JNCS
Journal of Nepal Chemical Society June/July 2022, Vol. 43, No. 1, 36-43
ISSN: 2091-0304 (print)
DOI: https://doi.org/10.3126/jncs.v43i1.46962
RESEARCH ARTICLE

Biological and Chemical Studies of Essential Oil and Extracts of Rhizome of *Acorus calamus* Linn

Rajesh Timilsina, Pooja Tandukar, Ishwor Pathak*

Department of Chemistry, Amrit Campus, Tribhuvan University, Kathmandu, Nepal

**Corresponding Email:* pathakishwor14@gmail.com

Submitted: **12 June 2022**, Revised **25 June 2022**, Accepted **26 June 2022**

Abstract

The essential oil (EO)of the rhizome of *Acorus calamus* Linn. was isolated by using a clevenger apparatus and extractswere prepared by cold percolation technique using the solvents hexane and methane. The chemical constituents of EOwere analyzed by Gas Chromatography-Mass Spectroscopy (GC-MS) analysis. A total number of nine chemical compounds were identified and quantified occupying 100% of the total oil composition. The major chemical constituent was reported to be β-asarone (84.87%). Acid value, saponification value, and iodine number of the oil were measured and found to be 0.24 mg KOH/g, 0.42 mg KOH/g, and 31.75 g I_2/100gm, respectively. The antibacterial activity of the hexane and methanol extract was examined against two bacteria by the agar well diffusion method. The hexane extract showed antibacterial activity against *E. coli* with a zone of inhibition (ZOI) of 10 mm, and*B. subtilis* with ZOI of 7 mm. The methanol extract showed antibacterial activity against *B. subtilis* only, with a ZOI of 4 mm. Hexane and methanol extract also showed significant antifungal activity against fungi *C. albicans* with a ZOI of 6 mm and 5 mm, respectively. DPPH assay showed that the percentage of free radical scavenging activity increased with an increase in the concentration of the extract. The total phenolic content of the methanol extractof *A. calamus* was found to be 48.36 mg/g GAE.

Keywords: *A. calamus*, essential oil, antimicrobial, antioxidant, total phenolic content

Introduction

Natural products are organic compounds isolated from natural resources by the pathways of primary or secondary metabolism[1, 2]. Primary metabolites have an intrinsic function that is essential for the survival of the organism. Sugar, fatty acids, acetyl coenzyme A, protein, etc. are primary metabolites. Secondary metabolitessuch as flavonoid, terpenoid, alkaloid, and essential oil have an extrinsic function that mainly affects other organisms outside of the producers[2]. These secondary metabolites are not essential to survival but do increase the competitiveness of the organism within its environment [3,4].

Plant-derived compounds as potent drugs have been a part of human evolution and healthcare for thousands of years. The studies have shown that these compounds, especially secondary metabolites have anticancer, antibacterial, analgesic, antitumor, anti-inflammatory, antiviral, and many other activities to a greater or lesser extent [4]. Distinguished examples of these phytochemical compounds include flavonoids, phenols, glycosides, saponins, tannins, alkaloids, terpenoids, and some other endogenous metabolites. Plants have become more important with time due to the presence of bioactive compounds as phytochemicals ans EOsthat could lead to a potenitailapotential drug discovery [4, 5]. EO is natural oil typically obtained by distillation and hascharacteristicsodor of the plant

or other source from which it is extracted. They are volatile and do not leave permanent stains on filter paper [6, 7]. The important role of EO in the plant is to act as a defense organ and helpsignaling processes [7, 8]. For example, EOs take part in the action against micro-organisms, insects, and herbivores, and attract insects for their pollination, for dispersal of seeds,and also attract animals for the same purposes [9, 10, 11, 12].

Among thousands of medicinally important plants, *Acoruscalamus* (L), garnered interest due to its intrinsic therapeutic values. It is commonly known as a sweet flag and the local name (in Nepal) is "*bojho*" which belongs to the family Acoraceae. It is distributed over the world in subtropical and temperate regions. It is native to Asia and also present in Africa as well as America. In Nepal, it is mostly present in the terai and hilly regions. It is mostly distributed in damp and marshy places [12]. It is anherbaceous perennial plant with a rhizome that is long indefinite branched, smooth, brownish or pinkish, or pale green [12].The rhizomes are used for numerous medicinal purposes. The herb is used both internally and externally. In rheumatism, rheumatic fever, and inflamed joints, the paste applied externally alleviates the pain and swelling. The most known and basic susceptibility testing can be used for drug discovery, epidemiology, and prediction of therapeutic outcomes [13]. Most plants, for the protection against pathogens, produce and exude several secondary metabolites, which play an important role as a defense mechanism against various organisms [14-16]. The plant used for traditional medicine generally contains several compounds that may be a potential natural antimicrobial combination and which may serve as an alternative, effective, cheap, and safe antimicrobial agent for treatments of common microbial infections [17]. The potential of the plant extracts to inhibit the oxidation processes can be determined mostly by the DPPH assay method [18, 19]. *A.calamus* was characterized by a higher percentage of β-asarone which was the main compound followed bya higher percentage of camphene enriched (E)-ocimene, camphor, calarene, α -selinene, andα-cadinol. β-asarone is a chemical compound of the phenylpropanoid class [20]. There are two isomers, α (or trans) and β (or cis). As volatile fragrance oil, it is used in killing pests and bacteria. Some previous reports showed the antibacterial, and antifungal activity along with antioxidant activity by the EO and extract of the plant from different foreign countries, however, not much detailed research has been done on the *A. calamus* found in Nepal[21-30].

Herein, we have isolated the EO and prepared the hexane and methanol extracts of the *A. calamus*. Chemical constituents of the EO were evaluated by GC-MS analysis. Furthermore, acid value, saponification value, and iodine number of the oil were measured. Similarly, antimicrobial test, antioxidant activity test, and total phenolic content of the extracts were evaluated.

Materials and Methods

Plant materials

The rhizomes of *A. calamus* Linn. were collected preferentially from Kathmandu valley (Dharmasthali) in September 2019 and peeled out by a conventional mechanical procedure. Then the peel was weighed before subjecting to the Clevenger apparatus for EO extraction and the cold percolation technique for extraction.

Isolation of essential oil

The rhizome of *A. calamus* was loaded in a dry and clean round bottom flask and added an appropriate volume of distilled water to immerse the peels completely. The Clevenger apparatus was appropriately fitted with a condenser and the contents were heated at the temperature of 80-100 °C for two to three hours per sample. The distillate was collected and condensed.The presence of the oil in the condensate was confirmed by the cloudy appearance on the top of the distillate. The oil thus obtained was collected and subjected to further analysis.

Percentage yield

Rhizome of *A. calamus* was weighed before employing it into the Clevenger apparatus and the amount of oil extracted was measured. The percentage yield of extracted essential oil was calculated by using the formula:

$$\text{Percentage yield} = \frac{\text{Weight of the oil extracted}}{\text{Weight of raw material used to extract oil}} \times 100\ \%$$

GC-MS analysis

GC-MS analysis was carried out inthe Natural Product Research Laboratory, Department of Plant Resources, Thapathali, Kathmandu, Nepal. The type of detector column was RTX-5-MS with a dimension of 60m × 0.32 mm × 0.25 µm. The test method was DPR/7.2/01 and FFNSC 1.3, NIST 2017 library was used for analysis.

Acid value

The acid value of essential oil was calculated by following the standard protocol[31].

$$\text{Acid value} = \frac{56 \times 0.2 \times V}{1000 \times W}$$

where,

W = weight of fat or oil taken

V = volume of 0.2N KOH required to neutralize the fatty acid

Saponification value

The saponification value of essential oil was calculated by using the formula [31].

$$\text{Saponification value} = \frac{127 \times (V2\text{-}V1)}{1000 \times M}$$

where,

W = Weight of fat or oil taken

V = Volume of 0.5N oxalic acid required to neutralize the fatty acid

Iodine number

The iodine number was calculated by the formula [31].

$$\text{Iodine number} = \frac{127 \times (V2\text{-}V1)}{1000 \times M}$$

where,

V2 = Volume of the thiosulphate solution in a blank titration

V1 = Volume of the solution used after halogenation of the sample

M = Mass of fat or oil used

Experimental procedure for plant extract

The collected plant materials were dried under shade for seven days. The dried materials were grinded to powders and the obtained powder waspercolated in hexane and methanol separately (for three days) for the extraction process. After then, the filtration process was carried out and crude extracts were extracted by using a Rota-evaporator. The process was continued until the extract become crude.

Antimicrobial activities test

The culture broth of each strain (100 µL) was plated on a nutrient agar plate and kept for 15 min at 37 °C. After 15 minutes of incubation at 37 °C, 10 µL of the sample of *A. calamus*, methanol, and hexane extract was pipetted and dispensed on a filter paper disc kept on the nutrient agar plate and incubated overnight at 37 °C. After then, the antimicrobial activities of the *A. calamus* extract against *E. coli*, *B. subtilis*, and *C. albicans* strains were investigated the next day by analyzing the zone of inhibition [31].

DPPH scavenging activity

DPPH scavenging activity was assessed for the determination of the antioxidant power of extract of *A. calamus*. It was performed by measuring the absorbance of DPPH solution by UV- Visible spectrophotometer (BioTek, EPOCH 2). To measure the absorbance of differently concentrated solutions, extract samples were stored in dark at 4 °C for half an hour. Then the absorbance of the solution was measured at 517 nm. Before measuring the absorbance of the sample solutions, the absorbance of the blank solution was taken. Then the percentage inhibition was calculated by the formula:

$$\text{Percentage radical inhibition} = \frac{\text{Absorbance of blank -Absorbance of the sample}}{\text{Absorbance of the blank}} \times 100\%$$

Total phenol content

The total phenol content of methanol extract of *A. calamus* was determined by employing the Folin-Ciocalteau reagent as the oxidizing agent and gallic acid as standard.Thephenolic content in extract was expressed as milligram of gallic acid equivalent per gram of dry weight (mg GAE/g) of extract. Plant extract of different concentration solutions was taken

in a test tube. Folin-Ciocalteau (diluted 10 times with water) reagent solution was added into the same test tube. Sodium carbonate (7.5%) solution was also added to the same test tube. The test tube was incubated for 30 minutes at 40°C in a water bath to complete the reaction. Then, the absorbance of the resulting blue solution was measured at 760 nm using a spectrophotometer against a blank containing all reagents except the standard solution [25].

Results and Discussion

Chemical profile

The percentage yield of the sample employed for the extraction of essential oil was found to be 0.45% (V/W). The oil was cloudy white-colored viscous liquid floating as distillate in the Clevenger apparatus and the color slightly changed to pale yellow on cooling.The essential oil was analysed by GC-MS analysis which showed eleven compounds. The major compound present in the oilwas β-asarone (84.87%).

Table 1: *Different compounds found (by GC-MS) in the EO fromthe rhizome of A. calamus*

Peak No.	Retention time	Area %	Name
1.	37.387	2.54	β -methyl isoeugenol
2.	39.099	0.65	Caryophyllene oxide
3.	39.930	1.53	6-Epishyobunone
4.	40.038	0.56	β - Guaiene
5.	42.108	1.83	β - Isoelemicin
6.	43.729	0.88	Eudesma -4,7-dien-1.beta.-ol
7.	44.063	84.87	β - Asarone
8.	46.187	4.63	α - Asarone
9.	46.386	0.53	-
10.	47.393	1.33	-
11.	48.421	0.64	3-(2-Isopropyl-5-methyphenyl)-2-methyl-1-propanol

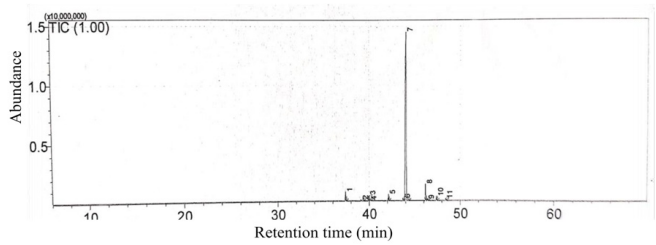

Figure 1: *Gas chromatogram of essential oil of rhizome of A. calamus*

The high abundance of β-asaronealong with the presence of other constituents in minor quantities is in accordance with the reports of previous findings. Literature showed that the β – asaroneis a chemical compound of the phenylpropanoid class with two isomers, α (or trans) and β (or cis) [29].β-asarone is an active bioactive compound that is used as a flavoring agent in the food and beverage industries. It is also used to treat mental disorders, prevent epilepsy, fight Alzheimer's diseases and relieve digestive troubles. As a volatile fragrance oil, it is used in killing pests and bacteria [29, 30, 32, 33].

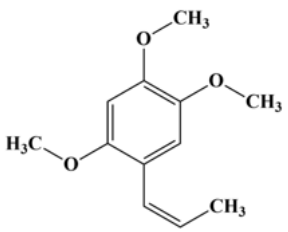

Figure 2: β – Asarone

Acid value, Saponification value, and Iodine number

The acid value of the essential oil was found to be 0.24 mg KOH/g. The acid valuemeasures the number of carboxylic groups and determines whether the oils are safe for human use or not. Similarly, the saponification value of the essential oil was found to be 0.42 mg KOH/g.Acid value and saponification values are in agreement with the reported value of the Chinese *A. Calamus*[29]. The value of the saponification number gives an idea about the average molecular weight of all the fatty acids in the oil as triglycerides [34, 35].Iodine numbers are often used to determine the amount of unsaturation in fatty acids. The iodine number of the *EO* was found to be 31.75 g I2/100 g which is almost comparable with the previous findings[29, 35].

Plant extracts

Antimicrobial activity

The results of antimicrobial activity of hexane and methanolextracts of *A. calamus* in terms of zone of inhibition are shown in tables 2 and 3, respectively.*A. calamus* extracts showed good antimicrobial activity against different types of bacteria. It was highly

effective against gram-positive bacteria than gram-negative bacteria.

Table 2: *Antimicrobial activity of hexane extract of A. calamus*

S.N	Micro-organisms	Reference culture	ZOI (mm) 400 mg/mL	Negative control (mm)
1.	*Bacillus subtilis*	ATCC 6051	7	0
2.	*Escherichia coli*	ATCC 8739	10	1
3.	*Candida albicans*	ATCC 2091	6	0

Table 3: *Antimicrobial activity of methanol extract of A. calamus*

S.N	Micro-organisms	Reference culture	ZOI (mm) 400 mg/mL	Negative control (mm)
1.	*Bacillus subtilis*	ATCC 6051	4	1
2.	*Escherichia coli*	ATCC 8739	2	2
3.	*Candida albicans*	ATCC 2091	5	1

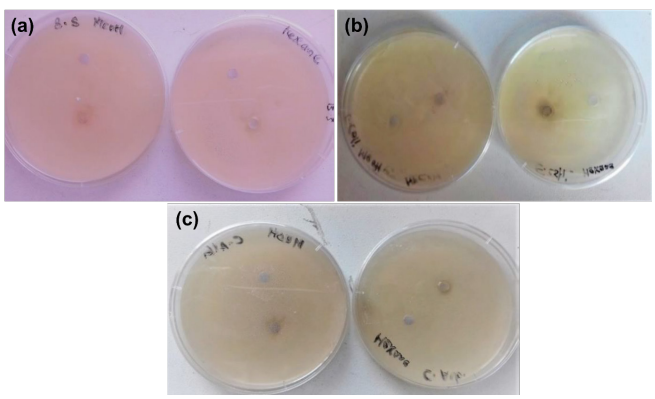

Figure3*: ZOI of methanol extracts against (a) B. subtilis, (b) E. coli, and (c) C. albicans*

Hexane extract of *A. calamus*(figure 3)showed high antibacterial activity against gram-negative bacteria *E. coli* (ZOI 10 mm) than gram-positive bacteria *B. subtillis* (ZOI 7 mm) while methanol extract of *A. calamus* showedhigh antibacterial activity only on a gram-positive bacteria *B. subtillis* (ZOI 4 mm). The methanol and hexane extract of *A. calamus* rhizome were examined against pathogenic fungi *C.albicans*which showeda zone of inhibition of 5 mm and 6 mm respectively. It was highly effective against gram-positive bacteria than gram-negative bacteria and the results are in accordance with the previous findings [36-39].

Antioxidant activity

The antioxidant activity of methanol extract of rhizome of *A. calamus* was determined by DPPH assay. Different absorbance values of extract of different concentrations were recorded and the percentage of radical scavenging was calculated.Percentage scavenging activity at different concentrations of the extracts is presented in table 4 and figure 4. The results showed that thefree radical percentage scavenging increased with a higher concentration of the extract of *A. calamus*. The antioxidant activity of the plant is owing to the presence of several active secondary metabolites in the extract which could inhibit the potential oxidative phenomenon. This antioxidant activity of the plant is also supported by the quantitative determination of phenolic content in the plant.

Table 4: *Absorbance and percentage scavenging values of extract of rhizome of A. calamus.*

Sample	Concentration (µg/mL)	Absorbance (nm)				Average Absorbance (nm)	Percentage Scavenged
Control		0.718	0.718	0.718	0.719	0.718	
Methanol Extract of *A. calamus*	5000	0.091	0.091	0.093	0.0917	0.091	87.326
	4000	0.123	0.12	0.123	0.122	0.122	83.008
	3000	0.201	0.204	0.203	0.203	0.202	71.866
	2000	0.411	0.412	0.41	0.411	0.411	42.758
	1000	0.525	0.524	0.525	0.525	0.524	27.019

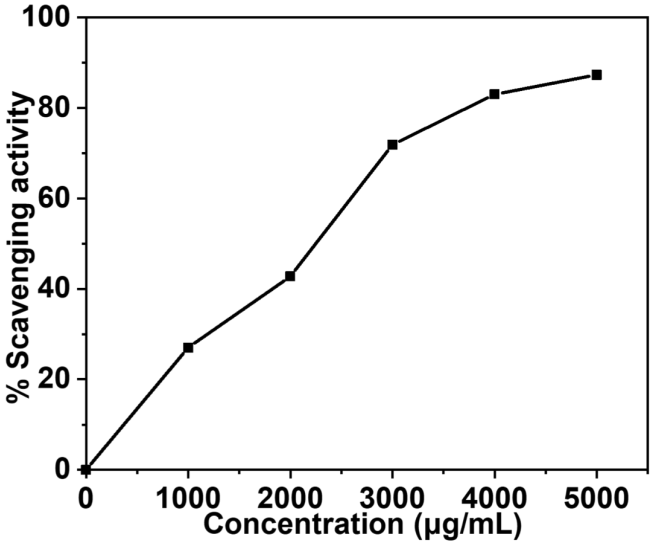

Figure 4: *Percentage radical scavenging capacity of methanol extract at different concentration*

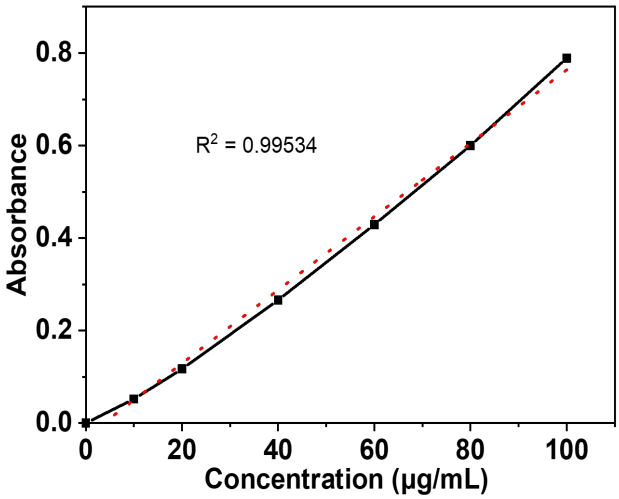

Figure 5: *Absorbance vs concentration of phenol standard (µg/mL)*

Determination of total phenolic content

Total phenols in compounds were determined by Folin-Ciocalteu colorimetric methodand were reported as Gallic acid equivalents by reference to the standard curve ($R^2 = 0.99534$). The total phenolic content of methanol extract of rhizome of *A. calamus* was found to be 48.36 mg/g GAE. The findings showed that the extent of antioxidant activity is directly proportional to the number of phenolic compounds present in that extract.The calculated phenolic content (48.36 mg/g GAE) of the methanol extract of the plant is slightly higher than that of the phenolic content present in the same plant from Indonesia reported by Susanah et al. (2018) [40]. This variation in the quantity of the phenolics in the same plants may be due to environmental variations which could affect the rate of metabolism for the production of metabolites. Phenolic, flavonoid compounds, as well as EO, have the potential to scavenge the possible oxidative process due to the presence of different functionalities [41-44].

Conclusions

The EO of the rhizome of *A. calamus* was extracted by hydrodistillation using a Clevenger apparatus. Quantitative analysis of essential oil by GC-MS analysis detected eleven compounds with major compound β-asarone. Acid value, saponification value, and iodine number of the extracted EO were evaluated.The methanol extracts of the rhizome showed effective antibacterial activity against *E. coli* and *B. subtilis*. Similarly, hexane extracts also showed effective antifungal activity against *C. albicans*. Antioxidant activity of methanol extract of *A. calamus* shows a high percentage scavenging capacity which is supported by the quantitative determination of phenolics in the plant. The results showed the plant act as a potent source of bioactive compounds. Hence, *A. calamus* Linn. would be a promising candidate for the extraction and isolation of bioactive compounds that could lead to natural product-based drug discovery. Further research is recommended to explore many other properties of this plant.

Acknowledgments

The authors havea deep sense of gratitude to the Department of Chemistry, Amrit Science Campus, Tribhuvan University, Kathmandu for the laboratory facility and the Department of Plant Resources, Thapathali, Kathmandu for GC-MS analysis.

References

1. T. J. Motley, Economic botany, the ethnobotany of sweet flag, *Acoruscalamus* (Araceae), The New York Botanical Garden, Bronx, NY 10458 U.S.A, 1994, 397–412.
2. R. S. Thorsen and M. Poulio, Traditional medicine for the rich and knowledgeable: challenging assumptions about treatment-seeking behavior in rural and peri-urban Nepal. Health Policy Plan, 2016, **31**, 314–324.
3. S. K. Rana, P. S. Oli and H. K. Rana, Traditional botanical knowledge (TBK) on the use of medicinal plants in Sikles area, Nepal Asian Journal of Plant Science Research, 2015, **5**, 8–15.
4. R. Loying, R. Gogoi, N. Sarma, A. Borah, S. Munda and S. K. Pandey, Chemical compositions, in-vitro antioxidant, anti-microbial, anti-inflammatory and cytotoxic activities of essential oil of *Acorus calamus* L. rhizome from North-East India, Journal of Essential OilBearing Plants, 2019, **5**(22), 1299-1312.(DOI: 10.1080/0972060X.2019.1696236).
5. G. C. Yen and P. D. Duh, Antioxidative properties of methanolic extracts from peanut hulls.Journal of the American oil chemistry society, 1993, **70**, 383.
6. J. B. Harborne,A Guide to Modern Techniques of plant analysis, Phytochemical methods. Chapman and Hall, London, 1998.
7. P. W. Grosvenor, A. Suptino and D. O. Gray, Medicinal plants from Rian province, sumatra, Indonesia, Part 2; antibacterial and antifungal activity,Journal of Ethnopharmacology, 1995,**45**, 97-111.
8. L. McGaw, A. K. Jager and J. V. Staden, Isolation of beta asarone, an antibacterial and antihelmintic compound from*Acoruscalamus* in South Africa,Journal of Botany, 2002, **68**, 31-35.
9. K. Das, R. K. Tiwari and D. K. Shrivastava, Techniques for evaluation of medicinal plant products as antimicrobial agents: current methods and future trends, Journal of Medicinal Plants Research, 2010, **4**, 104–111.
10. N. P. Manandhar,Medicinal plants used by Chepang tribes of Makwanpur district, Nepal, Fitoterapia, 1989, **1**(60), 61-68.
11. R. Tamang and D. Sedai, Documentation of ethnobotanical knowledge on plant resources used by Baram community in Arupokhari VDC, Gorkha District, Central Nepal, Bulletin of Deparment of Plant Resource, 2016,**30**, 283-285,Thapathali, Kathmandu, Nepal.
12. S. R. Baral and P. P Kurmi, A compedium of medicinal plants in Nepal, Mass Printing Press, Kathmandu, 2006.
13. P. K. Mukherjee and A. Wahile, Integrated approaches towards drug development of Ayurveda and other Indian system of medicines, Journal of Ethnopharmacology, 2006, **103**, 25–35.
14. G. Buchauer, L. Jirovetz andW.Jager,Fragrance compounds and essential oils with sedative effects upon inhalation,Journal of Pharmcological Science, 1993,**82**, 660-664.
15. M. B. Isman, O. Koul and A. Luczynski, Insecticidal and antifeedantbioactivities of neem oils and their relationship to Azadiractin content, Journal of Agricultural Food Chemistry, 1990,**38**, 1406.
16. N. N. Azwanida, A review on the extraction methods use in medicinal plants, principle, strength, and limitation, Journal of Medicinal Aromatic Plants, 2015, **4**, 196.
17. A. Sofowora, The present status of knowledge of the plants used in traditional medicine in western Africa: a medical approach and a chemical evaluation, Journal of Ethnopharmacology, 1980, **2**, 109–118.
18. J. N. Eloff, Which extract should be used for the screening and isolation of antimicrobial components from plants? Journal of Ethnopharmacology, 1998, **60**, 1–8.
19. J. B. Harborne, (3rd Ed), Phytochemical methods: a guide to modern techniques of plant analysis,Thomson Science, New York, NY: London, UK, 1998, p. 219.
20. S. L.Agarwal, P. C. Dandiya, K. P. Sing and R. B. Arora, A note on the preliminary studies of certain pharmacological actions of *Acoruscalamus* L, Journal of the American Pharmaceutial Association, 1956, **45**(9), 655-656.

21. A. Bahukhandi, S. Rawat, I. D. Bhatt and R.S.Rawal, Influence of solvent types and source of collection on total phenolic content and antioxidant activities of *Acorus calamus* L. National Academy Science Letters, 2013, **36**(1), 93-99.

22. K. R. Bhattarai, I. E. Maren and R. P. Chaudhary, Medicinal plant knowledge of the Panchase region in the middle hills of the Nepalese Himalayas, BankoJanakari,2008, **21**(2), 31-39.

23. R. Kumari, S. B. Agrawal, S. Singh and N. K Dubey, Supplemental ultraviolet-B induced changes in essential oil composition and total phenolics of *Acorus calamus* L. (sweet flag), Ecotoxicology and Environmental Safety, 2009, **72**(7), 2013-2019.

24. S. A Devi and D. Ganjewala, Antioxidant activities of methanolic extracts of sweet flag (*Acorus calamus*) leaves and rhizomes, Journal of Herbs, Spices and Medicinal Plants, **2011**, 17(1), 1-11.

25. Y. Cai, Q. Luo, M. Sun and H. Corke, Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer, Life Sciences, 2004, **74**(17), 2157-2184.

26. G. Divya, S. Gajalakshmi, S. Mythili and A. Sathiavelu, Pharmacological activities of *Acorus calamus*: a review, Asian Journal Biochemistry Pharmacy Research, 2010, **1**, 57-64.

27. S. Joshi and S. Bashyal, Study of the chemical constituents and antibacterial activity of essential oil of *Acorus calamus* L. rhizome of Rupendehi district of Nepal, Journal of Institute of Science and *Technology*, 2019, **23**(1), 57-60.

28. P. Chaubey, A. Praksah, K. Rai, R. Kumar and A. K. Pant, In vitro antioxidant activity and total phenolic content of rhizome of extracts from *Acorus calamus* Linn. Asian Journal of Chemistry, 2017, **29**(11), 2357-2360.

29. C. L. Lin, G. Y. Linand I. Z. Cai, Study on the chemical constituents of the volatile oils from *Acorus calamus* growing in Zhejiang Province, *Chinese Pharmacy*, 2012, **23**, 640-641.

30. T. J. Motley, The ethnobotany of sweet flag, *Acorus calamus* (Acoraceae), *Economic Botany*, 2002, **48**, 397-412.

31. S. D. Gautam, M. K. Prasad and D. P. Bhattarai, Fundamental Chemistry, 1st Edition, Heritage Publishers and Distributors Pvt. Ltd.,Nepal, 2013, pp.210-212.

32. V. S. Paithankar, S. L. Belsare, R. M. Charde and J. V. Vyas, *Acorus calamus*: an overview. Journal of Medicinal Plant Research, 2010, **4**, 2740-2745.

33. S. Pokrel and K. Chaulagain, Phytoconstituents and biological analysis of *Acorus calamus* rhizome of Sindhupalchowk District, Nepal, *Journal of Physical Science*, 2020, **17**, 104-109.

34. G. Samuelson, Drugs of Natural Origin: A Textbook of Pharmacognosy, Swedish Pharmaceutical Press. Sweden, 1999.

35. A. I. Alaajtal, F. E. Sherami, M. A. Elbagermi, Acid, peroxide, ester and saponification values for some vegetable oils before and after frying, AASCIT Journal of Materials, 2018, **4**(2), 43-47.

36. R. Valsaraj, P. Pushpangadan, U. Smitt, U. Adseren and U. Nymaan, Antimicrobial screening of selected medicinal plants from India,Journal of Ethnopharmacology, 1997, **58**, 75-83.

37. I. G. Vashi and H. C. Patel, Chemical constituents and antimicrobial activity of *Acorus calamus* Linn., Journal of Pharmaceutical Analysis, 1987, **12**, 49-51.

38. P. A. Wayne, Method for antifungal disk diffusion susceptibility testing of yeasts, Clinical and Laboratory Standard Institute, 1994, 44-47.

39. M. Yadav, S. Chatterji, S. K. Gupta and G. Watal, Preliminary phytochemical screening of six medicinal plants used in traditional medicine, International Journal of Pharmaceutical Sciences, 2014, **6**(5), 539-542.

40. R. W. Susanah, K. Retno and S. I. M. Dira, Total Phenolic and Flavonoid Contents and Antimicrobial activity of *Aocrus calamus* L. rhizome ethanol extract. Research Journal of Chemistry and Environment, **2018**, 22, 65-70.

41. M. Zuzarte and L. Salgueiro, Essential oils chemistry, Springer International Publishing. Switzerland, 2015.

42. H. Lohani, H. C.Andola, N. Chauhan and U. Bhandari, Variations of essential oil composition of *Acoruscalamus*: From Uttarakhand Himalaya, Journal of Pharmacological Research, 2012, **5**, 1246–1247.