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Evalution of Total Phenolics & Flavonoid Contents, Free Radical Scavanging Activity

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ABSTRACT: Acorus calamus Linn (Acoraceae) is a plant used for medicinal purpose, broadly conveyed in India and other eastern nations. The root, rhizome separates and fragment oil from the plants has been recently screened for its antioxidant potential. The antioxidant potential of methanolic extract is controlled by phytochemical screening estimation of all total phenolics and flavoniods and in vitro antioxidant activity viz. Hydrogen peroxide scavanging activity and nitric oxide scavanging activity. Phytochemical analysis of methanolic extract of A.Calamushowed the presence of phenolic ,flavanoids,alkaloids,carbohydrates,proteins,Tannins,diterpene,triterpene,saponnin ,steroids and glycosides. Ethanolic extract of A.Calamus shows the maximum antioxidant activity and can attributed to high polyphenolic content in the extract and ethanolic extract also shows higher reductive property. Standard procedure were applied to test the carbohydrates,saponins,tannins and also the confirmation of glycosides. IC_{50} values for Hydrogen peroxide scavanging activity and nitric oxide scavanging activity was estimated and found to be 81.81µg/ml and 43.90µg/ml respectively.

KEYWORDS: Acorus calamus Linn, antioxidant activity, H_2O_2 ,$IC_{50.}$NO

I. INTRODUCTION

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. There are number of biochemical reactions which are occur in our body which leads to the formation of free radicals who have been involved asmediators of many chronic and degenerative diseases [10]. Free radical scavanging property can be remove completely by the antioxidants and maintains the balance in the body [18] .

Antioxidants are compounds that inhibit the oxidation and oxidation is a chemical reaction which produces the free radicals which may lead to damage of the cell of organisam. Free radicals are basics of any biochemical procedure and constitute a basic pieceof vigorous life and digestion. The oxidants or free radicals species have an extremely short half-life, high reactivity, potential damage to biomolecules and harming action towards macromolecules like proteins [13] .

Various examinations are being occur worldwide to compete the valuable impacts of these antioxidant compounds on the human body. Antioxidants present in Acorus calamus Linn. Provide a shield against a few issues and the rhizome of the Acorus Calamus Linn rxtract posses antioxidant activities to scavenge free radical and the outcome from the expanded activity of free radicals and other destructive substances in the body [4] .

Acorus calamus Linn. regularly known as a sweet-smelling restorative plant this plant ha s belonging of Araceae family, it is a semi sea-going enduring sweet-smelling herb with crawling rhizomes.Moreover, its Acorus has been noticed to have an antioxidant agents and insusceptible energizerimpacts [6]. Acorus Calamus is a remarkable medicinal plant with extensive scope of biological activities and fascinating phytochemical constituents. In the field of ayurvedic medication, it is employ 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.[1][20] .

The deep investigation of the vital active elements which are present in the Acorus Calamus Lin. Plant causes the researchers to acknowledge the technique of activity of that antidote. The starter phytochemical screening of different therapeutic plants was accounted by numerous people who are working in that field. Acorus calamus is mostly known for its gainful, excellent and usage for medicinal purposes in Asia[16][17] . It is gathered on a business scale and for the most part, developed in the Asian locale. It was otherwise called "sweet banner", in light of its sweet aroma and is normally known as banners in English, since the late fourteenth century [19] . This traditional Ayurvedic medication was utilized for the treatment of memory misfortune and moreover utilized for the treatment of epilepsy,

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anorexia and mental diseases. In the current examination, methanolic extract of Acorus Calamus was utilized for in-vitro free radical scavanging potential and starter phytochemical assessment.

II. MATERIALS AND METHODS

Preparation of extract

The extraction was carried out in sufficient amount of methanol by dissolving 450 gm of powdered sample. The mixture is kept uninterrupted for 15 days. The mixture which was kept undisturbed was filtered through Whatman no.1 filter paper and by rotatory evaporator it was concentrateunder reduced pressure. Finally, at moderate temperature the extract was dried until it gets completely dried and the extract was stored for further phytochemical analysis.

Phytochemical screening

The Identification for presence of phytochemicals like phenolics, flavonoids, alkaloids, carbohydrates, proteins, tannins, diterpenes , triterpenes, saponins, glycosides was carried out under the preliminary quantitative analysis of the extract . All the chemical and reagents used are of analytical grade[9][10][15] . The wide phytochemical investigation of plant Acorus Calamus was done by Methanolic extract.

FT-IR analysis

FT-IR Spectroscopy is defined as Fourier transform infrared spectroscopy which is an analytical technique used to identify organic, polymeric,and also in inorganic materialand this method uses the infrared light to scan the samples in the extract. The methanolic extract of Acourus Calamus was obtaining the FT-IR spectra by using FT-IR spectrometer and the ranging of sample which was scanned lied at 4000cm to 1 cm[14].

Antioxidant Activity

Hydrogen peroxide scavanging activity:

Hydrogen peroxide is a naturally known oxidant on account of its capability of create the exceptionally potent hydrogen radicals[32] . This radical is the most potentially reactive oxidants by knowing to its capability of removal or adding of molecules of hydrogen to unsaturated hydrogen bonds of organic lipids. [7] . The deoxyribose method was used to determine Hydrogen peroxide scavenging activity[5] . According to this method 0.2ml of different concentrations (25 µg /ml, 50 µg/ml, 100 µg /ml, 150 µg /ml) of the 0.2 ml extract was taken and mixed together with 0.2 ml of 2-deoxyribose (50mM) , 0.2ml of EDTA (1.04mM), 0.8 ml of phosphate buffer was taken 0.8ml, 0.2 ml of ferric chloride (1mM), 0.2ml of ascorbic acid and 0.2ml of hydrogen peroxide. After incubation was carried out at 37° C for 1 hr, in the reaction mixture 2ml of thiobarbutyic acid(10%) was mixed into it. At last, the mixture was heated at 100^0 C for 20 minutes and then cooled. Absorbance was recorded at 534nm. The extract of Acorus calamus having hydrogen peroxide scavanging property based on thies formula :

% I = [Absorbance of (Control- test) / Absorbance of Control] ×100]

where, %I = percentage inhibition

Nitric oxide scavenging activity:

Nitric oxide scavenging activity was estimated by the method which is described by Garratt[2]. The extract with 1ml of different concentrations (25, 50, 100, 150 µg /ml) was mixed together with sodium nitroprusside which was prepared in buffer solution. After incubation at 25°C for 10 minutes, 1ml of Griess reagent was added to the reaction mixture. Absorbance was recorded at 543nm and as standard positive control Ascorbic acid was taken . The percentage inhibition of nitric oxide scavenging activity was calculated.

III. RESULT AND DISCUSSION

Methanolic extract of Acorus Calamus on Phytochemical screening showed the presence of phenolics, flavonoids, alkaloids, carbohydrates, proteins, tannins, diterpenes, triterpenes, saponins, steroids and glycosides. (Table 1).

S.no.	**Name of phytoconstituents**	**Absence / Presence**
1	Phenolics	+
2	Flavonoid	+
3	Alkaloids	+

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4	Carbohydrates	+
5	Proteins	+
6	Tannins	+
7	Diterpenes	+
8	Triterpene	+
9	Saponin	+
10	Steroids	+
11	Glycosides	+

Result

Phytochemical screening of methanolic extract of Acorus Calamus showed the presence of phenolics,flavonoids, alkaloids, carbohydrates, proteins, tannins, diterpenes, triterpenes, saponins, steroids andglycosides. (Table 1).

IV.PHYTOCHEMICAL QUALITATIVE ANALYSIS

Phytochemical qualitative analysis requisite to find out the phytoconstituent like phenolics, carbohydrate, tannins, saponin present in ethanolic extraction of Acorus calamus[29] .They shows the presence of alkaloids in the extract of plant and were carried by following standard procedures [25].

Test for carbohydrate: Molish reagent employes the phenols,α-napthenol and has abiltilty to detect carbohydrates. 3ml of extract was added with molish's reagent in quantity of 2ml and some drops of concentrated H_2SO_4. Formation of Purple or reddish colour in termination confirm the test of carbohydrate.

Test for saponins: Test for saponin is to shake aquous alcoholic plant extract in test tube . 2ml extract was added in 2ml of distilled H_2O in test tube for 10 min. so the presence of saponin was confirmed when a layer of froth began to frame up to 1 cm in testube.

Test for alkaloids: Existance of alkaloids can be determine by the Mayer's reagent when 2ml extract was added into 2 ml of concentrated HCL in mayer's reagent. Formation of grassy coloured precipitate shows the presence of alkaloids.

Test for flavonoids: Flavonoids can be determined by Alkaline reagent test .In the test tube 3 ml of extract and 3N sodium hydroxide was added and the existance of flavonoid was confirmed by yellow colour of precipitation .

Test for glycosides: 2ml of extract was added in test tube with chloroform 4ml and 10% of NH_3 solution in it .The formation of pink colour precipitate was show the confirmation of glycosides.

V.IDENTIFICATION OF FUNCTIONAL GROUPS

The Fourier transform infrared spectroscopy was done for the determination of functional groups of compounds present in Acorus calamus extract. The obtained FT-IR spectrum confirmed the presence of different functional groups which were appeared on different wavelengths as shown in (figure 2, table 2).

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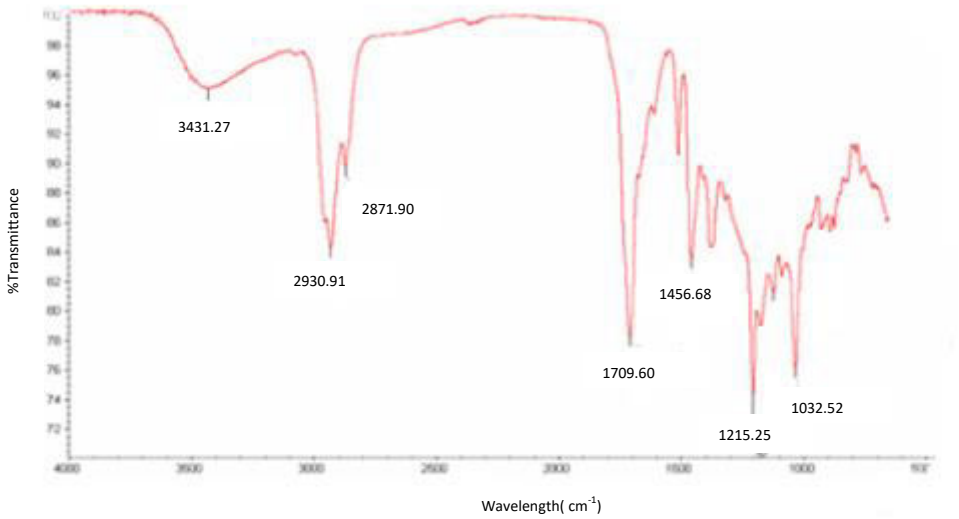

Figure 2: FT-IR spectrum of Acorus Calamus

Bonds	Characterstic absorption frequences (cm^{-1})	Functional groups
C-H Stretch	2930.91	Alkanes
C-H stretch	2871.90	Alkanes
H-bonded,O-H stretch	3431.27	Phenols,alcohols
C-C	1456.68	Aromatic ring
C=O	1709.60	α,β unsaturated ring aldehydes
C-O-C Stretch	1032.52	Ether linkage
C-N Stretch	1175.58	Aliphatic amines
C-N stretch	1215.25	Aliphatic amines

Table 2: FT-IR peak values and Functional groups present in the extract of Acorus Calamus

Total phenolic and flavonoid content

The rhizome extract of Acorus calamus contains the Total phenolic content which was found to be with the value of 27.5 mg of gallic acid/ g of dry weight. Phenolics compounds are considered to be having vigorous antioxidant activities and shows the reamarkabe role in scavenging free radicals becausedue to the presence of Hydroxyl group in their structure[11] . In the rhizome extract of Acorus Calamus the total flavonoid content was found to be 11.4 mg of quercetin/ g of dry weight. Flavonoids are also hinder the potential antioxidants. The properties of scavenging free radicals was somewhat depending upon the position of Hydroxyl group and some other features in their molecular structure[3].

Hydrogen peroxide scavanging property:

The methanolic extract of *Acorus Calamus* with the hydrogen peroxide scavanging activity is compared with the antioxidant activity of a well-known antioxidant L-Ascorbic acid by calculating the IC50values for both the cases. The IC50 value was found to be81.81µg/g as shown in (table 2, figure 2) and with respect to L-ascorbic acid it was also estimated and found to be 77.54µg/g as shown in (table 3, figure 3).

Concentration	Absorbance of	Absorbance of test	% inhibition	IC_{50}

	control			
25	0.81	0.537	33.70	81.81
50	0.81	0.427	47.28	81.81
100	0.81	0.369	54.44	81.81
150	0.81	0.291	64.07	81.81

Table 2: Hydrogen peroxide scavenging activity of Methanolic Extract of *Acorus Calamus.*

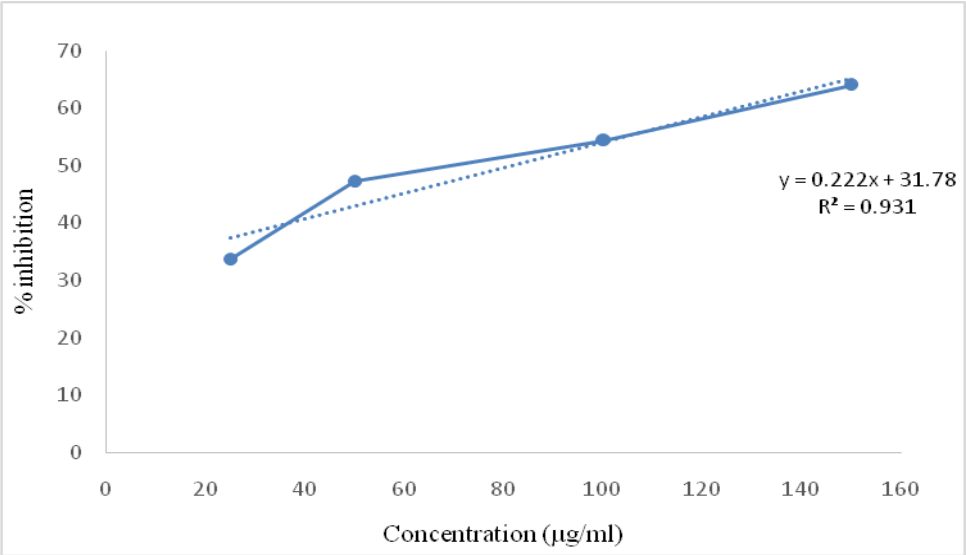

Figure 2: Chart representation of Hydrogen Peroxide scavenging activity of Methanolic Extract of *Acorus Calamus*

Concentration	Absorbance of control	Absorbance of test	% inhibition	IC_{50}
25	0.81	0.527	34.93	77.54
5	0.81	0.473	41.60	71.54
100	0.81	0.356	57.60	71.54
150	0.81	0.242	70.12	71.54

Table 3: % Inhibition of H_2O_2 by Ascorbic acid

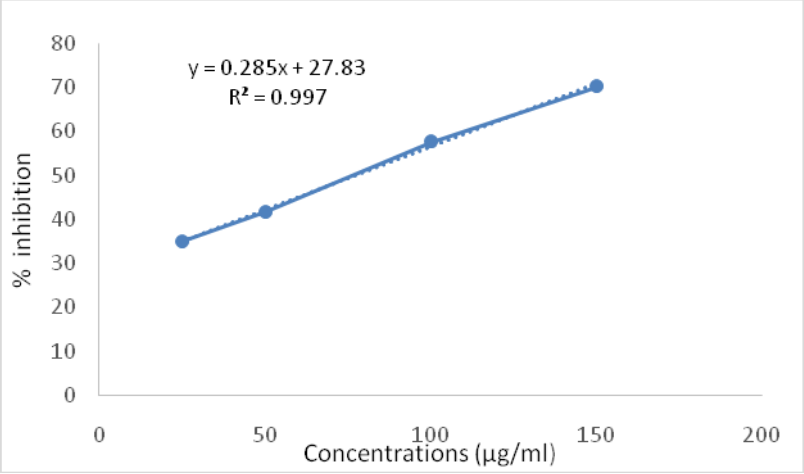

Figure 3: Chart representation of % inhibition of H_2O_2 by Ascorbic acid

Nitric Oxide Scavenging Activity:

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The methanolic extract of A*corus Calamus* with antioxidant activity was also compared with the antioxidant activity of well- known antioxidant L-ascorbic acid[37]. The IC50 values was calculated in both the cases. Rhizome extract of Acorus calamus with the IC50 value was found to be43.90µg/g as shown in (table 4, figure 4) and in comparison, with L-ascorbic acid IC50 value was found to be 29.25µg/g as shown in (table5, figure5).

Concentration	Absorbance of control	Absorbance of test	%inhibition	IC_{50}
25	0.412	0.224	45.63	43.90
50	0.412	0.192	53.69	43.90
100	0.412	0.176	57.28	43.90
150	0.412	0.140	66.01	43.90

Table 4: Nitric oxide Scavenging activity of Methanolic extract of *Acorus Calamus*

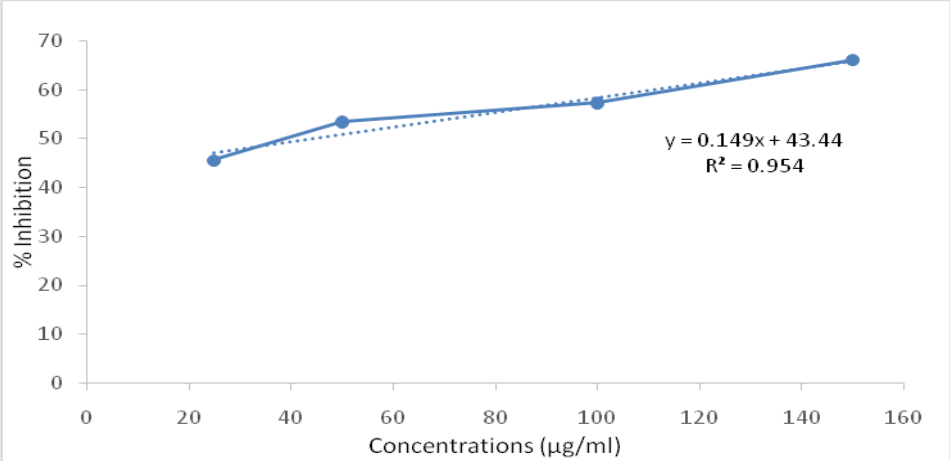

Figure 4: Chart representation of Nitric acid scavanging activity of Methanolic extract of *Acorus Calamus*

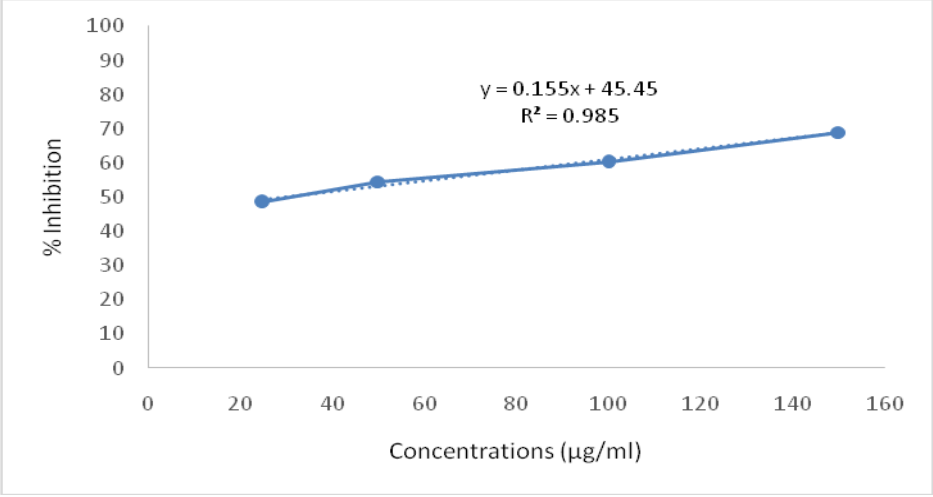

Figure 5: Chart representation of % inhibition of H_2O_2 by Ascorbic acid

From the results of these both methods has been revealed that the compounds exhibit strong free radical scavenging activity in contrast with L-ascorbic acid. It can also be recognized that these compounds shows significant values in healing of many degenerative diseases which are related to oxidative stress .

VI.CONCLUSION

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In the summary, the findings shoewed that extract of Acorus calamus having methanolic extract possess many phytochemicals also showed the high content of total phenolic which shows the strong free radical scavenging activity. The provided data from conducted experiments in this study supports the view that the methanolic extract of Acorus calamus which is enriched with potent antioxidant activity determined by Hydrogen peroxide scavenging method and Nitric oxide Scavenging method which confirms the use of this plant for medicinal purposes .

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