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Inhibition of α-amylase and α-glucosidase by raw and boiled garlic extracts for postprandial hyperglycemia management

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Abstract. *Alam MJ, Rahman NSS. 2025. Inhibition of α-amylase and α-glucosidase by raw and boiled garlic extracts for postprandial hyperglycemia management. Asian J Nat Prod Biochem 23: 122-130.* Diabetes Mellitus (DM) is a severe metabolic disorder that causes hyperglycemia due to impaired insulin function. Inhibition of carbohydrate-hydrolyzing enzymes, such as α-amylase and α-glucosidase, can reduce postprandial blood glucose levels. This study investigated the ability of garlic extracts (*Allium sativum*) to inhibit these important enzymes in Type 2 Diabetes Mellitus (T2DM). Raw and boiled Deshi and Indian garlic extracts were prepared and assessed for their ability to inhibit α-amylase and α-glucosidase activities using standard in vitro enzyme inhibition assays. The types of enzyme inhibition were analyzed through Lineweaver-Burk plots. Deshi raw garlic extract at 20 mg/mL inhibited the α-amylase enzyme by 52.49%, with an IC_{50} value of 17.62 mg/mL, while Deshi boiled garlic extract exhibited the lowest inhibition at the lowest concentration. Similarly, Deshi raw garlic extract showed the highest inhibition of α-glucosidase at the maximum concentration tested, whereas Indian boiled garlic extract demonstrated the lowest inhibition at the minimum tested concentration. Notably, raw and boiled garlic extracts inhibited α-amylase and α-glucosidase activities by 70% at concentrations comparable to acarbose, a commercial antidiabetic medication and the mode of inhibition for both enzymes by the both types of garlic extracts were evaluated as competitive inhibition. The findings demonstrate that both raw and boiled garlic extracts exhibit inhibitory action against α-glucosidase and α-amylase, indicating their potential for managing postprandial hyperglycemia. These results support the development of garlic-based nutraceuticals or dietary interventions for the management of T2DM.

Keywords: Acarbose, *Allium sativum*, antidiabetic activity, enzyme inhibition activity, nutraceuticals

INTRODUCTION

Traditional medicine has long turned to nature, with medicinal plants being central to the discovery and development of modern drugs. These plants are valued for their broad pharmacological benefits and low side effects. Historically, plant extracts have been essential in treating various diseases for their antidiabetic, antioxidant, antimalarial, antibacterial, antiviral, antifungal, antithrombogenic, and enzyme-inhibitory properties (e.g., amylase, lipase, invertase) (D'Britto et al. 2012).

Diabetes Mellitus (DM) is a metabolic disorder resulting from inadequate insulin production and/or secretion by the pancreas (Balamurugan et al. 2014; Zafar et al. 2022). Additionally, insulin resistance in body cells can lead to increased blood glucose levels (Khatun et al. 2015). According to the World Health Organization (WHO), over 347 million people worldwide have diabetes, a number projected to rise to 549 million by 2030 (Rowley et al. 2017; Thakre et al. 2017). Diabetes symptoms include weight loss, polyuria, polyphagia, polydipsia, fatigue, slow wound healing, and pruritus (Thakre et al. 2017). Chronic diabetes can cause severe complications like diabetic retinopathy, cardiovascular disease, stroke, poor circulation (resulting in amputations), and renal failure (Sathishkumar et al. 2015).

Carbohydrates are the primary source of energy for the body. Digestive enzymes including α-glucosidase, α-amylase, and invertase break down starch and other dietary carbohydrates into monosaccharides, primarily glucose (Behl and Kotwani 2017; Paul et al. 2021). This enzymatic process can cause rapid blood glucose spikes, complicating T2DM management. Inhibitors of α-glucosidase and α-amylase can help lower blood glucose after carbohydrate-rich meals, supporting glycemic control.

Current antidiabetic treatments include thiazolidinediones, insulin analogs, α-glucosidase inhibitors, sulfonylureas, and meglitinides (Gellad et al. 2013). Acarbose reduces both α-glucosidase and α-amylase activity, whereas voglibose and miglitol specifically reduce α-glucosidase activity. Despite their effectiveness in managing diabetes, these medications are associated with significant side effects (Thakre et al. 2017). About 80% of people with diabetes live in low- to middle-income countries (IDF 2015), where medication costs hinder effective management. As a result, researchers are exploring potent enzyme inhibitors from natural sources like bacteria, plants, and marine algae (Fatmawati et al. 2011; Konishi et al. 2012; Orhan et al. 2013; Panwar et al. 2015). While few plant extracts have shown activity against all the major digestive enzymes related to DM, the majority have shown inhibitory actions against either α-amylase or α-glucosidase (Debnath et al. 2020; Sani et al. 2020, 2024; Mahmud et al. 2023; Sarker et al. 2024). Traditionally known medicinal plants have been explored for their hypoglycemic potential, and the bioactive

compounds isolated from these plants appear highly promising (Rahmani 2015). Since α-amylase hydrolyzes complex carbohydrates into glucose—contributing to postprandial hyperglycemia—inhibiting this enzyme remains a key strategy in the development of improved antidiabetic drugs (Subramanian et al. 2008).

Garlic (*Allium sativum* L.), a widely cultivated member of the family *Alliaceae*, is commonly used as a culinary spice (Vazquez-Armenta et al. 2016). Traditionally, garlic has been used to treat ailments like diabetes, colds, and heart disease. Garlic oil extracts possess antidiabetic and antioxidant properties (Mnayer et al. 2014; Phan et al. 2019). Additionally, ethanolic extracts of *A. sativum* have demonstrated α-amylase inhibitory activity (Nickavar and Yousefian 2009). Raw garlic improves insulin sensitivity, and lowers blood sugar more effectively than cooked garlic. Raw or minimally process garlic significantly lowers fasting blood glucose and HbA1c levels in people with metabolic syndrome or type 2 diabetes (Zhao et al. 2024). Organosulfur content varies among garlic types, affecting their enzyme inhibition capacity (Lu et al. 2023; Wu et al. 2023).

Boiling garlic can alter its ability to inhibit carbohydrate-hydrolyzing enzymes (α-amylase and α-glucosidase), potentially impacting glycemic control. This study aimed to investigate how raw and boiled garlic, especially Deshi and Indian varieties, influence health benefits, and therapeutic potential, given their local prevalence and distinct bioactivity. However, in most comparison studies investigating enzyme inhibition have been conducted using *in vivo* models, particularly in diabetic rats. In contrast, the present study utilized an *in vitro* enzyme inhibition assay to more accurately assess the inhibitory potential of raw and boiled garlic (*A. sativum*) extracts on key digestive enzymes- α-glucosidase and α-amylase- associated with diabetes. This approach may offer a novel therapeutic strategy for diabetes management and also provides insight into the mode of enzyme inhibition.

MATERIALS AND METHODS

Collection and preparation of samples

This study utilized two types of garlic (*A. sativum*): Deshi and Indian varieties, both procured from local markets in Sylhet, Bangladesh. All bulbs were purchased in triplicate from different markets and only visually uniform, healthy, and undamaged bulbs were selected. Samples were processed under identical lab conditions immediately after purchase. Upon collection, the outer layers of the garlic bulbs were manually removed. The cloves were then separated and thoroughly cleaned with tap water, followed by two to three washes with distilled water to eliminate dirt and other impurities. The cleaned cloves were wiped with tissue paper to remove any residual water and allowed to air dry at room temperature. Consequently, the dried cloves were divided into two groups. One group was immersed in boiling water (100±1°C) for 10 minutes, while the other was set aside as raw. The boiled samples were then air-dried. Both raw and boiled samples from the Deshi and Indian varieties (*A. sativum*) were stored separately in four labeled containers for later use.

Chemicals/reagents

α-Glucosidase and α-amylase were purchased from The Merck Group, Darmstadt, Germany. Analytical-grade reagents, solvents, and chemicals were used in this investigation. Acarbose, a commercially available antidiabetic medication marketed under the brand name Sugatrol® (The Pacific Pharmaceuticals Ltd.), utilized as the experimental positive control.

Preparation of garlic extracts

Extracts from all samples (raw and boiled (Simmered in water at 100±1°C for 10 minutes), Deshi and Indian garlic) were prepared using a previously published method with minor modifications (Yibchok-Anun et al. 2006). Briefly, the samples were ground using a mortar and pestle with ice-cold acid-ethanol. After filtration, the homogenates were centrifuged at 8,000 rpm for 10 minutes. The pH of the resulting supernatants was adjusted to 3.0, followed by incubation at 4°C for 24 hours. After incubation, the mixtures were centrifuged again at 6,000 rpm for 10 minutes. The resulting pellet was washed with 80% acetone and allowed to air dry. Ice-cold acid-ethanol was chosen to facilitate the extraction of sulfur-containing compounds, while acetone was used for its ability to purify the extract by removing non-polar impurities. Finally, the air-dried pellet was dissolved in a 10 mM Tris–HCl buffer (pH 8.0). All extracts were stored at -20°C until further use.

α-Amylase inhibition assay

The α-amylase inhibition activity of the extracts was carried out by a previously reported method with slight modifications, employing 3,5-dinitrosalicylic acid (DNSA) (Poovitha and Parani 2016). Briefly, the extracts or acarbose at different doses (200-2000 µg/mL) were mixed with α-amylase (1 IU/mL) in test tubes. 20 mM sodium phosphate buffer (pH 6.9) was added in each reaction mixture, and the mixture was then pre-incubated for ten minutes at 25°C. Starch solution (as the substrate) was then added to initiate the reaction, followed by another 10-minute incubation at 25°C in a thermostatic water bath.

To stop the reaction, 3,5-dinitrosalicylic acid was added, and the mixture was placed in a thermostatic water bath at 100°C. After boiling, the solution was cooled to room temperature and diluted with distilled water at a 1:5 ratio. The inhibitory activity of α-amylase was determined by measuring the Optical Density (OD) at 540 nm using a spectrophotometer (Shimadzu, Japan). Following formula was used to figure out the percentage of inhibition of α-amylase.

Inhibition of α-amylase (%) = [(Enzyme Activity of Control - Enzyme Activity of Test) / Enzyme Activity of Control] x 100.

α-glucosidase inhibition assay

The extracts' α-glucosidase inhibition assay was carried out by a previously reported method with minor modifications, employing *p*-nitrophenyl-α-D-glucopyranoside

(pNPG) as the substrate (Poovitha and Parani 2016). Briefly, α-glucosidase (1 IU/mL) was mixed with the extracts or acarbose at various concentrations (200-2000 µg/mL) in test tubes and pre-incubated for 20 minutes at 37°C in a thermostatic water bath. Subsequently, 10 mM *p*-nitrophenyl-α-D-glucopyranoside was added to each tube, and the mixture was incubated in a thermostatic water bath again at 37°C for 30 minutes. The reaction was terminated by adding sodium carbonate, and the absorbance was measured at 405 nm using a spectrophotometer (Shimadzu, Japan). The formula below use used to calculate the percentage of α-glucosidase inhibition.

Inhibition of α-glucosidase (%) = [(Enzyme Activity of Control – Enzyme Activity of Test) / Enzyme Activity of Control] × 100.

Analysis of the mode of enzyme inhibition

The mechanism of α-amylase and α-glucosidase inhibition was analyzed following a previously reported method (Poovitha and Parani 2016). The enzyme solution (1 IU/mL) was incubated for 10 minutes at 25°C in a thermostatic water bath with either the acarbose (10 mg/mL), extracts (10 mg/mL), or phosphate buffer (pH 6.9) in order to perform the α-amylase assay. Reaction was initiated using the same procedure as described in 'α-amylase inhibition assay' section. The assay for α-glucosidase was conducted in a similar manner. Acarbose (10 mg/mL), extracts (10 mg/mL), or phosphate buffer (pH 6.9) were incubated in a thermostatic water bath with the enzyme solution (1 IU/mL) for 10 minutes at 37°C. Reaction was performed as described in the 'α-glucosidase inhibition assay' section. To determine the mode of inhibition, a double reciprocal plot (1/V vs. 1/[S]) was constructed using the reaction velocity (V) and substrate concentration ([S], ranging from 0.5-25 mh/mL). The type of enzyme inhibition was identified by analyzing the Lineweaver–Burk plot based on Michaelis–Menten kinetics.

Statistical analysis

All experiments were performed in triplicate (n=3) for each concentration to ensure both physiological consistency and statistical accuracy. Results are expressed as the mean ± Standard Deviation (SD), offering a reliable indication of central tendency and variation, thereby reinforcing the validity and reproducibility of the findings. Linear regression analysis, performed using Excel-generated plots, was used to calculate the IC_{50} values (the concentration required to inhibit 50% of enzyme activity) for both α-amylase and α-glucosidase.

RESULTS AND DISCUSSION

α-amylase inhibition activity of Deshi and Indian raw and boiled garlic extracts

This study evaluated the potential antidiabetic effects of Deshi and Indian garlic extracts by measuring their α-amylase inhibitory activity and comparing the results to those of the conventional antidiabetic drug, acarbose. To further understand the strength of α-amylase inhibition, IC_{50} values were also determined.

α-amylase inhibition by Deshi raw and boiled garlic extracts

The α-amylase inhibitory activity of raw and boiled Deshi garlic extracts was evaluated at various concentrations. Among the raw garlic extracts, the highest inhibition was observed at 20 mg/mL, with an inhibition rate of 52.49±3.17% and an IC_{50} value of 17.62 mg/mL (Figure 1, Table 1). The lowest inhibition was recorded at 2 mg/mL, showing 11.23±0.45% activity. For the boiled garlic extracts, the maximum inhibition was also observed at 20 mg/mL, yielding 29.36±0.987% inhibition in an IC_{50} value of 34.05 mg/mL. The lowest inhibition in this group was 5.54±1.34% at 2 mg/mL. Acarbose, used as a positive control, demonstrated the highest α-amylase inhibition (70.77±2.58%) at 20 mg/mL in an IC_{50} value of 9.03 mg/mL. The lowest inhibition by acarbose was 19.22±0.764% at 2 mg/mL (Figure 1, Table 1).

α-amylase inhibition by Indian raw and boiled garlic extracts

The α-amylase inhibitory potential of raw and boiled Indian garlic extracts was assessed across a range of concentrations. Among the raw garlic extracts, the highest inhibition was observed at 20 mg/mL, with an inhibition rate of 46.59±1.12% and an IC_{50} value of 22.00 mg/mL (Figure 2; Table 1). The lowest inhibition for the same extract was recorded at 2 mg/mL, showing 10.53±1.06% activity. In the case of boiled Indian garlic, the maximum inhibition was also found at 20 mg/mL, with a value of 31.55±0.89% and an IC_{50} of 32.96 mg/mL. The lowest inhibitory activity for this extract was 6.66±1.67% at 2 mg/mL (Figure 2; Table 1).

α-glucosidase inhibition activity of Deshi and Indian raw and boiled garlic extracts

The α-glucosidase inhibition activity of Deshi and Indian garlic extracts was evaluated and compared with that of acarbose. IC_{50} values for each extract were also determined to better understand their inhibitory effects.

α-glucosidase inhibition by raw and boiled Deshi garlic extracts

The percentage of α-glucosidase inhibition was measured using both raw and boiled Deshi garlic extracts. Among the raw garlic extracts, the highest inhibition (46.73±0.90%) was observed at 20 mg/mL, with an IC_{50} value of 21.77 mg/mL (Figure 3, Table 2). The lowest inhibition (8.81±3.54%) was seen at 2 mg/mL. For the boiled garlic extracts, the highest inhibition at 20 mg/mL was 24.45 mg/mL, corresponding to the IC_{50} value. As a positive control, acarbose was tested at the same concentrations. At 20 mg/mL, acarbose exhibited the highest α-glucosidase inhibition of 68.86±1.82%, with an IC_{50} value of 10.22 mg/mL.

The α-glucosidase inhibitory activity of raw and boiled Indian garlic extracts was evaluated at various concentrations. The raw garlic extract at 20 mg/mL exhibited the highest inhibition of 42.15%, with an IC_{50} value of 25.70 mg/mL (Figure 4, Table 2). The lowest

inhibition for the raw extract was 9.77% at 2 mg/mL. Among the boiled garlic extracts, the maximum inhibition of 37.51% was also observed at 20 mg/mL, with IC_{50} value of 26.21 mg/mL. The lowest inhibitory activity in this group was 4.67% at 2 mg/mL (Figure 4, Table 2).

Enzyme kinetics and mode of inhibition

To determine the type of enzyme inhibition exerted by the garlic extracts, enzyme kinetics studies were conducted using Lineweaver–Burk plots. The effects of both raw and boiled Deshi and Indian garlic extracts on α-amylase and α-glucosidase activities were analyzed and compared with the control.

Mode of α-amylase inhibition by raw and boiled Deshi garlic extracts

Using the Lineweaver–Burk plot, the K_m values were determined for the raw and boiled Deshi garlic extracts as well as for the control (phosphate buffer). While V_{max} remained constant, the K_m values for the buffer and raw garlic extract were 19.43 and 34.97, respectively (Figure 5). Similarly, V_{max} remained unchanged for both samples, while the K_m values for the buffer and boiled garlic extract were 19.43 and 27.04, respectively. This increase in K_m with no change in V_{max} indicates that both raw and boiled Deshi garlic extracts inhibit α-amylase through a competitive inhibition mechanism.

Mode of inhibition of α-amylase assessed by raw and boiled Indian garlic extract

The K_m values for raw and boiled Indian garlic extracts, along with the control (phosphate buffer), were determined using Lineweaver–Burk plots. While the V_{max} values remained constant across all samples, the K_m values for the buffer and raw Indian garlic extract were 31.86 and 19.43, respectively (Figure 6). In a separate experiment, V_{max} again remained unchanged, while the K_m values for the buffer and boiled Indian garlic extract were 19.43 and 29.29, respectively. The observed increase in K_m with a constant V_{max} indicates that both raw and boiled Indian garlic extracts act as competitive inhibitors of α-amylase.

Mode of inhibition of α-glucosidase assessed by Deshi raw and boiled garlic extract

The K_m values for both raw and boiled Deshi garlic extracts, along with the control (phosphate buffer), were determined using Lineweaver–Burk plots. For the buffer and raw Deshi garlic extract, the K_m values were 16.92 and 30.06, respectively, while V_{max} remained constant for both (Figure 7). Similarly, for the buffer and boiled Indian garlic extract, the K_m values were 25.75 and 16.92, respectively, with V_{max} again remaining unchanged. The increase in K_m coupled with an unchanged V_{max} in the presence of both raw and boiled Indian garlic extracts indicates that the inhibition follows a competitive mechanism.

Table 1. IC_{50} values of α-amylase inhibition activity under the treatment of different varieties of garlic extracts. Among the extracts, Deshi raw garlic extract demonstrated the highest efficacy, reaching about half the activity of the positive control, acarbose

Type of extracts	IC_{50} (mg/mL)
Deshi Raw Garlic	17.62
Deshi Boiled garlic	34.05
Indian Raw garlic	22.0004
Indian Boiled Garlic	32.96
Acarbose	9.03

Table 2. IC_{50} values of α-glucosidase inhibition activity under the treatment of different varieties of garlic extracts. All the extracts demonstrated inhibition levels ranging from approximately half to two-thirds of that shown by the standard inhibitor, acarbose

Type of extracts	IC_{50} (mg/mL)
Deshi Raw Garlic	21.76
Deshi Boiled garlic	24.45
Indian Raw garlic	25.70
Indian Boiled Garlic	26.21
Acarbose	10.22

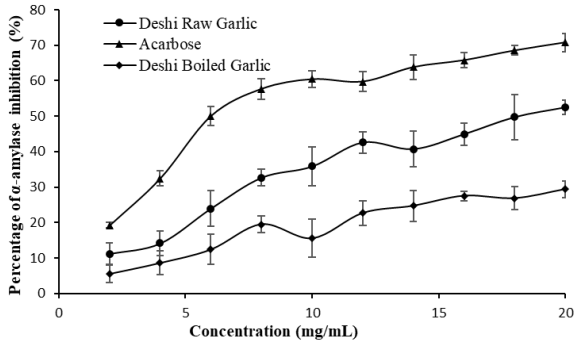

Figure 1. α-Amylase inhibition by Deshi raw and boiled garlic extracts. The extract concentrations ranged from 2-20 mg/mL. The inhibition of α-amylase by Deshi raw garlic extract was greater than that of the Deshi boiled garlic extract and approximately two-thirds that of the positive control, acarbose

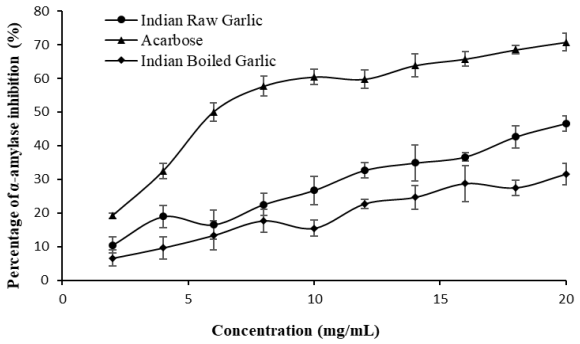

Figure 2. α-Amylase inhibition by Indian raw and boiled garlic extracts. The experimental concentrations of the extracts ranged from 2 to 20 mg/mL. The inhibition occurred in a concentration-dependent manner. At a concentration of 20 mg/mL, Indian raw garlic extract inhibited α-amylase more effectively than the Indian boiled garlic extract, with inhibition reaching approximately two-thirds of that observed with the positive control, acarbose

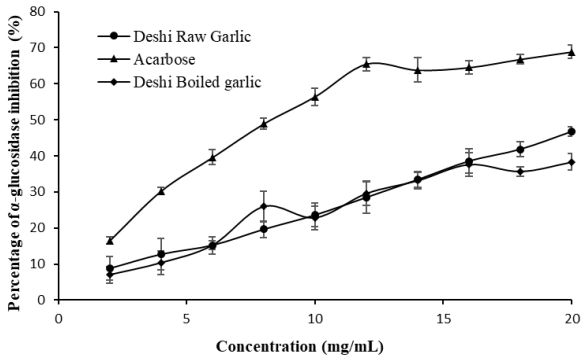

Figure 3. Concentration-dependent α-glucosidase inhibition by Deshi raw and boiled garlic extracts. Both Deshi raw and boiled garlic extracts exhibited comparable levels of α-glucosidase inhibition, whereas the positive control, acarbose, demonstrated nearly twice the inhibitory activity.α-glucosidase inhibition by raw and boiled Indian garlic extracts

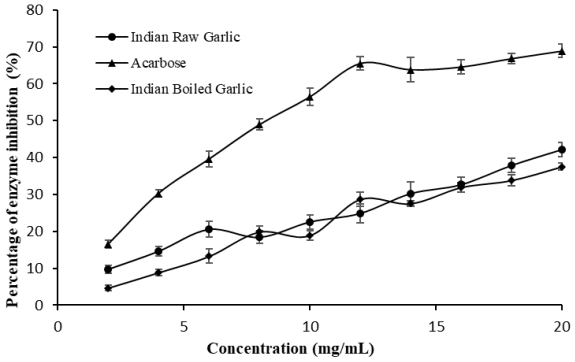

Figure 4. Concentration-dependent α-glucosidase inhibition by Indian raw and boiled garlic extracts. Indian raw and boiled garlic extracts exhibited comparable α-glucosidase inhibitory effects, achieving approximately two-thirds of the inhibition observed with the standard reference inhibitor, acarbose

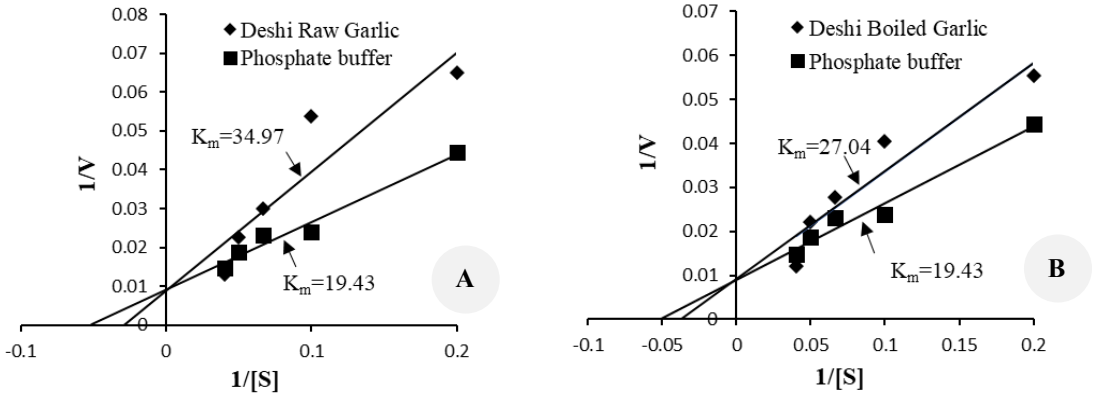

Figure 5. Lineweaver–Burk plot for the analysis of mode of α-amylase inhibition by A. Deshi raw and B. Deshi boiled garlic extracts. At a constant substrate concentration ([S]), the observed increase in K_m values of α-amylase in the presence of both Deshi raw and boiled garlic extracts suggests a pattern consistent with competitive inhibition. This indicates that compounds within the extracts may be competing with the substrate for binding to the enzyme's active site

Mode of inhibition of α-glucosidase assessed by Indian raw and boiled garlic extract

Using Lineweaver–Burk plots, the K_m values for both Indian raw and boiled garlic extracts, as well as the control (phosphate buffer), were determined. The K_m values for the Deshi raw garlic extract and buffer solution were 28.75 and 16.92, respectively, while V_{max} remained constant (Figure 8). Similarly, the K_m values for the Indian boiled garlic extract and buffer solution were 23.12 and 16.92, respectively, with V_{max} also unchanged for both samples. Since V_{max} remained constant and K_m increased in the presence of both Indian raw and boiled garlic extracts, the inhibition was classified as competitive in both cases.

Discussion

DM is a major global health issue, with its prevalence rising steadily in both developed and developing nations. In Bangladesh alone, around 10 million people are currently living with either type I or type II diabetes. DM is a long-term metabolic disorder that affects carbohydrate metabolism. One effective way to manage this condition, particularly type II diabetes, is through the use of glycosidic enzyme inhibitors. These compounds inhibit key digestive enzymes such as α-amylase, α-glucosidase, and invertase, which play important roles in carbohydrate breakdown (Kim et al. 2004). As oral hypoglycemic agents, these inhibitors help regulate blood sugar levels by slowing the digestion and absorption of carbohydrates (Iwakura et al. 2003). This results in delayed glucose uptake and a lower rise in blood glucose after meals (Iwakura et al. 2003). α-Amylase initiates the breakdown of starch into smaller sugar units by hydrolyzing complex polysaccharides into oligosaccharides and disaccharides. These are subsequently broken down by α-glucosidase into monosaccharides. The resulting simple sugars are then absorbed through the small intestine into the hepatic portal vein, contributing to increased postprandial blood glucose levels (Teng and Chen 2017).

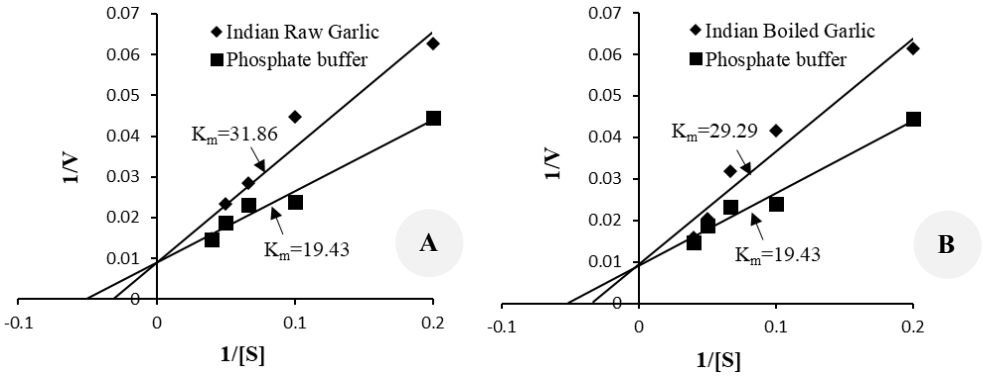

Figure 6. Lineweaver–Burk plot for the analysis of mode of α-amylase inhibition by A. Indian raw and B. Indian boiled garlic extracts. At a constant substrate concentration ([S]), the observed increase in the K_m values of α-amylase in the presence of both raw and boiled garlic extracts indicates a pattern consistent with competitive inhibition. This suggests that constituents in the extracts may be interfering with substrate binding by competing for the enzyme's active site

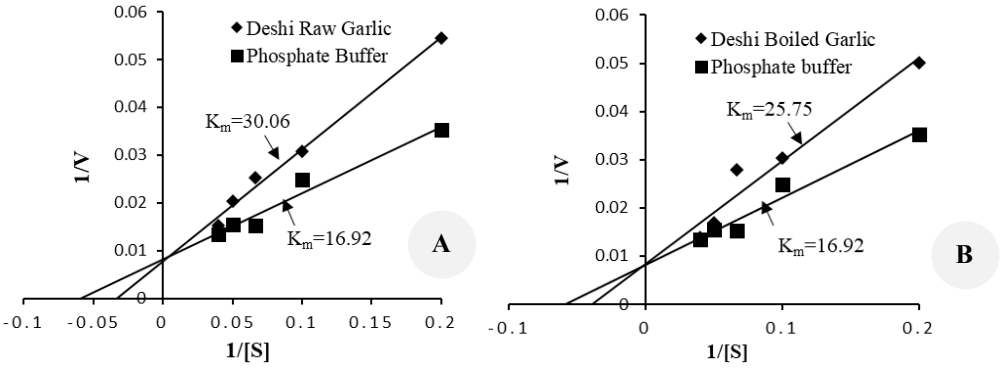

Figure 7. Lineweaver–Burk plot for the analysis of mode of α-glucosidase inhibition by A. Deshi raw and B. Deshi boiled garlic extracts. The elevated K_m values of α-glucosidase observed in the presence of both Deshi raw and boiled garlic extracts, under constant substrate concentration ([S]), indicate a decreased affinity between the enzyme and its substrate—an effect characteristic of competitive inhibition. This suggests that bioactive compounds in the garlic extracts are likely competing with the substrate for binding at the enzyme's active site

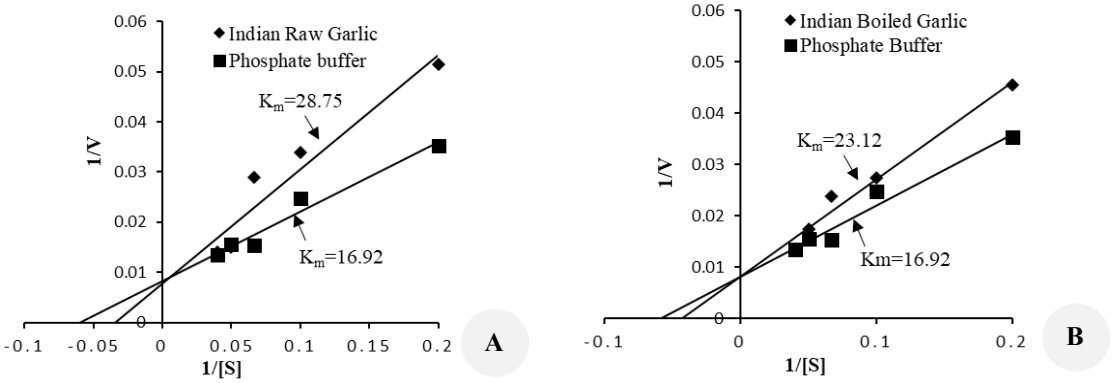

Figure 8. Lineweaver–Burk plot for the analysis of mode of α-glucosidase inhibition by A. Indian raw and B. Indian boiled garlic extracts. The plots were constructed by assessing enzyme activity across a range of substrate concentrations ([S]) while varying the concentrations of garlic extracts. In both cases, the Lineweaver–Burk analysis revealed an increase in the apparent Michaelis constant (K_m) at a constant substrate concentration, which is characteristic of competitive inhibition. This suggests that bioactive compounds present in both Indian raw and boiled garlic extracts compete with the substrate for binding at the active site of the α-glucosidase enzyme

Synthetic medications such as sulfonylureas, α-glucosidase inhibitors, insulin analogues, meglitinides, and thiazolidinediones are commonly used to manage diabetes. However, prolonged use of these drugs may lead to adverse effects, including liver dysfunction, diarrhea, skin irritation or itching, weight gain, fatigue or dizziness, increased risk of anemia, swelling in the legs or ankles, and even potential cancer risks. Additionally, the high cost of these medications presents a significant challenge, especially in low- and middle-income countries, which bear more than 80% of the global diabetes burden. As an alternative, plant-based enzyme inhibitors have attracted growing interest due to their affordability, fewer side effects, and promising antidiabetic properties. More than 400 plant species have been identified globally for their ability to lower blood glucose levels. Among them, garlic (*A. sativum*) stands out for its bioactive compounds with known hypoglycemic effects. Using medicinal plant extracts to inhibit enzymes like α-amylase and α-glucosidase offers a potential natural strategy for managing diabetes while minimizing the side effects associated with synthetic drugs (Kim et al. 2004).

Acarbose, miglitol, and voglibose are enzyme inhibitors currently used in the management of DM. Acarbose functions by inhibiting both α-amylase and α-glucosidase, whereas miglitol and voglibose selectively inhibit α-glucosidase only (Yibchok-Anun et al. 2006). Although these inhibitors have demonstrated effectiveness in controlling postprandial blood glucose levels, their use is often limited due to gastrointestinal side effects, making them less suitable for long-term therapy. Additionally, the high cost of these drugs poses a financial challenge—particularly in low- and middle-income countries, where approximately 80% of the global diabetic population resides. As a result, significant research efforts have been directed toward identifying alternative α-amylase and α-glucosidase inhibitors from natural sources such as plants, bacteria, marine algae, and fungi.

In this study, the antidiabetic potential of two garlic (*A. sativum*) varieties—Deshi and Indian—was evaluated in both raw and boiled forms through α-amylase and α-glucosidase inhibition assays, along with enzyme kinetic analyses. Previous research has demonstrated that garlic extracts possess significant antidiabetic effects, particularly in diabetic rat models (Mahmoud and Abdalla 2011; Masjedi et al. 2013; Jiang et al. 2024). Our results showed that the raw Deshi garlic extract exhibited the highest α-amylase inhibition, achieving 52.49% inhibition at a concentration of 20 mg/mL, with an IC_{50} value of 17.62 mg/mL. This inhibitory effect was comparable to that of acarbose, a standard antidiabetic drug, which showed 70.77% inhibition with an IC_{50} of 9.03 mg/mL at the same concentration. On the other hand, the boiled Deshi garlic extract demonstrated the lowest inhibition, with only 5.54% inhibition at 2 mg/mL. A similar trend was observed in the α-glucosidase inhibition assay, where raw garlic extracts consistently outperformed their boiled counterparts.

Previous studies have reported that aqueous garlic extracts possess significant in vitro inhibitory activity against α-amylase and α-glucosidase (Teng and Chen 2017). In our study, we utilized acetone extracts of both raw and boiled garlic varieties to assess their antidiabetic potential. Consistent with the findings from the α-amylase inhibition assay, the raw Deshi garlic extract exhibited the highest α-glucosidase inhibition, achieving 46.73% inhibition at 20 mg/mL, with an IC_{50} value of 21.77 mg/mL.

While the Deshi boiled garlic extract showed the lowest inhibition against α-amylase in our earlier results, a different pattern emerged in the α-glucosidase assay. Here, the Indian boiled garlic extract recorded the lowest inhibition, with only 4.67% inhibition at 2 mg/mL and an IC_{50} value of 26.21 mg/mL. Overall, raw Deshi garlic extracts demonstrated the strongest inhibitory activity against both α-amylase and α-glucosidase, highlighting their potential as a natural antidiabetic agent.

Although varying concentrations of different garlic extracts exhibited differing degrees of enzyme inhibition, acarbose—used as the positive control in this study—showed consistent inhibitory activity against both enzymes. Specifically, acarbose demonstrated 70.77% inhibition of α-amylase and 68.86% inhibition of α-glucosidase. These results highlight the effectiveness of acarbose as a reference inhibitor. However, the garlic extracts showed around two-third inhibition of α-amylase and α-glucosidase in comparison to positive control, acarbose, underscores their potential as natural alternatives or complementary agents to conventional antidiabetic drugs. To gain further insight into the mechanism of enzyme inhibition, Lineweaver-Burk plots were analyzed. Both raw and boiled garlic extracts were tested as potential inhibitors, using 20 mM phosphate buffer as the control. The resulting Lineweaver-Burk plots were used to determine the mode of inhibition, distinguishing between competitive and non-competitive inhibition patterns.

The mode of α-amylase inhibition by different garlic extracts (Deshi and Indian, in both raw and boiled forms) was determined through Lineweaver-Burk plot analysis. The K_m values for Deshi raw, Deshi boiled, Indian raw, and Indian boiled garlic extracts were found to be 34.97, 27.04, 31.86, and 29.29, respectively. In comparison, the control (20 mM sodium phosphate buffer) showed a K_m value of 19.43. Similarly, the inhibitory mode of α-glucosidase was assessed using Lineweaver-Burk plots. The K_m values for Deshi raw, Deshi boiled, Indian raw, and Indian boiled garlic extracts were 30.06, 25.75, 28.75, and 23.12, respectively, while the control exhibited a K_m value of 16.92.

In both enzyme assays, the presence of garlic extracts led to an increase in K_m values without significant changes in V_{max}, indicating a competitive mode of inhibition for both α-amylase and α-glucosidase. In both cases, the inhibition type was initially assessed as "competitive inhibition." However, further experiments involving multiple substrate concentrations and varying inhibitor levels are needed to definitively confirm the nature of the inhibition.

Raw garlic's inhibitory effects on carbohydrate-digesting enzymes are likely mediated by sulfur-containing compounds such as allicin and its derivatives, which may

interact with thiol groups in enzyme active sites, disrupting substrate binding (Wu et al. 2023). A study demonstrated that melanoidins from black garlic non-competitively inhibit α-amylase and α-glucosidase, with stronger activity linked to fluorescent groups (Wu et al. 2023). In silico docking has shown compounds like methionol and caffeic acid to have higher binding affinity to α-glucosidase than standard drugs like miglitol, indicating potential competitive inhibition (Sadeghi et al. 2021). Fermentation has also been shown to enhance garlic's inhibitory activity, with variability observed based on garlic origin (Ye et al. 2023). Additionally, thermal processing alters garlic's biochemical profile by deactivating alliinase and reducing allicin content, though other bioactive sulfur or melanoidin compounds may form (Lu et al. 2023). Reviews highlight that garlic's pharmacological properties are highly dependent on variety, cultivation, and processing methods, which influence the composition and activity of its sulfur compounds (Batiha et al. 2020). While raw or standardized garlic extracts may serve as adjuncts in managing postprandial hyperglycemia in T2DM, significant limitations remain—particularly in translating in vitro effects to in vivo outcomes due to variability in bioavailability, metabolism, and efficacy (Batiha et al. 2020; Sadeghi et al. 2021; Lu et al. 2023).

These findings suggest that garlic extracts, particularly in their raw form, exhibit significant inhibitory activity against key digestive enzymes, α-amylase and α-glucosidase, which play crucial roles in carbohydrate metabolism. The observed dual inhibition of both enzymes highlights the potential of garlic extracts to regulate blood sugar by slowing carbohydrate digestion and glucose absorption. This combined effect supports garlic's therapeutic potential in managing diabetes and controlling postprandial blood glucose spikes. However, in this study, we were unable to identify the specific active compound(s) in garlic responsible for competing with the enzyme substrates at the active site. Further investigation is needed to characterize these inhibitory components. This underscores the potential of garlic extracts as natural therapeutic agents for managing postprandial hyperglycemia, particularly in individuals with Type 2 Diabetes Mellitus (T2DM).

In conclusion, the findings of this study demonstrate that both Deshi and Indian garlic extracts, especially in their raw forms, exhibit significant in vitro inhibitory activity against α-amylase and α-glucosidase—key enzymes involved in carbohydrate digestion. These inhibitory effects, identified as competitive in nature, highlight garlic's potential as a functional food or nutraceutical for managing postprandial hyperglycemia and T2DM. These findings are based on observed data and require further experimental validation, such as molecular docking and/or compositional profiling, to confirm the underlying mechanism, while future investigations should aim to isolate and characterize the active bioactive compounds, assess their in vivo efficacy, and develop optimized formulations for potential clinical applications.

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