IJPSR (2015), Vol. 6, Issue 12 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 15 June, 2015; received in revised form, 20 July, 2015; accepted, 03 October, 2015; published 01 December, 2015

PARTIAL PURIFICATION AND ANTIOXIDANT ACTIVITIES OF METHANOL EXTRACTS OF *ACORUS CALAMUS.*

S. G. Funde

Dr. Shree Nanasaheb Dharmadhikari Arts, Commerce, & Science College Gove, Tal - Roha , Dist – Raigad Maharastra, India.

Keywords:

Acorus calamus, Antioxidant activity, DPPH, FRAP, Polyphenol

Correspondence to Author:
S. G. Funde

Dr. Shree Nanasaheb Dharmadhikari Arts, Commerce, & Science College Gove, Tal - Roha , Dist – Raigad Maharastra, India.

Email: fundesharad@gmail.com

ABSTRACT: *In vitro* antioxidant activity test was carried out on partially purification of methanol extract of *Acorus calamus*. Partially purification was done to obtain ten fractions. Partial purification was carried out by using hexane: ethyl acetate solvent system. These fractions were studied for the polyphenolics and antioxidant activity. S6 –S9 fractions shows maximum radical scavenging activity, ferric reducing antioxidant potential in S3, S7 and S9 and antioxidant properties by phosphomolybdenum method higher reduction of metals observed in S1 (34.18), S4 (33.95), S5 (33.02), S6 (31.33), S7 (27.9) and S10 (35.53) mM of ascorbic acid / gm of fraction.

INTRODUCTION: In biological system still have interest on free radicals reaction mechanism and induced diseases like cardiovascular diabetics cancer etc. In many diseases production of ROS is higher. Protection of ROS mediated cell damage could be possible by giving perfect drug to disease or natural antioxidant molecule treatment. Fruit vegetable and medicinal plant natural products have good antioxidant effect in biological system. *Acorus calamus L (Areace)* commonly known as sweat flag herb. Which prefer swampy or marshy habitat for growth. It has grass like long slender leaves, yellowish green to brown flower and rhizome. Plant observed in Asia, North America and Europe. Different plant part is used in formulation of the ayurved medicine.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.6(12).5279-83

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.6(12).5279-83

Rhizome use in emetic, kil lice nervous system, throat infection diarrheal, smallpox, respiratory, gastrointestinal track, gout rheumatism and mental disorder treatment [1, 2]. Leaves used wound to kil lice and stem for cough, cold, and toothache. Root and bark is used as antidotes for snake bite. *Acorus calamus* plant material have different type of extracts which showed different activity, alcoholic extract had anti-inflammatory, CNS depressant behavioral changes, anticancer, antiulcer, cytoprotective, hypolipidenic, antimicrobial, acetylcholine inhibitory activity [3, 4]. Aqueous extract antidiharial behavior changes, CNS depressant hypnotic effect aqueous-alcohol combined solvent extract have hypolipedimic activity in rat [5, 6, 7, 8].

The second step in biological activity related study of the drug discovery is partial purification of crude drug. For purification several techniques of column chromatography are available. Amongst that silica gel column, high pressure thin layer and high pressure liquid chromatographic techniques are widely used for purification of the extract.

In present study methanol was selected for the large scale extraction because of the easy avibality. This was further purified (partially) using silica gel column column chromatography. Partially purified fractions used for polyphenolic and antioxidant study.

MATERIALS AND METHOD:

Materials:

Methanol (MeOH), Hexane, Ethyl acetate, Silica gel (mesh size 60-120) purchased from Qualagen. Gallic acid, Ascorbic acid, Hydrochloric acid(HCl), Ferrous sulphate($FeSO_4$), Sulfuric acid(H_2SO_4), Sodium phosphate, Ammonium molybdate, Ammonium ferrous sulphate ($NH_3(FeSO_4)$), Diphenyl picryl hydrazine (DPPH), Ferric chloride($FeCl_3$), Folin ciocalteu reagent(FC reagent), (TPTZ), Merck and Sigma (Germany).

Extraction protocol:

Acorus calamus whole plant powder (100 g) was mixed with methanol (1.5 L) over a period of 24 hr. at 37 ^{0}C stirring. The extract was evaporated to dryness on a rotary evaporator at 35 ^{0}C. recidual yeild obtained was 28.9 g.

Partial purification of extract by silica gel column chromatography:

The dried residue (15 gm) was mixed (homogenized) with silica gel 60 - 120 and ethyl acetate was added prepare slurey and then dried in IR lamp. After that it was subjected to column chromatography , using a 44 × 4.5 cm glass column filled with silica gel (mesh size: 60-120). The extract dried powder was layered at the top of the column. After bedding down of the gel material, fractionation was conducted by successive changing polarity of hexane: ethyl acetate (0%:100%).

Ten fractions were collected by observing and comparing TLC pattern. The TLC plate's pattern (Merck 0.25 mm silica gel plates 1 mm (20 cm × 20 cm)) developed in a ethyl acetate and hexane solvent system and visualized in Iodine chamber. Solvent was removed by rotary evaporation in vacuo at 37 ^{0}C and dried fractions were suspended in methanol and DMSO (1mg/ mL). Which was used for polyphenolics and antioxidant and activity study.

Determination of polyphenolic content:

Total polyphenolic content was analyzed by the Folin–Ciocalteu method [9]. The reaction mixture (3.6 mL) contains 20µL of sample, 3.2 ml of distilled water, 100µL of FC reagent; 300 µL of 60µg of saturated sodium carbonate was incubated at 37^{0}C for 30 min in water bath the absorbance was read at 765 nm was measured in triplicate. Gallic acid (100µg/mL) was used for calibration of standard curve. The results were expressed for polyphenolics as mg of gallic acid equivalent (mg GAE)/g of fraction.

Antioxidant Activity:

Total antioxidant potential by phosphomolybdate method:

Total antioxidant potential of sample was determined by the Phosphomolybdate method [10]. Final volume of the reaction 3.010 ml containing 3.0ml of (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) 10 µL of the respective fractions incubated at 95^{0}C for 90 min. After the samples were cooled at room temperature, the absorbance of the aqueous solution of each fraction was measured at 695 nm against blank which contains 10µL of solvent. Antioxidant potential of fractions were expressed as mM of Ascorbic acid /g of dry wt. of fractions. The estimation was carried out in triplicate.

$$\text{mM of ascorbic acid} = \frac{(\text{O.D. of Standard} - \text{O.D. of Sample})}{(\text{O.D. Standard})} \times \text{Conc. of Stdandard}$$

Ferric-reducing antioxidant potential assay:

The FRAP assay was carried out according to the procedure of Benzie and Strain with modification [11]. FRAP reagent was prepared from sodium acetate buffer (300 mMol/L, pH 3.6), 10 mMol/L TPTZ solution in 40 mMol/L HCl and 20 mMol/L $FeCl_3$ in 40 mMol/L HCl solution in proportions of 10:1:1 (v/v), respectively. The FRAP reagent was stored in cooled condition at 0-4 ^{0}C for five days. Before use the reagent was warmed to 37 °C in a water bath. 25µL of each fractions was added to 1.475 mL of sodium acetate buffer and 1.5 mL of FRAP reagent. The absorbance of the reaction mixture was then recorded at 593 nm after 5 min. The standard curve was constructed using $FeSO_4$ solution (10–100 µMol/L). The results were expressed as µMol Fe (II)/g fraction.

$$\mu M \text{ of } Fe(II) = \frac{(O.D. \text{ of Standard} - O.D. \text{ of Sample})}{(O.D \text{ Standard})} \times \text{Conc. of Standard}$$

Free radical scavenging ability by DPPH radical:

The DPPH assay was carried out by 96 well plate methods [12]. DPPH assay is based on the measurement of the scavenging ability of antioxidants towards the stable DPPH radical. The free radical DPPH, which shows absorption at 517 nm, is reduced to the corresponding fractions when it reacts with hydrogen donors. Stock solution of DPPH 1 mM was prepared in methanol and working solution 0.1mM prepared in methanol. 10 µg of partially purified fractipon was added in 100 µL of methanol and 100 µL of 0.1mMol of DPPH. The mixture was shaken and allowed to stand at room temperature in the dark for 10 min. The decrease in absorbance of the resulting solution was monitored at 517 nm at 10 min. The results were expressed in % of inhibition by following formula.

$$\% \text{ of inhibition } = \frac{(O.D. \text{ of control} - O.D. \text{ of sample})}{(O.D \text{ control})} \times 100$$

RESULT:

Total polyphenols:

The contents of total polyphenolics of fractions are given in **Fig. 1** The amount of total phenolic compounds of different polarity fractions are ranged from 9.24 to 343.43 mg gallic acid/gm fractions. The S6 fraction (50 – 65% hexane: ethyl acetate) highest quantity of polyphenolics were as other polyphenolics obtained in various fractions are as follow S6 > S7 =S10 >S9 > S4> S3= S8 >S5 >S2 and S1.

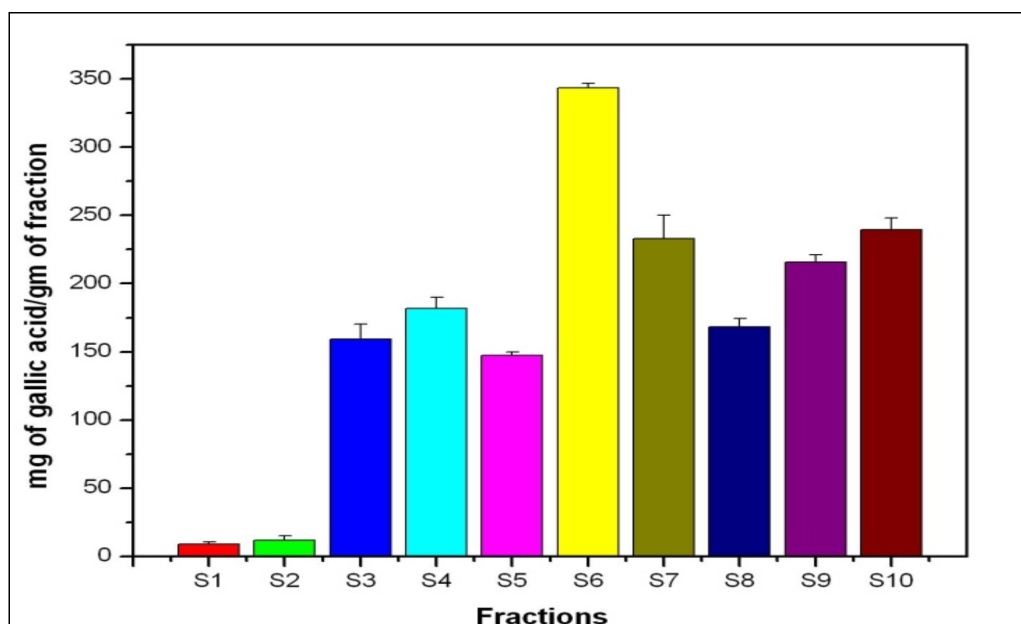

FIG.1: TOTAL PHENOLIC CONTENTS OF *A. CALAMUS* FRACTIONS.
Data are shown as mean±S.D. one way ANOVA $p = < 0.001$ which represents the significance n (sample size) = 3.

Antioxidant activities:

DPPH is a stable nitrogen-centred free radical the colour of which changes from violet to yellow upon reduction by either the process of hydrogen or electron donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers. % inhibition ranked from 12.2 % for S4 fraction to 84.92 % for fraction S9 (**Fig. 2**). S9 (84.92 %), S8 (79.96 %), S7 (83.28%) and S6 (66.47%) fractions has high DPPH scavenging property.

The results of Phosphomolybdate antioxidant activity of various fractions are represented in **Fig. 3** S10, S3, S4, S5, S1 and S6 fractions are the most effective phosphomolybdate antioxidant activity. The results showed for FRAP values were higher in fractions samples compared to other fraction. This showed that in S7, S1, S9, and S3 fractions was more efficient in isolating antioxidants in plant material compared remaining fraction.

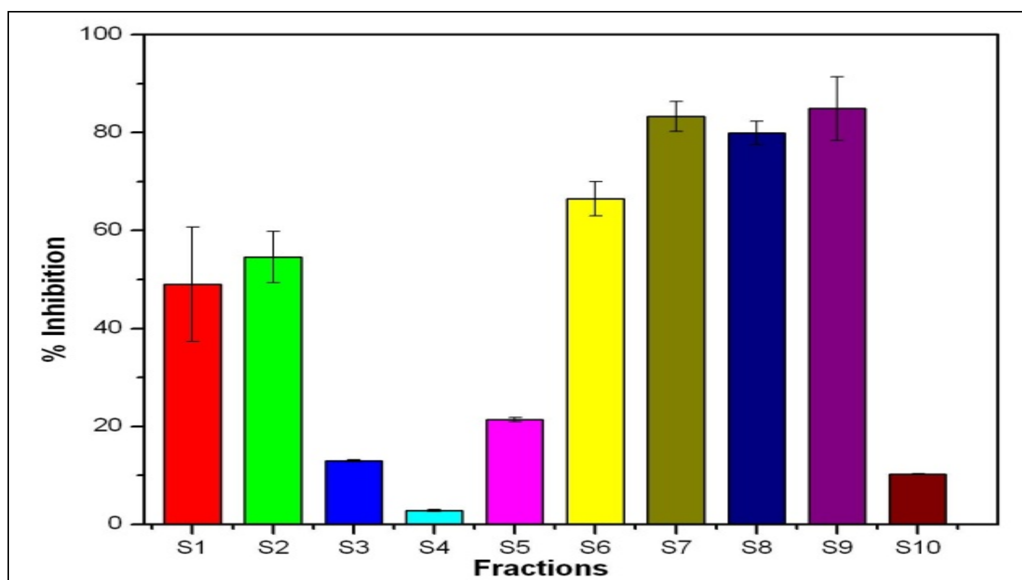

FIG.2: RADICAL SCAVENGING ANTIOXIDANT ACTIVITY OF *A. CALAMUS* FRACTIONS.
Data are shown as mean±S.D. one way ANOVA $p = < 0.001$ which represents the significance, n (sample size) = 3.

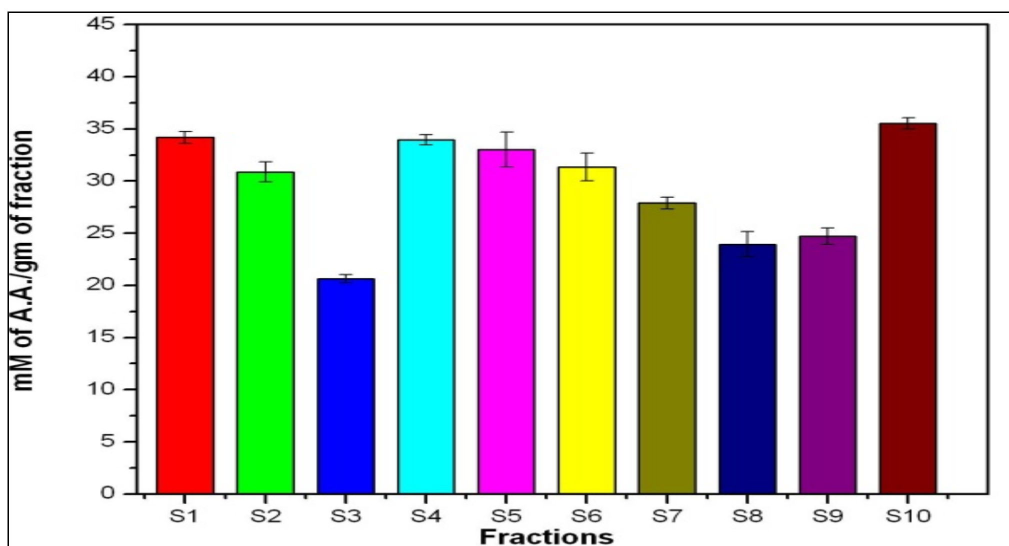

FIG.3: METAL REDUCING ANTIOXIDANT ACTIVITY OF *A. CALAMUS* FRACTIONS.
Data are shown as mean±S.D. one way ANOVA $p = < 0.001$ which represents the significance n (sample size) = 3.

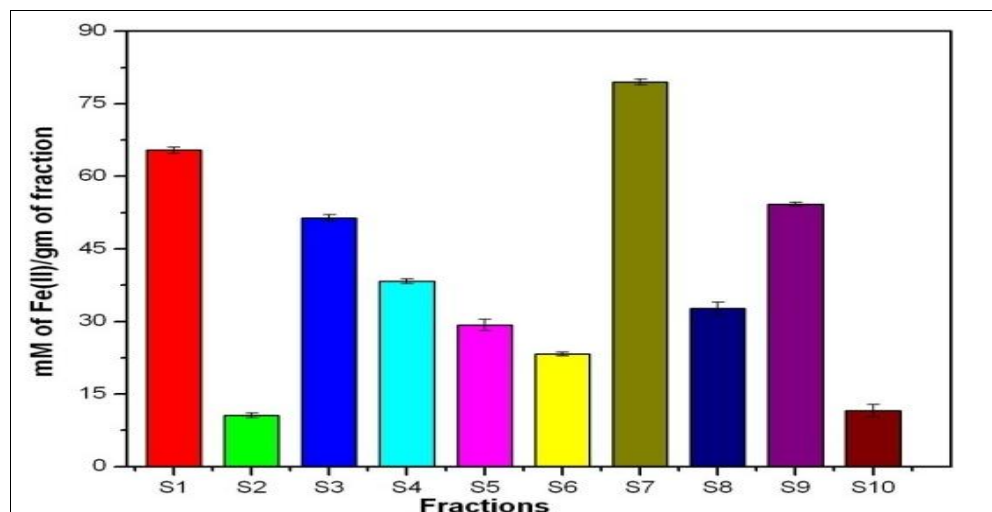

FIG.4: METAL REDUCING ANTIOXIDANT ACTIVITY OF *A. CALAMUS* FRACTIONS.
Data are shown as mean±S.D. one way ANOVA $p = < 0.001$ which represents the significance, n (sample size) = 3.

DISCUSSION: In this study, an investigation on in vitro antioxidant activities of Indian medicinal plant, *A. calamus* was carried out to verify the claimed traditional uses of the plant. Results have revealed that the hexane: ethyl acetate fraction of the whole plant was the most active in both experimental models. Bioactivity guided fraction through successive column chromatography procedures led to the isolation of a partially purified fractions of active compounds. Antioxidant studies of these fraction supports for *A. calamus* antimicrobial and hyperlipidemia activity in rat [13, 14]. Polyphenolics content fractions have free radical scavenging ability. Past finding had been proved that polyphenolics are directly proportional for the free radical scavenging ability. Presence of the different antioxidant activity in different fraction can suggest the synergetic effect of the crude extract, in traditional medicine for treatments [15, 16]. Further isolation of the active compounds on the basis of pharmacological activity is beneficial for new finding of drugs.

CONCLUSION: For systematic investigation of active component of alcoholic extracts for partial purification and its application is important. Crude alcoholic extracts of *Acorus calamus* was used for the partial purification. Partial purified fractions were analyzed for antioxidant activities. Various fractions have remarkable activity for antioxidants. Most of the fractions contain more than single activity. Therefore such fractions can offer promising leads for the discovery of potent antioxidants activities that can have therapeutic use globally.

REFERENCES:

1. A.Morina, L.Fio, P. M. Neva, M.Syalfinaf, N.Masayuki, G.Irfan, M. H. P.Agus, S.Rochmah, K.Mamoru, Termiticidal activity of *Acorus calamus* Linn. rhizomes and its main constituents against Coptotermes curvignathus Holmgren. *Journal of Asia-Pacific Entomology,* 2015(18), 1, 47-50
2. S. B. Rajput, M. B. Tonge, S. M. Karuppayil, An overview on traditional uses and pharmacological profile of *Acorus calamus* Linn. (Sweet flag) and others *Acorus s*pecies *Phytomedicine,* 2014, 21, 3, 268-276.
3. A. Muthuraman, N. Singh. Acute and sub-acute oral toxicity profile of Acorus calamus (Sweet flag) in rodents. *Asian Pacific Journal of Tropical Biomedicine,* 2012, (2), 2, S1017-S1023.
4. S.P. Jain, Tribal remides from Saranda forest Bihar India-I. Inti J crude drug res., 1989, 29-32.
5. L. Badam, *In vitro* studies on the effects of *Acorus calamus* extract and beta arsinone on herpes viruses, Derghaya Int., 1995; 16-18
6. C. X. Zhou, D. Q., Y. Y. Yan, H. S. Wu, J. X. Mo, L. S. Gan, A new anti-diabetic sesquiterpenoid from, *Acorus calamus Chinese Chemical Letters,* 2012; 23(10): 1165-1168
7. 7)A. Muthuraman, N. Singh, Neuroprotective effect of saponin rich extract of *Acorus calamus* L. in rat model of chronic constriction injury (CCI) of sciatic nerve-induced neuropathic pain. *Journal of Ethnopharmacology,* 2012; (142)3: 723-*731*
8. P. Patel, J. Vaghasiya, A. Thakor, J. Jariwala. Antihypertensive effect of rhizome part of Acorus calamus on renal artery occlusion induced hypertension in rats. *Asian Pacific Journal of Tropical Disease,* 2012; (2): S6-S10.
9. L. R.Fukumoto, G.Mazza; Assessing antioxidant and prooxidant activities of phenolic compounds. J. Agric. Food Chem., 2000; 3597–3604.
10. M. P.Germano, D. E. R.Pasquale, V. Angelo, S.Catania, V.Silvaria, C.Costa, Evaluation of extracts and isolated fraction from Capparis spinosa L. Buds as an antioxidant source. J. Agric. Food Chem., 2002; 1168–1171.
11. S. G. Funde Phytochemicals evaluation, anticancer, antioxidant and antimicrobial activity of *Acorus calamus* different solvent extracts. Journal of Chemical and Pharmaceutical Research, 2015(7): 6, 495-504.
12. B.Bozin, N.Mimica-Dukic, I.Samojlik, A.Goran, R. Igic, Phenolics as antioxidants in garlic (*Allium sativum L.*, Alliaceae). Food Chemistry. 2008; 925–929.
13. P. K.Mukherjee, V.Kumar, M. Mal, P. J.Houghton, *Acorus calamus*: Scientific validation of ayurvedic tradition from natural resources. Pharmaceutical Biology. 2007; 651–666.
14. A.E. Raja, M. Vijayalakshmi, G. Devalarao, *Acorus calamus linn.* : Chemistry and biology. Research J. Pharm. and Tech. 2009; 1- 6.
15. E. Joyce, B. Cristina, P. M. Rebeca, N. B. Lidiane, C. D. S. Luiz, F. J. Ary, Synergism between plant extract and antimicrobial drugs used on *Staphylococcus aureus* diseases Mem. Inst. Oswaldo Cruz, 2006; 101.
16. G.G.F. Nascimento, J. Locatelli, P.C. Freitas, G.L. Silva, Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Braz J Microbiol* 2000, 247-256.

How to cite this article:
Funde SG: Partial Purification and Antioxidant Activities of Methanol Extracts of *Acorus Calamus.* Int J Pharm Sci Res 2015; 6(12): 5279-83.doi: 10.13040/IJPSR.0975-8232.6(12).5279-83.

All © 2013 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **ANDROID OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)