IN-VITRO ANTI-DIABETIC ACTIVITY AND PHYTOCHEMICAL SCREENING OF ETHANOLICEXTRACT OF *CALOTROPIS GIGANTEA* (Linn)

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ABSTRACT

INTRODUCTION

Diabetes Mellitus (DM) is metabolic disorder characterized by hyperglycaemia due to defect insulin release from pancreas and insulin resistance. The present study was aimed to screen the phytoconstituent present in leaf extract and evaluate α- amylase inhibitory activity of ethanolic extract of *Calotropis gigantea* leaf. *Calotropis gigantea* also known as crown flower is rich in phytochemicals like alkaloids, saponins, steroids, flavonoids and glycosides. It is used for asthma, bronchitis and dyspepsia, dried whole plant is a good tonic.

MATERIAL AND METHODS

About 58.45g coarse powder of *Calotropis gigantea* leaves was extracted with ethanol using Soxhlet apparatus. Then the phytochemical screening of ethanolic extract was done. Different concentration (0.1mg/ml to 1mg/ml) of ethanolic extracts of leaves, acarbose, sitagliptin and metformin was prepared, and all the concentration were evaluated for α- amylase inhibitory activity through spectrophotometric method.

RESULTS

Maximum inhibition of α- amylase was shown by acarbose at concentration of 1mg/ml and found to be 78.787 ± 0.00240 % whereas plant extract at concentration of 1mg/ml showed inhibition of α- amylase activity 54.6296 ± 0.00282 %. Percentage inhibition of α- amylase activity by plant extract was found to be dose dependent.

CONCLUSION

It was concluded that *Calotropis gigantea* leaves possess some bioactive compounds which were responsible for controlling blood glucose level by inhibiting α- amylase and its identification, isolation and characterization may lead to development of newer drugs with lesser side effects.

KEYWORDS

Anti-diabetic, Metformin, Sitagliptin, Acarbose, α- amylase and *Calotropis gigantea.*

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INTRODUCTION

Diabetes meliitus (DM) is considered as one of the five leading causes of death in the world. There are an estimated 143 million people. Diabetes is a long-term condition of the metabolism of proteins, carbohydrates, and fat that results from an absolute or relative lack of insulin production. The key to correcting diabetic complications including neuropathy, microvascular issues, and cataracts and enhancing patients with diabetes quality of life is effective blood glucose management.[1]

Asclepiadaceae family member *Calotropis gigantea*, often known as the "Crown Flower," is a milky shrub that grows up to 1-3 meters tall and is distributed all throughout India. It is also known as "madar" in Hindi. Leaves of *Calotropis gigantea* blade: oblong to widely obovate, 5-30X 2.5-15.5 cm; opposite-decussate, simple, sub sessile, exstipulate. In particular, the powdered root is utilized in the treatment of asthma, bronchitis, and dyspepsia, and the dried whole plant is a good tonic. *Calotropis gigantea* is also employed in the ayurvedic system of medicine.[2] *Calotropis gigantea* activity may result from an improvement in peripheral glucose metabolism due to increase insulin release.[3]

It showed special attention because of the presence of secondary metabolites (cardiac glycosides, flavonoids, terpenoids, alkaloids etc.) and its long use as traditional medicine and displayed number of immune pharmacological activities such as anti-inflammatory, hepatoprotective, anti-fungal, free radical scavenging activity.[4]

The primary endonuclease enzyme found in the pancreas, amylase, oversees breaking down starches and absorbing glucose. Acarbose is one of its inhibitors, which prevents the release of glucose in the blood, producing an anti-diabetic action. Because of the presence of phytochemicals, primarily flavonoids, saponins, and alcohols, the alcoholic extracts of C. gigantea leaves may have a percentage inhibitory impact on α-amylase. The tannins have been shown to promote glucose transporter 4 (GluT4), a major mediator of glucose removal from the circulation and an important regulator of whole-body glucose homeostasis, as well as phosphorylation of the insulin receptors. Additionally, it aids in the inhibition of the primary gene involved in adipogenesis, lowering blood sugar levels without increasing adiposity. Additionally, flavonoids have been shown to protect against the advancement of diabetes mellitus by scavenging free radicals in the body and maintaining cell integrity and function. The potent inhibitor activities is generally due to the phytochemicals present in it.[5] The clinically approved inhibitor, i.e., acarbose, metformin, and miglitol, has several side effects associated with gastrointestinal problems such as flatulence and diarrhoea. Hence, we use acarbose, metformin and sitagliptin as standard drugs for the study. Various traditional medicines and phytoherbs having alpha-amylase inhibitory activity are well known for their role in the prevention and treatment of diabetes till date. Some plant-derived constituents with antidiabetic properties have been isolated and shown to have high potential and lower side effects than clinically approved synthetic drugs.[6] Therefore, it is still worth further investigation for the development of more effective inhibitors towards α-amylase.

MATERIAL AND METHODS

Collection and authentication of plant material

Leaves of *Calotropis gigantea* was collected from different places near Universal College of Medical Science Bhairahawa, Rupandehi. Herbarium will be made and sent to the Agriculture College of Pakhlihawa, Rupandehi for its identification. The certificate of plant identification was issued by Assistant Professor Dr. Puspa Raj Poudel Department of Horticulture and Plant Protection (IAAS, Paklihawa Campus) on date January 10, 2023.

Study site and duration of study

The study is carried out for four months (February 2023 to June 2023) after taking approval from Institutional Review committee of Universal College of Medical Sciences (UCMS/IRC/196/22) on date November 22, 2022.

Preparation of extract

The fresh leaves of *C. gigantea* were harvested, properly cleaned with distilled water, and totally shade dried. After using an electrical grinder to crush the leaves into a fine powder, sieve size 80 was used to filter the material. With the use of petroleum ether, chloroform, and 90% ethanol, the fine powder plant material was gradually extracted utilizing a Soxhlet device and a continuous hot percolation procedure. To get a dried extract, the solvent was evaporated at reduced pressure. The percentage yield was calculated on a dry basis.[7]

$$\text{Percent yield of extract} = \frac{\text{Weight of the extract after evaporationg solvant and drying x100}}{\text{Dry weight of plant material}}$$

Phytochemical Screening

Standard techniques for phytochemical screening were used to find bioactive substances like alkaloids, tannins, phenols, steroids, flavonoids, and saponins.[8]

Inhibition of α-amylase enzyme

An earlier approach that had been published was somewhat modified for the α- amylase inhibition experiment. 500 gm of 0.20 mM phosphate buffer (pH 6.9) containing alpha -amylase (0.5 mg/ml) solution was added to 500 gm of test samples. The mixture was then incubated at 25°C for 10 minutes. With 1.0 ml of 3, 5 di-nitro salicylic acid color reagent, the process was stopped. The test tubes were then cooled to room temperature after 5 minutes of incubation in a boiling water bath. After adding 10 ml of distilled water to the reaction mixture, the absorbance at 540 nm was measured using a UV-VIS spectrophotometer after validation with potassium dichromate solution. Without using plant extract, the absorbance of the control sample was determined and served as a negative reference. The experimental extract and acarbose, metformin, and sitagliptin were tested with varying concentration. The results were expressed as % inhibition of enzyme activity and calculated according in the following equation as decrease in the absorbance of the extract from control.[9]

$$\%\ \text{Inhibition of α-amylase activity} = \frac{\text{Absorbance of (control-extract)}}{\text{Absorbance of Control}} \times 100$$

Statistical Analysis

The in-vitro tests were carried out in triplicate, and the mean findings were reported. ANOVA was used to establish the statistical difference between the control and treatment groups, and Dunnett's Multiple Comparison Test was used for the post hoc analysis. The values of $p<0.05$ was considered as significant.

RESULTS

Yield Percentage
Weight of crude drug = 58.45
Weight of Extract = 8.345

$$\text{Yield Percentage} = \frac{\text{Weight of extract}}{\text{Weight of crude drug}} \times 100$$

$$= \frac{8.345}{58.45} \times 100$$

$$= 14.27\%$$

Table 1. Phytochemical screening of calotropis gigantea leaves extract.

S.N.	Phytochemical Constituents	Name of the test	Results	Obderved color
1	Alkaloids	Mayer's test	+	-Cream colored precipitate
		Wagner's test	+	-Reddish
2	Terpenoids	Salkowski test	+	-Reddish
3	Saponins	Frothing test	+	-Foam appearance
4	Carbohydrate	Fehling test	+	-Red precipitate
5	Tannins	Ferric chloride test	+	-Greenish to black
6	Flavonoid	Sulphuric acid test	+	-Yellow color disappear

In the present study % inhibition of α-amylase activity was determined by ethanolic leaves of Calotropis gigantea and standard drug metformin, sitagliptin, and acarbose, where % inhibition of α-amylase at a concentration 1mg/ml by acarbose, sitagliptin, metformin and plant extract were found to be 78.78±0.0024 %, 73.091±0.00304 %, 69.69±0.00042%, 54.629±0.00282 % respectively. Analysing the plot of % α-amylase inhibition as function of all concentration prepared IC 50 values was calculated. IC 50 value of acarbose was found to be least 571.048143 whereas plant extract was found to be more 853.9139. Acarbose showed highest significant α-amylase inhibitory activity (*p<0.01) followed by sitagliptin and metformin (*p<0.05).

Table 2. Percentage of inhibition of α-amylase activity by different concentration of standard drugs and plant extract and IC 50 value standard drugs and plant extract

Concentration (mg/ml)	α- amylase inhibition							
	Acarbose %inhibition	IC50 µg/ml	Sitagliptin %inhibition	IC50 µg/ml	Metformin %inhibition	IC50 µg/ml	Extract %inhibition	IC50 µg/ml
0.1	12.121±0.003		11.023±0.018		6.060±0.000		12.962±0.004	
0.2	27.272±0.035		26.126±0.022		18.181±0.02		20.370±0.014	
0.4	36.363±0.137	571.048	41.094±0.120	609.095	27.272±0006	733.163	30.092±0.141	853.9139
0.8	69.696±0.011		63.125±0.000		51.515±0.00		49.537±0.004	
1	78.787±0.002		73.091±0.003		69.696±0.00		54.6296±0.00	

Comparison of percentage inhibition of α-amylase and IC 50 value between standard and extract at varying concentration from 0.1mg/ml to 1 mg/ml. The results were expressed in % inhibition ± SD. The significant value between plant extract and standard was calculated by one way ANOVA followed by Dunnet multiple comparison test. *p<0.05, *p<0.01

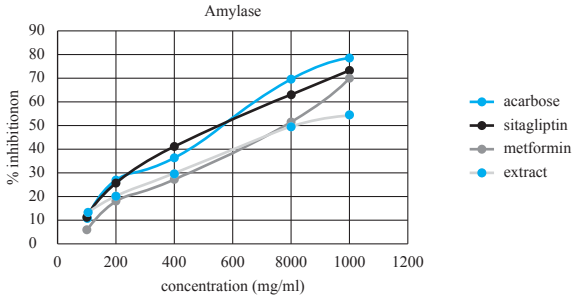

Figure 1. % Inhibition of α-amylase activity at different concentration of plant extract and standard drug used.

DISCUSSION

In the present in-vitro study, *C. gigantea* leaves ethanolic extracts was used to evaluate anti-diabetic activity. Preliminary phytochemical screening of the extract of *C. gigantea* showed presence of alkaloids, flavonoids, saponins, tannins and glycosides.

In earlier days it was found that the extract of *C. gigantea* flowers can be employed as an anti-diabetic agent since it is a potential natural source of antioxidants and may be more important as a therapeutic agent in preventing or slowing diabetes action. The current analysis on *C. gigantea* leaves using ethanol as a botanical confirms the presence of numerous biologically active compounds and their potential functional groups. Additionally, a variety of therapies are already being used with these leaves. The current study may serve as a springboard for more phytochemical and pharmacological research needed to isolate the novel active components from the leaves and create new medications to treat incurable conditions.[10]

Earlier Madhavan SA et al[11] concluded that α-Amylase activity was found at average level in extract in comparison to standard acarbose (IC50 184.56 µg/ml) but our recent study revealed the IC50 value of 571.048 mg/ml. The IC50 value is differ from the published data it may be due to the source of acarbose, and the grade of chemical used (Lab grade).

Gyawali R et al[12] in 2022 founded that greater anti- hyperglycemic activity at oral doses 100, 200, 400 mg draws out as per kg body weight. These doses resulted in percent drop in blood glucose level of 21.35, 25.39, and 28.54 which is supported by streptozocin whereas my recent study showed that metformin shows the similar effect as compared to the earlier study because we have taken the same plant and applied same method. Choudhary et al in 2011 observed the IC 50 value of standard drug and extract which is 47.1 mg/ml and 47.1 mg/ml respectively but my recent activity showed the IC 50 value as 571.048143 mg/ml and 853.9139 mg/ml. It may be due to different method used in the study.[13]

CONCLUSION

Present in-vitro study revealed that ethanolic extract of *Calotropis gigantea* leaves to be effective in inhibiting activity of α-amylase which regulates the glucose level. It was concluded plant leaves possessed certain medicinal values which may be generated the lead molecules for development of new drugs which act as alternative to treat diseases.

CONFLICT OF INTEREST

None

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