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Research Paper

Phytochemical Screening and Determination of Antioxidant Activity of Rhizome of *Acorus Calamus*

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Abstract
Phytochemical screening is an important step which leads to the isolation of new and novel compounds. Plant *Acorus calamus* plays a pivotal role in folk medicine. The present study was conducted with the main purpose of phytochemical screening and evaluation of antioxidant properties *rhizome extracts of A. calamus*. Rhizome of *A. calamus* was subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with methanol. The major phytochemicals found in methanol extracts of rhizome of *A. calamus* were, alkaloids, flavonoids, glycosides, proteins & amino acids, reducing sugars, saponins, steroids, phenolic compounds/tannins, and terpenoids. quantitative estimation of phytochemicals in methanolic extract of rhizome of *A. calamus* revealed that flavonoid quantity found to be highest (34.98 GAE) followed by total phenols (25.80 GAE), tannins (3.01 GAE) and carbohydrates (8.68 GAE). the IC_{50} values exhibited by methanolic extracts of rhizome of *A. calamus* was found to 6.01 µg/mL. In conclusion, this preliminary study confirms that the *A. calamus* has vide variety of secondary metabolites. Biological activity such as antioxidant properties of methanolic extracts of rhizome of *A. calamus* depicted that *A. calamus* could be potential drug agent of folk medicines.
Keywords: *Acorus calamus*, Rhizome, Phytochemicals, Antioxidant, Folk medicine

Introduction

Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are a group of species that accumulate different active principles, useful in treating various human or animal diseases. They 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] Phytochemicals are naturally occurring in different parts of the medicinal plants that have defense mechanism and protect from various diseases.[2] The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents which produce definite physiological action on the human body and these bioactive substances include alkaloids, carbohydrates, terpenoids, steroids, flavonoids, tannins, etc.[3]

Free radicals are continuously being produced in our body as a result of various metabolisms. Some amount of free radicals is very much necessary for body host defense system, signalling mechanism and in the induction of a mitogenic response. But the persistence of these free radicals even after their activity results in deleterious effects. These free radicals act on important biomolecules like nucleic acids (mutations), lipids (membrane lipid peroxidation), proteins (oxidation) and carbohydrates resulting in various diseases. Over 70 degenerative diseases (Alzheimer's disease, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis (ALS), memory loss, depression, arthritis, cancer, ageing etc..) are caused due to free

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radicals.[4-6] Herbal medicines are the materials which are derived from one or more plant which possess some curative values to prevent human body from common diseases.[7] Free radical scavenging property can be removed completely by the antioxidants and maintains the balance in the body.[8]

Acorus calamus L., often recognized as "sweet flag" or "calamus," is a flowering plant indigenous to Japan, China, India, Sri Lanka, Southern Russia, Europe, Burma, Mongolia and the United States.[9,10] It belongs to Acoraceae family, commonly grown in temperate and sub-temperate locations across the globe.[11] *A. calamus* is a deciduous, spreading, marginal aquatic perennial that features iris-like, sword-shaped leaf blades (to 3/4" wide) typically growing in basal clumps to 30" tall. It is a sterile triploid. Insignificant tiny greenish flowers appear in elongated inflorescences (spadixes to 2-4" long without showy spathes), which appear in late spring. Flowers may give way to tiny fleshy berries. Foliage and rhizomes are sweetly fragrant when bruised, hence the common name (Figure 1 and figure 2).

Figure 1: Showing *A. calamus* plant

Figure 2: Showing *A. calamus* rhizome

A. Calamus is a remarkable medicinal plant with extensive scope of biological activities and fascinating phytochemical constituents. In the field of ayurvedic medication, it is employed for the treatment of skin emissions, epilepsy, mental sicknesses, neuralgia, malignant growth dyspepsia, and bronchial catarrh, irregular fevers. It exists a multiple phytoconstituents present in restorative plants, for example, amino acids, alkaloid, phenol, tannins, carboxylic acid, terpenes and many inorganic acids.[12] With this view points, in the present we mainly aimed for

phytochemical extraction, screening and determination of antioxidant activities of rhizome parts of *A. calamus*.

Materials and Methods

Collection of plant material

The rhizomes *A. calamus* were purchased from the local market of Chikkaballapura, Karnataka, India. The rhizomes were prayed with ethanol, and then shade dried at room temperature for 10 days. The dried rhizomes were crushed to fine powder with help of electric grinder and stored in airtight containers for further analysis.[13,14]

Extraction

Approximately 50 g of dried and coarsely powdered rhizomes of *A. calamus* were subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with 550 mL of methanol. All the extracts were concentrated by distilling the solvent in a rotary flash evaporator. The extracts were preserved in airtight containers and stored at room temperature until further use.

Phytochemical Screening

Chemical screening was carried out on the methanol extracts of rhizomes of *A. calamus* by using standard procedure to detect constituents as described by sofora,[15] Trease and Evans[16] and Harborne.[17]

Test for Alkaloids

Approximately 0.2g of extract was warmed with 2% H_2SO_4 (2.0ml) for two minutes. The reaction mixture was filtered and few drops of Dragendrof's reagent was added to the filtrate. Orange red precipitation showed the presence of alkaloids moiety.

Test for Tannins and Phenolic Compounds

The extract of rhizomes of *A. calamus* in small quantity was mixed with water and heated on water bath and filtered. To the filtrate, few drops of ferric chloride ($FeCl_3$) was added. A dark green colouration indicate the presence of tannins.

Test for Glycosides

About 0.6g of extract was hydrolyzed with HCl and neutralized with NaOH solution and few drops of Fehling's solution A and B were added. Formation of red precipitate indicates the presence of glycosides.

Test for Reducing Sugars

The extract was shaken with distilled water and filtered. Few drops of Fehling's solution A and B were added and boiled for few minutes. Formation of an orange red precipitate confirms the presence of reducing sugar.

Test for Saponins

About 0.2g of extract was shaken with 5 mL of distilled water and then heated to boil. Frothing (appearance of creamy miss of small bubbles) showed the presence of saponins.

Test for Flavonoids

0.2g of extract was dissolved in diluted 10%NaOH and few drops of 2M HCl was added. A yellow solution that turns into colorless indicate the presence of flavonoids.

Test for Steroids

2 mL of acetic anhydride was added to 0.5g of extract and then added 2 mL of H_2SO_4. The change of color from violet to blue or green or red showed the presence of steroids.

Test for Terpenoids

0.3g of extract was mixed with 2 mL of chloroform ($CHCl_3$) and 3 mL of concentrated 6M H_2SO_4 was carefully added to form a layer. Reddish brown coloration at the interface was formed which indicate positive results for the presence of terpenoids.

Test for Proteins and Amino acids

To the 0.3g of extract few drops of 0.2% ninhydrin solution was added and heated for 5 minutes. Blue colouration indicate the presence of proteins.

Quantitative Estimation of Phytochemicals

Total phenolics

The concentration of total phenolics in the methanol extract of rhizome of *A. calamus* was determined by the Folin-Ciocalteu assay that involves reduction of the reagent by phenolic compounds, with concomitant formation of a blue complex, its intensity at 725nm increases linearly with the concentration of phenolics in the reaction medium.[18] The phenolic content of the extract was determined from calibration curve and were expressed in mg gallic acid equivalent/g of extract powder.

Carbohydrates

Carbohydrate content of all the extracts at 100µg concentration was determined by the phenol-sulphuric acid method.

Total flavonoid

Aluminum chloride colorimetric method was used for flavonoids determination.[19] The content was determined from extrapolation of calibration curve which was made by preparing gallic acid solution (0-0.8 mg/ml) in distilled water. The concentration of flavonoid was expressed in terms of mg gallic acid equivalent/g of extract powder.

Tannins

The tannin concentration was determined for each extract variety following a modified version of the vanillin-HCl method.[20]

Antioxidant Assay

The modified literature protocol of Blois was used for antioxidant assay.[21,22] Briefly 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution (1mL;1mM) was prepared in methanol and mixed with sample solution (3mL, containing 20-100ug) in methanol. The control was also run which contains only methanol. The hydrogen atom or electron donation abilities of extract and standards were measured from the bleaching of the purple-colored methanol solution of 2, 2-diphenyl1-picrylhydrazyl (DPPH). The absorbance was measured at 517 nm after 30 min incubation. Decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical-scavenging activity. Scavenging of free radicals by DPPH as percent radical scavenging activities (%RSA) was calculated by using the formula; DPPH% = (Control abs – Extract abs / Control) × 100. The IC_{50} value was determined by using linear regression equation *i.e.*, Y = Mx + C; Here, Y = 50, M and C values were derived from the linear graph trendline.

Results

The major phytochemicals found in methanol extracts of rhizome of *A. calamus* were, found to be alkaloids, flavonoids, glycosides, proteins & amino acids, reducing sugars, saponins, steroids, phenolic compounds/tannins, and terpenoids (Table 1).

Table 1: Photochemical screening of methanolic extracts of rhizome of *A. calamus*

Chemical Components	**Methanolic Extract of Rhizome of *A. calamus***

Alkaloids	+
Flavonoids	+
Glycosides	+
Proteins and Amino acids	+
Reducing sugar	+
Saponins	+
Steroids	+
Phenolic compounds	+
Tannins	+
Terpenoids	+

Moreover, quantitative estimation of phytochemicals in methanolic extract of rhizome of *A. calamus* revealed that flavonoid quantity of found to highest (34.98 GAE) followed by total phenols (25.80 GAE), Tannins (3.01 GAE) and carbohydrates (8.68 GAE) (Table 2).

Table 2: Quantitative estimation of phytochemicals present rhizome of *A. calamus*

Chemical Components	**Methanolic Extract of Rhizome of *A. calamus***
Total phenolics	25.80 GAE
Total flavonoids	34.98 GAE
Tannins	3.01 GAE
Carbohydrates	8.68 µg

Furthermore, the IC_{50} values exhibited by methanolic extracts of rhizome of *A. calamus* was found to 6.01 µg/mL (Table 3).

Table 3: Antioxidant activities of methanolic extracts of *A. calamus*

S. No.	**Methanolic Extract of Rhizome of *A. calamus***	**IC_{50} (µg/mL)**
1	Rhizome	6.01

Discussion

Active research has been driven in recent years on plant-based products due to their biologically beneficial effects emanating from antioxidant activities of phenolic phytochemicals. The plant

products over synthetic compound in the treatment of diseases are needed because of no deleterious effects on man. India is a home to a variety of traditional medicine system that relay to a very large extent on native plant species for their raw drug materials. Therefore, there is a need to look backwards towards folk medicines which can serve as novel therapeutic agent. These free radical intermediates and ROS escape from the site of reaction and act on various biological molecules such as lipids, nucleic acids, proteins and carbohydrates, thus causing deleterious changes in their structure and function and finally leading to cell death.[23]

Different phytochemicals have various protective and therapeutic effects which are essential to prevent diseases and maintain a state of well-being. Our study results on the qualitative analysis of the rhizome extract revealed the presence of phytochemical constituents such as alkaloids, flavonoids, glycosides, proteins & amino acids, reducing sugars, saponins, steroids, phenolic compounds/tannins, and terpenoids. Moreover, quantitative estimation of phytochemicals in methanolic extract of rhizome of *A. calamus* revealed that flavonoid quantity found to be highest (34.98 GAE) followed by total phenols (25.80 GAE), tannins (3.01 GAE) and carbohydrates (8.68 GAE).

The phenolic compounds contain hydroxyls that are responsible for the radical scavenging effect mainly due to redox properties. These results gives a reason for the activity of these plants as antioxidant and how these plants extracts enable to scavenge the free radicals. Tannin content was found to be 3.01 mg gallic acid equivalent/g of extract powder. Tannins are another major group of polyphenols in our diets and usually subdivided into two groups: (i) hydrolysable tannins and (ii) condensed tannins. Researchers and food manufacturers have become more interested in
polyphenols due to their potent antioxidant properties, their abundance in the diet, and their credible effects in the prevention of various oxidative stress associated diseases.

free radical scavenging activity by DPPH method was seen in methanolic extract of *A. calamus* rhizome. The IC_{50} value, which is the amount of extract needed to scavenge 50 % of DPPH radical for the acetone extract of *A. calamus* rhizome, was found to be 6 µg. DPPH is a stable radical that has been used to evaluate the antioxidant activity of rhizome extract. Antioxidant reacts with DPPH, which is a stable free radical, and converts it to α, α-diphenyl-β-picryl hydrazine. The degree of discoloration indicates the scavenging potentials of the antioxidant extract. The activity of extracts is attributed to their hydrogen donating ability. Increasing the number of hydroxyl or catechol groups increases radical scavenging activity. In presence of other H-donating groups (sulfhydryl, amide) in molecule also accelerates this activity.

The results obtained in the present study are encouraging as this study evidenced the vide variety of secondary metabolites present in the methanolic extracts of rhizome of *A. calamus* and methanol fractions *A. calamus* have shown considerable antioxidant properties. Hence this study supplies as evidence-based study for rhizome of *A. calamus* could be exploited in the management of various ailments.

Conclusions

In conclusion, this preliminary study confirms that the *A. calamus* has vide variety of secondary metabolites. Biological activity such as antioxidant properties of methanolic extracts of rhizome of *A. calamus* depicted that *A. calamus* could be potential drug agent of folk medicines. However further studies need to be conducted to elucidate the mechanism of action of various secondary metabolites present in *A. calamus* against various ailments.

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