J. Soils and Crops 33 (2) 328-334, December 2023

ISSN 0971-2836 (Print)
ISSN 2582-2756 (Online)

PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF METHANOLIC RHIZOME EXTRACT OF *ACORUS CALAMUS* COLLECTED FROM IMPHAL WEST DISTRICT OF MANIPUR

M.R. Khan[1], Bhaigyabati Thoudam[2], Mrinamala Devi Yengkhom[3], Dinendra Sharma, Leihaothabam[4] and Jinus Singh Senjam[5]

ABSTRACT

The present study was carried out in the year 2022 at Institutional level Biotech Hub, Pravabati College, Mayang Imphal, to analyze the phytochemical constituents *viz*., total phenolic content, total flavonoid content and antioxidant activity in the crude methanolic rhizome extract of *A. calamus*. Preliminary phytochemical screening indicates the presence of most of the phytoconstituents except oil and phlobatannins. Results indicate that the amount of total phenolic and total flavonoid content in crude methanolic rhizome extract were moderate and expressed in terms of gallic acid equivalent and quercetin equivalent respectively. In antioxidant activity, reducing power assay and total antioxidant activity showed a dose dependent manner. Result indicates the presence of most of the phytochemicals in crude methanolic rhizome extract of *A. calamus* with moderate amount of total phenolic content and total flavonoid content in the extract and in antioxidant assay, antioxidant activity increases with increase in concentration, which may be due to the presence of phenolic and flavonoid components in the extract. We suggested that, such potential high value and low maintenance plants can be adopted by the poor grower by cultivating with the main crops as inter cropping to reduce the pressure of high maintenance of fertilizer, irrigation, pesticide and labour inputs in paddy cultivation and other crops, and thus huge amount of income can be generated and possible to enhance revenue in the state by selling the end product to the pharma industry and to improve health care sector. Thus, it will be a win-win situation both for agricultural sector and pharma industry.

(Key words: Phytochemicals, *Acorus calamus*, phenolic, flavonoid, antioxidant)

INTRODUCTION

Traditional medicine plays an important role in primary healthcare for most population of the world. Asia has a long history of using herbal medicines. Plants used in traditional medicine contain a wide range of substances that can be used to treat various ailments. These chemical substances are responsible for the medicinal properties of plant and are refer to as bioactive compounds. Among them alkaloids, flavonoids, steroids, tannins, terpenoids and phenolic compounds are the most important bioactive compounds (Harborne, 1988, Kumar *et al*., 2010).

Additives derived from plants are also utilized in food and cosmetic industries due to their preservative nature because of the presence of phytochemical constituents which have antioxidant and antimicrobial properties (Brijesh *et al*., 2009, Beya *et al*., 2021). The antioxidant properties of medicinal plants depend on the plant, its variety, environmental conditions, climatic and seasonal variations, geographical regions of growth, degree of ripeness, growing practices, and many other factors such as postharvest treatment and processing.

In addition, antioxidant property of plant depends on composition and concentration of present antioxidants, such as phenolic compounds (Skrovankova *et al*., 2012). *Acorus calamus* (Linn.) belongs to the family Araceae (Acoraceae) is commonly known as sweet flag or calamus, Vacha in Sanskrit and *Okhidak/O-Hidak* in Manipuri. *A. calamus* is a semi-aquatic, perennial, aromatic herb with creeping rhizomes. The plant's rhizomes are brown in colour, twisted, cylindrical, curved, and shortly nodded. The leaves are radiant green, with a sword-like structure, which is thicker in the middle and has curvy margins. Rhizome of this plant is mainly responsible for its various therapeutic potentials. Moreover, rhizome has medicinal properties against bugs, moths and lice (Mukherjee *et al*., 2007, Joshi, 2016). A number of active constituents from leaves, rhizomes

1. Assoc. Professor, Dept. of Botany, Lilong Haoreibi College, Lilong, Manipur, India
2&3. Asstt. Professor, Dept. of Botany, Pravabati College, Mayang Imphal, Imphal West, Manipur, India
4. Asstt. Professor, Dept. of Botany, Pravabati College, Mayang Imphal, Imphal West, Manipur, India. Corresponding author
5. Associate Dean, School of Horticulture, Pandit Deen Dayal Upadhyay Institute of Agricultural Sciences, Kameng Village Campus and Utlou, Manipur, India

and essential oils of *A. calamus* have been isolated and characterized. Of the constituents, alpha and beta-asarone are the predominant bioactive components (Rajput *et al*., 2014). Seeing its various medicinal property and ethnomedicinal uses, the present study was carried out to analyze the phytochemical constituent, total phenolic content, total flavonoid content and antioxidant activity of *Acorus calamus* collected from Imphal West district of Manipur.

MATERIALS AND METHODS

Plant sample

Acorus calamus was collected from Imphal-West district of Manipur, Northeast India. Authentication of the plant sample was done in Manipur University, Department of Life Sciences (Botany) (001340MUMP). Rhizome of the plant were washed with tap water and then rinsed with distilled water, shade dried and ground into fine powder.

Soxhlet extraction

40 g of dried rhizome powder was extracted using 400 ml of methanol by soxhlation until the solvent become colourless in main chamber of the soxhlet extractor. The extract was evaporated to dryness and crude extract was obtained. The crude extract was screened for the phytochemical constituents, total phenolic content, total flavonoid content and antioxidant activity.

Phytochemical screening was carried out for crude methanolic rhizome extract of *A. calamus* using standard protocol (Audu *et al*., 2007, Edeoga *et al*., 2005, Kokate, 2005, Tiwari *et al*., 2011, Rathod and Valvi, 2011, Bag *et al*., 2016, Bhaigyabati *et al*., 2017). For the extracting of valuable bioactive compounds from various natural sources Soxhlet extraction has been used widely (Redfern *et al*., 2014; Rakhee *et al*., 2018).

Determination of total phenolic content

Total phenolic content in crude methanolic rhizome extract of *A. calamus* was determined using Folin-Ciocalteu reagent (Spanos and Wrolstad, 1990, Chakraborthy and Ghorpade , 2010, Bag *et al*., 2016, Bhaigyabati *et al*., 2017). 2.5 ml of 10% Folin-Ciocalteu reagent and 2 ml of Na_2CO_3 (2% w/v) were added to 0.5 ml of (3 replicates) extract solution (1mg ml^{-1}). The resulting mixture was incubated at 45^0C for 15 minutes. The absorbance of sample was measured at 760 nm using Visible Spectrophotometer. Gallic acid (10-50 µg ml^{-1}) was used as a standard compound.

Total phenolic content in the crude methanolic rhizome extract in gallic acid equivalent (GAE) was calculated by the following formula:

$$T = (C \times V)/M$$

Where, T = total content of phenolic compounds, mg g^{-1} plant extract in GAE; C = concentration of gallic acid established from the calibration curve, µg ml^{-1}; V = volume of extract, ml; M = weight of the plant extracts, g.

Estimation of total flavonoid content

Total flavonoid content in the crude methanolic rhizome extract was determined by Aluminium chloride colorimetric method. Studies have reported Quercetin to be suitable reference for determination of total flavonoid content in plant sample extract. Therefore standard Quercetin solutions of various concentrations were used to make the calibration curve.

10 mg of quercetin was dissolved in 100 ml methanol and then diluted to 6.25, 12.5, 25, 50, 80, and 100 ìg ml^{-1} using methanol. Stock solution of extract was prepared by dissolving 100 mg of extract in 5ml methanol and transferred to 10 ml volumetric flask and made up the volume with methanol. 10% aluminium chloride and 1M potassium acetate were prepared using distilled water.

The assay was determined using 0.5 ml of extract stock solution and each dilution of standard quercetin taken separately in test tubes. To each test tube 1.5 ml methanol, 0.1 ml aluminium chloride solution, 0.1ml potassium acetate solution and 2.8 ml distilled water were added and mixed well. Blank sample was prepared in similar manner by replacing aluminium chloride solution with distilled water. All the prepared solutions were filtered through Whatmann filter paper if necessary before measuring their absorbance. Absorbance was taken at 415 nm against the suitable blank solution (Akbay *et al*., 2003, Kaufman *et al*., 1999).

Estimation of reducing power

Various concentrations (30-150 ìg ml^{-1}) of the methanolic rhizome extract from the stock solution of (1mg ml^{-1}) in methanol were prepared in different test tubes. Ascorbic acid (1mg ml^{-1}) at various concentrations was used as standard. To each tube, 2.5 ml of phosphate buffer and 2.5 ml of 1% potassium ferricyanide were added. This mixture was kept at 50^oC in water bath for 20 minutes. After cooling, 2.5 ml of 10% trichloroacetic acid was added and centrifuged at 3000 rpm for 10 mins (whenever necessary). The upper layers of solution (2.5 ml) were transferred into fresh tubes and to each tube, 2.5ml of distilled water and 0.5ml of freshly prepared 0.1% ferric chloride solution were added. The absorbance was measured at 700 nm. Blank was prepared in similar manner excluding samples. Increased absorbance of the reaction mixture indicates increase in reducing power (Oyaizu, 1986, Bag *et al*., 2016, Bhaigyabati *et al*., 2017). Here in this method, as per little modification from the Bhalodia *et al.* (2013); Khatoon *et al*. (2013), is based on the principle that to form potassium ferrocyanide (Fe^{2+}), those substances which have reduction potential were reacted with potassium ferricyanide (Fe^{3+}), and which then react with ferric chloride to form ferric–ferrous complex that has an absorption maximum at 700 nm. For rapid screening method of antioxidant potential this method is a convenient (Adebayo *et al*., 2012).

Determination of total antioxidant activity

The phosphomolybdenum method was used to evaluate the total antioxidant activity of the extract (Prieto *et al*., 1999, Bag *et al*., 2016, Bhaigyabati *et al*., 2017).

Antioxidants can reduce Mo (VI) to Mo (V) and the green phosphate / Mo (V) compounds at acidic pH, which have an absorption peak at 695 nm, were generated subsequently. 0.3 ml of the sample methanolic extract (1mg ml^{-1}) as well as ascorbic acid (1mg ml^{-1}) was mixed with 3.0 ml of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) separately. Reaction mixture was incubated at 95ºC for 90 min under water bath. Absorbance of all the mixtures was measured at 695 nm after cooling. Total antioxidant activity is expressed as the number of equivalents of ascorbic acid in micrograms (AAE). Total antioxidant activity was calculated by using the formula: Total antioxidant = O.D. of test sample x concentration of standard in ìg x made up volume of sample

RESULTS AND DISCUSSION

Phytochemical constituents present in methanolic rhizome extract of *A. calamus* are listed in Table 1. Preliminary phytochemical screening shows the presence of most of the phytochemicals in methanolic rhizome extract of *A. calamus* except oil and phlobatannins.

Table 1. Phytochemicals present in methanolic rhizome extract of *A. calamus*

Phytochemical constituents	Tests	Crude methanolic rhizome extract of *A. calamus*
Amino acids	Ninhydrin test	+
Alkaloids	Mayer's test	+
Carbohydrates (reducing sugar)	Benedict's test	+
	Fehling's test	+
Proteins	Biuret test	-
	Xanthoproteic test	+
Flavonoids	Alkaline reagent test	-
	Lead acetate test	+
Phenolic compounds	Lead acetate test	+
	Ferric Chloride test	+
Steroids and Terpenoids	Salkowski's test	+
Saponins	Froth test	+
Tannins	Lead acetate test	+
	Ferric chloride test	+
Cardiac glycosides	Keller-killiani test	+
Oil		-
Phlobatannins		-

Key: + = presence and - = absence

Phytochemical studies have reported the presence of glycosides, flavonoids, saponins, tannins, polyphenolic compounds, mucilage, and volatile oil. Presence of glucoside, alkaloid and essential oil containing calamen, clamenol, calameon, asarone and sesquiterpenes has also been reported. Phytochemical analysis of the methanol extract of *A. calamus* shown to contain glycosides, flavonoids, saponins, tannins, polyphenolic, essential oils, and terpenes (Elayaraja *et al*., 2010, Barua *et al*., 2014, Muthuraman *et al*., 2011). Preliminary phytochemical screening is usually performed for the identification of substantial phytochemicals that may be involved in the antioxidant activity of plant extracts (Anandakirouchenane *et al*., 2013, Basma *et al*., 2011, Das et *al*., 2012).

In the present study, total phenolic content and total flavonoid content were analyzed in the crude extract as they are the primary compounds responsible for antioxidant activity. Standard curve of gallic acid and quercetin is shown in Figures 1 and 2 respectively. Total phenolic content and total flavonoid content in crude methanolic rhizome extracts of *A. calamus* was obtained from the respective standard curve. Table 2 indicates the total phenolic and total flavonoid content in the crude extract.

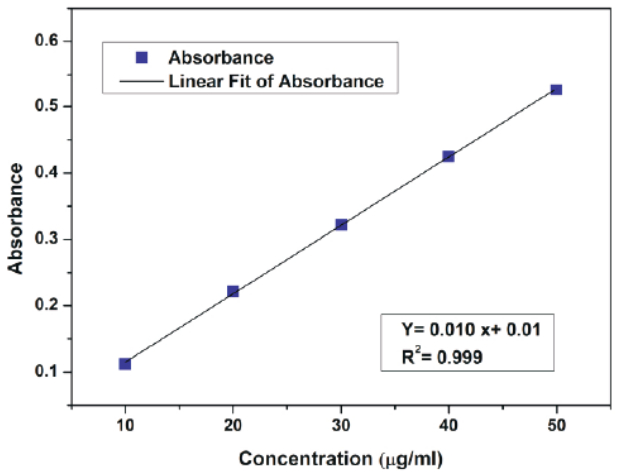

Figure 1. Standard curve of gallic acid

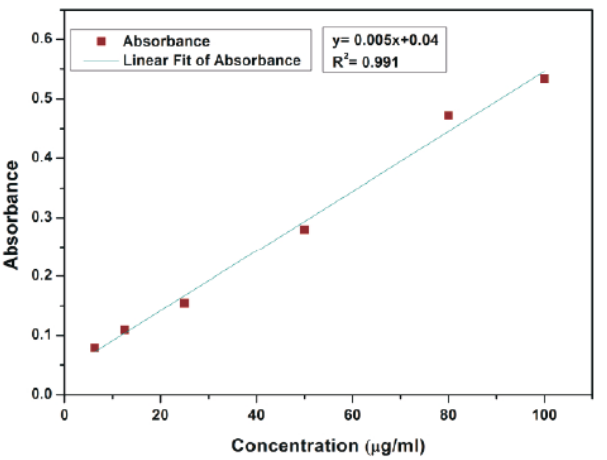

Figure 2. Standard curve of quercetin

From the standard curve, concentration value of extract was obtained and total flavonoid content (TFC) was calculated by using the following formula (Chang *et al*., 2002).

$$TFC = \frac{R * D.F * V * 100}{W}$$

Where R - Result obtained from the standard curve, D.F - Dilution factor, V - Volume of stock solution 100 - For 100 g dried plant and W - Weight of plant used in the experiment

Table 2. Total phenolic and total flavonoid content in crude methanolic rhizome extract of *A. calamus*

Sample	Total phenolic content in mg g^{-1} of extract (in GAE)	Total flavonoid content in µg 100 g^{-1} of dried extract (in QE)
	Methanol extract	Methanol extract
A. calamus rhizome	1.79 ± 0.02	8.26 ± 0.40

Assays were performed in triplicates. Values are expressed as means ± SD

The total phenolic content in methanolic rhizome extract of *A. calamus* was found to be 1.79 ± 0.02 mg g^{-1} (GAE). Phenolic compounds act as free radical terminators through scavenging or chelating process (Saikia and Upadhyaya, 2011). They have wide bioactivity including antioxidant properties/activity, due to the presence of hydroxyl functional group and are responsible for the radical scavenging effect mainly due to redox properties (Rice-Evans *et al*., 1997).

Total flavonoid content in crude methanolic rhizome extract of *A. calamus* was found to be 8.26 ± 0.40 µg 100 g^{-1} (QE).

According to Rita *et al*. (2019) the contents of flavonoid and phenolic compounds in ethyl acetate fraction were the highest, followed by n-butanol, n-hexane, and water fractions respectively. Total flavonoid contents of ethyl acetate, butanol, n-hexane, and water fractions were successively 2978.34, 399.07, 142, 23, and 14.80 mg QE/100g, while total phenolic contents of those fractions were 1820.51, 329.71, 237.81, and 74.36 mg GAE 100 g^{-1}, respectively.

Flavonoids have been shown to exhibit their actions through effects on membrane permeability, and by inhibition of membrane-bound enzymes such as the ATPase and phospholipase A2 (Li *et al*., 2003). Flavonoids serve as health promoting compound as a results of its presence as anion radicals (Hausteen, 1983). The compounds such as flavonoids, which hold hydroxyl groups, are responsible for the radical scavenging activity in the plants (Das and Pereira, 1990, Younes, 1981). It has been acknowledged that flavonoids show significant antioxidant action on human health and fitness. It is known that flavonoids act through scavenging or chelating process.

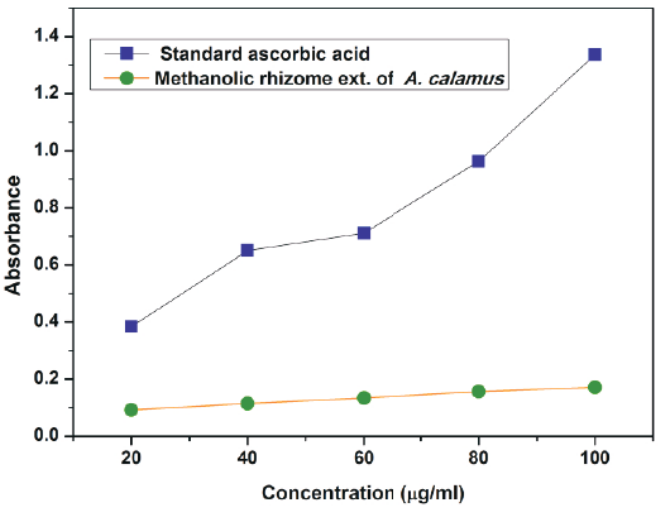

Figure 3. Reducing power of standard and crude methanolic rhizome extract of *A. calamus*

Reducing power assay indicates an increasing order for standard as well as for the plant sample extract and is shown in fig.3. Reducing power of the extract may be contributed by bioactive compounds in the extract which possess electron donating abilities. Presence of reducers causes the conversion of the Fe^{3+} complex to the ferrous (Fe^{2+}) form which serves as a significant indicator of its antioxidant capacity (Yildirim *et al*., 2002). Total antioxidant activity was analyzed in the crude rhizome extract and found that with the increase in concentration total antioxidant activity increased and expressed in µg ml^{-1} of extract in ascorbic acid equivalent (AAE). Table 3 indicates the total antioxidant activity showed by various concentration of crude methanolic rhizome extract of *A. calamus.*

Table 3. Total antioxidant activity in crude methanolic rhizome extract of *A. calamus*

Concentrations (µg ml^{-1})	Total antioxidant activity in µg/ml of extract (in AAE) for crude methanolic rhizome extract of *A. calamus*
30	2.26 ± 0.02
60	2.88 ± 0.01
90	3.69 ±0.03
120	4.24 ± 0.02
150	4.76 ± 0.02

Assays were performed in triplicates. Values are expressed as means ± SD

Highest total antioxidant activity of 4.76 ± 0.02 µg ml^{-1} of extract (in AAE) was noted in the highest concentration (150 µg ml^{-1}) used in the assay. The total antioxidant activity assay also indicates a dose dependent manner.

Studies have shown that *Acorus calamus* exhibits free radical scavenging, reducing power and metal chelating property in dose dependent manner (Imam *et al*., 2013).

From the study, it can be inferred that phytochemical screening of crude methanolic rhizome extract of *A. calamus* shows the presence of most of the phytochemicals. These phytochemicals content would be a potential to be used in various diseases treatments. In addition to this, this potential climate resilient plants (Saoji *et al.*, 2022) can be used in income generating crops by intercropping (Kharche *et al*. 2022; Singh *et al.*, 2021) with the main crops like rice and other fruits crops. In Manipur, traditional paddy crops cultivation is declining day by day, due to the factors like high costs of maintenance, lack of irrigation facilities, labour, fertilizer and pesticide and thus these factors can be lessen by planting this high value and low maintenance crops to generate more revenue for the state by large scale cultivation and development of end produce to be sold in other industry in substitute of traditional farming. Apart from this, in our study, the total phenolic content and total flavonoid content were moderate and in antioxidant assay, antioxidant activity increased with the increased in concentration, which may be due to phenolic and flavonoid contained in the extract. Further studies can be carried out for the isolation of bioactive compounds from *A. calamus* rhizome.

REFERENCES

Adebayo, E. A., J.K. Oloke, A.A. Ayandele and C.O. Adegunlola. 2012. Phytochemical, antioxidant and antimicrobial assay of mushroom metabolite from *Pleurotus pulmonarius* – LAU 09 (JF736658). J. Microbiol. Biotech. Res. **2**:366–374.

Akbay, P., A.A. Basaran, U. Undeger and N. Basaran, 2003. *In vitro* immunomodulatory activity of flavonoid glycosides from *Urtica dioica* (L.), Phytother Res. **17**: 34-37.

Anandakirouchenane, E., I. C. Sarath and B. Kadalmani, 2013. An investigation on preliminary phytochemical and safety profiles of methanolic root extract of *Curculigo orchioides*, J. Pharm. Res. **7**: 692-696.

Atanasov ,A.G., B. Waltenberger, E.M.P. Wenzig, T. Linder, C. Wawrosch, P. Uhrin, V. Temml, L. Wang, S. Schwaiger, E.H. Heiss, J.M. Rollinger, D. Schuster, J.M. Breuss, V. Bochkov, M.D. Mihovilovic, B. Kopp, R. Bauer, V.M. Dirsch, and H. Stuppnerb, 2015. Discovery and resupply of pharmacologically active plant-derived natural products: A review, Biotechnol. Adv. **33**(8): 1582–1614.

Audu, S.A., I. Mohammed and H.A. Kaita, 2007. Phytochemical screening of the leaves of *Lophira lanceolate* (*Ochanaceae*), Life Sci. **4**(4):75-79.

Bag, G.C., P.G. Devi and T.H. Bhaigyabati, 2016. Phytochemical screening and antioxidant activity of *Meyna laxiflora* species found in Imphal West District of Manipur, Int. J. Pharm. Sci. Rev. Res. **36**(1):137-143.

Barua, C.C., S. Sen, A.S. Das, A. Talukdar, N.J. Hazarika, A.G. Barua, A.M. Barua and I.A. Barua, 2014. Comparative study of the in vitro antioxidant property of different extracts of *Acorus calamus* (Linn.), J. Nat. Prod. Plant Resour. **4**: 8-18.

Basma, A.A., Z. Zakaria, L. Yoga and L. Sreenivasan, 2011. Antioxidant activity and phytochemical screening of the methanol extracts of *Euphorbia hirta (*L.), Asian Pac. J. Trop. Med. **4**: 386-390.

Beya, M.M.M.E., Y. Sultanbawa, H. Smyth and L.C. Hoffman, 2021. Plant-Based Phenolic Molecules as Natural Preservatives in Comminuted Meats: A Review, Antioxidants (Basel), **10**(2): 263.

Bhaigyabati, T., P. G. Devi, N.R. Devi and G.C. .Bag, 2017. Antioxidant activity, total phenolic and total flavonoid content of *Oenanthe javanica* Blume (D C) collected from Imphal west district, Int. Res. J. Pharm. **8**(6):63-68.

Bhalodia, N. R., P. B. Nariya, R. N. Acharya, V. J. Shukla, 2013. In vitro antioxidant activity of hydro alcoholic extract from the fruit pulp of *Cassia fistula* (Linn.), Ayu. **34**(2):209-14.

Brijesh K. Tiwari, Vasilis P. Valdramidis, Colm P. O' Donnell, Kasiviswanathan Muthukumarappan, Paula Bourk, and P. J. Cullen, 2009. Application of Natural Antimicrobials for Food Preservation, J. Agric. and Food Chem. **57** (14), 5987-6000

Chakraborthy, G.S. and P.M. Ghorpade, 2010. Free radical scavenging activity of *Abutilon indicum* (Linn) Sweet stem extracts, Int. J. Chem. Tech. Res. **2**(1):526-531.

Chang, C., M. Yang, H. Wen and J. Chern, 2002. Estimation of total flavonoid content in Propolis by two complementary colorimetric methods, J. Food Drug Anal. **10**(30): 178-182.

Das, N.P. and T.A. Pereira, 1990. Effects of flavonoids on thermal autooxidation of Palm oil: structure-activity relationship, J. Am. Oil Chem. Soc.**67**: 255-258.

Das, S., R. Datta and S. Nandy, 2012. Phytochemical screening and evaluation of anti-inflammatory activity of methanolic extract of *Abroma augusta* (Linn), Asian Pac. J. Trop. Med. **2**:114-117.

Edeoga, H.O., D.E. Okwu and B.O. Mbaebie, 2005. Phytochemical constituents of some Nigerian medicinal plants, Afr. J. Biotechnol. **4**(7):685-688.

Elayaraja, A., M. Vijayalakshmi and G. Devalarao, 2010. *In vitro* free radical scavenging activity of various root and rhizome extracts of Acorus *calamus* (Linn.), Int. J. Pharma Bio. Sci. **1**(4): 301-304.

Harborne, J.B. 1988. Phytochemical Methods, A Guide to Modern Techniques of Plant Analysis. Chapman and Hall, London, 70.

Hausteen, B. 1983. Flavonoids, a class of natural products of high pharmacological potency, Biochem. Pharm. **32**: 1141-1148.

Imam, H., Z. Riaz, M. Azhar, G. Sofi and A. Hussain, 2013. Sweetflag (*Acorus calamus* Linn.): An incredible medicinal herb, Int. J. Green Pharm. **7**: 288-96.

Joshi, R.K. 2016. *Acorus calamus* (Linn.) phytoconstituents and bactericidal property, World J. Microbiol. Biotechnol. **32**(10):164.

Kaufman, P.B., L. J. Cseke, S. Warber, J.A. Duke and H. L. Brielmann, 1999. Natural products from plants. CRC press. New York, pp. 20-22.

Kharche, P.P., U.S. Surve, A.D. Tumbare, 2022. Economics of different integrated farming system models, under irrigated conditions of Western Maharashtra, J. Soils and Crops, 32(1):201-207.

Khatoon, M., E. Islam, R. Islam, 2013. Estimation of total phenol and *in vitro* antioxidant activity of *Albizia procera* leaves. BMC Res. Notes, **6**:121.

Kokate, C.K., 2005. A text book of Practical Pharmalognosy. 5th ed. Vallabh Prakashan, Delhi, pp. 07-111.

Kumar, S.S., A.S. Akram, T.S.F. Ahmed and M.S.M. Jaabir, 2010. Phytochemical analysis and antimicrobial activity of the ethanolic extract of *Acorus calamus* rhizome, Orient. J. Chem. **26**(1): 223-227

Kurmukov, A.G. 2013. Phytochemistry of Medicinal Plants. In: Eisenman, S., Zaurov, D., Struwe, L. (eds) Medicinal Plants of Central Asia: Uzbekistan and Kyrgyzstan. Springer, New York, NY.

Li, H., Z. Wang and Y. Liu, 2003. Review in the studies on tannins activity of cancer prevention and anticancer, Zhong-Yao-Cai., **26**(6): 444-448.

Mukherjee, P. K., V. Kumar, M. Mal and P. J. Houghton, 2007. *Acorus calamus*: validation of Ayurvedic tradition from natural resources, Pharm. Biol. **45**(8): 651-666.

Muthuraman, A., N. Singh and A.S. Jaggi, 2011. Protective effect of *Acorus calamus* (L.) in rat model of vincristine induced painful neuropathy: an evidence of anti-inflammatory and anti-oxidative activity, Food Chem. Toxicol. **49**(10):2557-63.

Noreen, H., N. Semmar, M. Farman, J.S.O. McCullagh, 2017. Measurement of total phenolic content and antioxidant activity of aerial parts of medicinal plant *Coronopus didymus*, Asian. Pac. J. Trop. Med. **10**(8):792-801.

Oyaizu, M. 1986. Studies on Products of Browning Reactions: Antioxidative Activities of Products of Browning Reaction Prepared from Glucosamine, Jpn. J. Nutr. Diet. **44**(6): 307-315.

Prieto, P., M. Pineda and M. Anguilar, 1999. Aguilar Spectrophotometric quantification of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E, Anal. Biochem. **269**:337-341.

Rajput, S.B., M.B. Tonge and S.M. Karuppayil, 2014. An overview on traditional uses and pharmacological profile of *Acorus calamus* Linn. (Sweet flag) and other Acorus species, Phytomed. **21**(3): 268-276.

Rakhee, Jigni Mishra, Raj K. Sharma and Kshipra Misra. 2018. Characterization Techniques for Herbal Products, In Editor(s): Kshipra Misra, Priyanka Sharma and Anuja Bhardwaj, Management of High Altitude Pathophysiology, Academic Press, Pages 171-202, ISBN 9780128139998.

Rathod, V.S., and S.R. Valvi, 2011. Antinutritional factors of some wild edible fruits from Kolhapur District, Recent Res Sci Technol. **3**(5):68-72.

Redfern, J., M. Kinninmonth, D. Burdass, J. Verran, 2014. Using soxhlet ethanol extraction to produce and test plant material (essential oils) for their antimicrobial properties, J. Microbiol. Biol. Educ. **15**(1):45-6.

Rice-Evans, C.A., N.J. Miller and G. Paganga, 1997. Antioxidant properties of phenolic compounds, Trend Plant Sci. **4**:152-159.

Rita, W.S., I.M.D. Swantara and G.A.P. Utami, 2019. Antimicrobial activity of *Acorus calamus* L. rhizome extract and its total flavonoid and phenolic contents. AIP Conference Proceedings 2155, 020054.

Saikia, L.R. and S. Upadhyaya, 2011. Antioxidant activity, phenol and flavonoid content of some less known medicinal plants of Assam, Int. J. Pharma Bio Sci. **2**(2): 383-387.

Saoji, B.V., B.S. Morwal, Y.D. Charjan. 2020. Climate smart integrated farming systems model for resource management and rural employment in Vidarbha region of Maharashtra, J. Soils and Crops, **30**(2):273-278.

Singh, A., Ram Pratap and Jagdeep Singh, 2021. Response of maize to integrated nutrient managment under irrigated area of Punjab, J. Soils and Crops, **31**(2):237-241.

Skrovankova, P.S., L. Misurcova and L. Machu, 2012. Antioxidant activity and protecting health effects of common medicinal plants, Adv. Food Nutr. Res. 67:75-139.

Spanos, G.A. and R.E. Wrolstad, 1990. Influence of processing and storage on the phenolic composition of Thomson seedless grape juice, J. Agric. Food. Chem. **38**:1565-1571.

Tiwari, P., B. Kumar, M. Kaur, G. Kaur and H. Kaur, 2011. Phytochemical screening and extraction: a review, I. Pharm. Sciencia. **1**(1):98-106.

Yildirim, A., A. Mavi, M. Oktay, A.A. Kara, O.F. Algur, V. Bilaloglu, 2000. Comparison of antioxidant and antimicrobial activities of tilia (*Tilia argentea* Des f Ex DC), sage (*Salvia triloba* L.) and black tea (*Camellia sinensis*) extracts, J. Agri. Food Chem. **4**(10): 5030-5034.

Younes, M. 1981. Inhibitory action of some flavonoids on enhanced spontaneous lipid peroxidation following glutathione depletion, Planta Medica. **43**: 240-245.

Rec. on 01.04.2023 & Acc. on 20.04.2023