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Research Paper

Phytochemical Examination and in Vitro Free Radicl Scavinging Activity of Rhizome of Acorus Calamus Linn. Family Araceae

Chemistry

KEYWORDS : Acorus calamus linn. Rhizome Phytochemical screening, Anti-oxidant activity, DPPH

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ABSTRACT

Acorus calamus linn. Family-Araceae commonly called as 'Sweet flag' The preliminary phytochemical examination show the presence of terpenoids, steroids, saponins, flavonoid, carbohydrate, glycoside etc. The present study is directed to investigate the antioxidant potential of the plant. The antioxidant activity of Acorus calamus linn. (Araceae) were prepared and investigated for antioxidant potential against 2, 2 diphenyl-1-picrylhydrazyl (DPPH) free radical and compared with standard ascorbic acid. The methanolic extract with different concentrations (50, 100, 250, 500, 1000 µg/ml). The percentage of inhibition was found to be the IC50 value was 259.46 µg/ml. The result showing that Acorus calamus Linn. exhibits potential free radical scavenging. It was also concluded that methanolic extract showed towards the maximum antioxidant activity.

INTRODUCTION:-

Acorus calamus linn. Family Araceae Commonly called as 'Sweet flag' is a semi aquatic, pereanial, aromatic herb with creeping rhizomes, originating Asia[1.] *Acorus calamus* grows either as wild or cultivated crop throughout India ascending upto 1800 metres in the Himalayas[2]. It is a most valuable plant in the medical sciences almost throughout the India. In Ayurvedic science the use of sweet flag is effective against wide varieties of illnesses.The family Araceae comprises about 110 genera and more than 1800 species. The members of the family are rhizomatous or tuberous herbs. *Acorus calamus* Linn. Commercially occurs in both palled and unpaled forms. but now widely distributed in Europe, North America, and Africa etc. It is Cultivated throughout India, ascending to an altitude of about 2200 meters. The sweet flagoil present in this plant is a unique source of Oxygenated Sesquiterpenes of great structural variety[3]. Apart from terpenes, a few commonly occurring Steroids and xanthones have also been reported. The rhizome of the plant has medicinal value against bugs, moths, lice, emetic, stomatch, in dyspepsia[4.] The various pharmacological activity of *Acorus calamus* Linn. Such as analgesic[5], anticonvulsant[6], anti-inflammatory[7], was reported earlier. Most pharmacological activity of *Acorus calamus* Linn. Was reported by using rhizomes and roots. Indian Acorus oil had shown sedative-tranquaillizing action in rats, mice, cats, dogs and monkey[8]. Rhizomes and Roots extracts *Acorus calamus* Linn. Possess CNS depression, tranquillizing, inhibiting the spontaneous motor activity[9].

BOTANICAL DESCRIPTION OF *ACORUS CALAM*US:- *A. Calamus* **linn. Commonly known as sweet flag, belongs to the family Araceae (Adoraceae).** *Acorus calamus* **grows either as wild or cultivated crop throughout India ascending upto 1800 meters in the Himalayas. wild woody liane belongs to the Family Araceae .**

CLASIFICATION AND PLANT PROFILE

SCIENTIFIC CLASSIFICATION

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

MATERIALS AND METHODS

Collection and authentication of plant materials:-The fresh specimen and rhizomes of selected plant *A. calamus* linn. having medicinal value were collected from the Amarkantak district in Madhya Pradesh, India. The plant was identified and specimen were authenticated by Dr. Madhuri Modak Professor Deptt. of Botany Govt. M.V.M. Bhopal , the voucher specimen (Herbarium No .....) has been deposit in the department herbaria.

EXTRACTION AND PRELIMINARY PHYTOCHEMICAL SCREENING

I Extraction of rhizomes of *Acorus calamus*:-The rhizomes were dried in shade and used for analytical work. About 100 gm of shade dried rhizomes were made into powder form by using electrical grinder. The powdered material was filled in the thimble of soxhlet apparatus and exhaustively extracted with methanol (40^0c) for about 48 cycles.The concentrate extract was treated with methanol at (40^0c) for about 48 cycles and the solvent was distilled off at low temperature under vacuum and concentrated on water-bath to get thick extract. with water[10] .

Phytochemical screening:- Plant contain several constituents and some of them are present at very low concentrations. The modern chemical analytical procedure available only rarely do phytochemical examinations succeed in isolating and characterizing all secondary metabolites present in the plant extract. The preliminary phytochemical screening of the plant is carried out by testing of different class of compounds using standard methods to identify the compound showing in Table No. 01[11]. The chemical constituents of the extract were identified by qualitative analysis, which indicates the presence terpenoids, steroids, flavanoids, carbohydrates, reducing sugars, tanins and phenolic compounds, saponis, glycosides. All the chemicals and reagents used were of analytical grade.

ANTIOXIDANT ACIVITY

PROCEDORE:- The antioxidant activity of *Acorus calamus* linn. of Methanolic extract and the standard antioxidant ascorbic acid assessed on the basis of the radical scavenging effect of the stable 2, 2- diphenyl-1-picrylhydrazyl (DPPH) free radical activity according to the method described by Brand –William et, al. (1995). The methanol extract with different concentrations (50, 100, 250, 500, 1000, ug /ml) were prepared using methanol. Ascorbic acid was used as the standard in 1-100ug/ml solution. 0.004 % of DPPH solution was prepared in ethanol and 5 ml of this solution was mixed with 5 ml of extract solution and standard solution distinctly. These solution mixtures were kept in dark for 30 minute. The degree of DPPH purple decolourization to DPPH yellow indicated the scavenging effectiveness of extract. The absorbance of the combination was determined 517 nm using UV-Visible Spectrophotometer and ascorbic acid was served as a positive control. Lower absorbance of the reaction mixture indicates higher, free radical scavenging activity[12,13]. IC_{50} value of the methanolic extract of acorus calamus was found to be 259.46 µg/ml.

RESULTS AND DISCUSSION

DPPH test is based upon the ability of DPPH, a stable free radical, to decoloruize from purple in the presence of antioxidant. It is direct and dependable method for determining the radical scavenging action. Ascorbic acid was chosen as the standard antioxidant for this test. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for a noticeable deep purple colour. When DPPH accepts an electron donated by an antioxidant compound the DPPH becomes colourless, which is quantitatively measured from the

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changes in absorbance.

Scavenging activity of DPPH radical was found to rise with increasing concentration of the extract. Oxidative injury now appear as the fundamental mechanism causing a number of human neurological and other disorder such as autoimmune pathologies, inflammation, viral infection and digestive disorders including gastrointestinal inflammation and ulcer Aruoma, 2003. The present result suggest that all the tested plant extract have moderate to potent antioxidant activity,

Table no. 01. Phytochemical test of Methanolic extract

S. No.	Experiments	Methanolic extract
• Alkaloids		
1.1	Mayer's reagent test	-ve
1.2	Wagner's reagent test	-ve
• Carbohydrates		
2.1	Molish's test	+ve
2.2	Barfoed's test	+ve
• Reducing sugars		
3.1	Fehling's test	+ve
3.2	Benedict's test	+ve
• Flavonoids		
4.1	Alkaline reagent test	+ve
4.2	Lead acetate test	+ve
• Glycosides		
5.1	Borntrager test	-ve
5.3	Killer- Killiani test	-ve
• Tannin and Phenolic compound		
6.2	Lead Acetate test	+ve
6.3	Gelatin test	+ve
• Saponin		
7.1	Faom Test	+ve
• Test for Proteins and amino acid		
8.1	Ninhydrin test	-ve
8.2	Biuret test	-ve
• Test for Triterpenoids and Steroids		
9.1	Salwonski Test	+ve
9.2	Libberman and Burchard's test	+ve

Note:- +ve **=** Higher percent of Phytocostituents , -ve =absence of Phytocostituents.

1. Free radical scavenging assay (DPPH assay)

Table 2:- % Inhibition of DPPH by Ascorbic acid

S.No.	Conc. (µg/ml)	Absorbance (Control), A_c	Absorbance (Test), A_t	Inhibition	IC_{50} (µg/ml)
1.	50	0.796	0.361	54.65	30.27
2.	100		0.329	58.67	
3.	250		0.27	67.71	
4.	500		0.217	72.74	
5.	1000		0.124	84.42	

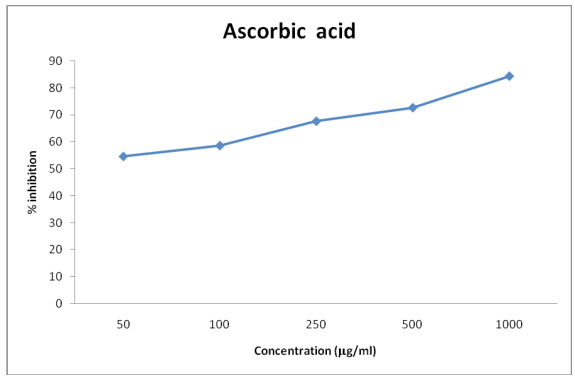

Fig.01: Standard curve of ascorbic acid. Graph represent regression curve of ascorbic acid by DPPH assay method

Table 2: % Inhibition of DPPH by Methanolic Extract

S. NO.	CONC. (MG/ ML)	ABSORBANCE (CONTROL), A_C	ABSORBANCE (TEST),A_T	% INHIBITION	IC50 (MG/ ML)
·	50	0.796	0.672	15.58	259.46
·	100		0.626	21.36	
·	250		0.569	28.52	
·	500		0.505	36.56	
·	1000		0.381	52.14	

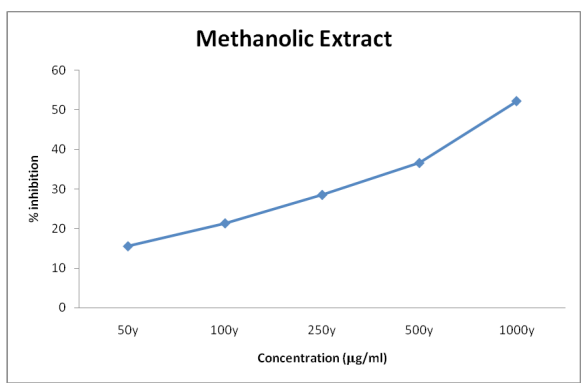

Fig.02: Graph represent regression curve of Methanolic Extract by DPPH assay method

CONCLUSION

The plant extract of *Celastrus paniculata* linn. has shown phytochemical result as in table no.1 revealed that methanolic extract contains terpenoids, tannins, saponins, flavonoids carbohydrates etc. The plant rhizomes can also serve as the natural source of antioxidants. The methanolic extract were prepared with different concentrations (50, 100, 250, 500, 1000 µg/ml). The percentage of inhibition was found to be 259.46 µg/ml. (IC_{50} value) Determination of the natural antioxidant constituents of the plant extracts will help to develop new drug. Further investigation of the isolation and examination of antioxidant components of the plant extract may lead to chemical group with potential for medicinal use.

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