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Chemical profiling and antioxidant activities of essential oil from the rhizomes of *Acorus calamus* L.

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ABSTRACT

Acorus calamus L. is an indigenous herb in Nepal. It belongs to family Acoraceae and grows in wetland with scented rhizomes. It is also known as Sweet flag in English and commonly as Bojho in Nepal. The present investigation reveals the chemical compositions and antioxidant activity of rhizome essential oil of *A. calamus*. The essential oil of rhizomes of *Acorus calamus* L. from Kaski district, Nepal was extracted by hydrodistillation method and volatile constituents were analyzed using Gas chromatography-Mass spectrometry technique. The antioxidant potential of essential oil was analyzed by 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) scavenging assay. A GC-MS analysis revealed the presence of *β*-asarone (22.38%), *α*-asarone (14.97%), 1-(4,6-dimethoxy-2,3-dimethylphenylethanone (14.24%), Isoelemicin (5.68%), cis-methylisoeugenol (4.26%), *α*-calacorene (4.16%), and other 20 minor components. From DPPH assay, half-maximal inhibitory concentration (IC_{50}) value of essential oil was found to be 109.83 µg/mL. These findings have strengthened the *A. calamus* is good source of compounds like *β*-asarone, *α*-asarone and can be used as potential antioxidants.

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1. Introduction

Acorus calamus L. belongs to the Acoraceae family, is an important medicinal plant of South Asia commonly recognized as Bojho (in Nepali), Sweet flag (in English). It is a well-known herbal drug usually used in conventional medicines [1]. It grows as an herb with thick rhizomes in wetlands, mainly marshes [2]. It is widely distributed in South Asia along with some countries of Europe.

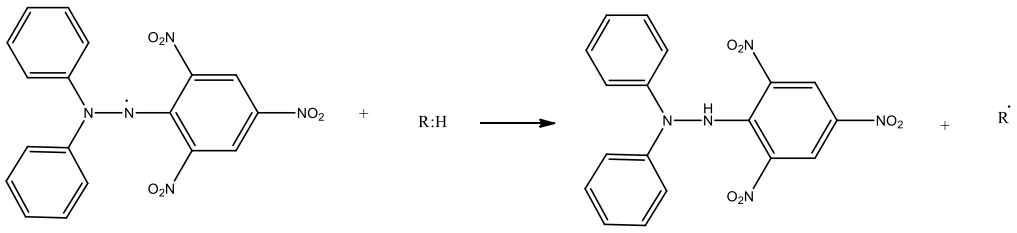
The calamus species are used in the treatment of mental ailments, dysentery, chronic diarrhea, bronchial catarrh and abdominal tumors [3]. More specifically, *A. calamus* is an indigenous herb used in the treatment of fever, cough, bronchitis, inflammation, tumor, skin diseases and some other bacterial and fungal infections [4,5]. As an analgesic, it is used for relief of toothache,

headache, cough, asthma, and bronchitis and as a sedative [6]. Additionally, it has significant antibacterial and antifungal properties [7]. The essential oil from the rhizome of *A. calamus* has been reported to have several biological activities such as antifungal, antibacterial [8] and its ethanolic extract has anti-cellular immunosuppressive properties [9].

Monoterpene hydrocarbons, sequestrine ketones, *α*-asarone (2,4,5-trimethoxy-1-propenyl benzene) and *β*-asarone are the major compounds of rhizome essential oil from *A. calamus* [10]. *β*-asarone (42.4% - 60.7%) as the major constituents followed by *α*-asarone (2.6% - 7.9%) have been reported from India [1]. However, *β*-asarone (40.59% - 76.33%) as the major constituent, *α*-asarone (1.29% - 10.48%) and some other minor compounds have been reported from Nepal [10]. The chemical constituents, yield, and activities are influenced by weather, soil conditions, time of harvest and some other minor factors [11]. Indian origin *A. calamus* has IC_{50} value 475.48 ± 0.08 µg/mL whereas 11.72 ± 0.03 µg/mL for standard [1]. The essential oil of *A. calamus* from different markets of Nepal exhibited the IC_{50}value 312.64±1.14 µg/mL [12].

Antioxidants are the compounds that can slow or retard the oxidation of an oxidizable material, even when used in a small amount (commonly 1−1000 mg/mL) as compared to the amount of material they must protect [13]. The DPPH (2,2-diphenyl-1-picrylhydrazyl) is a well-known radical and by DPPH assay antioxidant potential can be determined. Because of a strong absorption band centered at about 517 nm, the DPPH radical has a deep violet color in solution, and it becomes colorless or pale yellow when neutralized. This property allows visual monitoring of the reaction.

To the best of our knowledge, the chemical profile and antioxidant potential of rhizome essential oil of *A. calamus* from Kaski, Nepal has not been investigated yet. So, the objectives of this research are to find its volatile constituents by GC-MS and its role as an antioxidant.



DPPH, purple Antioxidants DPPH-H yellow

Fig. 1: Mechanism of antioxidants with DPPH radical.

2. Materials and Methods

Plant materials

Rhizomes of *A. calamus* L. were collected from the Togi village of Kaski district, Nepal in September 2018. The plant species was authenticated at National Herbarium and Plant Laboratories, Godavari, Nepal.

Extraction of essential oils

The rhizomes of *A. calamus* L. are washed with clean water and 200 g of it was subjected to

hydrodistillation using the Clevenger apparatus with 600 mL of distilled water. The extraction of essential oil was continued for 6 hours. The vapors of water along with volatile oils were condensed into a liquid by a condenser which was fitted with Clevenger. The essential oil was collected in the measuring tube of Clevenger. Because of immiscible nature, essential oil was separated from water and its volume was noted from which the percentage yield could be calculated. The oil was collected on a small glass bottle and a pinch of anhydrous sodium sulfate was added to remove moisture. Finally, the bottle was sealed, labeled and stored in the refrigerator until further analysis.

Gas chromatography-Mass spectrometry (GC-MS) analysis

The chemical composition of essential oil was analyzed by Gas Chromatography (Shimadzu GC 2010) having an RTX-5 MS column (60 m×0.32 mm×25µm) and using Helium as the carrier gas. The sample (100 µL) diluted with spectroscopic grade hexane in a ratio 1:10 was injected into the GC inlet maintaining constant flow rate of 0.68 mL min^{-1} and purge flow 3 mL min^{-1} in split mode. The initial column temperature was set at 40 ^{o}C. The qualitative analysis of oil was further continued in a Shimadzu GCMS-QP 2010 Plus. The MS Library used for comparison was FFNSC 1.3, NIST 2017. Oil components were identified based on their retention indices (RI) and by comparison of their mass spectral fragmentation patterns.

DPPH assay

This is a quick and easy method to analyze the scavenging potential of antioxidants. Free radical scavenging activity of essential oil was measured by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical, as previously described by Jamuna *et al*. [14]. For this, 100 mL solution of 0.1 mM concentration of DPPH was prepared and a stock solution of essential oil of 1 mg/mL.From the sample stock solution 50, 100, 150, 200, 250 µg/mL solutions were prepared. Then, to the sample solutions of different concentrations, 1mL DPPH solution was added. Finally, the absorbance at 517 nm was taken after incubation at room temperature for 30 minutes. Ascorbic acid was used as the standard for antioxidants.

The percentage of inhibition was calculated by using formula,

$$\% \text{ I} = \frac{\text{AC} - \text{AO}}{\text{AC}} \times 100\%$$

where, A_C = absorbance value of the control (1 mL methanol+1 mL DPPH solution),

A_O = absorbance value of the sample solution, and

I % = percentage of inhibition

The radical scavenging activities of essential oil were expressed in terms of their IC_{50} values.

3. Results and Discussion

Chemical profile

The yellow-colored essential oil was obtained and the percentage yield of oil was 0.9 %. The percentage yield of essential oil in this study somewhat different from the previously published reports from India and Nepal. The Gas Chromatogram of oil showed a total of 29 peaks corresponding to total 29 compounds. The oil was further continued in a Shimadzu GCMS-QP 2010 Plus for the qualitative analysis. The MS Library used for comparison was FFNSC 1.3, NIST 2017, and from it, the 26 oil components were identified based on their retention indices (RI) and by comparison of their mass spectral fragmentation patterns. *β*-asarone (22.38%), *α*-asarone (14.97%), 1-(4,6-dimethoxy-2,3-dimethylphenylethanone (14.24%), Isoelemicin(5.68%), cis-Methylisoeugenol (4.26%), *α*-calacorene (4.16%), are major components and other are minor constituents (less than 3%), shown in table 1. The major components of oil of Indian origin were reported to be *β*- asarone (43.4-60.7%), *α*-asarone (2.6-7.9%), shyobunone (3.4-6.3%) and *β*-isoelemicin (3.2-5.4%) [1]. In the same way, Nepalese origin *A. calamus* has major constituents *β*- asarone and α-asarone are found 40.59% and 1.29% respectively from Banke, 76.33% and 10.41% respectively from Salyan [10]. This deviation in percentage yield and chemical constituents can be explained in terms of

contribution from several factors including age, vegetative cycle stage, climate, seasons, soil composition, altitude, etc.

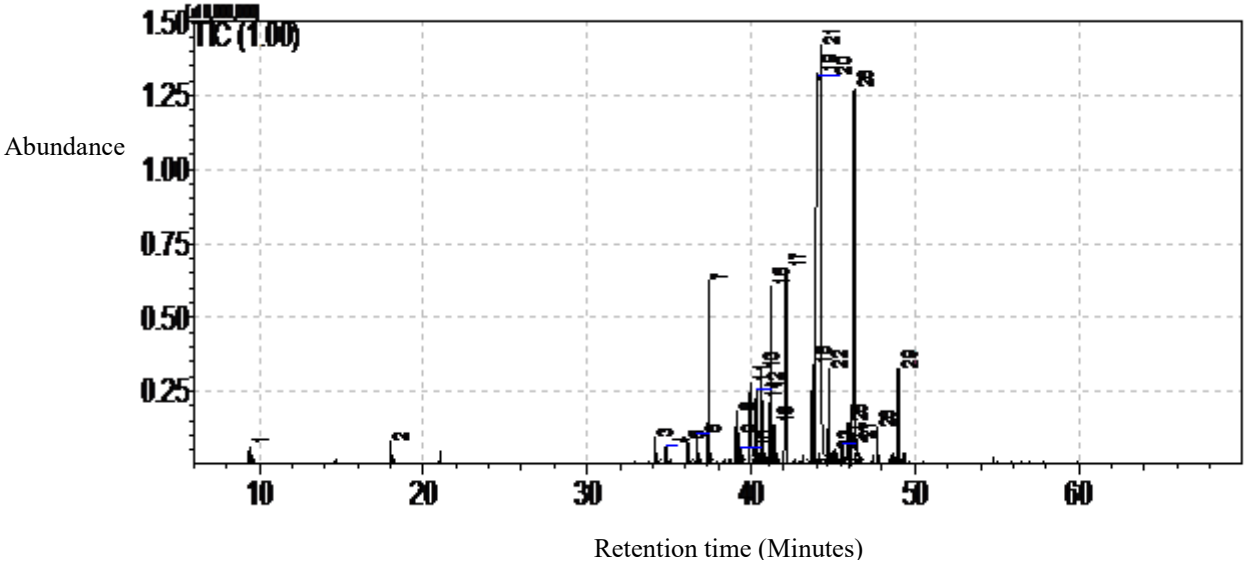


Fig. 2: Gas chromatogram of rhizome essential oil of *A. calamus.*

The abundant constituents *β*-asarone (1,2,4-Trimethoxy-5-[(*Z*)-prop-1-enyl] benzene) and *α*-asarone(1,2,4-Trimethoxy-5-[(*E*)-prop-1-enyl] benzene) are used in killing pests and bacteria [15]. Asarone is not metabolized to trimethoxyamphetamine, which has been reported by online vendors [16]. The Council of Europe Committee of Experts on Flavoring Substances concluded that *β*-asarone is clearly carcinogenic and has proposed limits for its concentration in flavorings such as bitters made from *A. calamus* [17]. *β*-Asarone exhibits anti-fungal activity by inhibiting ergosterol biosynthesis in *Aspergillus niger* [18].

Antioxidant activity

DPPH assay is fundamentally based on the capability of DPPH free radical which is discolored in the presence of antioxidants present its sample. The results of the antioxidant activity of this study are demonstrated below. The IC_{50} values of ascorbic acid and essential oil of *A. calamus* was calculated and shown below in table 2.

The half inhibitory concentration (IC_{50}) of *A. calamus* oil was found to be 109.83 µg/mL whereas that of standard ascorbic acid is 25.38 µg/mL. This implies that *A. calamus* oil has 0.231 times antioxidant potential than that of ascorbic acid. These results are in close agreement with the previous report suggested by *Bhandari et. al.,* [12] which revealed that the antioxidant property of *A. calamus* oil is 0.277 times than that of ascorbic acid. In the same way, this result also correlated with the report suggested by *Parki et al.* [1].

Table 1. The chemical composition of rhizome essential oil of *A. calamus.*

Peak	Retention time	Retention index	Area %	Name of the Compounds	Identification methods
1	9.397	831	0.36	Furfural	RI, MS
2	18.02	1046	0.41	β-Ocimene	RI, MS
3	34.158	1375	0.54	1,3-Dimethyl-8-propan-2-yltricyclo [4.4.0.02,7] dec-3-ene	RI, MS
4	34.794	1398	0.38	1-Ethenyl-1-methyl-2,4-bis(prop-1-en-2-yl) cyclohexane	RI, MS
5	36.138	1419	0.52	Isoledene	RI, MS
6	36.707	1434	0.68	Calarene	RI, MS
7	37.42	1455	4.26	*cis*-Methylisoeugenol	RI, MS
8	37.12	1463	1.59	2,3-5,6-Bis(1,5-octanediyl)-2,5-dibora-1,4-dioxane	RI, MS
9	39.282	1491	0.73	Viridiflorene	RI, MS
10	39.389	1497	0.40	α-Muurolene	RI, MS
11	40.323	1543	2.91	Cedrol	RI, MS
12	40.617	-	2.01	δ-Cadinene	MS
13	40.323	1537	2.21	9- Cedranone	RI, MS
14	40.617	1538	1.18	*(*2S,3S,6S)-6-Isopropyl-3-methyl-2-(prop-1-en-2-yl)-3-vinylcyclohexanone	RI, MS
15	40.676	1544	4.16	α-Calacorene	RI, MS
16	41.203	1550	0.97	Elemicin	RI, MS
17	41.409	1565	5.68	Isoelemicin	RI, MS
18	42.144	1614	2.30	Acorenone *B*	RI, MS
19	43.755	1617	22.38	*β*-Asarone	RI, MS
20	44.101	-	8.19	-	
21	45.182	1634	14.24	1-(4,6-dimethoxy-2,3-dimethylphenyl) ethanone	RI, MS
22	44.284	1635	2.48	Isolongifolol	RI, MS
23	44.747	1659	0.76	-	
24	45.097	1659	0.59	Cadin-4-en-10-ol	RI, MS
25	45.933	1663	1.1	6-Methyl-2-(4-methylcyclohex-3-en-1-yl) hepta-1,5-dien-4-ol	RI, MS
26	46.301	1679	14.97	α-Asarone	RI, MS
27	46.442	1683	0.91	(5-Methyl-8-propan-2-yl-3,4,4a,7,8,8a-hexahydronaphthalen-2-yl) methanol	RI, MS
28	47.723	-	0.9	-	
29	48.981	1771	2.18	4α-hydroperoxy-2,5,5,8α-tetramethyl-4-methylidene-7,8-dihydro-6H-chromene	RI, MS

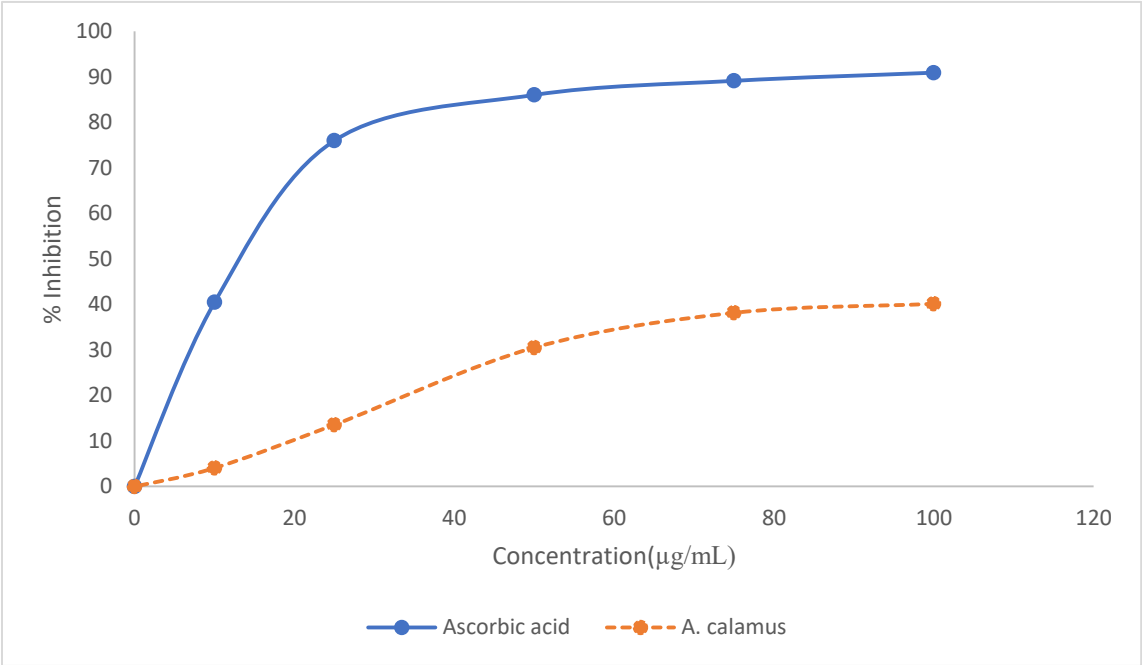


Fig. 3: Percentage Inhibition vs Concentration plot of Ascorbic acid and *A. calamus* esssential oil

Table 2: IC_{50} values of standard ascorbic acid and *A. calamus* essential oil.

S.N	Name	IC_{50} (µg/mL)
1.	Standard Ascorbic acid	25.38
2.	The essential oil of *A. calamus*	109.83

4. Conclusions

The rhizome essential oil of *A. calamus* from Kaski, Nepal was analyzed by GC and GC-MS and was found to be rich of *β*-asarone, *α*-asarone, asarone, *α*-calacorene and cis-Methylisoeugenol. Furthermore, essential oil of rhizome of *A. calamus* is a good source of antioxidants.

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