



Evaluation of *In Vitro* Anti-Diabetic and Anti-oxidant Potential of *Acorus calamus* Rhizome

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ABSTRACT: Aromatic volatile oils contained in the dried rhizome of an ethnomedicinal plant, *Acorus calamus* is rich in potent phytochemicals responsible for the various pharmacological activity. This study was aimed to investigate the traditionally claimed anti-diabetic potential by *in vitro* models and explore the anti-oxidant potential of *A. calamus* rhizome upon evaluating their total phenolic and flavonoid content. Pulverized fine powder of rhizome was subjected to methanol extraction and analyzed for their anti-diabetic activity by alpha-glucosidase enzyme inhibition assay using PNPG substrate and alpha-amylase inhibition assay using DNSA colouring agent. Anti-oxidant property of extract was evaluated by DPPH radical scavenging activity and Nitric oxide radical scavenging assay along with the estimation of antioxidant components like total flavonoid and total phenolic content. The result of the assay suggested that methanolic extract of *A. calamus* showed significant alpha-glucosidase inhibitory activity and alpha-amylase inhibitory activity with IC₅₀ value (half maximal inhibitory concentration) at 54.96 µg/ml and 45.15 µg/ml whereas standard acarbose showed 30.745 µg/ml respectively. The inhibitory effect of these enzymes by methanolic extract leads to the reduction in postprandial hyperglycemia in diabetic condition and also produced strong anti-oxidant activity with IC₅₀ value 192.99 µg/ml and 236.60 µg/ml determined by DPPH radical scavenging activity and nitric oxide radical scavenging activity respectively. The total phenolic and flavonoid content was found 32.3±0.072 GAE mg/g and 22.5±0.064 QE mg/g respectively. The results thus indicate that *Acorus calamus* possesses considerable anti-diabetic and antioxidant activity and the effective utilization of the plant can bring out a concomitant therapeutic agent. © 2020 iGlobal Research and Publishing Foundation. All rights reserved.

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INTRODUCTION

Nature has been the primary source of medicine since the beginning of human civilization. An impressive number of the modern drug has been isolated from natural sources and many of these isolations are based on traditional use of medicines.[1-2] Traditional medicine systems have continued playing an essential role as 80% of today's world population still rely on it for primary healthcare.[3] Collective work in the field of medicinal plants now aims to bring prevailing traditional remedies into rational use in the modern frame which can make a significant difference to treat numerous acute and chronic diseases.[4-6]

Diabetes mellitus is a group of metabolic disorders that are characterized by elevated glucose level in blood which is due to the total or relative decrease in production of insulin by

liver and insulin resistance by a target cell. Diabetes patients are more prone to the development of chronic health complications including renal failure, stroke, atherosclerosis, ischemic heart disease, retinopathy and wide array of a heterogeneous disease. As the prevalence of diabetes has increased dramatically in the last few decades, International Diabetes Federation (IDF) predicts 592 million diabetes patients in 2035 AD which as of 2013 is 382 million.[7] An important way to reduce postprandial hyperglycemia is inhibition of enzymes responsible for the metabolism of carbohydrates. Several synthetic drugs have been developed to show such effect but have also been reported to cause several side effect. Efforts have been focused to develop safer drug of natural origin with minimal side effects. Thus, to develop an ideal drug able to manage diabetes mellitus and its

complications, studies on a large number of plant is being carried out.

The dried rhizome of ethnomedicinal plant *Acorus calamus* contains yellow aromatic volatile oils having several potent phytochemicals which have been used to treat a number of metabolic disorder since ancient ages.[8] Traditionally *A. calamus* rhizome is used for appetite loss, bitter tonic, bronchitis, chest pain, diabetes, sedative, digestive disorder, nervous disorders, to promote circulation to the brain, sharpen memory and many other conditions.[9-10] Various investigations evaluated analgesic, anti-fungal, anti-tumor, anti-inflammatory anti-oxidant, anti-microbial, cytotoxic, anti-bacterial and anti-hepatotoxic activity of *A. calamus* rhizome with minimal studies on its anti-diabetic activity but the potentiality was yet to be explored.[11-16] This study was aimed to investigate the traditionally claimed anti-diabetic potential of *A. calamus* rhizome by *in vitro* models and also explore its anti-oxidant potential upon evaluating their total phenolic, flavonoid and tannins content.

MATERIALS AND METHODS

Collection of Plant

The plants were collected on the month of June-July from Goalpara area of Assam, India. They were thoroughly washed in running water and segregated from extraneous material. The plant was identified by its vernacular name and authenticated by Prof. (Dr.) S. K. Borthakur, HOD, Department of Botany, Guwahati University, Assam. (Voucher specimen No. GUBH17889).

Reagents and Chemical Used

Sodium chloride, sodium carbonate, 3,5- dinitrosalicylic acid (DNSA) and p-nitrophenyl- α -D Glucopyranoside (PNPG) were purchased from Himedia Laboratories, India. Standard drug acarbose was gifted by Aurobindo Pharma Limited, India. α -amylase and α -glucosidase was purchased from Sigma-Aldrich, India. Methanol was purchased from Merck India. All the chemical and reagents used for qualitative and quantitative estimations were of analytical grades.

Extraction

The plant rhizomes were collected and cleaned to remove unwanted materials. The rhizomes were dried in the shed for 4 weeks. The air-dried rhizomes of *Acorus calamus* was pulverized to the fine powder and was extracted with methanol in the Soxhlet apparatus. The extract was concentrated by distillation and then the solvent was evaporated to dryness on the water bath.[17]

The colour of the extract was observed and the yield was determined using the formula:

$$\% \text{ yield} = (\text{Weight of extract/weight of powder taken}) * 100$$

Qualitative Analysis

Small amount of methanolic extract of *Acorus calamus* rhizomes was used to carried out the phytochemical screening to reveal the presence of different chemical constituents such as alkaloids, carbohydrates, tannins, flavonoids, glycosides, anthraquinones, coumarin, saponin, deoxy sugar, cardiac glycosides, phenols, proteins, steroids and terpenoids using a standard protocol as described in Phytochemical methods and Pharmacognosy.[18-19]

Quantitative Analysis

The total Phenolic content was determined using Foline-Ciocalteu reagent and expressed as mg gallic acid equivalent (GAE)/g dry extract.[20] The total flavonoid content of extracts was determined following a colourimetric method and values were expressed as mg quercetin equivalent (GE)/g dry extract.[21] The total tannin content in the extract was measured by Folin-Denis method as suggested by Schanderi.[22] Result was expressed as mg tannic acid equivalent per gram of dry weight (mg TE/g) of extract.

Evaluation of anti-diabetic activity

The α -amylase inhibition assay is carried out as illustrated by Miller using the chromogenic 3,5- dinitrosalicylic acid (DNSA) method and acarbose as standard positive control.[23] Pancreatic alpha-amylase (0.04 units) was mixed with 500 μ l of 0.02 M Sodium phosphate buffer (pH 6.9) containing 6mM Sodium chloride and 1ml of varying concentrations (25, 50, 100, 200, 400 mcg/ml) of extracts which were pre-incubated at 37 °C for 10 min. To the same, 500 μ l of 1% (w/v) starch solution prepared using buffer is added and incubated at 37 °C for 15 min. DNSA reagent (1 ml) is added to stop the reaction and placed in a boiling water bath for 5 min, cooled, diluted and measured at 540 nm.

α -glucosidase inhibitory activity was evaluated according to the chromogenic method described by McCue with some modification.[24] 0.5ml alpha-glucosidase (1 U/ml) was mixed with 2.5 ml phosphate buffer (50mM, pH= 6.8) and 1ml of varying concentrations (25, 50, 100, 200, 400 mcg/ml) of extracts was pre-incubated at 37 °C for 15 min. Then 1 ml of p-nitrophenyl- α -D Glucopyranoside (PNPG) (1mM) was added as a substrate and incubated further at 37 °C for 30 min. The reaction was stopped by adding 2.5ml sodium carbonate (0.1M). The yellow colour produced was read at 405nm using UV – visible spectrophotometer. The system without α -

glucosidase was used as a blank, and acarbose was used as a positive control. The enzyme inhibitory rates of samples were calculated as follows:

$$\text{Inhibition \%} = [(\text{control absorption} - \text{sample absorption}) / \text{control absorption}] * 100$$

The IC₅₀ values of samples were calculated and reported as the mean ± standard deviation (SD) of three experiments.

Table 1. Qualitative Analysis of Methanolic Extract of *Acorus Calamus*

S.N.	Phytochemicals	Extract of <i>A. calamus</i>
1.	Alkaloids	(+)
2.	Glycosides	(+)
3.	Anthraquinone	(-)
4.	Coumarin	(+)
5.	Saponin	(+)
6.	Deoxy sugar	(+)
7.	Cardiac glycosides	(+)
8.	Flavonoid	(+)
9.	Steroid	(+)
10.	Terpenoid	(+)
11.	Tannins	(+)
12.	Phenols	(+)
13.	Quinones	(+)
14.	Protein	(+)
15.	Carbohydrate	(+)

(+) Presence, (-) Absence

Table 2. α -Amylase Inhibitory Activity of Methanolic Extract of *A. Calamus* and Comparison with Standard Drug Acarbose.

S.N	Plant extract/ Standard drug	Concentration ($\mu\text{g/ml}$)	% Inhibition	IC ₅₀ value
1.	Methanolic extract of <i>A. calamus</i>	2	3.72±0.04	45.15
		4	6.23±0.07	
		8	9.9±0.02	
		16	17.54±0.03	
		32	34.32±0.04	
		64	71.28±0.03	
2.	Acarbose	2	3.85±0.05	30.745
		4	9.5±0.02	
		8	18.58±0.12	
		16	30.02±0.09	
		32	56.3±0.11	
		64	96.06±0.04	

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM.

Table 3: α -Glucosidase Inhibitory Activity of Methanolic Extract of *A. Calamus* and Comparison with Standard Drug Acarbose.

S.N	Plant extract/ Standard drug	Concentration ($\mu\text{g/ml}$)	% Inhibition	IC ₅₀ value
1	Methanolic extract of <i>A. calamus</i>	2	2.32±0.04	54.90
		4	6.33±0.03	
		8	8.93±0.06	
		16	14.02±0.11	
		32	28.33±0.03	
		64	58.89±0.07	
2	Acarbose	2	4.12±0.03	39.12
		4	7.45±0.03	
		8	14.9±0.04	
		16	22.1±0.04	
		32	42.28±0.08	
		64	79.06±0.02	

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM.

Evaluation of antioxidant activity

DPPH free radical scavenging activity and nitric oxide radical scavenging capacity of *A. calamus* rhizome extracts was determined to study the antioxidant activity. DPPH radical scavenging ability is determined in terms of hydrogen donating ability of extracts, using the stable DPPH radical.[25] The scavenging activity of extracts was calculated based on the percentage of DPPH radical scavenged using the following equation:

$$\% \text{ inhibition} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

Where, A_{sample} is the absorbance of a sample solution and A_{control} is the absorbance of the control solution (containing all reagents except the test sample).

The method of Yen was adopted to determine the nitric oxide radical scavenging activity of extracts of *A. calamus*.[26]

Statistical Analysis

All analyses were performed in triplicate and data were reported as means ± SD. Differences between experiments were analyzed using Student's t-test in Microsoft Excel 2013. The confidence limits used in this study were based on 95% (P<0.05).

RESULT AND DISCUSSION

The powdered form of methanolic extract weighing 48 g of *Acorus calamus* rhizome was extracted using Soxhlet apparatus. The extraction process was continued for 72 h. Solvent was evaporated to get the solvent-free extract and the

total yield was found to be 3.78%. The extract was reddish brown in colour and sticky in nature. Preliminary phytochemical analysis revealed the presence of alkaloids, glycosides, coumarin, saponin, deoxy sugar, cardiac glycosides, flavonoid, steroid, terpenoid, tannins, phenols, quinines, protein and carbohydrate as given in **Table 1**. The presence phytochemicals to the certain limit can vary on the basis of the geological region of collection.[27] Wide array phytochemicals present in the extract are to be responsible for the range of pharmacological response produced in the biological body.

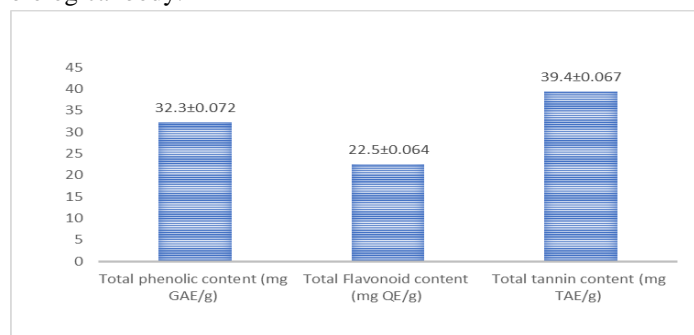


Figure 1. Total phenolic, flavonoid and tannin content of methanolic extract of *A. calamus*.

The result showed the total phenolic content of 32.3 ± 0.072 mg gallic acid equivalent (mg GAE/g). Similarly, total flavonoid content and total tannin content were found to be 22.5 ± 0.064 mg quercetin equivalent (mg QE/g) and 39.4 ± 0.067 mg tannic acid equivalent (mg TAE/g) respectively as given in **Figure 1**. These compounds play an important role in absorbing and neutralizing free radicals thus exhibit redox properties.[28] The presence of high content of phenol, flavonoid and tannin in methanolic extract of *A. calamus* suggests the potentiality of its antioxidant activity.

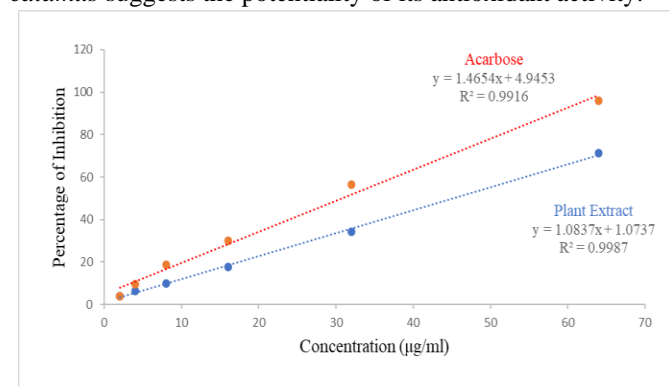


Figure 2: α-Amylase activity of acarbose vs methanolic extract of *Acorus calamus*.

The extract exhibited significant α-amylase inhibitory activity with IC₅₀ value at 45.15 µg/ml whereas standard acarbose showed at 30.745 µg/ml. The half maximal inhibitory concentration (IC₅₀) is a measure of the effectiveness of a

substance in inhibiting a specific biologic or biochemical function. α-glucosidase inhibitory activity of the methanolic extract was quantified with IC₅₀ value at 54.90 µg/ml when standard acarbose was of 39.12 µg/ml which has been shown in **Table 2** and **Figure 2** for α-amylase inhibitory activity and **Table 3** and **Figure 3** for α-glucosidase inhibitory activity. As α-amylase and α-glucosidase being potent carbohydrate metabolizing enzymes, the inhibition of these enzymes blocks the breakdown of carbohydrate and subsequent glucose absorption thus leading to a decrease in postprandial hyperglycemia and maintaining the blood-glucose level. Inhibition of these enzymes is a significant and well-focused therapeutic approach to control hyperglycemia till date in type II diabetes.[29] This effect may be due to the action of inherent polyphenols and flavonoids in the plant.

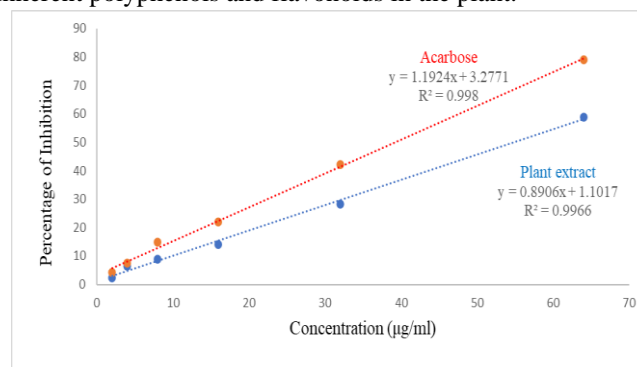


Figure 3: α-Glucosidase activity of Acarbose vs methanolic extract of *Acorus calamus*.

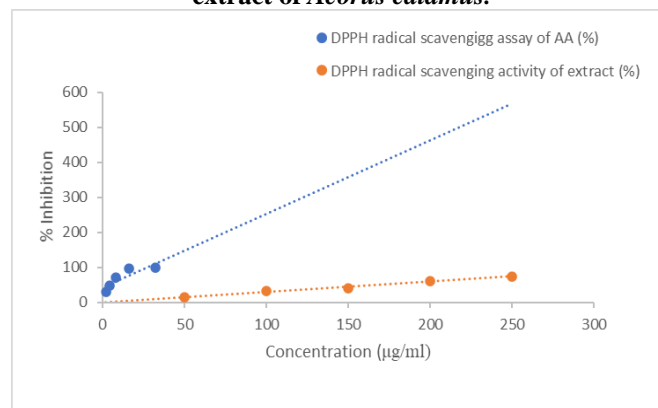


Figure 4: DPPH radical scavenging activity of root extract of *A. calamus* and comparison with standard Ascorbic acid.

Hydrogen donating ability in the compound present in the extract is responsible for the antioxidant activity of any plant. In DPPH radical scavenging activity, an anti-oxidant compound present in the abstract decolorizes the DPPH by donating the electron.[30] The acceptance of electron by DPPH from those compounds changes its colour from violet to yellow which can be measured spectrophotometrically at 517 nm.[31] Thus, the ability of plant extract to scavenge DPPH is calculated and the degree of discoloration is an indication of

the scavenging ability of the antioxidant extract. Extract of the rhizome of *A. calamus* possesses strong anti-oxidant activity with IC₅₀ value 192.99 µg/ml as determined by DPPH radical scavenging activity which is represented graphically in **Figure 4** comparing with DPPH scavenging potential of potent anti-oxidant, ascorbic acid.

Nitric oxide is a gaseous free radical present in the body and is associated with the number of physiological processes and plays a role in immune, respiratory, neuromuscular function and regulation of cell-mediated toxicity.[32] Metabolic products of a nitric oxide such as peroxynitrite are highly reactive and can induce toxic reactions and can lead to protein tyrosine nitration, lipid peroxidation and DNA modification.[33] Extract of the rhizome of *A. calamus* also possesses strong anti-oxidant activity with IC₅₀ value 236.60 µg/ml as determined by nitric oxide radical scavenging activity and the relative comparison of antioxidant potential is shown in **figure 5**.

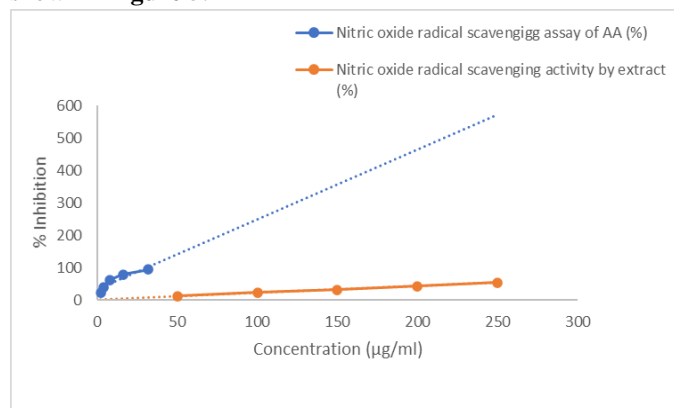


Figure 5: Nitric oxide radical scavenging activity of root extract of *A. calamus* and comparison with standard Ascorbic acid.

The presence of the high amount of free radicals and reactive oxygen species in the body results to oxidative stress which can lead to pathogenesis of wide array of degenerative diseases such as diabetes mellitus.[34-35] Thus, the independent anti-diabetic activity is further synergized by the anti-oxidant activity. Polyphenols and flavonoids which are present in high amount are to be responsible for the significant anti-oxidant and anti-diabetic property exhibited by the plant extract of *A. calamus*.

CONCLUSION

The results thus indicate that *Acorus calamus* possesses considerable anti-diabetic and antioxidant activity with notable polyphenols and flavonoids content. Further studies are needed for the identification and isolation of individual phytochemicals responsible for the activity and the effective

assessment and utilization can bring out a concomitant therapeutic agent.

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DATA AVAILABILITY

Not declared.

CONFLICTS OF INTEREST

Nil

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