VOLUME 17 January 2020 eISSN : 2382-5340 ISSN : 2091 - 0762

Journal of Physical Sciences

BIBECHANA

Editor-in-Chief

Devendra Adhikari

Professor, Physics

MMAMC, T.U.

Published by

Department of Physics

Mahendra Morang Adrash Multiple Campus

T.U., Biratnagar

www.nepjol.info/index.php/BIBECHANA

BIBECHANA
ISSN 2091-0762 (Print), 2382-5340 (Online)
Journal homepage: http://nepjol.info/index.php/BIBECHANA
Publisher: Department of Physics, Mahendra Morang A.M. Campus, TU, Biratnagar, Nepal

Phytoconstituents and biological analysis of *Acorus calamus* rhizome of Sindhupalchowk District, Nepal

Shanta Pokhrel*, Kalpana Chaulagain
Department of Chemistry, Tri-Chandra Multiple Campus, Tribhuvan University, Nepal
∗E-mail: shantabhattarai2014@gmail.com

Article Information
Received: October 25, 2019
Accepted: December 2, 2019

Keywords:
Acoruscalamus rhizome,
Antibacterial activity,
Antioxidant activity

ABSTRACT
Phytochemical and biological activities of methanolic and hexane extract of *Acorus calamus* rhizome were carried out. Phytochemical analysis showed the presence of flavonoid, glycoside, saponin, resin and carbohydrates which are responsible for the antibacterial activities. The antibacterial potential was studied against *Staphylococcus aureus* (gram positive bacteria) and *Escherichia coli, Salmonella typhi* (gram negative bacteria) using Agar Well Diffusion Method. The activity was shown by both methanolic and hexane extract of *Acorus calamus* rhizome against both Gram positive and Gram negative bacteria. Antioxidant activity of methanolic extract was evaluated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity showed potent antioxidant activities with IC_{50} value 3.74 µg/mL slightly higher than standard ascorbic acid (IC_{50} = 3.56 µg/mL).

DOI: https://doi.org/10.3126/bibechana.v17i0.24674

1. Introduction

Acorus calamus Linn. (familyAraceae) commonly referred to as calamus or sweet flag, **Bojho** in Nepali, is a well-known medicinal plant.The rhizomes were utilized extensively by the Chinese, Indians and American Indians as well as by other cultures, and many of these uses continue to this day [1] including in Nepali traditional medicine [2]. People of Far Western Region (Baitadi and Darchula) of Nepal utilize the juice of the *Acorus calamus L*. rhizome as an anthelmintic and it is chewed to treat coughs, cold and sore throat [3,5].

Medicinal plants, possessing secondary metabolites, are potential sources of curative drugs with the very long list of chemicals and its curative nature. Plant products are used as main source of medicine throughout the world for treating various human ailments [6]. The interest to study the plants is because of their medicinally and pharmacologically important active ingredients. Plant produces a plethora of natural products, such as alkaloids, flavonoid, glycoside, steroid, saponin and resin which has often been correlated with medicinal and pharmacological properties of the plants. The

rhizome, roots and essential oil distilled from have been reported to possess several important biological activities including antimicrobial [7-10], anticellular and immunosuppressive [11]. The aim of present study was to investigate the phytochemical constituents of extracts with methanol and hexane solvents, their antioxidant activity and antibacterial activities of extract *Acorus calamus* rhizomes against different strains of bacteria.

2. Experimental

Collection and preparation of plants extracts

The *Acorus calamus* rhizome was collected from the Sindhupalchowk, Nepal. The collected rhizomes were washed by fresh water to remove other contamination like dust, soil, insect larvae, eggs etc. then clean plant were air dried in shade for about a month, then obtained dried leaves were grinded to power and store in clean plastic bag. The powdered form (60 g) of plants rhizomes was extracted by Soxhlet method in n-Hexane, and methanol solvents (250 mL) at room temperature. The solvent used for Soxhlet extraction, which has undergone several cycle for about 8-10 hours was collected and obtained solvents were individually concentrated by rotary evaporator under reduced pressure, maintaining temperature lower than the boiling point of respective solvents used. These extracts were stored in the refrigerator until use in vile tubes.

Phytochemical screening

Analysis of crude methanolic and hexane extracts of *Acorus calamus* rhizome for various phytochemical constituents were carried out using standard protocols [12]. The analysis of the presence of main group of natural constituents present in the different plant extract was done by the color reaction using different specific reagents.

Antibacterial activity

Antibacterial activity of the plant extract was performed by agar well diffusion method. Effectiveness of antimicrobial substance was evaluated by determination of zone of inhibition (ZOI) [13]. The microbial strains *staphylococcus aureus cocci* ATCC 25923 (gram-positive) and *Escherichia coli* ATCC 25922 and *Salmonella typhi* (gram-negative) bacteria were obtained from MED-MICRO Nepal Lab, Kathmandu. The 50 µL of the working solution of the plant extract, DMSO as negative control (NC) and 25 µL of Ofloxacin (antibiotic- ear and eye drop) as positive control (PC) at the same time in separated well (6 mm) were loaded into the respective wells with the help of micropipette. The plates were then left for half an hour with the lid closed so that the extract diffused into media. The plates were incubated overnight at 37 ℃. After 24 hours of incubation, the plates were observed for the presence of inhibition of bacterial growth that indicated by a clear zone around the wells. The size of the zone of inhibition was measured and the antibacterial activity expressed in term of the average diameter of zone of inhibition in millimeters. The ZOI were measured with the help of millimeter ruler and mean was recorded.

Antioxidant activity

The free radical scavenging activity of samples and standard ascorbic acid solution in methanol was determined based on their ability to react with stable 1, 1-diphenyl-2-picrylhyrazyl (DPPH) free radical [14].

The IC_{50} (50% inhibitor concentration) value is indicated as the effective concentration of the sample that is required to scavenge 50% of the DPPH free radicals. IC_{50} values were calculated using the inhibition curve by plotting extract concentration *vs* the corresponding scavenging effect. Different concentrations of test samples of 20, 40, 60, 80 and 100 µ/mL were made from stock solutions. Then 2 mL of all the concentration of test solution were mixed 2 mL of 2 mM DPPH solution. The test tubes were shaken vigorously for the uniform mixing then the solutions was kept for 30 minutes in dark place at room temperature. The control was prepared as above but without the plant extracts (methanol + DPPH). After 30 minutes absorbance of the entire sample was measured at 517 nm using a UV-visible spectrophotometer. The antioxidant activity of the samples was expressed as IC_{50} (inhibitory concentration), which was defined as the concentration (in µg/mL) of sample required to inhibit the formation of DPPH radicals by 50%. Ascorbic acid was used as positive control. Free

radical scavenging activity was calculated by using following equation (1):

$$\text{Radical Scavenging (\%)} = \frac{A_0 - A_s}{A_0} * 100 \quad (1)$$

where, A_o = Absorbance of the control (DPPH solution + methanol)

A_s = Absorbance of test sample

The % scavenging was then plotted against concentrations used and from the graph IC_{50} was calculated.

3. Results and Discussion

Phytochemical screening

The different phytochemicals in the crude methanol and n-hexane extracts were identified by the color reaction with different reagents. The results obtained for methanolic and n-hexane extract of the plant is tabulated below (Table 1).

A preliminary study has reported the rhizome extracts contains large number of bioactive secondary molecules like alkaloids, tannins, carbohydrates, flavonoids, glycoside (Table-1). The presence of these components in this species is an indication that it may have some medicinal potential. The rhizome of *Acorus calamus* are used traditionally for treatment of emetic, stomachic, dyspepsia, colic, remittent fevers, nerve tonic, in bronchitis, dysentery in children, insectifying and in snake bite [6]. These results are consistent with reported in the literature [6] with slight difference. This is due to the variation in altitude of plants and different environmental conditions.

Table 1: Phytochemical screening of various extracts of *Acorus calamus.*

S.N.	Phytochemicals	n-Hexane Extracts	Methanol Extracts
1.	Alkaloids	-	+
2.	Flavonoids	+	+
3.	Steroids	-	+
4.	Glycosides	-	+
5.	Tannins	+	+
6.	Carbohydrates	-	-
7.	Phenol	-	-

(+) sign indicated the presence of phytochemicals whereas (-) sign indicated the absence of phytochemicals

Antibacterial assay

The diameter of zone of inhibition (ZOI) produced by the plant extracts on particular bacteria was measured for the estimation of their antibacterial activity. The Methanol and hexane extracts of rhizome of *Acorus calamus* were studied. Results obtained from the antibacterial analysis of different extracts are tabulated (Table 2).

Figure 1 (a, b, c) show the results of antibacterial activity of methanolic and hexane extract of *Acorus calamus* rhizome. It is clear that the extract shows higher antibacterial activity against *S. aureus* than the *Salmonella typhi* and *E. coli.* Hexane extracts shows higher antibacterial activity against *S. aureus* than the *Salmonella typhi* and *E. coli* gives ZOI value 15 mm, 11 mm and 11 mm respectively in 50% concentration. But in crude hexane extract of *Acorus calamus* only show antibacterial activity against *S. aureus* gives ZOI value 14 mm and others do not show antibacterial activity.

Table 2: Antibacterial analysis of methanol and hexane extracts of *Acorus calamus* rhizome.

S.N.	Plant extract (Solid)	Bacteria	ZOI (mm) of Extracts at Concentration 100 mg/ mL		ZOI (mm) of Description Ofloxacin as Control at 100 mg/mL
			50%	**Crude**	
1.	**Hexane**	*E. coli*	11	0	30
		S. aureus	15	14	28
		S. typhi	11	0	26
			10%	**1%**	
2.	**Methanol**	*E. coli*	10	0	30
		S. aureus	12	10	28
		S. typhi	0	12	26

Methanol extracts of *Acorus calamus* against *Staphylococcus aureus and Salmonella typhi* gives ZOI value 10 mm and 12 mm respectively in 1% concentration. But in 10% concentration of methanol extracts against *Staphylococcus aureus and*

Escherichia coli gives ZOI value 10 mm and 12 mm and other are inactive. Hexane extract shows highest zone of inhibition against *Staphylococcus aureus* 15 mm in 50% concentration than that in methanol (12 mm in 10% concentration). According to Chhatopadhyay *et al.* the rhizome of *A. calamus* exhibited 16 mm zone of inhibition, which support the present finding [16].

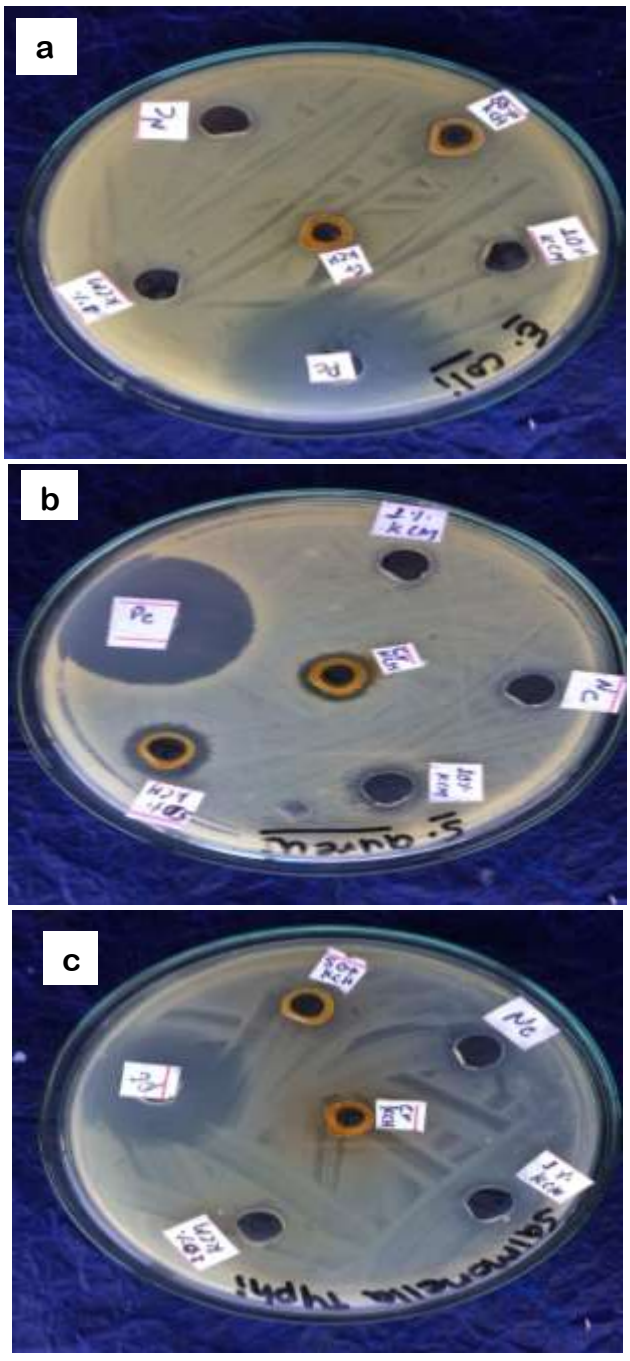

Fig. 1: Antibacterial screening of methanol and hexane extract of *Acorus calamus* rhizome against (a) *Escherichia coli*, (b) *Staphylococcus aureus,* (c) *Salmonella typhi.*

Antioxidant activity

The antioxidant activity of the methanolic solution of different samples were explored by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay.

DPPH assay

Antioxidants are the compounds which terminate the attack of reactive species and reduce the risk of disease [16]. Most of the plants have antioxidant property. In this study, the antioxidant activity of each plant extract was measured by using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), is a very stable free radical with purple color. It is scavenging by antioxidants through the donation of proton forming the reduced DPPH.

The DPPH radical assay was performed for methanol extract of *Acorus calamus* rhizome by using ascorbic acid as standard. In this assay the mixture of DPPH with different concentration of extract solution and ascorbic acid were separately incubated at room temperature and absorbance was recorded at 517 nm by spectrophotometer. Calibration curve was constructed by measuring the absorbance of ascorbic acid in order to calculate IC_{50} value. The value obtained from plant extract was compared with ascorbic acid. The observed absorbance with the different concentration of ascorbic acid was plotted in the graph as shown below.

The comparison of percentage radical scavenging at different concentration between plant extract and ascorbic acid as standard was shown in Table (3) and Figure (2).

The comparison of percentage radical scavenging at different concentration between plant extract and ascorbic acid as standard was shown in graph.

The antioxidant potential is in an inverse relation with IC_{50}value, lower value of IC_{50} indicates high antioxidant potential. The IC_{50} values of the plant extracts along with the standard ascorbic acid is tabulated below. It is found that the IC_{50} value of *Acorus calamus* rhizome in methanol extract is 3.74 µg/mL and that of standard ascorbic acid is 3.56 µg/mL. By comparing the reference standard ascorbic acid, the methanol extract showed less

antioxidant activity. Kumar reported that the chloroform extract of *Acorus calamus* showed more antioxidant property and is potent DPPH free radical scavenger [17]. World Health Organization (WHO) mentioned that medicinal plants would be the best source to a variety of drugs. About 80% of individuals from developed countries use natural medicine. Alkaloids and tannin have antioxidant capacities because they have been found to possess antioxidant and free radical scavenging effect [17].

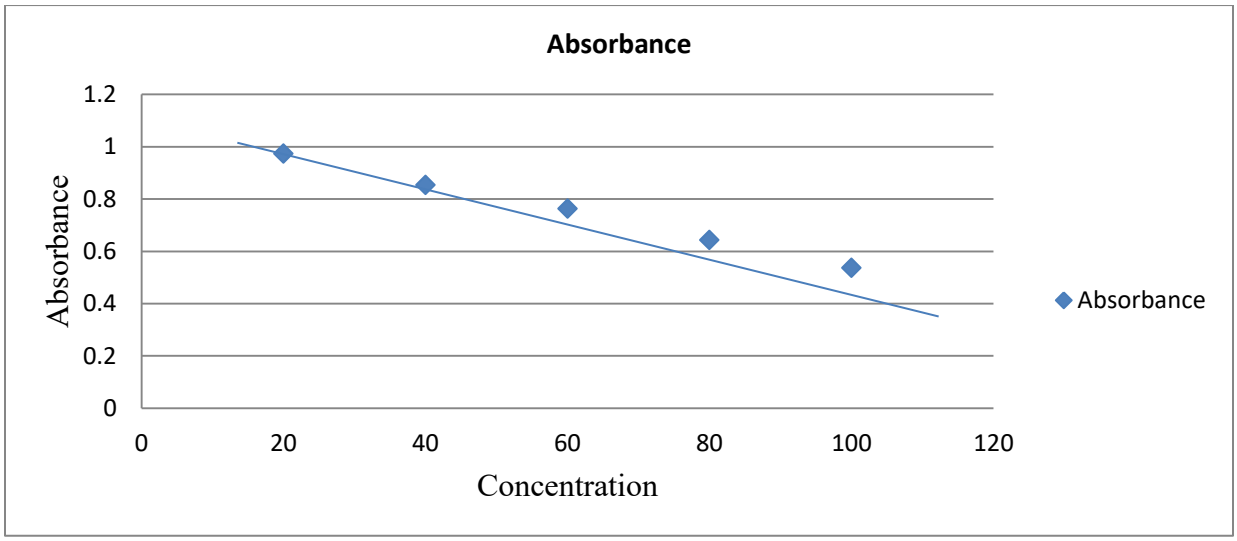

Fig. 2: Graph of absorbance *vs* concentration of ascorbic Acid.

Table 3: Percentage of radical scavenging with different concentrations

	Radical scavenging (%)				
Sample	Concentration (µg/mL)				
	20	40	60	80	100
MR	13.21	19.38	27.50	58.66	72.59
Ascorbic Acid	13.12	23.75	31.87	42.58	52.05

where,
MR =Methanol Rhizome Extract

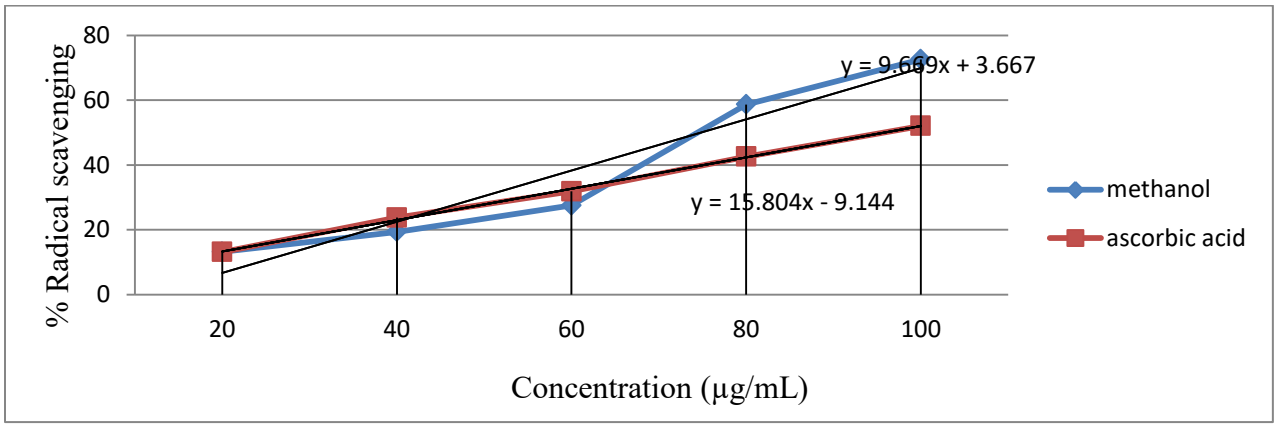

Fig. 3: A plot of percentage radical scavenging activity *vs* concentration of methanol extract and ascorbic acid

Table 4: Comparison of IC_{50} values of methanol extract and ascorbic acid.

S. No	Sample	IC_{50}(µg/mL)
1.	Standard Ascorbic acid	3.56
2.	Methanol rhizome extract	3.74

4. Conclusion

Phytochemical screening of different rhizome extract of *Acorus calamus* showed the flavonoids and tannins were present in both the extracts while alkaloids, glycosides and steroids were only in methanol extract. The phytochemical screening and antibacterial assay of the plants indicated positive application of these plant extracts for medicinal use wherein their antioxidant property is an added advantage. More studies on this plants help to study the use of the plant.

Acknowledgements

Authors are thankful to Department of Chemistry, Tri-Chandra Multiple Campus, Tribhuvan University, Kathmandu, Nepal for providing laboratory facilities to conduct experimental work. We are thankful to Med-Micro Nepal Lab, Thapathali, Kathmandu, Nepal for antibacterial tests.

References

[1] T. J. Motley, The ethnobotany of sweet flag, *Acorus calamus* (Araceae), Econ. Bot. 48 (1994) 397-412.

[2] Anonymous, Thai Herbal Pharmacopoeia Volume II, Department of Medical Sciences, Ministry of Public Health, Thailand, Prachachon Co., Ltd., Bangkok, 2000.

[3] P. Satyal, P. Poudel, A. Poudel, N. S. Dosoky, D. M. Moriarity, B. Vogler, and W.N. Setzer. Chemical compositions, phytotoxicity and biological activities of *Acorus calamus* essential oils from Nepal, Nat Prod Commun. 8 (2013) 1179-1181.

[4] R. C. Uprety, H. Asselin, E. K. Boon, S. Yadav, K. K. Shrestha, Indigenous use and bio-efficacy of medicinal plants in the Rasuwa District, Central Nepal, J Ethnobiol Ethnomed. 13 (2011) 463-492.

[5] S. Joshi, Bashyal S, Study on the chemical constituents and antibacterial activity of essential oil of *Acorus calamus L*. Rhizomes of Rupendehi district (Nepal), J Inst Sci Technol 23 (2018) 57-60

[6] R. Balakumbahan, K. Rajamani and K. Kumanan, *Acorus calamus*: An overview, J Med Plant Res 4 (2010) 2740-2745,

[7] L. J. McGaw, A. K. Jager, J. Vanstaden, Isolation of β-asarone, an antibacterial and anthelmintic compound, from *Acorus calamus* in South Africa, South African J. Bot. 68 (2002) 31-35

[8] H. S. Lee. Pesticidal constituents derived from piperaceae fruit, Agric Chem Biotechnol, 48 (2005) 65-74

[9] J. Y. Lee, J. Y. Lee, B. S. Yun, B. K. Hwang, Antifungal activity of β-asarone from rhizome of *Acorus gramineus*, J. Agric. Food Chem. 52 (2004) 777-780.

[10] S. Phongpaichit, N. Pujenjob, V. Rukachaisirkul, M. Ongsakul, Antimicrobial activities of the crude methanol extract of *Acorus calamus* Linn., Songklanakarin J. Sci. Technol. 27 (2005) 517-523.

[11] S. Mehrotra, K. P. Mishra, R. Maurya, R.C. Srimal, V.S. Yadav, R. Pandey, V. K. Singh, Anticellular and Immunosuppressive properties of ethanolic extract of *Acorus calamus* rhizome, Int. Immunopharmacol. 3 (2003) 53-61.

[12] I. Culie, Methology for analysis of vegetable drugs, practical manuals on industrial utilization of medicinal and aromatic plant, Bucharest, Phytochemistry 63 (1982) 97-104.

[13] M. Valko, D. Leibfritz, J. Moncol, M. T. D. Cronin, M. Mazur, and J. Telser, Free radicals and antioxidants in normal physiological functions and human disease, Int. J. Biochem. Cell Biol. 39 (2007) 44-84.

[14] G. Funde, Phytochemicals evaluation, anticancer, antioxidant and antimicrobial activity of *Acorus calamus* different solvent extracts, J Chem Pharm Res 7 (2015) 495-504.

[15] M. Saxena, J. Saxena, Phytochemical screening of *Acorus calamus* and *Lantana camara*, Int Res J Pharm 3 (2012) 324-326.

[16] M. Chhatopadhyay, S. R. Ali and S. Aboud, Antimicrobial activities of aqueous and methanolic extracts from *Acorus calamus* used in the treatment of wound infection isolates. Int J Infect 2 (2010) 18-25.

[17] S. Kumar, Studies on antioxidant and antimicrobial activity of *Acorus calamus* and *Myristica fragrans*. Int. J. Appl. Res. 2 (2016) 292-297.