	69.2	70.0	75.9	71.4	73.2	M^+ 208; M/z208,193,165
14	α-asarone	1675	4.7	4.4	3.4	3.2	6.1	4.0	2.2	5.2	3.6	4.1	3.5	4.3	2.3	5.3	5.1	3.4	4.6	3.1	2.5	2.8	M^+ 208; M/z208,193,165
15	shyobunone	1506	5.3	2.3	2.4	1.7	2.9	3.6	2.5	2.6	1.9	3.3	3.5	3.3	3.5	1.8	1.5	2.3	2.6	1.8	2.4	2.9	M^+ 220; M/z 150,83,81
16	asaronaldehyde		2.4	-	-	-	-	-	-	-	-	-	-	0.6	-	-	-	-	-	-	-	-	M^+ 196; M/z196,181,150
17	*cis*- ocimene	1032	-	-	-	-	0.2	0.4	-	-	-	-	0.1	-	-	-	-	0.2	-	-	-	-	M^+ 136; M/z 93,91,92
	Total		88.3	89.1	90.6	90.1	90.2	86.0	93.2	89.5	88.7	91.0	89.4	91.1	90.6	91.3	93.1	88.4	89.0	94.3	89.7	88.6	

Where, KI= Kovats Index1= Chhoi, 2= Kotsari, 3= Devlat, 4=Saraikhet, 5= Jaitpur, 6=Paithani, 7=Chaukhutiya, 8=Rikherikhal, 9=Jadaukhand, 10=Bageshwar, 11= Someshwar, 12=Palpur, 13=Naulakot, 14=Walka, 15=Gumti, 16=Champawat, 17=Patkot, 18=Gairsen, 19=Fatehpur, 20=Khedagaon, R= Rhizome, ACREO = A. calamus rhizomes essential oil., MF=mass fragment

investigation with the previous report might be possibly due to the altitudinal variation. The bioactive constituents *viz*; 2-allyl-5-ethoxy-4-methoxyphenol, 4-terpineol, lysidine, epieudesmin, spathulenol, furylethyl ketone, borneol, nonanoic acid, 2,2,5,5-tetramethyl-3-hexanol, galgravin, bornyl acetate, retusin, (9*E*,12*E*,15*E*)-9,12,15-octadecatrien-1-ol, geranylacetate, butyl butanoate, sakuranin, camphor, acetic acid, isoelemicin, acetaphenone, *α*-ursolic acid, dehydroabietic acid, methyl ether, isoeugenol, apigenin 4,7-dimethylether, linalool, dehydro di-isoeugenol, elemicin and linolenic acid, 1 *β*,7 *α*(H)-cadinane-4 *α*,6 α,10 *α*-triol (1), 1 *α*,5 *β* -guaiane-10 *α*-O-ethyl-4 *β*,6 *β*-diol (2), and 6 *β*,7 *β*(H)-cadinane-1 *α*,4 *α*, 10 *α*-triol (3) have been reported in *A. calamus* because of its rich ethnobotanical history and traditional uses against various ailments like, fever, asthma, bronchitis, cough and mainly for digestive

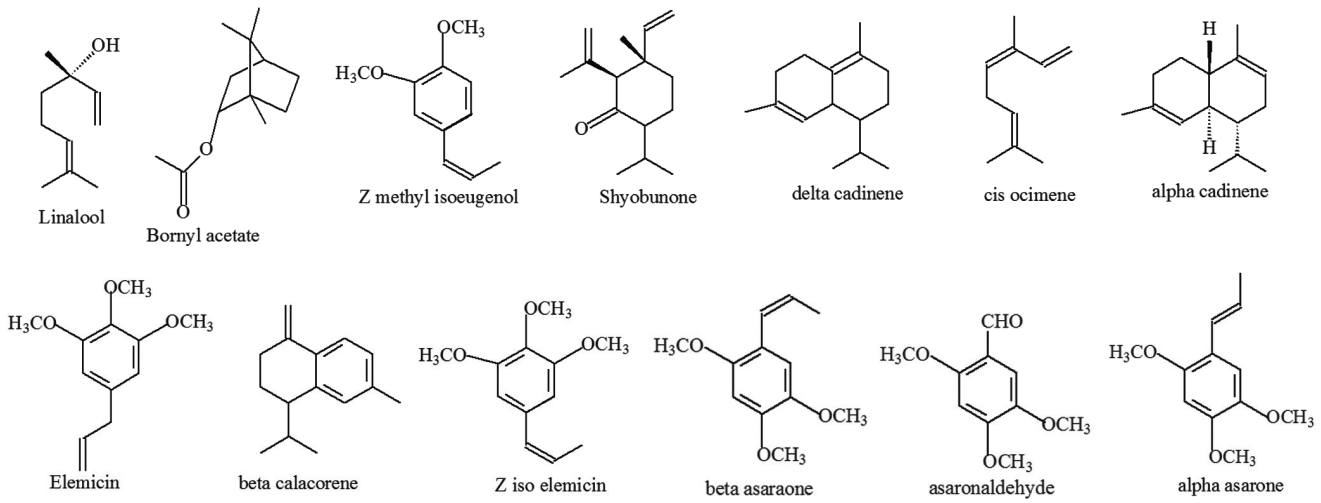

Fig. 1 — Structures some of terpenoids present in the essential oils of *Acorus calamus*.

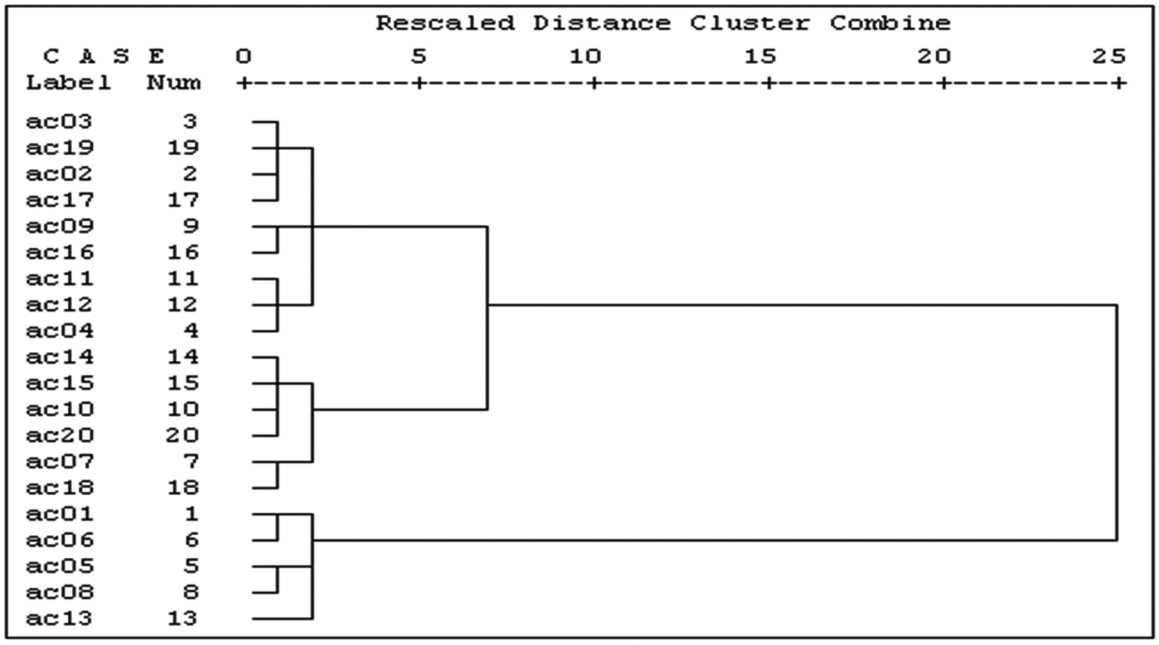

Fig. 2 — Dendogram obtained from the cluster analysis of essential oil components of *Acorus calamus* L. population using Ward method with square Euclidean distance measure.

problems such as gas, bloating, colic, and poor digestive function. The herb has also been reported to be used in Ayurveda and Unani systems of medicine[5-8].

To verify and characterise the variation of *A. calamus* oils and to identify the various possible chemotypes in the populations of *A. calamus*, their compositions were determined by cluster analysis, as shown in (Fig. 2). The dendrogram exhibited a distinct separation of all the populations with *β*-asarone as the major constituent. The essential oils from the populations (ac01, ac05, ac06, ac08 and ac13) contained 62.3–65.6% *β*-asarone. The second chemotype was composed of populations ac02,ac03,ac04,ac09,ac11,ac12,ac16,ac17 and ac19 contained 68.7–71.4% *β*-asarone content whereas the populations ac07,ac10,ac14,ac15,ac18 and ac20 possess > 71.4% *β*-asarone content. The present study also revealed that the major constituent *β*-asarone is positively correlated, as mentioned above, with the total amount of essential oil (Table 3).

A previous study showed that the essential oil of *A. calamus* rhizomes collected from Quebec, Canada

Table 3 — Correlation between the total EOs content and main constituents of essential oil of *A. calamus* Rhizomes

	Total	β-asarone	α-asarone	*Z*-methyl isoeugenol	Shyobunone	*Z*-isoelemicin
Total	1					
β-asarone	0.664**	1				
α-asarone	-0.095	-0.272	1			
Z-methyl isoeugenol	0.179	-0.218	-0.366	1		
Shyobunone	-0.445*	-0.628**	0.045	-0.045	1	
Z-isoelemicin	0.405	0.038	-0.167	0.471*	0.048	1

** Correlation is Significant at α=0.05, ** Correlation is Significant at α=0.01, EOs= essential oils

Table 4 — Antioxidant activity of rhizomes essential oils in *Acorus calamus*

	Antioxidant Assay		
Sample name	DPPH assay IC_{50} (µg/mL)	Metal chelating IC_{50}(µg/mL)	FRAP assay RP_{50} (µg/mL)
ac01	28.52±0.029^{e}	35.64±0.344^{d}	36.06±0.732^{h}
ac02	27.37±0.060^{de}	29.57±0.148^{ab}	25.78±0.436^{c}
ac03	46.05±0.147^{j}	135.18±2.059^{ab}	41.63±0.420^{k}
ac04	32.82±0.022^{g}	104.69±2.188^{j}	44.02±1.023^{l}
ac05	40.21±0.237^{i}	55.96±0.437^{h}	42.24±0.852^{k}
ac06	32.53±0.030^{fg}	82.96±0.270^{i}	27.22±0.226^{d}
ac07	61.96±0.552^{l}	57.78±0.072^{h}	33.48±0.522^{g}
ac08	24.63±0.046^{bc}	129.46±3.029^{l}	31.19±0.458^{f}
ac09	29.93±0.433^{ef}	56.47±0.212^{h}	27.95±0.869^{d}
ac10	40.59±0.041^{i}	48.10±0.041^{g}	37.14±0.350^{hi}
ac11	22.28±0.067^{b}	159.26±1.694^{m}	21.41±0.286^{b}
ac12	28.65±0.162^{e}	39.33±0.655^{e}	39.94±0.265^{j}
ac13	45.58±0.208^{j}	29.55±0.363^{ab}	37.75±0.228^{i}
ac14	25.75±0.020^{cd}	43.34±0.138^{f}	27.48±0.122^{d}
ac15	36.65±0.146^{h}	30.92±0.153^{bc}	26.86±0.198^{cd}
ac16	22.27±0.046^{b}	130.59±0.675^{l}	46.05±0.044^{m}
ac17	57.11±0.308^{k}	120.63±0.785^{k}	61.19±0.157^{n}
ac18	32.44±0.031^{fg}	33.92±0.131^{cd}	31.39±0.137^{f}
ac19	34.59±0.096^{gh}	44.16±0.706^{f}	29.42±0.336^{e}
ac20	32.98±0.113^{g}	49.85±0.385^{g}	33.33±0.200^{g}
Catechin*	14.06±0.028^{a}	-	-
Citric Acid*	-	27.43±0.078^{a}	-
BHT*	13.91±0.042^{a}	-	8.96±0.057^{a}

*=Standard antioxidant, (-) = 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).

contained preisocalamendiol (18.0%), acorenone (14.2%), shyobunone (13.3%) and cryptoacorone (7.5%)[30] while the calamus oil extracted from Nepal contained *β*-asarone (78.1–86.9%) as a major constituent[31]. The essential oil composition in the present study is different from those reported in previous studies[32-34]. Previously, the *β*-asarone essential oils from the *A. calamus* have been reported by us for their biological activities like antibacterial and anthelmintic activity[18-19]. However, the study was done in individual accessions, while in present communication we have reported chemical diversity in twenty different accessions along with their *in vitro* antioxidant activity. It has been reported that the essential oils of *A. calamus* can be used as an ideal food additive in place of synthetic ones based on its strong broad-spectrum antifungal activity against food-infesting fungi, antioxidant efficacy, and anti-aflatoxigenicity, non-phytotoxicity as well as non-mammalian toxicity[35].

The antioxidant potential *of A. calamus* exhibited strong to good DPPH scavenging activity in a dose-dependent manner (Table 4). The rhizome essential oil of *A. calamus* exhibited dose-dependent free radical scavenging, and their IC_{50} value was highest in ac11 (22.28 µg/mL) and lowest in ac07 (61.96 µg/mL). However, the IC_{50} value of strong antioxidant BHT (butylated hydroxyl toluene) was 13.91 µg/mL. The metal chelating assay of plant essential oil has

been summarized in Table 4. The essential oil of ac13 exhibited the highest metal chelating assay along with the minimum IC_{50} value (IC_{50} = 29.55 µg/mL), whereas ac11 showed the lowest chelating activity (IC_{50} = 159.26 µg/mL). The reducing power of a compound may also act as a remarkable indicator of its antioxidant potential[36]. In this study, the reducing activities of Fe^{3+} to Fe^{2+} exhibited antioxidant ability in terms of their RP_{50} values (Table 4). All the essential oils exhibited antioxidant activity in a dose-dependent manner compared to the standard antioxidant BHT (RP_{50} = 8.96 µg/mL).

Molecular docking studies

Molecular docking is a technique used in medicinal chemistry and drug discovery to anticipate the interactions between two molecules. Docking has been reported to be useful in drug design and investigating the optimum confirmation of ligand-protein complexes based on their relative orientation. The primary bioactive components of *A. calamus* from various accessions docked with the active site of an enzyme involving the crystal structure of bovine xanthine oxidase (PDB ID: 3NRZ). The binding energy, ΔG, for each bioactive compound against the different protein targets, inhibition constant, Ki, and residual interactions with vanderwaal, pi-alkyl, and pi-sigma interactions are listed in Table 5. Shyobunone, the essential oil component, exhibited the lowest binding energy of -7.04 kcal/mol, followed by (Z)-methyl isoeugenol (-6.23 kcal/mol) and *β*-asarone (-6.0 kcal/mol) with pdb id: 3NRZ, the protein responsible for antioxidant activity. The binding energies of elemicin and *α*-asarone were found to be -5.76 and -5.37 kcal/mol, respectively. It is possible that the significant results observed were due to the synergistic action of the chemical components of *A. calamus* essential oils. There was not just one major compound that was found to be solely responsible for the antioxidant activities, but rather a bioactive mixture of essential oils. There was a direct link found between the computed binding energy, G, and the inhibition constant for each ligand-protein interaction. According to the aforementioned computed results, the main chemical constituents present in *A. calamus* essential oil had the lowest binding energies for major compounds like (Z)-methyl isoeugenol, *β*-asarone, and shyobunone, which were present in all essential oils of different accessions and were consistent with the previously reported *in-vitro* results[12-15]. The strongest ligand-protein interactions, as indicated by the lowest binding energies, are shown in 3D and 2D docked conformations in Fig. 3, together with the residual interactions that constitute vanderwaals, pi-alkyl and pi-sigma interactions, and other interactions. According to the literature, the docking scores predict better outcomes for proteins that bind in the catalytic location with a higher number of hydrogen bonds compared to other interactions[37]. These interactions demonstrated the binding complex's power and catalytic ability.

Table 5 — Binding energy and interactions of tested compounds on interaction with PDB files of target proteins

Compounds	ΔGb (kcal/mol)	Ki (µM)	I. E. (kcal/mol)	Residual interactions		
				Van der Waals	Pi-alkyl	Pi sigma
1. (PDB ID: 3NRZ)						
β-asarone	-6.00	40.24	-7.19	VAL591,TYR592, CYS593,VAL791, VAL764,PHE763, ARG790, GLU1065	ARG793, LYS792	ILE596
α-asarone	-5.37	114.90	-6.57	LYS269,ASP828, GLU831, LEU605, MET826,ARG824, ARG31, ASP594, ARG32	ARG606, CYS825, PRO675, PHE604, LEU41	-
(Z)-methyl isoeugenol	-6.23	26.90	-7.13	ILE787,GLN773, THR1068,ASN1069, GLU1065,LYS754, GLU759,GLU761, ARG786,ASN785	LEU1030, TYR 1024, SER1064 (pi-donor hydrogen bond)	LEU788
shyobunone	-7.04	6.86	-7.94	TYR592,ILE596, CYS593,VAL791, VAL764,ARG790, GLU1065, LYS754	LYS792, PHE763	-
elemicin	-5.76	60.18	-7.25	VAL764,GLU1065, SER1064,GLU759, LYS754, ILE596, PHE763	LYS 792	ARG790 (pi-cation)

Gibbs free energy, ΔG; Estimated inhibition constant, Ki; Intermolecular energy, I.E.; Binding energy, B.E. Root mean square deviation, R.M.S.D.; Residual interactions represent amino acids interactions forming vanderwaals, pi-alkyl, pi-sigma interactions with the ligand.

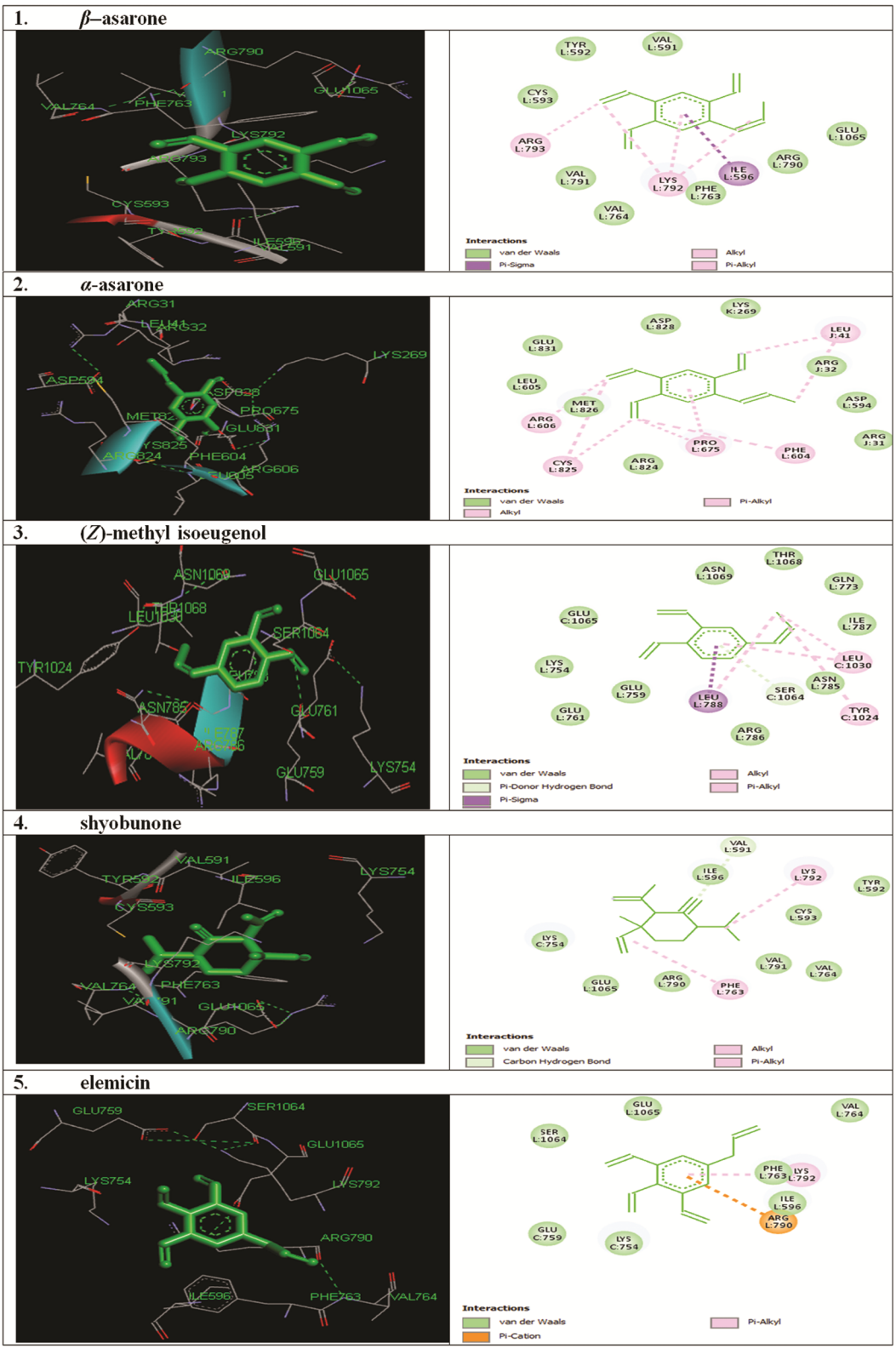

Fig. 3 — Schematic illustration of the three-dimensional (left) and two-dimensional (right) interactions based on the lowest binding energies and strongest interactions between major compounds of *A. calamus* essential oils and the amino acid residues of xanthine oxidase protein with PDB ID: 3NRZ involved in the antioxidant activities.

Conclusion

The present study reveals the chemical diversity and antioxidant activity of the essential oils of *A. calamus* rhizomes collected from different ecological niches of Uttarakhand, India. The development of herbal antioxidants using essential oils enriches the scientific knowledge of traditional folk herbal remedies. Phenyl propanoids, *viz.* *β*-asarone, *α*-asarone, and *Z-isoelemicin,* were identified as active components in *A. calamus*, besides monoterpenoids and sesquiterpenoids like asaronaldehyde, *Z*-methylisoeugenol, shyobunone, *β*-elemene, and kessane. The essential oil analysis of all twenty accessions of *A. calamus* exhibited differences in qualitative and quantitative make-up of the essential oil constituent because of different ecological locations. All the essential oils exhibited good antioxidant activity, possibly due to the presence of dominating phenylpropanoids, mono and sesquiterpenoids or the synergistic effect of other major/minor constituents. Based on the study, it can be inferred that *A. calamus* can play a significant role as a source of natural herbal nutraceuticals and phenylpropanoids after its proper clinical trials.

Conflict of interest

The authors declare no conflict of interest.

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