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ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACT OF LEAVES AND RHIZOMES OF *ACORUS CALAMUS* L.

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ABSTRACT

The present study was to investigate the antioxidant activity of methanolic extract of leaves and rhizomes of *Acorus calamus* L. by using DPPH (2, 2 diphenyl-1-picrylhydrazyl). Both the extracts exhibit potential free radical scavenging activity. The rhizome extract displayed stronger 2, 2 diphenyl-1-picrylhydrazyl free radical scavenging activity comparing to leaves extract. Free radical scavenging activities of methanolic extract of leaf and rhizome of *A. calamus* showed the scavenging property of 68% and 95% respectively. Total phenolic content in each extracts were also determined. Total phenolic content was found to be 17.1±0.04 and 17.3±0.88 mg gallic acid/g of extract respectively. The study revealed the correlation between the total phenolic content and antioxidant activitiy to free radical scavenging property of extracts of leaves and rhizomes of *A. calamus*.

Keywords: *Acorus calamus*, Antioxidant, DPPH, Methanol,Phenolic content.

INTRODUCTION

Acorus calamus Linn. (syn. *Acorus odoratus*) commonly known as Sweet Flag or Calamus, belongs to the family Araceae (Adoraceae) that comprises about 110 genera and more than 1,800 species. The common names of *Acorus calamus* which are used in different parts in India are Bach (Hindi), Vasambu (Tamil), Baje (Kannada), O-hidak (Manipuri) and Vasa (Telugu). It is found commonly on the banks of streams and in damp marshy places. *Acorus calamus* Linn. is a semi aquatic perennial herb with creeping and extensively branched, aromatic rhizome. The scented leaves and more strongly scented rhizomes have traditionally

been used medicinally. The rhizomes are considered to possess anti-spasmodic, carminative and anthelmintic properties and also used for treatment of epilepsy, mental ailments, chronic diarrhea, dysentery, bronchial catarrh, intermittent fevers and tumors. It is listed as an insecticide, an antifungal agent, an antibacterial agent and a fish toxin (Anonymous, 1975). *A. calamus* is a native of eastern countries and also it is indigenous to the marshes of the mountains of India. It is cultivated throughout India, ascending to an altitude of about 2200 metres. It is also found in marshy tracts of Kashmir, Shirmaur (Himachal Pradesh), Karnataka, Manipur and in Naga Hills.

Medicinal properties of medicinal plants have been investigated from ancient times. Many medicinal plants are potential sources of antioxidant compounds which protects the cells against the damaging effects of reactive oxygen species. Medicinal plants contain large amounts of antioxidants which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Many of these phytochemicals possess significant antioxidant capacities (Anderson et al., 2001). Reactive oxygen species (ROS) such as hydrogen peroxide, hydroxyl radical and superoxide anion play a major role in various degenerative diseases. Antioxidant compounds can delay or inhibit oxidation of lipids and other molecules by inhibiting oxidative chain reactions (Emmons 1999).The antioxidant property is mainly due to phenolic compounds (Shahidi *et al*., 1992). Recently interest has increased for exploring untapped reservoirs of medicinal plants. A great numbers of studies have been reported toward biological activities of traditional medicinal plants. Natural antioxidants neutralize the free radicals effect in the human body and retard the progress of many diseases (Oskoueian et al., 2011). Hence the present study was to investigate total phenolic content and antioxidant activity of *Acorus calamus.*

MATERIALS AND METHODS

Plant Extract preparation

Acorus calamus was collected during the month of September from Imphal west district. The collected plant was washed thoroughly in running tap water and the leaf and rhizome were dried in shaded condition. The dried samples were coarsely grinded separately for extraction. Each of the dried powdered samples was soxhlet extracted with methanol for 96hrs. The extracts were concentrated by evaporation.

Determination of total phenolic content

Dilutions of Sample (0.5ml) were oxidised with 2.5mL Folin-Ciocateau for 5 minutes at room temperature. Then the reaction was neutralized with 2mL Sodium Carbonate. The absorbance of the resulting blue colour was measured at 650nm with the spectrophotometer after incubation for 2hr at room temperature in dark. Quantification was done on the basis of the standard curve of gallic acid. The entire tests were carried out in triplicate. Results were expressed as gallic acid equivalent (GAE), i.e., mg gallic acid/g of extract.

Determination of free radical scavenging activity

The free radical scavenging activity was measured by DPPH method with slight modification (Warjeet *et al.*, 2010). DPPH radical scavenging assay were performed to study the antioxidant potency of methanol extract of leaf and rhizome of *A.calamus*. Each sample (3mL at 0.025g/ mL) was mixed with a DPPH solution (45µg/ mL, Sigma) in HPLC grade methanol (Merck), vortexed well at room temperature and left standing exactly for 10 min. The UV/VIS absorbance was measured at 517 nm serving the methanol without DPPH solution as blank solution. A reference solution (125µg/ mL) of Butylated hydroxyl toluene (BHT, Sigma) in methanol was used taking 100% radical scavenging activity. The scavenging percentage was calculated using the equation:

$$\% \text{ Scavenging} = (A_0 - A_5) \times 100 / (A^*_0 - A^*_5)$$

where, A_0, A_5 are the absorbance values of DPPH + Sample solution at 0.0 min and after 0.5 min, respectively; A^*_0, A^*_5 are the absorbance values of DPPH + BHT at 0.0 min and after 0.5 min.

Statistical Analysis

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

RESULT AND DISCUSSION

There is always an interest to study the antioxidant property of medicinal plants especially used in traditional folk medicine. Antioxidant activity of the plant extract is often associated with the phenolic component present in them. Free radical scavenging activity was measured by DPPH method. The reduction of the DPPH by the process of either proton or electron donation can be monitored spectrophotometrically, as the DPPH upon reduction changes colour from violet to yellow. The DPPH antioxidant assay is based on the ability of DPPH a

stable free radical, to decolorize in the presence of antioxidants. Therefore, the substances capable of reducing DPPH could be considered as an antioxidant(Kris 2002).

Using this method antioxidant capacity of methanolic extract of leaf and rhizome of *Acorus calamus* were assessed. Free radical scavenging activities of methanolic extract of leaf and rhizome of *Acorus calamus* showed the scavenging property of 68% and 95% respectively. A strong correlation has been observed between total phenolic content and antioxidant activity. Relation between phenolic content and antioxidant activity has been reported (Kohakenon 1999). Phenolic compounds present in the plants are known for their ability of scavenging free radical (Devi et al 2011). Both roots and leaves of *A. Calamus* have shown antioxidant, antimicrobial and insecticidal activities (Srinivasan et al 2001). Total phenolic content was found to be 17.1±0.04 and 17.3±0.88 mg gallic acid/g of extract respectively. High phenolic content 12.6mgGAE/100Gdw has been investigated in *A. calamus*(Wojdylo et al, 2007) *Acorus calamus* is the source of various types of compounds having diverse biological activities.

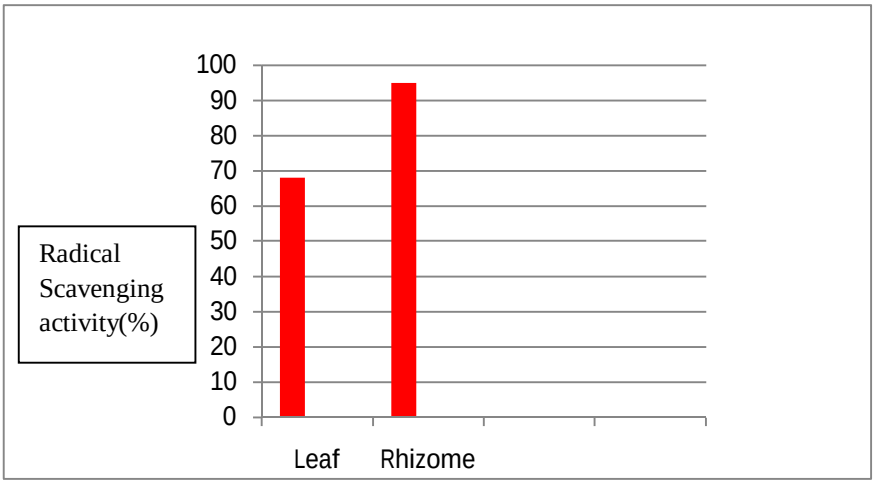

Figure 1: DPPH Scavenging activity of leaf and Rhizome of *Acorus calamus* Linn.

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