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PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTIDIABETIC POTENCY OF ROOTS OF *DEBREGEASIA LONGIFOLIA*

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ABSTRACT

Background: Despite the availability of modern therapeutic interventions, diabetes mellitus (DM) remains a substantial public health concern in the 21st century. The exploration of novel phytomedicines is an increasingly expanding field of research.

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

Materials and Methods: A standardized approach was employed in this study to investigate the materials and methods used in the research. Root extracts of *Debregeasia longifolia* 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 Debregeasia longifolia. Furthermore, the *in vitro* antidiabetic activity of

these root extracts was assessed, using alpha-amylase inhibition assay and non-enzymatic glycosylation of hemoglobin as an indicator in an *in vitro* model.

Results: The study's findings indicate the presence of phytochemicals such as carbohydrates, tannins, flavonoids, phenols, and others in *Debregeasia longifolia* 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 *Debregeasia longifolia* 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: *Debregeasia longifolia*, Phytochemicals, Diabetes Melitus, Antidiabetic activity

1. INTRODUCTION

Diabetes mellitus is a chronic medical condition characterized by a combination of genetic and environmental factors that result in either insufficient 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 **[1]**. Currently, the global population affected by diabetes is estimated to be approximately 463 million individuals, and projections suggest that this number may increase to 578 million by 2030 and further escalate to 700 million by 2045 **[2]**. Traditional herbal remedies, containing a diverse range of phytoconstituents with therapeutic properties, have been employed for centuries to address various health conditions **[3]**. *Debregeasia longifolia*, commonly known as Orange Wild Rhea, is a tall shrub that can reach heights of up to 5 meters and is typically found in moist regions of Indochina, western China, Myanmar, and Sri Lanka. In light of this, the present study is aimed at investigating the phytochemical composition and anti-diabetic potential of root extracts derived from *Debregeasia longifolia*.

2. MATERIALS AND METHODS:

2.1 Plant Collection and Authentication:

The fresh roots of plants, *Debregeasia longifolia* were obtained from Chilliangaij Jowai West Jaintia hills district along the Jowai main road in Meghalaya, India. After drying, the specimens were stored in airtight containers. The Botanical Survey of India at Shillong, Meghalaya, identified and authenticated plant herbarium specimens and received authenticity certificates.

2.2 Extraction:

The extraction process involved subjecting 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 Phyto- chemical Screening:

The preliminary phytochemical studies involve the testing of various chemical groups present in the extract. To determine the chemical composition of plant extracts **[4]**. The crude extracts underwent qualitative testing to determine the presence of 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 500 µL 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 500 µL 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.

*Percentage inhibition = Absorbance of control - absorbance of sample/ 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.

*Percentage inhibition = Absorbance of control - absorbance of sample / Absorbance of control * 100*

3. RESULTS

3.1 Phytochemical Screening

The results of the study revealed that the ethanolic extracts of *Debregeasia longifolia* root (DLR) were devoid of alkaloids, cardiac glycosides, and carbohydrates. In contrast, the petroleum ether extracts of DLR exhibited the presence of alkaloids, oils and fats, flavonoids, and steroids. The chloroform extracts of DLR indicated the presence of cardiac glycosides, flavonoids, and saponins. Additionally, the ethyl acetate extracts of DLR contained alkaloids, oils and fats, flavonoids, and steroids, as summarized in **Table 1**.

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 *Debregeasia longifolia* **(Table 2 & Figure 1)** and standard drug **(Table 3 & Figure 2)** acarbose ranges from 12.5 µg/mL to 400µg/ml.

Concentration-dependent inhibition was noted for various concentrations of the tested extracts and also the standard. the IC50 value of *Debregeasia longifolia* root extract was 377.35 ± 0.88 (76.72 ± 1.45% inhibition at 800µg/mL). The standard positive control Acarbose exhibited an IC50 value of 112.70 ± 1.18µg/mL (79.45 ± 1.25 % inhibition at 400µg/mL).

3.2.2 In-vitro non-enzymatic glycosylation of hemoglobin method

The inhibitory activity of *ethanolic roots* extract of *Debregeasia longifolia* **(Table 4 & Figure 3)** and standard drug **(Table-5 &Figure-4)** ranges from 12.5 µg/mL to 400µg/ml.

Concentration-dependent inhibition was noted for various concentrations of the tested extracts and also the standard. the IC50 value of *Debregeasia longifolia* root extract was 346.49 ± 1.19 (66.72 ± 1.14% inhibition at 800µg/mL). The standard positive control Acarbose exhibited an IC50 value of 112.70 ± 1.18µg/mL (77.72 ± 1.07% inhibition at 400µg/mL).

Table 1: Phytochemical screening of *Debregeasia longifolia* 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

Table 2: α-amylase inhibition by the *ethanolic roots* extract of *Debregeasia longifolia*

S. No.	Conc. of plant extract(µg/ml)	% inhibition	IC_{50} (µg/ml)
1.	25	30.54 ± 1.25	377.35 ± 0.88
2.	50	35.98 ± 3.05	
3.	100	41.98 ± 2.34	
4.	200	43.92 ± 1.08	
5.	400	53.02 ± 2.25	
6.	800	76.72 ± 1.45	

n = 3, Values are expressed as ± SEM

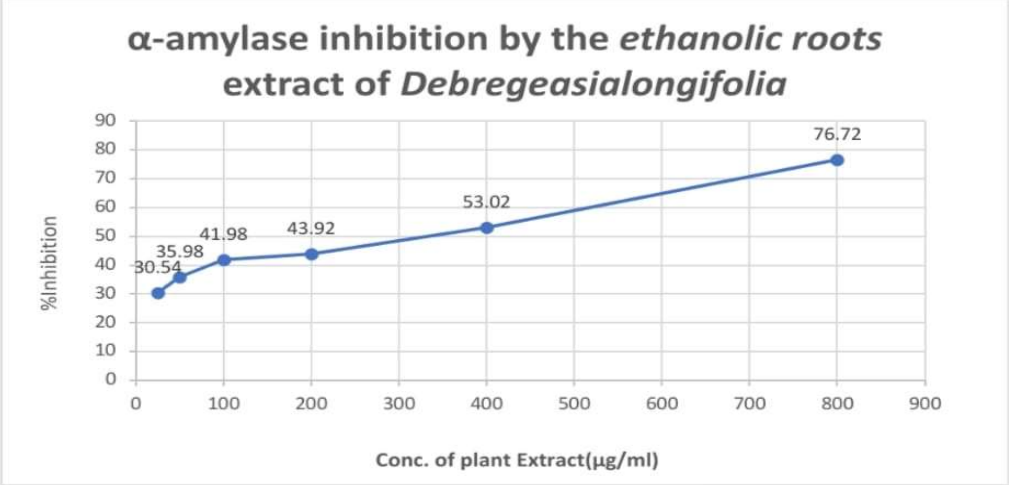

Figure 1: α-amylase inhibition by the *ethanolic roots* extract of *Debregeasia longifolia*

Table 2: 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

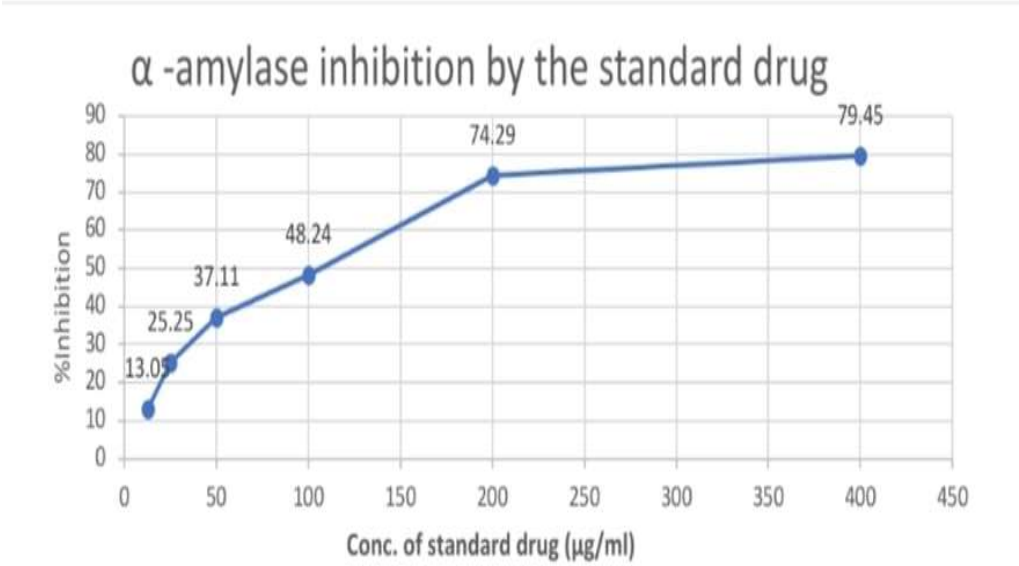

Figure 2: α -amylase inhibition by the standard drug (acarbose)

Table 2: Non-enzymatic glycosylation by the *ethanolic roots* extract of *Debregeasia longifolia*

S. No.	Conc. of plant extract (µg/ml)	% inhibition	IC_{50} (µg/ml)
1.	25	23.54 ± 2.69	346.49 ± 1.19
2.	50	32.98 ± 1.45	
3.	100	38.98 ± 0.89	
4.	200	44.92 ± 3.35	
5.	400	51.02 ± 2.78	
6.	800	66.72 ± 1.14	

n = 3, Values are expressed as ± SEM

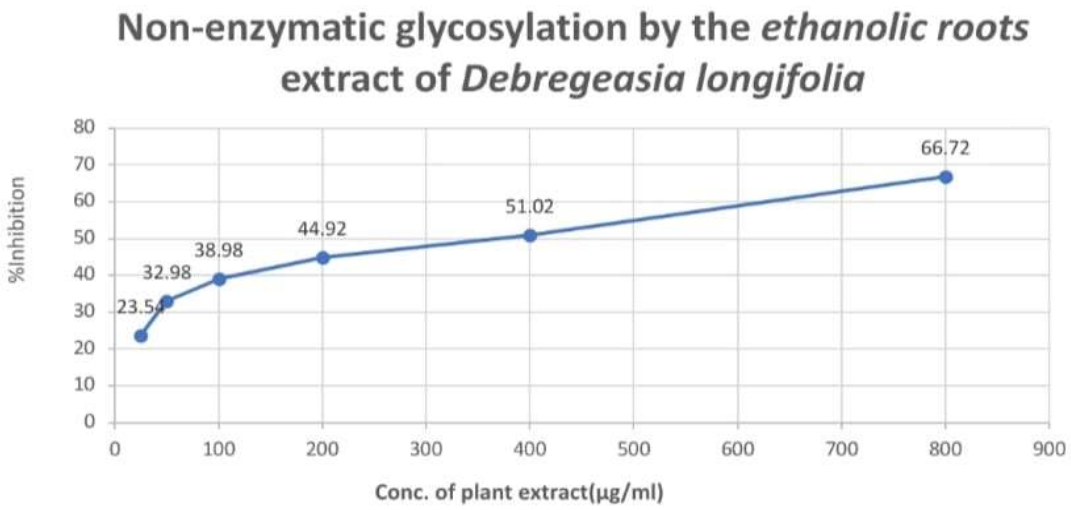

Figure 3: Non-enzymatic glycosylation by the ethanolic roots extract of *Debregeasia longifolia*

Table 3: Non-enzymatic glycosylation by the standard drug

S. No.	Conc. of standard drug (µg/ml	% inhibition	IC_{50} (µg/ml)
1.	12.5	40.13± 1.38	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

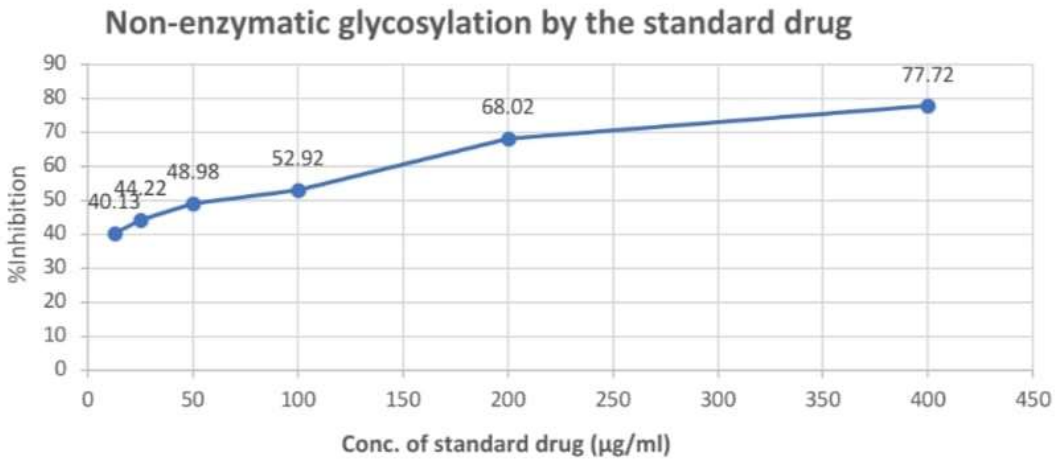

Figure 4: Non-enzymatic glycosylation of Standard drug

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 **[4, 5]**. Plant-derived compounds, such as alkaloids, terpenoids, polysaccharides, and glycosides, have demonstrated promising antidiabetic properties in the treatment of hyperglycemia **[6, 7]**. In the current study, phytochemical analysis revealed the presence of diverse phytoconstituents in different extracts of *Debregeasia longifolia* roots. Notably, the ethanolic extracts of *Debregeasia longifolia* 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 and compare the *in vitro* antidiabetic effects of ethanolic extracts obtained from *Debregeasia longifolia* roots. This evaluation was conducted using the alpha-amylase inhibition assay and the measurement of non-enzymatic glycosylation of hemoglobin **[8-12]**. The results indicated that *Debregeasia longifolia* root extracts exhibited significant inhibitory

activity on alpha-amylase inhibition assay and non-enzymatic glycosylation of hemoglobin, with an IC50 value of alpha-amylase inhibition was 377.35 ± 0.88 (76.72 ± 1.45% inhibition at 800µg/mL) and IC50 value 446.49 ± 1.19 and 73.94 ± 0.09% inhibition at 800µg/mL. Further research is warranted to isolate and identify the specific compound responsible for the observed antidiabetic activity in *Debregeasia longifolia* 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 **[13]**. Additionally, the rhizome of the plant serves as an antibacterial agent, and the phenolic components in this plant exhibit antihepatitic and anti-HIV properties **[1]**.

5. CONCLUSION:

In conclusion, this study substantiates the traditional claims regarding the therapeutic efficacy of *Debregeasia longifolia* root extract. Our finding properties compelling evidence that the root extract of *Debregeasia longifolia* 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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7. CONFLICT OF INTEREST:

The authors have no conflict of interest for the publication of this paper.

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