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Research Article

PHYTOCHEMICAL SCREENING OF METHONOLIC RHIZOMES EXTRACT OF *ACORUS CALAMUS* (FAMILY-*ARACEAE*) FOR ITS *IN-VITRO* ANTIOXIDANT ACTIVITY

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ABSTRACT

The present study is directed to investigate Phytochemical examination, in- vitro antioxidant activity. The antioxidant activity of methanolic rhizomes extract of *Acorus calamus* linn. belonging to family-Araceae was determined by the following radical scavenging assays namely nitric oxide scavenging assay, Hydrogen Peroxide Scavenging Assay methods. In nitric oxide radical scavenging activity, the percentage of inhibition was found to be 376.2 µg/ml. In Hydrogen Peroxide Scavenging, the percentage of inhibition was found to be 182.4µg/mL. the concentration range from (50, 100, 150, 250, µg/ml). Phytochemical screening reveals the presence of saponins, tannins, terpenoids, and flavonoids. These results were an indication of antioxidant potential of the *Acorus calamus* linn. rhizomes extract and may be responsible for some of the therapeutic uses.

Keywords: *Acorus calamus,* antioxidant, methanolic extract, NO, H_2O_2 Scavenging assay.

INTRODUCTION

Herbal medicines are plant derived material or preparations which contain raw or processed ingredient from one or more plants or its parts, with therapeutic value and used as dietary supplements to fight or prevent common diseases in various systems of medicine such as Ayurveda,Unani and Sidha**.** Natural products from folk remedies have contributed significantly in the discovery of modern drugs and can be alternative source for the discovery of antioxidant constituents based drugs with novel structures and better safety and efficacy profiles.

Antioxidants or inhibitors of oxidation are compounds which retard or prevent the oxidation and in general prolong the life of the oxidizable matter[1]. Free radicals are fundamentals to any biochemical process and represent an essential part of aerobic life and metabolism. Majority of the diseases and disorders are mainly linked to oxidative stress due to free radicals[2]. The oxidants or free radicals are species with very short half life, high reactivity and damaging activity towards macromolecules like proteins, DNA and lipids. These species may be either Oxygen derived (ROS) orNitrogen derived (RNS). The most common reactive oxygen species include superoxide anion(O_2), hydrogen peroxide (H_2O_2), peroxyl radicals (ROO) and reactive hydroxyl radicals (OH). The nitrogen derived free radicals are nitric oxide (NO), peroxy nitrite anion (ONOO), Nitrogen dioxide (NO_2) and Dinitrogen trioxide (N_2O_3). Numerous studies are being conducted worldwide to study the beneficial effects of these antioxidant compounds on the human body. Antioxidants present in *Acorus calamus* lnn. help protect against several disorders that result from the increased action of free radicals and other harmful substances in the body.

Acorus calamus linn. *Acorus calamus* linn. commonly known as sweet flag is an aromatic medicinal plant belonging to the Araceae family, it is a semi aquatic perennial aromatic herb with creeping rhizomes[1] . In addition its Acorus has also been noted to have antioxidant and immune stimulant effects. *Acorus Calamus* is an important medicinal plant with wide range of biological activities and interesting phytochemical constituents. In ayurvedic medicine, it is used for the treatment of skin eruptions, epilepsy, mental ailments, neuralgia, cancer dyspepsia and bronchial catarrh, intermittent fevers[2] . The different phytocontituents present in medicinal plants such as flavonoid, alkaloid, phenol and tannins, carboxylic acids, terpenes and amino acids and inorganic acids. These phytoconstituents present specific distinctiveness and properties to plants[3]. Many plants harvested in wild in India

are used by local people as medicine-next to their source of food, for shelter and various livelihood needs. *Acorus calamus* Linn. (family: Araceae) commonly known as Sweet flag, Sweet Sedge, Myrtle Flag is a semi-aquatic, perennial, aromatic and marshy herb with creeping rhizomes originating in Asia but now widely distributed in Europe, North America and Africa. It is also found indigenously in the marshy tracts of Kashmir, Shirmaur (Himachal Pradesh), Manipur and in Naga Hills of India[4]. The rhizome, root and leaf yield a light brown to brownish yellow volatile aromatic oil known as calamus oil. It is a semi aquatic perennial plant of Acoraceae having scented rhizomes and tapered reed-like leaves. [Figure 1]. The rhizomes are considered the officinal part of the plant and have been reported to possess tranquilizing, antimicrobial, anti diarrheal, antidyslipidemic, neuroprotective, anti-inflammatory and analgesic activities. The different pharmacological activities of *Acorus calamus* such as low-grade mentally retarded children[5-6]. Anticonvulsant[7]. Rhizomes extracts of *Acorus calamus* linn. possess CNS depressant, tranquilizing, inhibiting the spontaneous motor activity[8] .For the manufacturing of modern drugs advanced chemical intermediates needed are also obtained from plants[9]. Recently it has been reported that Acorus calamus(AC) has antistressor activity and prevents stress induced changes in the rat brain by its antioxidant activity[10] .

Botanical Description Of *Acorus Calam*us:- *A. Calamus* linn. Commonly known as sweet flag, belongs to the family Araceae (Adoraceae). *Acorus calamus* linn. is a herbaceous perennial with a long indefinite branches cylindrical rhizome which is about 3-4 inch in diameter smooth, pale green, browns. Its leaf scars are brown. It possesses slender roots. grows either as wild or cultivated crop throughout India.

SCIENTIFIC CLASSIFICATION

Kingdom:	Plantae
Family:	Araceae
Genus:	Acorus
Species:	Calamus

MATERIALS AND METHODS

Collection and identification of plant material: The Plant *Acorus Calamus* ware collected from Amarkantak, Madhya Pradesh, India. The plant was identified and authenticated by Dr. Madhuri Modak Professor Deptt. of Botany Govt. M.V.M. Bhopal, (M.P.) the voucher specimen (Herbarium No. 3050-191.02-X.1) has been deposit in the department herbaria.

Extraction: The Plant samples were washed thoroughly in running tap water to remove soil particles and adhered debris followed by sterile distilled water. The dried plant rhizomes of *Acorus calamus* linn. was grounded by electrical grinder. till the fine powder in a mixer grinder and weighed accurately. The powdered material was subjected to solvent extraction with methanol by Soxhlet for 96 hours. The resulting mixture was filtered and evaporated in a shaker water-bath; temperature maintained at 55-65^{0}C. The extracts were concentrated using water bath set at 60°C. After that, the respective extracts were weighed and percentage extractive values were determined.[11-13]. The obtained dried crude extract was used for phytochemical analysis.

Phytochemical screening:

The phytochemical evaluation of the plant is carried out by testing of different class of compounds using standard methods to identify the compound showing in Table no.5. The preliminary phytochemical investigations were carried out with the methanolic extract of *Acorus* calamus linn. Rhizomes of plant for qualitative identification of phytochemical constituents using standard conventional protocol. All the chemicals and reagents used were of analytical grade[14-16].

Antioxidant activity Experiments Procedure:

1. Hydrogen peroxide (H_2O_2) scavenging activities: is a biologically important oxidant because of its ability to generate the hydroxyl radical which is extremely potent. The ability of the hydroxyl radical to remove or add hydrogen molecules to unsaturated hydrogen bonds of organic lipids makes it potentially one of the most reactive oxidants in biological systems. It's very short half-life (1×10^{-9} at 37°C), however, restricts its diffusion capability and potency. The procedure involved UV-spectrophotometric determination of Hydrogen peroxide radical scavenging. Three solutions i.e. Standard, Test and Control were prepared.

Preparation of Standard Ascorbic acid solutions: Different concentrations (50–250µg/ml) of the ascorbic acid were prepared in distilled water. 1ml of each solution of ascorbic acid was mixed with 2ml of 0.1 M phosphate buffer solution and 600µl of 100mM H_2O_2 solution. After 10 minutes absorbance of different concentration of ascorbic acid solutions was taken at 230nm.

Preparation of Test solutions: Various concentrations (50, 100, 150, 200, 250, µg/ml) of the Extract were prepared in distilled water. 1ml of each solution of extract was mixed with 2ml of 0.1 M phosphate buffer solution and 600µl of 100mM H_2O_2 solution. After 10 minutes (approximately) absorbance of different concentration of extract solutions were taken at 230nm.

Preparation of Control solution: For control 2ml of 0.1 M phosphate buffer solution was mixed with 600µl of 100mM H_2O_2 solution. After 10 minutes absorbance of control was taken at 230nm. Percentage Hydrogen peroxide radical scavenging activity of plant aq.extract and ascorbic acid was calculated by using the formula:

$$I\% = Ac - (At - Ab)/Ac \times 100$$

Where, I% = Percentage inhibition.,Ac = Absorbance of control (0.1 M phosphate buffer solution and H_2O_2) ,At =Absorbance of ascorbic acid / plant extract with H_2O_2 after 10 min.,Ab=Absorbance of ascorbic acid / plant extract without H_2O_2 .[17]. The results were expressed as percentage (%) inhibition exhibited by the test substances and the standard (Figure 1-2) IC50 value was calculated in each case. Hydrogen peroxide (H_2O_2) by the methanolic extract of *Acorus calamus* linn. was found to be 182.4µg/ml. Table no. 02 .

Nitric oxide scavenging activity:

Nitric oxide radical scavenging activity was determined according to the method reported by (**Garrat).** Sodium nitro prusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined by

the use of the Griess Illosvoy reaction. 2 mL of 10 mM sodium nitroprusside in 0.5 mL phosphate buffer saline (pH 7.4) was mixed with 0.5 mL of extract at various concentrations and the mixture incubated at 25^{0}C for 180 min. From the incubated mixture 0.5 mL was taken out and added into 1.0 mL sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. finally, 1.0 mL naphthylethylenediaminedihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min before measuring the absorbance at 540 nm was measured with a spectrophotometer. The nitric oxide radicals scavenging activity was calculated[18]. Methanolic extract of *Acorus calamus* exhibited good nitric oxide scavenging activity. Methonolic extract of the plants inhibited nitrite formation in concentration dependent manner. This may be due to the presence of antioxidant principles in the extract, which complete with oxygen to react with nitric oxide. The results were expressed as percentage (%) inhibition exhibited by the test substances and the standard (Figure 3-4) IC_{50} value was calculated in each case. Nitric oxide by the methanolic extract of *Acorus calamus* linn. was found to be 376.2µg/ml. While for ascorbic acid it was found to be 302.0 µg/ml. and Table no. (3-4)[19-20].

RESULTS AND DISCUSSION

The preliminary phytochemical studies it showed the presence of alkaloids, flavonoids, saponins and tannins also present in the methanolic rhizomes extract of *Acorus calamus*. The antioxidant activity of *Acorus calamus*. leaves extract was compared with L-Ascorbic acid which is a well known antioxidant. The rhizomes extract of *Acorus calamus*. showed prominent IC_{50} value of 376,2 µg/ml by Nitric oxide method (Fig no-4), was estimated by L-Ascorbic acid it was found to be IC_{50} value of 302.0µg/g. Hydrogen Peroxide method was found to be IC_{50} value of 182.4µg/g (Fig no-2) was estimated by L-Ascorbic acid it was found to be IC_{50} value of 116.2µg/g (Fig no-1).The IC_{50}value was determined for each compound. From results of Hydrogen Peroxide and Nitric oxide methods, it found that compound displayed strong antioxidant activity compared to the ascorbic acid and it suggested that these compounds could have great importance as therapeutic agents in preventing or slowing the progress of aging and age associated oxidative stress related degenerative diseases. It was found that the methanolic extract of *Acorus calamus*. showed significant antioxidant activity.

Table 5: Preliminary Phytochemical screening of Extract of seeds of *Acorus calamus* linn.

Sr. No.	Name of Phytoconstituents	Presence/Absence
1	Terpenoides	+ve
2	Flavonoids	+ ve
3	Tanins	+ ve
4	Saponins	+ ve
5	Steroids	+ ve
6	Carbohydrates	+ ve
7	Phenolic compounds	+ ve
8	Reducing sugars	+ ve
9	Oil	+ ve

Key: + = Present and - = Absent

Table 1: % Inhibition of H_2O_2 by Ascorbic acid

S. No.	Conc. (µg/ml)	Absorbance (Control), A_c	Absorbance (Test), A_t	% Inhibition	IC50 (µg/ml)
1.	50	0.71	0.427	39.86	116.2
2.	100		0.372	47.61	
3.	150		0.322	54.65	
4.	200		0.269	62.11	
5.	250		0.191	73.10	

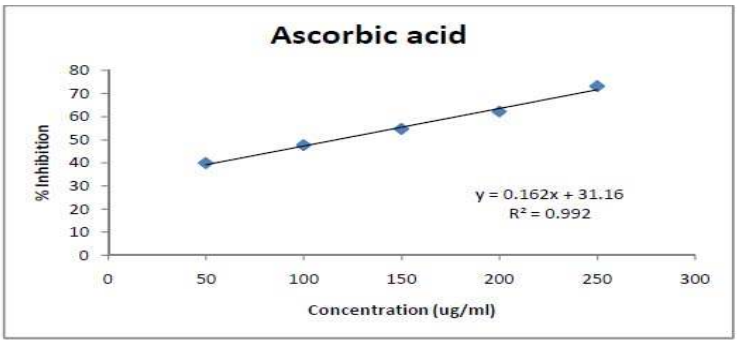

Fig.01: Standard curve of ascorbic acid. Graph represent regression curve of ascorbic acid by H_2O_2 method

Table 2: % Inhibition of H_2O_2 by Methanolic Extract

S. No.	Conc. (µg/ml)	Absorbance (Control),A_c	Absorbance (Test),A_t	% Inhibition	IC50 (µg/ml)
1.	50	0.71	0.472	33.52	182.4
2.	100		0.427	39.86	
3.	150		0.391	44.93	
4.	200		0.342	51.83	
5.	250		0.289	59.30	

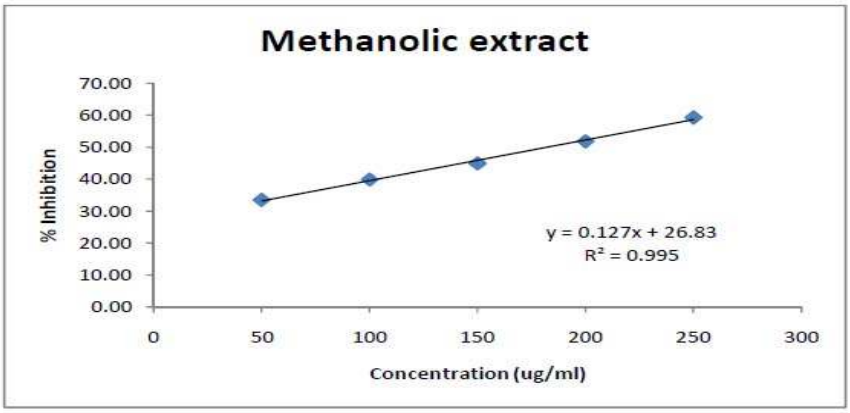

Fig.02: Graph represent regression curve of Methanolic Extract by H_2O_2 assay method

Table 3: % Inhibition of NO by Ascorbic acid

S. No.	Conc. (µg/ml)	Absorbance (Control),A_c	Absorbance (Test),A_t	% Inhibition	IC50 (µg/ml)
6.	50	0.543	0.157	25.39	302.0
7.	100		0.122	31.83	
8.	150		0.103	35.33	
9.	200		0.073	40.86	
10.	250		0.052	44.72	

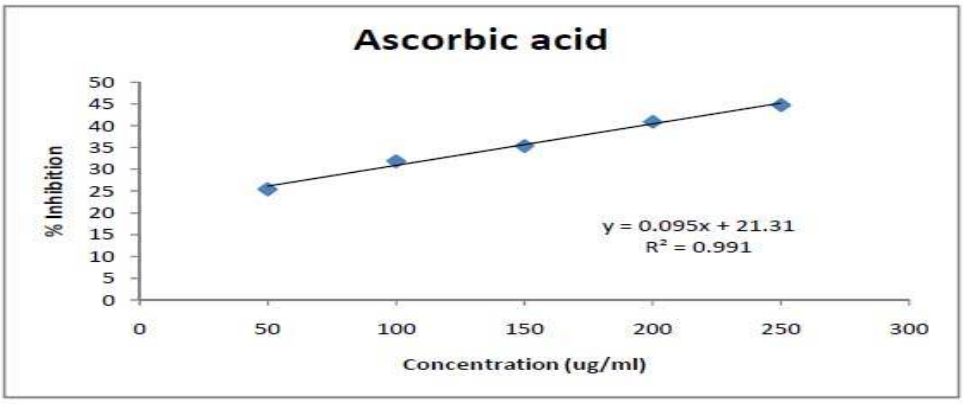

Fig.03: Standard curve of ascorbic acid. Graph represent regression curve of ascorbic acid

Table 4: % Inhibition of NO by Methanolic Extract

S. No.	Conc. (µg/ml)	Absorbance (Control),A_c	Absorbance (Test),A_t	% Inhibition	IC50 (µg/ml)
6.	50	0.543	0.185	20.23	376.2
7.	100		0.151	26.49	
8.	150		0.127	30.91	
9.	200		0.102	35.52	
10.	250		0.089	37.91	

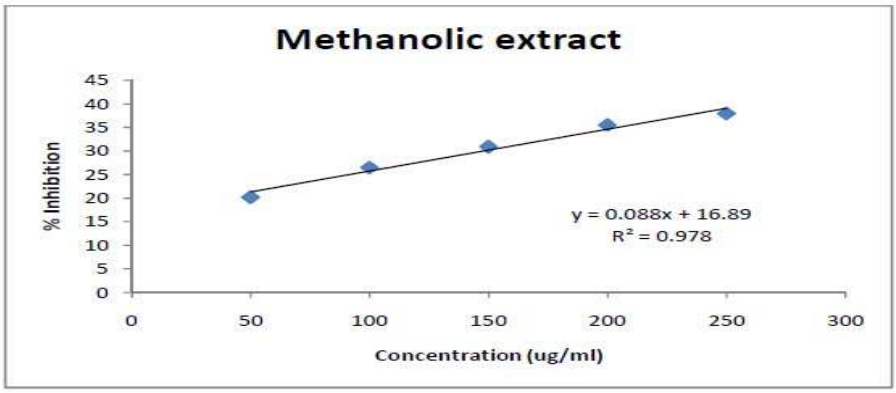

Fig.04: Graph represent regression curve of Methanolic Extract by NO assay method

CONCLUSION

There has been an increasing interest in the role of plants as therapeutic agents as they are easily a vailable and are devoid of harmful side-effects as opposed to their synthetic counterparts. All the conducted experiments in the present study are based on crude extract and are considered to be preliminary and more sophisticated research is necessary to reach a concrete conclusion about the findings of the present study. The findings of this study support this view that plant is promising source of potential antioxidant activity and may be efficient as preventive agent in some diseases. The providing data can just enrich the existing comprehensive data of antioxidant activity of plant material. In vitro antioxidant activity was carried out with methanolic extract extract of *Acorus calamus*. by Hydrogen Peroxide scavenging assay method and Nitric oxide scavenging assay method.

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