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Research Article

Methanolic and ethanolic phytochemical screening of Sweet flag (*Acorus calamus* L.) rhizome

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Abstract

Acorus calamus is a medicinal plant with several ethno-medicinal properties. The present study was carried to screen the presence of major phytochemical groups. Phytochemical screening of methanolic and ethanolic rhizome extract showed the presence of carbohydrates, alkaloids, phenolic compound, protein, amino acid flavanoids and tannins. The total phenolic content in methanolic and ethanolic rhizome extracts in terms of gallic acid equivalent was 4.77.50 and 7.5 mg/g of extract powder respectively whereas the total flavonoid content of methanolic and ethanolic extract was 12.02 and 12.89 mg QE/g of extract respectively. Total antioxidant activity was found highest in ethanol extract (75.6 µg AAE/mg of extract) than methanol extract (37.2 µg AAE/mg of extract). Reducing power is also found highest in ethanol extract than methanol extract.

Keywords: *Acorus calamus*, phytochemical screening, methanol, ethanol.

INTRODUCTION

Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs[1]. The most important of these bioactive constituents of plants are alkaloids, tannins, flavanoids and phenolic compounds[2]. Nearly 80%of the world's population in developing countries mainly depends on natural products for their health needs[3]. Mother earth has bestowed to the mankind and various plants with healing ability for curing the ailments of human being. This unique feature has been identified since pre historic times. The WHO has also estimated that 80% of the world population meets their primary health care needs through traditional medicine only. India is the eighth largest country having rich plant diversity with a total of around 47,000 species, of which more than 7500 species are being used as medicinal plants. Plant products are used as main source of medicine throughout the world for treating various human ailments[4]. About 50% of the present day medicines in the United States of America are derived from natural sources especially from various plants[5]. The use of traditional medicine in both developing and developed countries is significantly increasing in recent times. For discovery and development of novel drugs, scientists are looking forward to the alternative sources and in last few decades, medicinal plants have been extensively studied for their bioactive principles to develop new lead molecules for pharmaceutical use therefore aim of the present study be to find out the phytochemical active constituents of plants extracts of *Acorus calamus.*

Acorus calamus. (AC) Linn. (Araceae), commonly known as "sweet flag" or "calamus", is a species of semiaquatic, perennial, aromatic herb with creeping rhizomes. The plant is found in the northern temperate and subtropical regions of Asia, North America, and Europe. The plant prefers swampy or marshy habitats. In India, the plant is found growing wild as well as cultivated up to an altitude of 2200 m in the Himalayas. It is plentiful in the marshy tracts of Kashmir, Himachal Pradesh, Manipur, and Naga hills, and is regularly cultivated in Karnataka[6]. It is a traditional indigenous herb generally used in the treatment of cough, bronchitis, gout, tumours, haemorrhoids, skin diseases, numbness, and general debility. It possesses a wide range of pharmacological activities, such as anti-diabetic central nervous system depressant, anti-inflammatory, antioxidant, antispasmodic, antibacterial, antifungal, and cardiovascular and insecticidal agent[7].

MATERIALS AND METHODS

Plant Extract preparation

Acorus calamus was collected from five valley district of Manipur. The collected plant was washed thoroughly in running tap water and then rinsed with distilled water and dried in shaded condition. Completely dried rhizome was grinded into powder

Soxhlet extraction

40g powdered rhizome of *A. calamus* was extracted with 400ml each methanol and ethaanol respectively by soxhlation until the solvent become colourless in main chamber of the soxhlet extractor. The extracts were evaporated to dryness and crude extracts were obtained.

PHYTOCHEMICAL SCREENING

The phytochemical tests were carried out for the above mentioned plants extract using the standard procedures

1. Test for alkaloids

Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

2. Tests for carbohydrates

Benedict's test: Extract was filtered. Filtrate was treated with Benedict's reagent and heated gently. Formation of orange red precipitate indicated the presence of reducing sugars.

Fehling's test: Filtrate was mixed with equal volume of Fehling's A and Fehling's B solutions and heated. Formation of brick red precipitate of cuprous oxide indicated the presence of reducing sugars.

3. Test for amino acids

Ninhydrin test: Amino acids and proteins when boiled with few drops of 5% solution of ninhydrin, violet colour appear.

4. Test for proteins

Xanthoproteic test: The extract was treated with a few drops of conc. nitric acid. Formation of yellow colour indicated the presence of proteins.

Biuret test: To the test solution 4% NaOH solution and few drops of 1% CuSO4 solution were added, appearance of violet colour indicates the presence of protein.

5. Test for flavonoids

Alkaline reagent test: To the test solution, a few drops of sodium hydroxide solution were added. Formation of intense yellow colour which turns to colourless by addition of few drops of dilute acetic acid indicated the presence of flavonoids.

6. Test for phenolic compounds

Lead acetate test: To the test solution, a few drops of 10% lead acetate solution were added. Formation of white precipitate indicated the presence of phenolic compounds.

7. Test for tannins

Lead acetate test: To the test solution, a few drops of 10% lead acetate solution were added. Precipitate formation indicated the presence of tannin.

Ferric chloride test: To the test solution, a few drops of mferric chloride solution were added. An intense green, purple, blue or black colour indicated the presence of tannin.

8. Test for steroids and terpenoids

Salkowski's test: Extract was treated with chloroform and filtered. The filtrate was treated with few drops of conc. sulphuric acid, shaken well and allowed to stand. Appearance of red colour in the lower layer indicated the presence of steroids. Formation of reddish brown colour of interface after addition of conc. sulphuric acid to the side carefully (without shaking) indicated the presence of terpenoids.

9. Test for saponins

Froth test: Extract was added to 2-3 ml of distilled water. The mixture was shaken vigorously. Formation of foam indicated the presence of saponin.

10. Test for cardiac glycosides

Keller Killiani test: To the test solution, 2ml of glacial acetic acid containing a few drops of FeCl3 solution was added. 1ml of conc. H2SO4 was added along the side of the test tube carefully. A brown ring at the interface indicated the presence of deoxysugar of cardenoloides. A violet ring may appear beneath the brown ring, while in the acetic acid layer, a greenish ring may also form just gradually throughout the layer indicating the presence of cardiac glycosides.

11. Test for oil and fat

A small quantity of the extract was pressed between the two filter papers. Oil stain on the filter papers indicated the presence of oil.

12. Test for phlobatannin

Extract was boiled with 2 ml of 1% hydrochloric acid. No red precipitate was formed indicating the absence of phlobatannin.

13. Test for diterpenes

Copper acetate test: Extract was dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicated the presence of diterpenes.

Determination of Total Phenolic Content

The amount of phenol content in methanol and ethanol extracts *Acorus calamus* rhizome was determined with Folin-Ciocalteu reagent[15]. 2.5 ml of 10% Folin-Ciocalteu reagent and 2 ml of Na2CO3 (2% w/v) were added to 0.5 ml of the sample (3 replicates) of rhizome extract solution (1mg/ml). The resulting mixture was incubated at 45°C for 15 min. The absorbance of sample was measured at 760 nm using UV Visible Spectrophotometer (UV-2700). Gallic acid (50-300 µg/ml) was used as a standard compound. The gallic acid standard calibrationcurve was established by plotting concentration (µg/ml) versus absorbance (nm) (y= 0.009675X + 0.004840; R2= 0.9584), where y is absorbance at 760 nm and x is concentration (Figure 1). Total phenolic content in the plant extract was expressed as gallic acid equivalent (mg of gallic acid equivalent/g of sample) and was calculated by the formula:

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

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

Estimation of total flavonoid content

Aluminium Chloride Colorimetric Method

The basic principle of Aluminium chloride colorimetric method is that Aluminium chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5

hydroxyl group of flavones and flavonols. In addition it also forms acid labile complexes with the orthodihydroxyl groups in the A- or B-ring of flavonoids. Quercetin is reported to be suitable for building the calibration curve (Figure 2). Therefore standard Quercetin solutions of various concentrations were used to build up the calibration curve. In this method, quercetin was used to make the calibration curve. 10 mg of quercetin was dissolved in respective solvent (methanol and ethanol) and then diluted to 10,30,50,70 and 90µg/ml. A calibration curve was made by measuring the absorbance of the dilutions at 415 nm (λmax of quercetin) with a spectrophotometer (UV-2700). Aluminium chloride, 1% and potassium acetate, 1M solutions were prepared. Stock Solution of Extracts: 100 mg of each extract was accurately weighed and transferred to 10 ml volumetric flask and made up the volume with methanol. Preparation of Test Solutions: 0.5ml of each extract stock solution, 1.5 ml methanol, 0.1 ml aluminium chloride, 0.1 ml potassium acetate solution and 2.8 ml distilled water were added and mixed well. Sample blank was prepared in similar way by replacing aluminium chloride with distilled water. Sample and sample blank of all extracts were prepared and their absorbance was measured at 415 nm. All prepared solutions were filtered through Whatmann filter paper before measuring[8, 9]. Total Flavonoids content of the extracts was calculated from the regression equation of calibration curve (y = 0.002763X + 0.03923; R2 = 0.9671) and expressed as mg quercetin equivalent (QE) per gram extract (mg/g) and calculated by the formula

TF = (C x V)/M

Where, TF = total flavanoid content, mg/g plant extract, in QE; C = concentration of Quercetin established from the calibration curve, µg/ml; V = volume of extract, ml; M = weight of the plant extract.

Estimation of reducing

The reducing potential of the rhizome extract was determined by the method of Oyaizu M[10]. Different concentration of the extracts were prepared and mixed with 2.5 ml phosphate buffer and potassium ferricyanide and the mixture was kept at 50°C in water bath for 20 min. Followed by 2.5 ml of 10% trichloroacetic acid was added after cooling and centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5ml) was mixed with distilled water (2.5ml) and freshly prepared 1% ferric chloride solution (0.5ml). The absorbance was measured at 700 nm using Uv spectrophotometer. Control was prepared in similar manner excluding samples. Ascorbic acid (0.5mg/ml) at various concentrations was used as standard. Increased absorbance of the reaction mixture indicates increase in reducing power.

Determination of total antioxidant activity

The phosphomolybdenum method was used to evaluate the total antioxidant activity of the extracts[11]. 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 methanolic and ethanolic extract sample (1mg/ml) as well as ascorbic acid (0.5mg/ml) was mixed with 3.0ml of the reagent solution (0.6M sulphuric acid, 28nM sodium phosphate and 4nM ammonium molybdate) separately. Reaction mixture was incubated at 95°C for 90min 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 microgram per millilitre of extract. Total antioxidant activity was calculated by using the formula.

Total antioxidant = O.D. of test x concentration of standard in µg x made up volume of sample

Statistical Analysis

All sample determinations were conducted in triplicates and the results were calculated as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical csreening of methanol and ethanol extract of *Acorus calamus* rhizome reveals the presence of phytoconstituents as listed in table 1.

Table 1: Phytochemical constituents of *Acorus clamus* rhizome

Phytochemicals	Test performed	Solvent	
		Methanol	Ethanol
Amino acids	Ninhydrinm	+	+
Alkaloids	Mayre's test	+	+
Carbohydrate	Benedict's test	+	+
	Fehling's test	+	+
Proteins	Xanthoproteic test	+	+
	Biuret test	-	-
Flavanoids	Alkaline reagent test	-	-
	Lead acetate test	+	+
Steroiods and terpenoids	Salkowski's test	+	+
Saponins	Froth test	+	+
Cardiac glycosides	Keller Killiani test	+	+
Phenolic compounds	Lead acetate test	+	+
	Ferric chloride test	+	+
Tannins	Lead acetate test	+	+
	Ferric chloride test	+	+
Oils	Translucent test	-	-
Phlobatanins	Hcl test	-	-

Key + = presence, - = absence

Almost all the phytochemicals were present in methanol and ethanol extracts of *A. Calamus rhizome*. Different phytochemicals have various protective and therapeutic effects which are essential to prevent diseases and maintain a state of well being. The qualitative analysis of the rhizome extract showed the presence of phytochemical constituents such as alkaloids, carbohydrates, flavonoids, tannins, anthocyanins and phenolics[12].

Total Phenolic Content

The total phenolic content in methanolic and ethanolic rhizome extracts in terms of gallic acid equivalent is 4.77.50 and 7.5 mg/g of extract powder respectively. Result indicates that ethanol extract of rhizome of *A. Calamus* showed higher total phenolic content than methanol (Table2) which shows that total phenolic content varies according to the solvent use in extract. Similar finding also reported by Amit Bahukhandi et al. 2013 [13], that total phenolic content varies significantly among the different solvent extracts. Variation in total phenolic content in different solvents is due to the fact that solvents play an important role in extraction of phenolic compounds mainly due to their diverse and complex nature.

Total flavanoid content

Total flavonoid content of methanolic and ethanolic extract was 12.02 and 12.89 mg QE/g of extract respectively. The data in table 2 shows no much difference of flavanoid content between methanol and ethanol extract. However ethanolic extract shows higher flavanoid content than methanolic extract which also shows that the total flavanoid contents were greatly influenced by the solvent used in extract. G. S. Suhartati Djarkasi et al, 2019[14] also reported that the the extraction of flavonoids is significantly influenced by solvent treatments on karimenga leaves and rhizomes where the highest flavonoid content is derived in methanol extract compare to acetone, ethyl acetate, and hexane solvents which are non-polar solvents.

Table1: Total phenolic and flavanoid content in *A. Calamus* rhizome extract.

Solvent	Total Phenolic content (mg GAE/g of extract)	Total flavanoid content (mg QE/g of extract)
Methanol	4.77± 0.22	12.02± 2.95
Ethanol	7.51± 0.32	12.89± 2.76

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

Total antioxidant activity:

The total antioxidant assay indicates a significant difference ($p< 0.05$) between two solvent i.e methanol and ethanol used in extract with ethanol extract having higher activity than methanol extract (Table.1). However, the total antioxidant activity increased with the increased in concentration of extract in both the solvent. The total antioxidant activity of both methanolic and ethanolic extracts of rhizome were found highest (37.2 µg AAE/mg and 75.6 µg AAE/mg respectively) in highest concentration 90 µg/ml and lowest (25.5 µg AAE/mg and 30.0 µg AAE/mg respectively) in lowest concentration 10 µg/ml (Table1). Amit Bahukhandi et al. (2013)[13] reported that Antioxidant activity was significantly influenced by solvent type and the highest antioxidant activity was found in acetone extract (average value ABTS 10.70; DPPH 5.56; FRAP 5.14 mM AAE/ 100 g dw) followed by acetonitrile and methanol. G.C. Bag et al. (2016)[15] also reported the total antioxidant activity assay also indicates a dose dependent manner with methanol extract having higher activity than aqueous extract.

Table 2: Total antioxidant of *A. calamus*

Concentration (µg/ml)	Total antioxidant activity in µg AAE/mg of extract	
	Methanol	Ethanol
10	25.5 ± 0.002	30.0 ± 0.003
30	28.8 ± 0.003	44.7 ± 0.004
50	32.7 ± 0.003	55.8 ± 0.005
70	35.1 ± 0.003	60.6 ± 0.005
90	37.2 ± 0.003	75.6 ± 0.006

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

Determination of reducing power

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Figure 1 shows the dose dependent reducing power activity of *Acorus calamus* methanol and ethanol rhizome extract at different concentration using the potassium ferricyanide reduction method. The concentration of extract ranged from 10-90µg level. As the concentration of extract is increased the reducing power also increased in both the solvent and attained maximum at 100µg concentration. Increasing absorbance indicates an increase in reductive ability. Ethanoilc extracts showed higher reducing power than methanolic extracts. The reducing power activity is due to the presence of reductones (phenolics). As reducing power assay measures the electron donating capacity of an antioxidant, it is associated with the presence of reductones. Reductones exhibit antioxidant action by breaking the chain reactions by donating a hydrogen atom and also reported to react with certain precursor of peroxide thereby preventing peroxide formation[16, 17, and 18]. G.C. Bag et al 2016[15] reported that the total antioxidant activity assay also indicates a dose dependent manner with methanol extract having higher activity than aqueous extract.

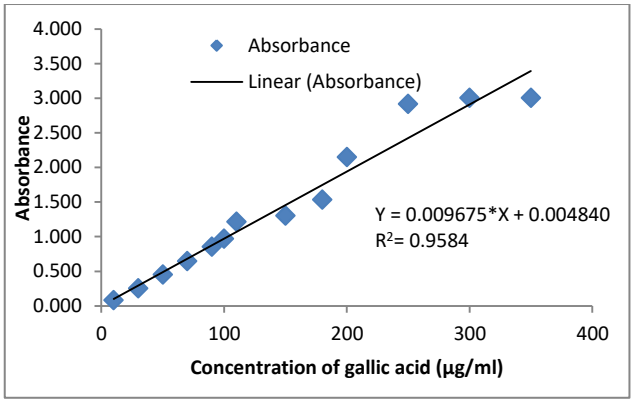

Figure 1: Standard curve of gallic acid (µg/ml)

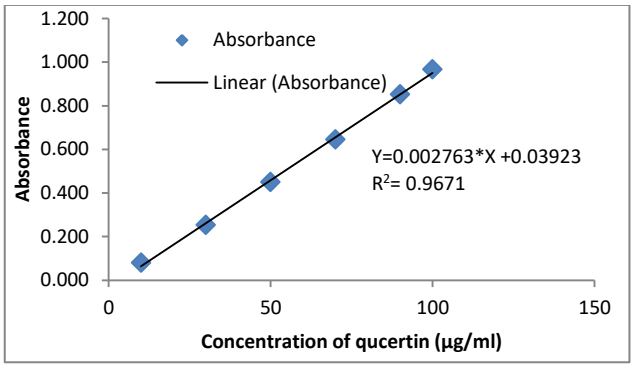

Figure 2: Standard curve of quercetin (µg/ml)

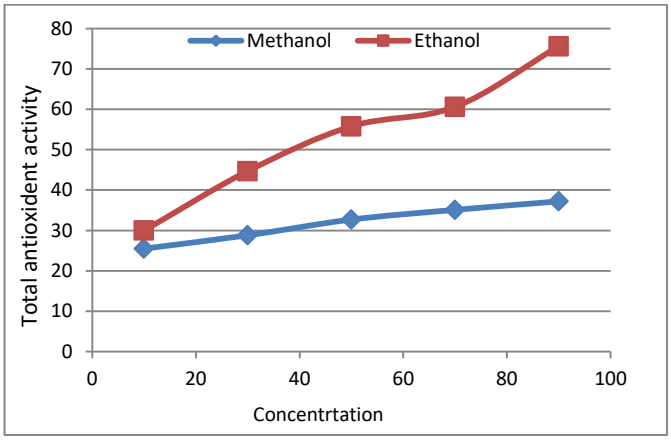

Figure 3: Total antioxidant activity of methanolic and ethanolic extract of *A. Clamus*

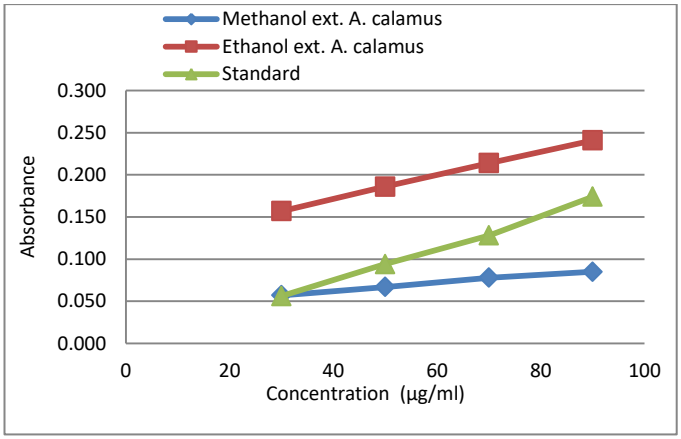

Figure 4: Reducing power of methanolic and ethanolic extracts of *A. calamus*

CONCLUSION

Phytochemical screening showed the presence of almost all the primary and secondary metabolites in meythanolic and ethanolic extract of *A. Calamus* rhizome. The present study indicates rhizome of A. Calamus possess antioxidant activity. Ethanol was found to be a better solvent for extracting phytochemicals from *A. Calamus.* Total phenolic content was highest in ethanol extract than methanol extract. Total antioxidant activity and reducing power was highest in ethanolic rhizome extract. Both total antioxidant and reducing power increased with increases in concentration of the extract.

Conflict of Interest: None

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REFERENCES

1. Ncube NS, Afolayan AJ, Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. African Journal of Biotechnology 2008; 7(12):1797-1806. https://doi.org/10.5897/AJB07.613

2. Hill AF. Economic botany a text book of useful plants and plant products. 2nd edn. Mcgraw-Hill book company Inc, NY. 1952.

3. Ramakrishna Y, Manohor AI, Mamata P, Shreekant KG. Plants and novel antitumor agents: A review. Indian Drug. 1984; 21:173-85.

4. Balakumbahan R, Rajamani K, Kumanan K. Acorus calamus: An overview. Jour Med Plants Res. 2010; 4: 2740-2745.

5. Copping LG. Crop protection agents from nature. In: Natural products and analogins: The Royal Society of Chemistry, Cambridge, 1996, 501.

6. Cavazza G. Les chimiotypes parmi les plantes aromatiques cas du calamus polyploide. Ann Fals Exp Chins, 1976; 69:833-844.

7. Archana Parki, Pinky Chaubey, Om Prakash, Ravendra Kumar and Anil K. Pant. Seasonal Variation in Essential Oil Compositions and Antioxidant Properties of Acorus calamus L. Accessions. Medicines, 2017; 4:81. https://doi.org/10.3390/medicines4040081 PMid:29117116 PMCid:PMC5750605

8. Kaufman PB, Cseke LJ, Warber S, Duke JA, Brielmann, Natural products from plants, CRC press, New York, 1999, pp. 20-22. https://doi.org/10.1201/9780849331343

9. Braca A, Tommasi ND, Bari LD, Pizza C, Politi M, Morelli I, J Nat Prod, 2001; 64:892-895. https://doi.org/10.1021/np0100845 PMid:11473417

10. Oyaizu M. Studies on products of browning reactions: antioxidant activities of products of browning reaction prepared from glucosamine. J Nutrit; 1986; 44:307-15. https://doi.org/10.5264/eiyogakuzashi.44.307

11. Prieto P, Pineda M, Aguilar M. Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E1. Analytical Biochem, 1999; 269:337-341 https://doi.org/10.1006/abio.1999.4019 PMid:10222007

12. Nanda B L, Sayeeda Nigar Sultana G and Thulasi T Radhakrishnan.Determination of phytochemicals and antioxidant activity of acorus calamus rhizome. Journal of Drug Delivery & Therapeutics; 2014; 4(6):117-121 https://doi.org/10.22270/jddt.v4i6.1005

13. Amit Bahukhandi, Sandeep Rawat, Indra D. Bhatt, Ranbeer S. Rawal . Influence of Solvent Types and Source of Collection on Total Phenolic Content and Antioxidant Activities of Acorus calamus L. Natl. Acad. Sci. Lett, January-February 2013; 36(1):93-99. https://doi.org/10.1007/s40009-012-0109-8

14. G. S. Suhartati Djarkasi, Lana E. Lalujan, Erny Nurali and Maria F. Antioxidant Activity of Karimenga (Acorus calamus). AIP Conference Proceedings 2019; 2155:020051. https://doi.org/10.1063/1.5125555

15. G.C. Bag, P. Grihanjali Devi, Th. Bhaigyabati. Phytochemical Screening and Antioxidant Activity of Meyna laxiflora Species Found in Imphal West District of Manipur. Int. J. Pharm. Sci. Rev. Res., January - February 2016, 36(1):137-143

16. Olayinka A, Aiyegoro AI, Okoh. Preliminary phytochemical screening and in vitro antioxidant activities of the aqueous extract of Helichrysum longifolium DC, BMC Complementary and Alternative Medicine, Oxford University Press, 2010; 10:21. https://doi.org/10.1186/1472-6882-10-21 PMid:20470421 PMCid:PMC2877649

17. Oktay M, Gulcin I, Kufrevioglu OI. Determination of in vitro antioxidant activity of fennel (Foeniculum vulgare) seed extracts, Lebensum. Wiss. U. Technol, 2003; 36:263-271. https://doi.org/10.1016/S0023-6438(02)00226-8

18. Chanda S, Dave R . In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview, Afr J Microbiol Res., 2009; 3(13):981-996.