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INVITRO ALPHA AMYLASE INHIBITORY AND ANTI-INFLAMMATORY ACTIVITY OF THE LEAVES EXTRACT OF DENSEFLOWER KNOTWEED *(Persicaria glabra)*

M. M. Rahman[1*], Z. R. Moni[2] and M. M. Rahman[3]

[1]Department of Applied Nutrition and Food Technology, Faculty of Biological Sciences, Islamic University (IU), Kushtia; [2]Nutrition Unit, Bangladesh Agricultural Research Council (BARC), Farmgate, Dhaka; [3]Gucchagram project, Ministry of Land. Bangladesh.

Abstract

The primary objective of this study was to examine the inhibitory effects of *Persicaria glabra* on α-amylase and its potential anti-inflammatory properties. The α-amylase inhibitory activity of leaves extracts from *P. glabra* was assessed using the 3,5-dinitrosalicylic acid (DNSA) methodology, while the anti-inflammatory activities were evaluated using the egg albumin denaturation method. The methanolic and aqueous extract of *P. glabra* exhibited strong inhibitory activity against α-amylase and demonstrated anti-inflammatory properties. The methanolic extract exhibited an IC50 value of 5.43 mg/mL for α-amylase inhibitory activity, while the aqueous extract had an IC50 value of 6.01 mg/mL. In terms of anti-inflammatory activity, the IC50 values for the methanolic and aqueous extracts were 128.04 µg/mL and 95.96 µg/mL, respectively. The phytochemicals have been found in *P. glabra* that demonstrated strong inhibition of α-amylase and possess anti-inflammatory properties, which could have significant implications in the fields of medical and veterinary science.

Keywords: Anti-inflammatory*, Persicaria glabra,* α-Amylase.

Introduction

Type 2 diabetes mellitus (T2DM) is a multidimensional chronic condition characterized by impaired functioning of pancreatic β cells and resistance to insulin, resulting in high blood sugar levels (DeFronzo, 2004). The disease remains a significant global health issue and economic burden as a result of contemporary lifestyle and heightened carbohydrate intake. The frequency may increase, leading to a substantial effect on the population of developing nations due to the lack of effective and affordable treatment options for DM.

In the presence of diabetes, untreated chronic hyperglycemia leads to an increase in the formation of reactive oxygen species (ROS) in both the mitochondria and non-mitochondrial components. This process enhances the activation of protein kinase C (PKC) iso forms, hexosamine pathway flux, polyol pathway flux, and advanced glycation end products (AGE) that contribute to oxidative damage caused by hyperglycemia (Moussa,

* Corresponding author: hmmrmahfuz111@gmail.com

2008). A current therapeutic objective in the treatment of type 2 DM is inhibiting α-glucosidase and α-amylase to reduce the absorption of glucose in the gut (Sim *et al.*, 2010). Alpha-amylase, also known as α-1,4-glucan-4-glucanohydrolases, is a vital enzyme generated by the pancreas and salivary gland. It is essential for intestinal mucosa breakdown of complex carbohydrates into oligosaccharides and disaccharides. Alpha-glucosidase digests these sugars into monosaccharides. Clinically utilized alpha-amylase and glucosidase inhibitors can cause hypoglycemia, diarrhea, gas, and colon bloating, limiting its effectiveness in diabetes management (Evans and Rushakoff, 2002). Hence, it is crucial to seek out supplementary and alternative treatments that have low negative implications and can be used in conjunction with the management of DM (Evans and Rushakoff, 2002). Inflammation is a complex physiological reaction of the body to damage, infection, or destruction, which is marked by increased temperature, redness, pain, swelling, and disrupted physiological processes. The process is initiated by the liberation of chemical mediators from damaged tissue and migratory cells (Sangeetha and Vidhya, 2016). Inflammation serves as the primary catalyst for the development of Type 2 Diabetes Mellitus (T2DM). This occurs due to the influence of the aforesaid risk factors and the persistent activation of pro-inflammatory cytokine pathways in the specific tissues affected by insulin-related processes, such as adipose tissue, muscle mass, and the liver(Pradhan *et al.*, 2001; Marques-Vidal *et al.*, 2012; Sakashita *et al.*, 2015; Tsalamandris *et al.*, 2019). Even a slight deviation in glucose levels is associated with inflammatory mechanisms and consequences connected to type 2 diabetes (T2D) (Löbner and Füchtenbusch, 2004). Inflammation has also been connected with additional illnesses related to type 2 diabetes mellitus (T2DM), including atherosclerosis and blood clotting, metabolic syndrome, heart failure, cardiometabolic diseases, renal diseases, and malignancies(Chen *et al.*, 2012; Sarvottam and Yadav, 2014; Adar *et al.*, 2015; Ellulu *et al.*, 2016). Modern medicine has benefited from natural products. Traditional medicine is being evaluated globally through study on plant species and their strong medicinal ingredients. The plant kingdom's diversity may yield novel anti-inflammatory compounds. Herbal medications are considered effective, affordable, and have less serious side effects (Chandra *et al.*, 2012). *P. glabra* is a member of the polygonaceae family. The Bengali name for this plant is Bihagni, also known as Sada Kukri. The aerial parts of the plant include several physiologically active terpenoids and flavones. The plant's leaves, flowers, stems, and seeds contain many significant components that can be utilized for different reasons (Runa *et al.*, 2019). The current investigation aimed to assess the potential *in vitro* anti-inflammatory impact of *P. glabra* extract on protein denaturation and determine the *invitro* α - amylase inhibitory activity.

Materials and Methods

Chemicals

All chemicals used were of analytical reagent grade. The compound used is 3,5-dinitro salicylic acid (DNSA), and the enzyme used is α-amylase obtained from Sigma, USA. The compound Dimethylsulfoxide (DMSO), and methanol were acquired from Sigma-Aldrich (Germany). The acquisition of diclofenac sodium and other chemicals were collected from HS Scientific, located at Hatkhola, Dhaka, Bangladesh. A freshly laid hen egg was purchased at a local market.

The study was conducted from October to December 2023, at the Department of Applied Nutrition and Food Technology, Islamic University, Kushtia, Bangladesh.

Collection of plant materials

Fresh leaves of *P. glabra* were collected from the Islamic University, Kushtia campus and were carefully rinsed and dried in a shady area at room temperature until their weight stabilized. Then, each dried leaf was ground into a fine powder using an electric grinder. The powder was sieved to make it uniform and fine. The powder was then packaged and stored in a dark, dry place for future use.

***P. glabra* plant** ***P. glabra* plant's dried leaves**

Fig. 1. *P. glabra* plant (left) and dried leaves (right)

Preparation of extracts

The leaf powder was immersed in methanol and distilled water for 24 hours, separately. Then strained through muslin cloth and collected in conical flask. Subsequently, the filtrates underwent a second filtration process using Whatman filter paper (No. 1) and were then subjected to vacuum concentration in a rotary evaporator at a temperature of 40 °C.

α-Amylase Inhibitory Assay

The α-amylase inhibition assay was conducted employing the 3,5-dinitrosalicylic acid (DNSA) technique (Wickramaratne, *et al*., 2016). *P. glabra* leaf extract was dissolved in a small amount of 10% DMSO and then in a buffer solution at pH 6.9 (Na_2HPO_4/NaH_2PO_4 at 0.02 M, NaCl at 0.006 M). This yielded values of test solution 0.5 to 15 mg/mL (w/v). A 2unit/ml α-amylase solution was mixed with an extract in a 200 µl volume. The mixture was incubated at 30 °C for 10 minutes. Then, 200 µl of 1% starch solution in water (w/v) was added to each tube and incubated for 3 minutes. To stop the reaction, add 200 µl of DNSA reagent, which contains 12 g of sodium potassium tartrate tetrahydrate in 8.0 mL of 2 M NaOH and 20 mL of 96 mM 3,5-dinitrosalicylic acid solution. The mixture was heated for 10 minutes at 85–90 °C in a water bath. The solution was diluted with 5 mL of distilled water after cooling to room temperature. The solution's absorbance was measured at 540 nm using a UV-Visible spectrophotometer (Hitachi U-

2900, Japan). A blank solution was produced by replacing plant extract with 200 µl of buffer, resulting in 100% enzyme activity. A control reaction using plant extract without enzyme solution at each concentration was also produced. A positive control sample was produced using Acarbose at doses from 100µg/ml to 5µg/ml. To calculate α-amylase inhibitory activity, the percentage of inhibition was calculated using the following equation: The % inhibition of α-amylase was compared to the extract concentration. The graph yielded IC50 values.

$$\%\ \alpha\ amylase\ inhibition\ = 100 \times \frac{Abs\ of\ control - Abs\ of\ Sample}{Abs\ of\ control}$$

Evaluation of in vitro anti-inflammatory activity

The *in-vitro* anti-inflammatory activity was studied using egg albumin denaturation method (Chandra *et al.*, 2012). The reaction mixture was prepared combining 0.2 mL of egg albumin from fresh hen egg, 2.8 mL of phosphate buffered saline (PBS) with a pH of 6.4, and 2 mL of extract at different concentrations to achieve final concentrations of 31.25, 62.50, 125, 250, 500/; and 1,000 µg/mL. A control was provided using an equivalent amount of double-distilled water. Subsequently, the mixtures were placed in an incubator (Froilabo) and incubated at a temperature of 37°C for 15 minutes. Following this, the mixtures were subjected to a heat treatment at a temperature of 70°C for 5 minutes. Following the chilling process, the absorbance of the sample was measured at a wavelength of 660 nm using a double beam spectrophotometer (Hitachi U-2900, Japan). The vehicle was used as a reference to account for any background absorbance. The reference drug, diclofenac sodium, was used at final concentrations of 78.125, 156.25, 312.5, 625, 1250, and 2500 µg/mL. It was processed in a similar manner to determine absorbance. The proportion of protein denaturation inhibition was determined using the following formula:

$$\%\ inhibition = 100 \times (At / Ac - 1)$$

Where, A_t = absorbance of test sample, A_c = absorbance of control

The IC50 value was calculated by generating a graph that plotted the percentage of inhibition in relation to the treatment concentration, compared to the control.

Result and Discussion

α-Amylase Inhibitory Assay

The potential of leaves extract of *P. glabra* to inhibit α-amylase activity was examined at six different concentrations (0.5, 1, 2, 5, 10, and 15 mg/mL). Individual dose-response calibration curves were developed for methanol and water extracts of *P. glabra* leaves. The percentage of α-amylase inhibition and the IC50 values were calculated based on the dose-response calibration curves for each extract. The α-amylase inhibitory activities of the leaf extracts were methanol (IC50 5.43 mg/mL; $R^2$0.910) > water (IC50 6.01 mg/mL; $R^2$0.9288). The standard positive control Acarbose showed an IC50 of 46.13 µg/mL (R^2 0.9796). The conducted α-amylase inhibitory investigations revealed that the extracts of *P. glabra* leaves exhibited substantial inhibitory properties. The IC50 value of methanol extracts was comparable to that of Acarbose, an extensively utilized and

commercially available anti-diabetic medication. These α-amylase inhibitors, also known as starch blockers, hinder or delay the absorption of starch into the body by primarily obstructing the hydrolysis of 1,4-glycosidic bonds in starch and other oligosaccharides, resulting in the prevention of their conversion into maltose, maltriose, and other simple sugars (Dineshkumar *et al*., 2010).The methanol extract likely contains polar molecules that exhibit α-amylase inhibitory activity. Further investigation and isolation of these pure active compounds is recommended.

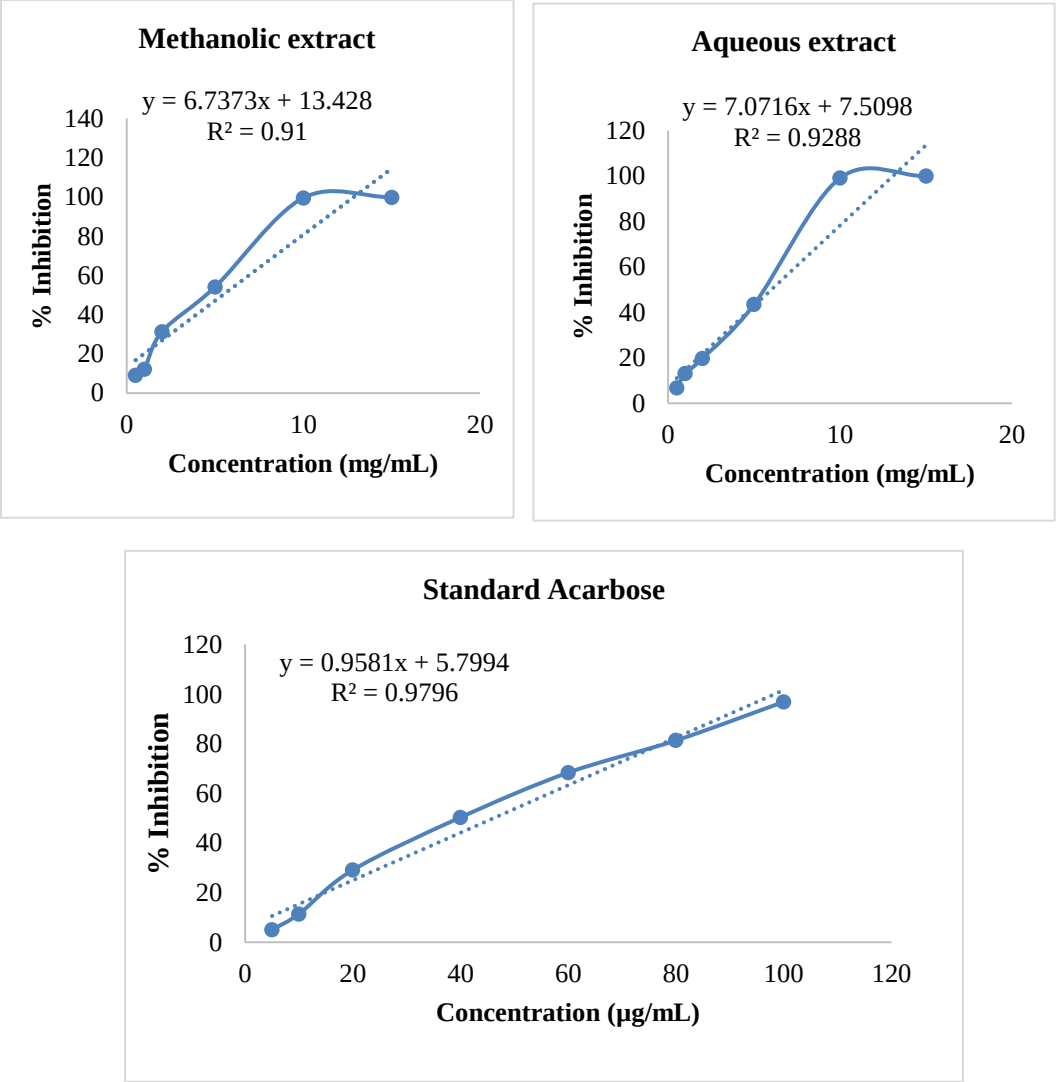

Fig. 2. Calibration curve for α-amylase inhibition assay

In vitro anti-inflammatory activity

The current study aimed to assess the *in-vitro* anti-inflammatory properties of the leaf extracts of *P. glabra* by examining its impact on the denaturation of egg albumin. The findings are succinctly presented in Table 1. The current results demonstrate that test samples effectively inhibit protein denaturation (specifically albumin) in a concentration-dependent manner, covering a range of 31.25 to 1,000 µg/mL. The reference drug utilized was diclofenac sodium, with a concentration range of 78.125 to 2,500 µg/mL. It showed suppression of protein denaturation that varied depending on the concentration. However, the effects of both extracts were shown to be slightly reduced compared to diclofenac sodium. This was additionally validated by comparing the IC50 values of the two. The IC50 value of methanolic extract and aqueous extract were 128.04 µg/mL and 95.96 µg/mL respectively, while the IC50 value of diclofenac sodium was 38.59 µg/mL. Denaturation of tissue proteins is an established factor that contributes to the development of inflammatory and arthritic disorders. The production of auto antigens in some arthritic disorders may result from the denaturation of proteins within the body (Opie, 1962; Umapathy *et al.*, 2010). The observed changes in absorbance of the test samples compared to the control samples suggest that extracts and the reference medication diclofenac sodium effectively prevented the denaturation of the protein albumin caused by heat (Jagtap *et al.*, 2011).

Table 1. Effect of leaves extract of *P. glabra* on protein denaturation

Concentration (µg/mL)	% Inhibition	
	Methanolic extract	Aqueous extract
31.25	16.208	14.679
62.5	20.795	30.428
125	39.144	53.67
250	106.42	129.36
500	221.1	282.26
1000	481.04	496.33

Conclusion

The study revealed that the leaf extracts of *P. glabra* demonstrate significant α-amylase inhibitory action, particularly in the crude methanolic extract. The leaves of *P. glabra* have the potential to be utilized into ayurvedic decoctions for the management and treatment of diabetes mellitus. From this study, it can also be inferred that *P. glabra* leaves extract has a significant anti-inflammatory impact, specifically against protein denaturation. Additional conclusive research is required to determine the specific mechanisms and components responsible for its antidiabetic and anti-inflammatory effects.

Author's contribution

M. M. Rahman executing, investigating and formal data analysis of the research, M. M. Rahman, Z. R. Moni and M. M. Rahman writing-review, editing the original manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding publication of this manuscript.

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