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RESEARCH ARTICLE

DETERMINATION OF PHYTOCHEMICALS AND ANTIOXIDANT ACTIVITY OF ACORUS CALAMUS RHIZOME

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ABSTRACT

Acetone extract of Acorus calamus rhizome was assessed for phytochemical components qualitatively and quantitatively and for its antioxidant activity. The results revealed that the rhizome extract contained alkaloids, carbohydrates, flavonoids, tannins, anthocyanins and phenols. Steroids and saponins were absent in the extract. Phenolics, flavonoids and proanthocyanidins were found to be more in Acorus calamus rhizome extract from quantitative analysis followed by total sugars and tannins respectively. Acorus calamus rhizome extract showed the concentration dependent reducing power activity, Free radical scavenging potential and inhibition of lipid peroxidation respectively.

Key words: Phytochemicals, Antioxidant activity, Lipid peroxidation, Reducing power activity, Acorus calamus rhizome.

INTRODUCTION

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 degenarative 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 radicals[1,2,3]. In our body there are antioxidant defense systems, which include both enzymes such as glutathione peroxidase, superoxide dismutase, catalase and low molecular weight compounds such as uric acid, bilirubin, α-lipoic acid, vitamin-E, vitamin-C, carotenoids, ubiquinones etc. But due to imbalance between oxidants and antioxidants diseases develop. Therefore to quench the overload of these free radicals the exogenous antioxidants are of importance. The fruits, vegetables and the folk medicinal plants used are rich in phytochemicals such as phenolics, flavonoids, terpenes, alkaloids etc. These phytochemicals also acts as antioxidants and are known to have protective or disease preventive properties. The implication of oxidative stress in the etiology of several chronic and degenerative diseases suggests that antioxidant therapy represents a promising avenue for treatment. In the future, a therapeutic strategy to increase the antioxidant capacity of cells may be used to fortify the long term effective treatment. . Recently, phenolics have been considered powerful antioxidants *in vitro* and proved to be more potent antioxidants than Vitamin C and E and carotenoids. It has been proposed that the antioxidant properties of phenolic compounds can be mediated by the following mechanisms: (1) scavenging radical species such as ROS/RNS; (2) suppressing ROS/RNS formation by inhibiting some enzymes or chelating trace metals involved in free radical production; (3) upregulating or protecting antioxidant defense. Due to some common side effects of synthetic drugs (NSAIDs and steroidal) such as gastric irritation, ulceration, bleeding, renal failure, interstitial nephritis, hepatic failure, headache, thrombocytopenia, hemolytic anemia, asthma exacerbation, skin rashes, and angioedema. Its use is considered to be unsafe. Hence, treatment of inflammatory diseases by herbal drugs has keen interest to the researchers. In India, the use of different parts of several medicinal plants to cure specific ailments has been in vague from ancient times. Therefore, now there is a need to look back towards the traditional medicine which can serve as novel therapeutic agent. A lot of researches are going on worldwide directed towards finding natural antioxidants of plants origins. Studies have shown that many of these antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, and antiviral activities[4,5]. Acorus calamus belongs to family Acoraceae It is used in Ayurveda, Folk, Tibetian, Unani, Sidha and Modern medicine. The rhizomes of Acorus calamus is used in the form of powder, paste and decoction to treat diarrhoea, epilepsy, oedema, scrotal enlargement, skin diseases, headache, alopecia, wound, eye diseases, colic, piles, indigestion, acid gastritis, heart-diseases, rat-poisoning, diseases of mouth and as rejuvinative.

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A.calamus has been used as an aphrodisiac in ancient Egypt and in India, for more than 2,500 years today. This wonder herb has been used for a variety of purposes throughout the world, by different people suffering from different ailments and disorders. While in Europe A.calamus was used as a stimulant for one's appetite or to aid one's digestion, in North America the herb was used in the form of decoction for fevers, colics, and stomach cramps , while rhizome was chewed to help ease tooth ache. The powdered form was taken to treat congestion. As a matter of fact, the calamus has been used extensively in Western herbal medicine to provide effective relief from digestive problems such as flatulence , bloating , and weak digestive function. In Ayurvedic medicine too, calamus has been used to treat patients suffering from digestive disorders, as well as for 'rejuvenating' the brain and the nervous system of the user. Calamus, particularly 'A. calamus var. americanus', also known as one of the best antispasmodics, relieves intense spasms of the intestines. Calamus helps and relieves distended and uncomfortable stomachs, and also treats the intense headaches that are generally related to a weak digestion. Taken in small amounts, the drug can help reduce and relieve acidity of the stomach, while larger amounts would increase deficient acid production. This is a good example of the way in which the same drug, when used in different dosages, would produce entirely different results, and can therefore be used to treat different ailments.The aim of the present study is to screen the rhizome of Acorus calamus plant the wonder herb which has various medicinal property qualitatively and quatitatively for its phytochemicals and its antioxidant property.

MATERIALS AND METHODS

Collection of plant material: Acorus calamus rhizomes were purchased from the market. The rhizomes were powdered and stored in airtight containers until further studies.

Preparation of Extract: 10 g of Rhizome powder was extracted in 20ml of acetone by stirring using a magnetic stirrer at cold condition for 4 hours. The extracts were centrifuged at 10,000rpm for 10 minutes and then filtered through Whatman no.1 filter paper for removal of particulates. The residues obtained were re-extracted with another 20ml of acetone and again the process was repeated. The acetone extracts of rhizome were pooled, concentrated under vaccum at 40°C. The yield was calculated and expressed as percentage of w/w.

Preliminary phytochemical screening:

Qualitative analysis of phytochemicals present in the acetone extract of Acorus calamus rhizome was carried out by the standard procedures with little modifications [6,7].

TABLE 1: QUALITATIVE ANALYSIS OF ACORUS CALAMUS RHIZOME EXTRACT.

Tests	*Acorus calalamus rhizome*
Alkaloids	
Mayers test	+
Wagners test	+
Dragendroffs test	+
Carbohydrates	
Molisch tests	+
Fehling test	+
Benedict test	+
Flavonoids	
Shinoda test	+
Lead acetate test	+
Alkaline reagent test	+
Saponins	
Foam test	-
Froath test	-
Steroids	
Salkowski's test	-
Tannins	
Gelatin test	+
Anthocyanin	+
Phenolics	+

DETERMINATION OF TOTAL PHENOLICS BY FOLIN-CIOCALTEAU ASSAY:

The concentration of total Phenolics in the acetone extract of Acorus calamus rhizome 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[8]. The phenolic content of the extract was determined from calibration curve and were expressed in mg gallic acid equivalent/g of extract powder.

DETERMINATION OF TOTAL SUGARS BY PHENOL-SULPHURIC ACID METHOD:

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

ESTIMATION OF TOTAL FLAVONOIDS

Aluminum chloride colorimetric method was used for flavonoids determination[9]. The content was determined from extrapolation of calibration curve which was made

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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

DETERMINATION OF TOTAL PROANTHOCYANIDINS:

Total proanthocyanidin was determined for all the three acetone extracts based on the procedure of Sun et al[10]. Total proanthocyanidin content was expressed as gallic acid equivalent (mg/g) from the standard curve.

DETERMINATION OF TANNINS:

The tannin concentration was determined for each extract variety following a modified version of the vanillin-HCl method[11].

DETERMINATION OF REDUCING POWER:

The reducing power of all the three acetone extracts was evaluated according to the method of Oyaizu [12].

ANTIOXIDANT ACTIVITY BY DPPH METHOD:

2, 2-Diphenyl -1- picrylhydrazyl radical (DPPH·) was used as a stable radical for assessing antioxidant activity as described by Blios[13]. Reduction of DPPH by an antioxidant or by a radical species results in a loss of absorption at 517nm. Thus the degree of discoloration of the solution indicates the scavenging efficiency of the added substances. Determination of antioxidant activity of Acorus calamus rhizome by the DPPH method was done at 100µg concentration. Changes in the absorbance of the samples were measured at 517 nm.Radical scavenging activity was expressed as the inhibition percentage and was calculated using the formula:

Percentage of radical scavenging activity = Control OD-sample OD/Control OD

ANTIOXIDANT ACTIVITY BY TBA METHOD:

Antioxidant activity of acetone extract of Acorus calamus rhizome was performed using thio barbituric acid (TBA) according to the protocol of Halliwell and Gutteridge[14]. Lipid peroxidation induced by ferric chloride resulted in the production of malondialdehyde (MDA), lipid peroxide. Thio-barbituric acid (TBA) reacts with malondialdehyde (MDA) to form a di-adduct, a pink chromogen, which can be detected spectrophotometrically at 532nm.

Table 2: THE QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF ACETONE EXTRACT OF acorus calamus rhizome

Phytoconstituents	Acorus calamus acetone extract
Phenolic	23.6 mg GAE
flavonoids	32.6 mg GAE
Proanthocyanidins	80.5 mg GAE
Tannins	2.5 mg GAE
Sugar	8.5 µg

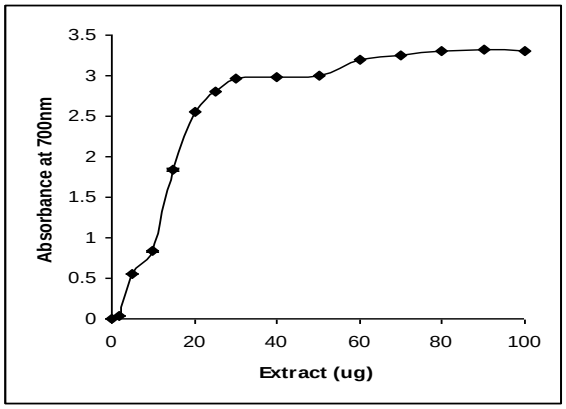

Figure 1: Reducing power activity of acetone extracts of Acorus calamus rhizome at different concentrations .

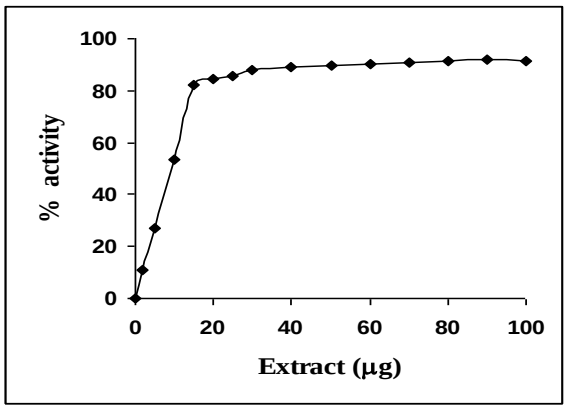

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Figure 2: Dose dependent radical scavenging activity of acetone extract of Acorus calamus rhizome by DPPH method.

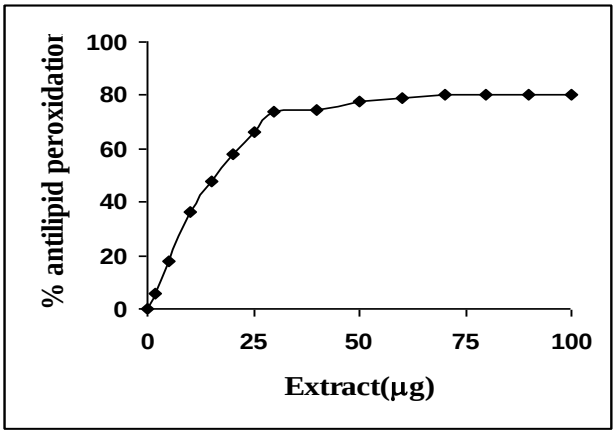

Figure 3: Dose dependent inhibition of lipid peroxidation by Acorus calamus rhizome acetone extract.

RESULTS AND 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

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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[15].

The Acorus calamus rhizome was purchased from market. The dried rhizome was powdered. The powder in the range of 10g was used for extraction using acetone. They were extracted on a magnetic stirrer for fixed amount of time. After extraction the extracts were centrifuged at 10,000 rpm for 10' mins and the supernatant obtained was filtered through what man No.1 filter paper. The residue left behind was re-extracted with another 20 ml of solvents and filtered. The filtrate obtained from the extracts was pooled and concentrated under vacuum at 40^{o}C. The percentage of yield (w/w) was calculated for the acetone extract of rhizome and is 2.88 %.

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. At the same time, the phytochemical constituent such as saponins and steroids was found to be absent as depicted in Table 1.

Phenolics, flavonoids and proanthocyanidins were found to be more in Acorus calamus rhizome extract from quantitative analysis followed by total sugars and tannins respectively. Total phenolics, Flavonoids, Proanthocyanidins, Tannins and Sugars were estimated for the acetone rhizome extract of Acorus calamus at 100 µg concentration as shown in Table 2. The results are as follows 23.6, 32.6 and 80.5 mg gallic acid equivalent/g of extract powder of phenolics, flavonoids and proanthocyanidins. It showed maximum amount of proanthocyanidins followed by the flavonoids and phenolics. proanthocyanidins serve among other chemical and induce defense mechanisms against plant pathogens and predators, such as in strawberries[16]. The acetone rhizome extract of Acorus calamus has 8.5 µg glucose equivalent/100µg of extract powder. 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 2.5 mg gallic acid equivalent/g of extract powder .Tannins are another major group of polyphenols in our diets and usually subdivided into two groups: (1) hydrolysable tannins and (2) 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

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 acetone rhizome extract at different concentration using the potassium ferricyanide reduction method. The concentration of extract ranged from 0-100µg level. As the concentration of extract is increased the reducing power also increased and attained maximum at 100µg concentration. The reducing power activity is due to the presence of reductones (phenolics).

Dose dependent free radical scavenging activity by DPPH method was seen in acetone extract of Acorus calamus rhizome as shown in Figure 2. The concentration of extract ranged from 0-100µg level. The IC_{50} value, which is the amount of extract needed to scavenge 50 % of DPPH radical for the acetone extract of Acorus calamus rhizome, was found to be 5µg. 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.

Dose dependent inhibition of lipid peroxide formation by acetone extract of Acorus calamus rhizome is as shown in Figure 3. The IC_{50} concentration is found to be 18µg for 50 % inhibition of lipid peroxide formation. Determination of the lipid peroxide content was carried out indirectly by means of derivatizing MDA with TBA at high temperature and acidic conditions. . In biological systems, MDA is a very reactive species and takes part in cross-linking of DNA with proteins and also damages the liver cells. The production of lipid peroxides by ferric chloride in liver homogenates were inhibited greatly by the acetone extract of Acorus calamus rhizome.

CONCLUSION:

Thus acetone rhizome extract of Acorus calamus had maximum amount of proanthocyanidins, polyphenolics and flavonoids which is directly related to their dose dependent antioxidant activity. Active research has been driven in recent years on plant based components due to their biologically beneficial effects emanating from antioxidant activities of phenolic phytochemicals. Thus an active molecule present in the acetone extract of Acorus calamus rhizome having both antioxidant and disease preventive property (anti-inflammatory activity, diabetes, CVD etc) may be useful in targeting the free radical mediated diseases.

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