PHYTOCHEMICAL SCREENING AND IN VITRO ANTIDIABETIC POTENCY EVALUATION OF *ARUNDINA GRAMINIFOLIA* ROOT EXTRACTS

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Abstract:

Background: *Exploring novel phytomedicines is an expanding field of research, despite the availability of modern therapeutic interventions, diabetes mellitus (DM) continues to pose a significant public health concern in the 21st century.*

Objective: *This study aims to conduct a preliminary screening of phytochemicals and assess the in vitro antidiabetic activity of root extracts from Arundina graminiifolia.*

Materials and Methods: *A standardized approach was employed in this study to investigate the materials and methods used in the research. Root extracts of Arundina graminiifolia were prepared using a Soxhlet apparatus with various solvents, including ethanol, chloroform, petroleum ether, ethyl acetate, and water. The study also sought to identify different types of phytochemicals present in the roots of Arundina graminiifolia. Furthermore, the in vitro antidiabetic activity of these root extracts was assessed, using non-enzymatic glycosylation of hemoglobin as an indicator in an invitro model.*

Results: *The study's findings indicate the presence of phytochemicals such as carbohydrates, tannins, flavonoids, phenols, and others in Arundina graminiifolia root extracts. These extracts exhibited noteworthy inhibitory activity on alpha-amylase and non-enzymatic glycosylation of hemoglobin, suggesting their potential as antidiabetic agents.*

Conclusion: *Based on the results obtained from this investigation, it can be concluded that Arundina graminiifolia root extracts exhibit significant antidiabetic properties in an in vitro model. Further research using an in vivo model is recommended to validate these findings.*

Keywords: *Arundina graminiifolia, Root extract, Phytochemicals, Antidiabetic activity*

1. Introduction

Diabetes mellitus is a chronic medical condition characterized by a combination of genetic and environmental factors that lead to inadequate insulin production by the pancreas or reduced responsiveness to the insulin produced. This condition is often associated with the development of various additional health complications (World Health Organization, 2021). Currently, an estimated 463 million individuals worldwide are affected by diabetes, and projections indicate that this number may rise to 578 million by 2030 and further increase to 700 million by 2045 (IDF, 2020). Traditional herbal remedies, containing a wide range of phytoconstituents with therapeutic properties, have been used for centuries to address various health conditions (Prabhakar & Doble, 2014; Jasmine et al., 2018; Gaonkar and Hullati, 2020). The reedy terrestrial tropical orchid species *Arundina graminifolia* generally grows in bunches. Its native habitat is either broad, grassy plains or steep, rocky terrain. Indonesia, the Philippines, New Guinea, Malaysia, Singapore, Sri Lanka, Thailand, and the Ryukyu Islands are all included in the distribution of this tropical Asian genus (Indian Biodiversity, 2022). Thus, the present study aimed to investigate the phytochemical and anti-diabetic activity from the root extracts of *Arundina graminifolia.*

2. Materials and Methods:

2.1 Plant Collection and Direct Extraction:

Fresh roots of *Arundina graminiifolia* were collected from Tripura, India. After drying, the specimens were carefully stored in airtight containers. Plant identification and authentication were carried out by the Guwahati University. Authenticity certificates were obtained for the herbarium specimens.

2.2 Extraction:

The extraction process involved subjecting the root powder to solvent extraction for a duration of 16 hours at a ratio of 1:5 (w/v). Specifically, 250 mL of ethanol was utilized in a Soxhlet apparatus. Following extraction, the solvent was evaporated under reduced pressure using a rotary evaporator at 40°C. The resulting extract was subsequently filtered through Whatman filter paper No. 2, employing a Buchner funnel. The pre-weighed extract was then dried in flasks for quantitative determination.

2.3 Phytochemical Screening:

Preliminary phytochemical analysis encompassed the examination of various chemical groups within the plant extract. Established methodologies, as outlined by Trease and Evans (1996) and Harborne (1987), were employed to ascertain the chemical composition of the crude extracts. Qualitative testing was conducted to identify the presence of specific chemical constituents.

2.4 Anti–diabetic Potential – Evaluation

The study investigated the in-vitro potential of selected plants for their anti-diabetic properties. This was achieved by conducting enzyme inhibition assays on the extracts using carbohydrate digesting enzymes, as well as employing the non-enzymatic glycosylation of hemoglobin method. Ethanol was used to prepare stock solutions of all the plant extracts.

2.4.1 Inhibition assay for α-amylase activity:

500 µL of test samples and reference medication were mixed with 500µL of 20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5 mg/mL) and also incubated at 25 °C for 10 min. Further, each tube was incubated at 25 °C for 10 min with 500µL of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9). 3,5dinitro salicylic (DNS) acid color reagent stopped the process. After 5 min inside a boiling water bath, the test tubes were cooled to room temperature. Add 10 mL distilled water to the reaction mixture and measure absorbance at 540 nm. Control samples (acarbose) without plant extract had 100% enzyme activity. By replacing the enzyme solution with buffer, a blank was made to measure the colored extracts' absorbance. at 540 nm. The percentage α-amylase inhibition was calculated from the three tests' mean absorption. The formula for percentage inhibition.

$$\textit{Percentage inhibition} = \textit{Absorbance of control - absorbance of sample} / \textit{Absorbance of control} * 100$$

2.4.2 *In-vitro* non-enzymatic glycosylation of haemoglobin:

1000 µL of test samples (50-800 mg/ml) were added to 1000 µL of 20 mM phosphate buffer (pH 6.9) containing hemoglobin (0.5 mg/ml), gentamycin (0.02%), and glucose (2%). After 72 hours, absorbance was then measured at 540 nm. Assay standard Trolax was employed. Control samples without plant extract have 100% enzyme activity. The mean absorption was used to compute hemoglobin glycosylation inhibition.

$$\textit{Percentage inhibition} = \textit{Absorbance of control - absorbance of sample} / \textit{Absorbance of control} * 100$$

3. Results

3.1 Phytochemical Screening:

The results of the study revealed that the ethanolic extracts of *Arundina graminiifolia* root (AGR) were presence of alkaloids, cardiac glycosides, and carbohydrates. In contrast, the petroleum ether extracts of AGR exhibited the presence of alkaloids, oils and fats, flavonoids, and steroids and absence of amino acids and proteins. The chloroform extracts of AGR indicated the presence of oils, fats, Flavonoids and steroids. Additionally, the ethyl acetate chemicals such as alkaloids Tannins phenolic compounds, Flavonoids and saponins as summarized in Table-1.

Table 1: Phytochemical screening of *Arundina graminiifolia* roots extract:

Phytoconstituents	Petrolium Ether extract	Chloroform extract	Ethyl acetate extract	Ethanol extract	Water Extract
Alkaloids	-ve	-ve	+ve	+ve	-ve

Cardiac Glycosides	-ve	-ve	-ve	+ve	-ve
Carbohydrates	-ve	-ve	-ve	+ve	-ve
Oils and Fats	-ve	-ve	-ve	+ve	+ve
Tannins and phenolic compounds	-ve	-ve	+ve	+ve	+ve
Amino acid and Proteins	-ve	+ve	-ve	-ve	-ve
Flavonoides	-ve	-ve	+ve	+ve	-ve
Saponins	-ve	-ve	+ve	+ve	-ve
Terpenoids	+ve	+ve	-ve	+ve	-ve
Steroids	-ve	+ve	-ve	+ve	-ve

‘‘ “+ve”- Present, “-ve”- Absent

3.2 Anti – diabetic potential

3.2.1 Evaluation of in-vitro α-amylase inhibitory activity:

In phytochemical studies, the ethanolic root extract showed more positive components, and after performing with all extract ethanolic extract shows more potent results so ethanolic extracts all data mentioned here. The inhibitory activity of *ethanolic roots* extract of *Arundina graminifolia* **(Table-2 & figure-1)** and standard drug **(Table-3 &figure-2)** acarbose ranges from 12.5 µg/mL to 400µg/ml.

Table 2: α-amylase inhibition by the *ethanolic roots* extract of *Arundina graminifolia*

S.No.	Conc. of formulation(µg/ml)	% inhibition	IC_{50} (µg/ml)
1.	25	11.22± 0.66	419.46 ± 1.14
2.	50	18.45 ± 0.86	
3.	100	27.31 ± 1.45	
4.	200	37.63 ± 2.14	
5.	400	49.22 ± 2.22	
6.	800	61.44 ± 3.45	

n = 3, Values are expressed as ± SEM

Figure 1: α-amylase inhibition by the *ethanolic roots* extract of *Arundina graminifolia.*

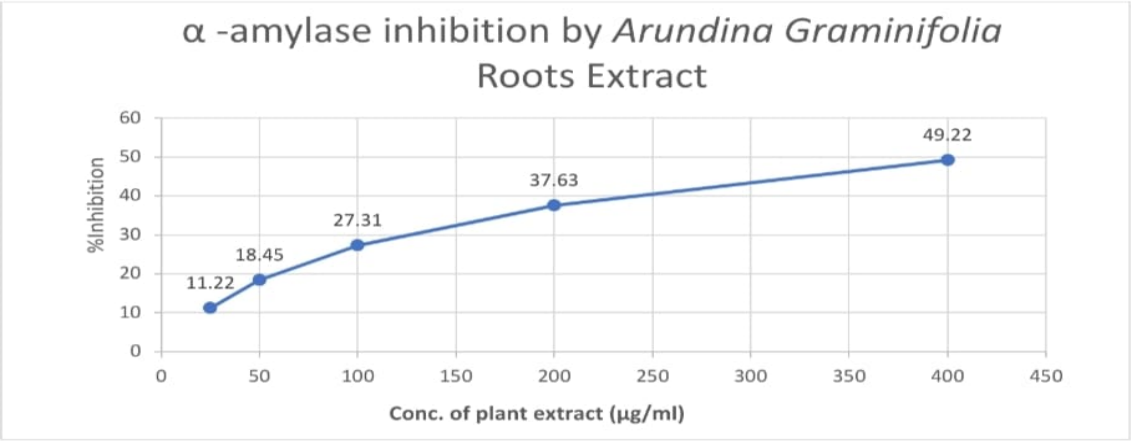
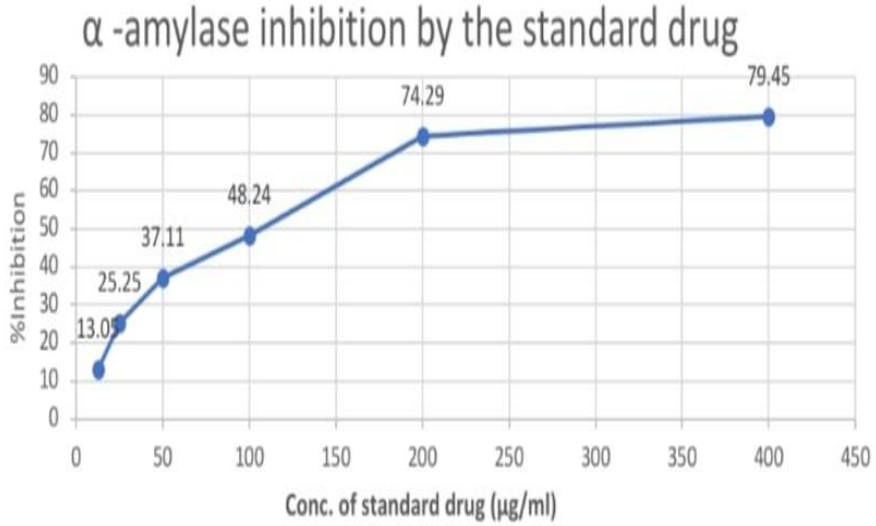


Table 3: α -amylase inhibition by the standard drug (acarbose)

S. No.	Conc. of standard drug (µg/ml)	% inhibition	IC_{50} (µg/ml)
1.	12.5	13.05± 0.55	112.70 ± 1.18
2.	25	25.25 ± 0.08	
3.	50	37.11 ± 1.69	
4.	100	48.24 ± 2.24	
5.	200	74.29 ± 3.25	
	400	79.45 ± 1.25	

n = 3, Values are expressed as ± SEM

***Figure* 2:** α -amylase inhibition by the standard drug (acarbose)



Concentration-dependent inhibition was noted for various concentrations of the tested extracts and also the standard. the IC50 value of *Arundina graminifolia* root extract was of 419.46 ± 1.14 and an inhibition rate of 61.44 ± 3.45% at a concentration of 800 µg/mL. The standard positive control Acarbose exhibited an IC50 value of 112.70 ± 1.18µg/mL (79.45 ± 1.25 % inhibition at 400µg/mL).

3.2 In-vitro non-enzymatic glycosylation of haemoglobin method

In phytochemical studies, the ethanolic root extract showed more positive components, and after performing with all extract ethanolic extract shows more potent results so ethanolic extracts all data mentioned here. The inhibitory activity of *ethanolic roots* extract of *Arundina graminifolia* **(Table-4 & figure-3)** and standard drug **(Table-5 & figure-4)** acarbose ranges from 12.5 µg/mL to 400µg/ml.

The ethanolic root extract of *Arundina graminiifolia* displayed inhibitory activity ranging from 25 µg/mL to 800 µg/mL, with an IC50 value of 312.35 ± 0.88 and an inhibition rate of 69.41 ± 1.12% at a concentration of 800 µg/mL. In comparison, the standard exhibited a range of inhibitory activity from 12.5 µg/mL to 400 µg/mL, with an IC50 value of 51.04 ± 2.65 µg/mL and an inhibition rate of 77.72 ± 1.07% at 400 µg/mL, according to the established protocol.

Table 4: Non-enzymatic glycosylation by the *ethanolic roots* extract of *Arundina graminiifolia.*

S.No.	Conc. of formulation(µg/ml)	% inhibition	IC_{50} (µg/ml)
1.	25	18.22± 1.12	312.35 ± 0.88
2.	50	27.63 ± 1.47	
3.	100	35.33 ± 2.66	
4.	200	41.89 ± 0.48	
5.	400	55.22 ± 0.95	
6.	800	69.41 ± 1.12	

n = 3, Values are expressed as ± SEM

Figure 3: *Non-enzymatic glycosylation by the ethanolic roots extract of Arundina graminiifolia.*

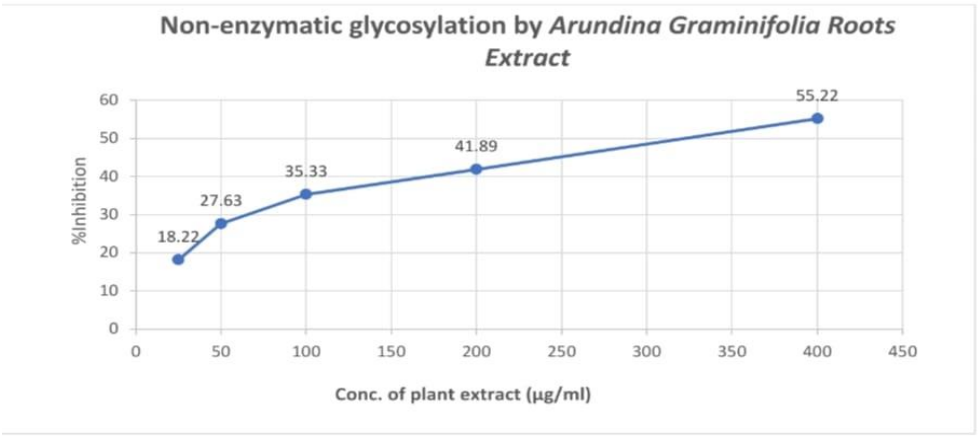
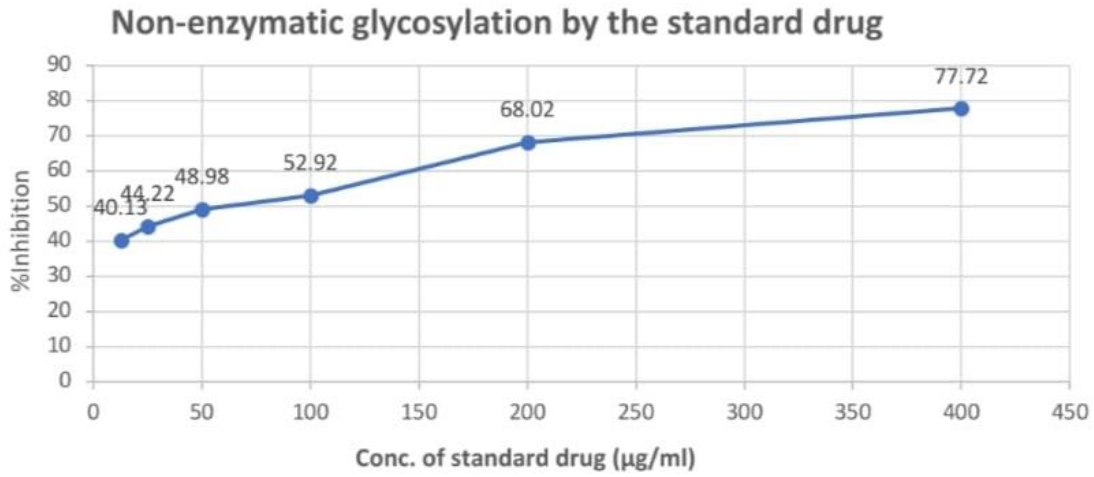


Table 5: Non-enzymatic glycosylation by the standard drug *(Trolax)*

S.No.	Conc of standard drug (µg/mL)	% inhibition	IC_{50} (µg/mL)
1.	12.5	35.54 ± 2.68	51.04 ± 2.65
2.	25	44.22 ± 1.03	
3.	50	48.98 ± 1.89	
4.	100	52.92 ± 3.25	
5.	200	68.02 ± 2.87	
6.	400	77.72 ± 1.07	

n = 3, Values are expressed as ± SEM

***Figure 4**: Non-enzymatic glycosylation of Standard drug (Trolax)*



4. Discussion

Diabetes mellitus primarily results from insufficient insulin secretion or impaired insulin action. The management of diabetes encompasses various strategies, including the promotion of insulin secretion and the inhibition of polysaccharide and disaccharide degradation (World Health Organization, 2019).

Plant-derived compounds, such as alkaloids, terpenoids, polysaccharides, and glycosides, have demonstrated promising antidiabetic properties in the treatment of hyperglycemia (Md. Mominur Rahman et al., 2022). In the current study, phytochemical analysis revealed the presence of diverse phytoconstituents in different extracts of *Arundina graminifolia* roots. Notably, the ethanolic extracts of *Arundina graminifolia* roots contained a significant number of constituents, including carbohydrates, tannins, alkaloids, and others. These constituents may contribute to the plant's multifaceted medicinal properties.The primary objective of this study was to assess the in vitro antidiabetic effects of ethanolic extracts obtained from *Arundina graminiifolia* roots. This evaluation was conducted using the alpha-amylase inhibition assay

and the measurement of non-enzymatic glycosylation of hemoglobin. The results indicated that *Arundina graminiifolia* root extracts exhibited significant inhibitory activity on non-enzymatic glycosylation of hemoglobin, with an IC50 value of 312.35 ± 0.88 and 69.41 ± 1.12% inhibition at 800µg/ml.

Further research is warranted to isolate and identify the specific compound responsible for the observed antidiabetic activity in *Arundina graminiifolia* roots. It's worth noting that the root decoction of this plant is frequently employed in traditional medicine to address conditions such as diabetes, hyperlipidemia, and hepatitis. Additionally, the rhizome of the plant serves as an antibacterial agent, and the phenolic components in this plant exhibit antihepatitic and anti-HIV properties (Debnath et al., 2016).

5. Conclusion

In conclusion, this study substantiates the traditional claims regarding the therapeutic efficacy of *Arundina graminifolia* root extract. Our findings provide compelling evidence that the root extract of *Arundina graminiifolia* demonstrates significant anti-diabetic properties in an in vitro model, with particular emphasis on the potent inhibition observed with the ethanolic extract. Therefore, it is imperative to undertake a comprehensive investigation aimed at identifying the active compound responsible for the ethnopharmacological activity of this plant through analytical studies. Additionally, the utilization of advanced technologies is essential to elucidate the precise mechanism of action.

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