Alpha amylase and alpha glucosidase inhibitory potentials of sinigrin: An in vitro and in silico approach

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Abstract

Type 2 diabetes mellitus (T2DM) is commonly characterized by postprandial hyperglycemia. One effective preventive treatment for hyperglycemia is the use of α-amylase and α-glucosidase inhibitors, which slow down the last steps of the breakdown of carbohydrates and so prevent glucose from entering the bloodstream. The use of synthetic α-amylase and α-glucosidase inhibitors have shown various undesirable effects. Hence, natural glucosidase inhibitors from plants have become more prominent for the treatment of diabetes because of their less side effects and effectiveness. Glucosinolates are one among the secondary metabolites which pos Among the glucosinolates, sinigrin is one which is well known for its anti-inflammatory, antibacterial, antifungal, anti-tumour, antioxidant, and wound-healing properties. Having known the pharmacological significance of sinigrin, the present study is aimed to evaluate the alpha amylase and alpha glucosidase inhibitory potential of sinigrin. The percentage inhibitory activity of sinigrin against alpha amylase and alpha glucosidase increased in a dose-dependent manner. The inhibition of α- amylase by sinigrin was found to be 9.5 % (20µM) 19.3% (40µM), 39 % 41 % (60µM), 62% (80µM), and 88% (100µM). The inhibition of α-glucosidase inhibitory potential was of sinigrin was 7.3 % (20µM) 16.9% (40µM), 33 % (60µM), 64% (80µM), and 83% (100µM) .The molecular docking studies of alpha-amylase and alpha-glucosidase with Sinigrin revealed their binding interactions and inhibitory potential. Sinigrin has a binding energy of -6.05 kcal/mol to alpha-amylase, indicating a moderate affinity. The key amino acid residues involved in stabilisation were Ser4, Asn5, Arg252, Tyr2, Asp402, Gln8, and Phe335, which contributed through normal hydrogen bonding, Pi-Sulfur, and Pi-Alkyl interactions. These associations indicate that sinigrin may interact with alpha-amylase, perhaps altering its enzymatic activity. The results on in vitro and in silico studies strengthen the antidiabetic nature of sinigrin.

Key words: *Sinigrin; Alpha amylase; Alpha glucosidase; In silico studies; Antidiabetic nature*

INTRODUCTION

Type 2 diabetes is the most common form of diabetes, accounting for approximately 90–95% of all cases [1]. It arises from a combination of insulin resistance and inadequate insulin secretion [2]. Insulin resistance in target tissues is evident through reduced glucose uptake by skeletal muscle cells and increased glucose production by the liver [3]. Treatment typically involves insulin injections for individuals with type 1 diabetes, while type 2 diabetes is generally managed through oral medications combined with lifestyle modifications [4]. Non-insulin therapeutic strategies aim to address the underlying pathophysiological mechanisms contributing to hyperglycemia [5]. These medications work by reducing glucose absorption, inhibiting hepatic gluconeogenesis, and preventing glucose reabsorption in the kidneys. Common examples include alpha-glucosidase inhibitors, metformin, and sodium-glucose co-transporter-2 (SGLT-2) inhibitors [6]. Nonetheless, despite their effectiveness in lowering blood glucose levels, these drugs are often associated with adverse side effects.

Alpha-glucosidase inhibitors are a non-invasive treatment option known for their relatively mild, short-lived, and dose-dependent gastrointestinal (GI) side effects, such as diarrhea, abdominal discomfort, and flatulence [7]. These drugs function by temporarily delaying carbohydrate absorption in the intestine, effectively reducing postprandial blood glucose spikes. This mechanism makes them particularly suitable for managing type 2 diabetes, which is closely associated with dietary patterns. Currently, only three alpha-glucosidase inhibitors—acarbose, miglitol, and voglibose are approved for clinical use, prompting increased research into the development of novel inhibitors with enhanced efficacy. In recent years, there has been a growing interest in harnessing natural products for therapeutic purposes, particularly in the prevention and management of type 2 diabetes. Historically, medicinal plants and traditional remedies have been widely used to treat a range of ailments, including diabetes.

Sinigrin, a prominent glucosinolate from the Brassicaceae family, is naturally present in the seeds of plants such as broccoli, Brussels sprouts, and black mustard (*Brassica nigra*). Notably, Indian mustard (*Brassica juncea*) has been reported to contain high concentrations of sinigrin. Glucosinolates in cruciferous plants of Brassicaceae family such as Brussels sprouts, broccoli, cabbage, and mustard seeds possess a such as antifungal, antibacterial, bioherbicidal, antioxidant, antimutagenic, anticancer, and anti-inflammatory properties [8-12] Traditionally, Indian mustard seeds and oil have been used for centuries in the treatment of various health conditions, including fever, inflammation, respiratory ailments (such as coughs and colds), joint pain, and for cranial cleansing Recent scientific evidence has sparked interest in further investigating sinigrin's therapeutic potential. However, due to the lack of comprehensive studies, the current research aims to evaluate the inhibitory effects of sinigrin on alpha-amylase and alpha-glucosidase enzymes.

MATERIALS AND METHODS

Evaluation of invitro anti diabetic potential

Inhibition of alpha amylase

100 µl of test samples (Sinigrin in concentration range of 20-100 µM) were collected. After adding 250 µl of α- amylase (1 mg/ml) in 0.2 M sodium phosphate buffer (pH 6.9), each tube was incubated for 20 minutes at 37°C. Each tube received 250µl of a 0.5% starch solution in 0.2 M sodium phosphate buffer (pH 6.9). The reaction mixtures were then incubated at 37°C for 15 minutes. The reaction was halted using 1.0 mL of 3,5 dinitrosalicylic acid. The test tubes were then immersed in a boiling water bath at 100°C for 10 minutes before cooling to room temperature. The reaction mixture was then diluted to 10 ml with distilled water, and absorbance was measured at 540 nm [13].

$$\% \text{ of alpha amylase inhibition} = \frac{\text{OD test} - \text{OD control}}{\text{OD test}} \times 100$$

Inhibition of alpha glucosidase

50 µl of 0.2 M sodium phosphate buffer (pH 6.8) and 50 µl of 0.1 U glucosidase were added to separate tubes. Incubate 50 µl of sample and standard (various concentrations) at 37°C for 5 minutes without mixing. Then 50 µl of p-nitrophenyl alpha-D-glucosidase was added, vortexed, and incubated at 37°C for 30 minutes. 50 µl sodium carbonate (0.1 M) was added. The absorbance was determined at 405 nm [14]

$$\% \text{ of alpha glucosidase inhibition} = \frac{\text{OD control} - \text{OD test}}{\text{OD control}} \times 100$$

***In silico* studies**

Protein preparation and optimization

The crystal structure of alpha amylase and alpha glucosidase from *Homo sapiens* were retrieved from RCSB protein data bank (http://www.rcsb.org/pdb). The Hydrogen atoms, solvation parameters and fragmental volumes to the protein were added and electronic charges were assigned to the protein atoms using kollman united atoms force field by using Auto Dock Tool (ADT) –2.0. 2.2.

Ligand preparation and optimisation:

Using Chemsketch Software the structure of the Sinigrin was drawn and 3-D structure were generated and optimized. The selected ligands were saved in MDL-MOL format which were subsequent converted using open babel molecular converter program and were saved in PDB format.

Molecular Docking and Visualization

The affinity of alpha amylase and alpha glucosidase against Sinigrin was determined using the AutoDock Tool (Morris et al. 1998). Many intermediary processes were completed, including the creation of pdbqt files for the protein and ligand, as well as grid preparation and grid mapping utilising grid boxes. The genetic algorithm was utilised as a search parameter. The output selected was Lamarckian GA. The protein-ligand interaction between alpha amylase and alpha glucosidase and Sinigrin was visualised with Biovia Discovery Studio Visualiser software

RESULTS AND DISCUSSION

Hyperglycemia linked to type 2 diabetes can be effectively managed by delaying, modulating, or inhibiting the activity of carbohydrate-hydrolyzing enzymes [15]. Among these enzymes, α-glucosidase plays a key role. It is located on the brush border of enterocytes in the jejunum and is responsible for catalyzing the hydrolysis of α-1,4 glycosidic bonds in unabsorbed oligosaccharides and disaccharides. This process converts them into monosaccharides—mainly glucose—which are then absorbed in the upper jejunum, contributing to elevated blood glucose levels. α-glucosidase inhibitors act by slowing down the digestion and absorption of dietary carbohydrates. They achieve this by interfering with the breakdown of linear and branched oligosaccharides—such as α-limit dextrins, maltose, and maltotriose—into glucose. Consequently, the amount of glucose entering the bloodstream is reduced, helping to control postprandial hyperglycemia (PPHG)

The adverse effects associated with α-glucosidase inhibitors are attributed to the fermentation of undigested carbohydrates by intestinal bacteria [16]. In addition to gastrointestinal discomfort, commonly used α-glucosidase inhibitors have also been linked to rare but concerning liver-related side effects [17]. Consequently, there has been a growing emphasis on the search for safer and more potent α-glucosidase inhibitors.

Figure 1 demonstrates sinigrin's alpha amylase inhibition potential, demonstrating that morin successfully inhibited the alpha amylase enzyme in vitro. The percentage inhibition of alpha amylase increased in a dose-dependent manner. Morin inhibited α-amylase by 9.5% at 20µM, 19.3% at 40µM, 39% at 60µM, 62% at 80µM, and 88% at 100µM.

Figure 1 α amylase inhibitory activity of sinigrin

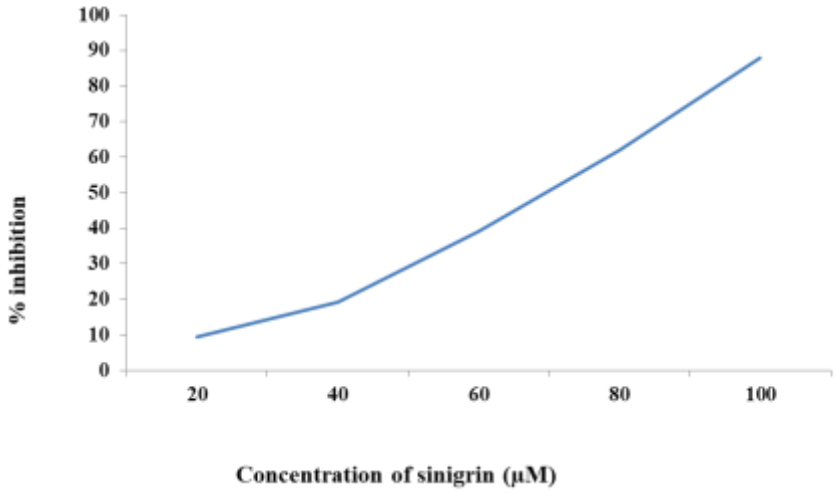
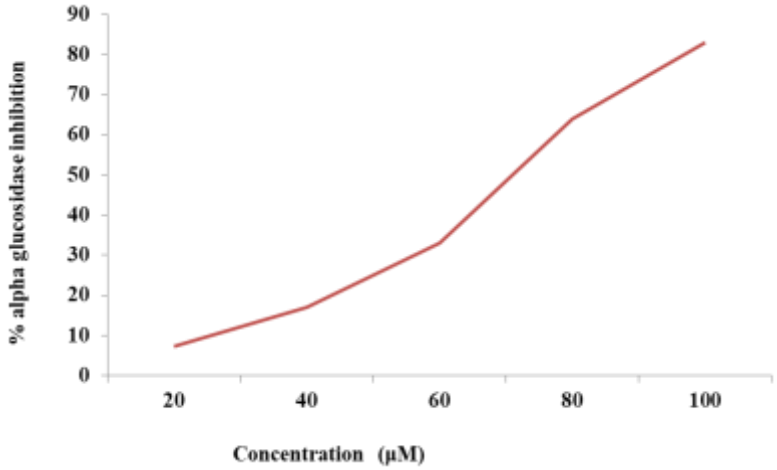


Figure 2 displays Sinigrin alpha glucose inhibitory potential, demonstrating that morin successfully inhibited the alpha glucosidase enzyme in a dose-dependent manner. Sinigrin inhibited α-glucosidase by 7.3% at 20µM, 16.9% at 40µM, 33% at 60µM, 64% at 80µM, and 83% at 100µM.

Figure 2: Alpha Glucosidase Inhibitory Activity of sinigrin



Diabetes mellitus is a metabolic disorder characterized by impaired insulin production or function, leading to persistent hyperglycemia. Effective management of elevated blood glucose levels remains a central focus in treating this condition. One proven therapeutic strategy involves the use of pharmaceutical agents that inhibit carbohydrate-metabolizing enzymes, thereby reducing postprandial hyperglycemia. We have recently reported for the antidiabetic effects of sinigrin in rat L6 myotubes [18] Sinigrin has been shown to inhibit both α-glucosidase and α-amylase enzymes, with a stronger inhibitory effect on α-glucosidase [19]. This enzymatic inhibition plays a key role in delaying the breakdown of starches and oligosaccharides, which in turn slows glucose absorption and helps mitigate postprandial spikes in blood sugar levels [20].

In silico studies

The primary goal of isolating natural products is to identify biologically active compounds with therapeutic potential. However, isolating secondary metabolites and evaluating their biological activities can be a time-consuming, labor-intensive, and costly process. This challenge can be mitigated through the use of *in silico* techniques. Among these, molecular docking stands out as a well-established and effective computational approach. It facilitates the elimination of inactive compounds by predicting how different optimized molecular conformations interact with a target protein. Additionally, molecular docking can provide insights into the potential mechanisms underlying the observed biological activities.

Protein structure retrieval

The crystal structures of human α-amylase (PDB ID: 3DHP) and α-glucosidase (PDB ID: 8EMR) were retrieved from the Protein Data Bank (PDB) to analyze their structural and functional characteristics. α-Amylase, a key enzyme in carbohydrate metabolism, features a $(\beta/\alpha)_8$-barrel catalytic domain along with three distinct subdomains—A, B, and C. Its structure reveals a conserved active site responsible for hydrolyzing α-1,4 glycosidic bonds in starch. The presence of chloride and calcium ions is essential for maintaining enzyme stability and enhancing catalytic efficiency. Similarly, the α-glucosidase structure provides critical insights into its substrate-binding pocket and catalytic mechanism, which facilitate the breakdown of complex carbohydrates into glucose. A detailed understanding of these structural features is vital for elucidating their roles in human metabolism and supports the rational design of pharmacological inhibitors for managing diabetes and other metabolic disorders.

Table 1

Targets	PDB ID	Resolution	Chain
Alpha amylase	3DHP	1.50 Å	A
Alpha glucosidase	8EMR	2.92 Å	A

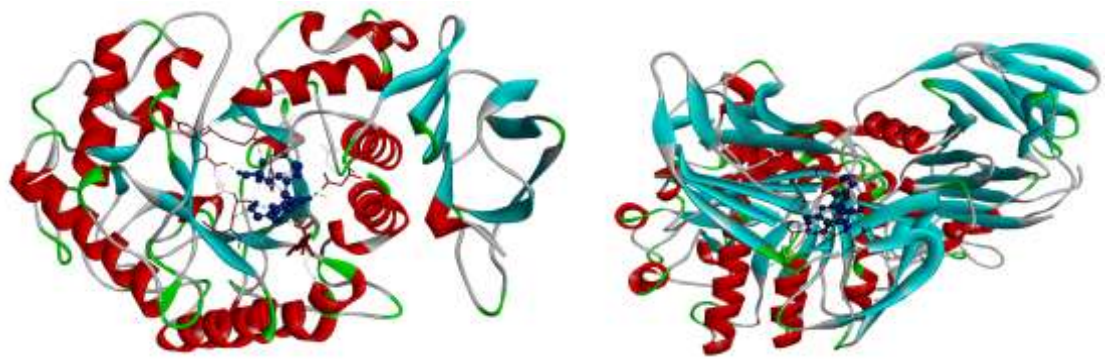


Figure 3

(a) 2D diagram between Alpha amylase with Sinigrin

(b) Alpha glucosidase structure visualized using Biovia Discovery Studio Visualizer

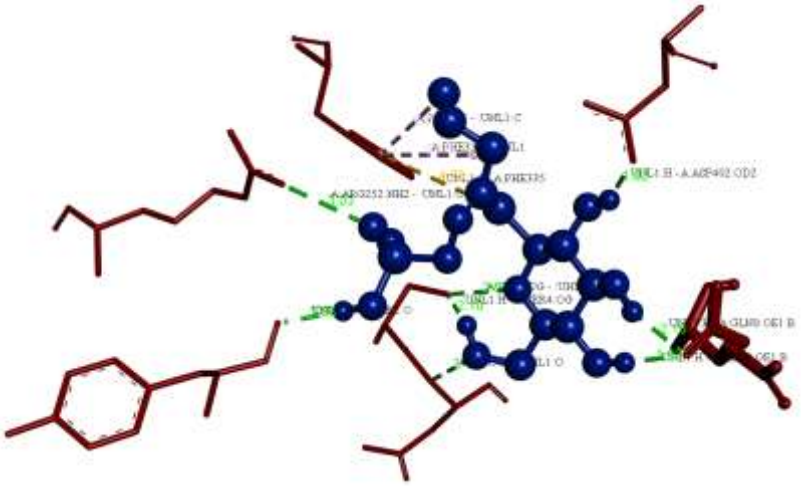


Figure 4 3D interactions: Alpha amylase (protein) –blue colour stick model; and Sinigrin ligand) red colour scaled ball and stick model; green dotted lines – Hydrogen bond interactions

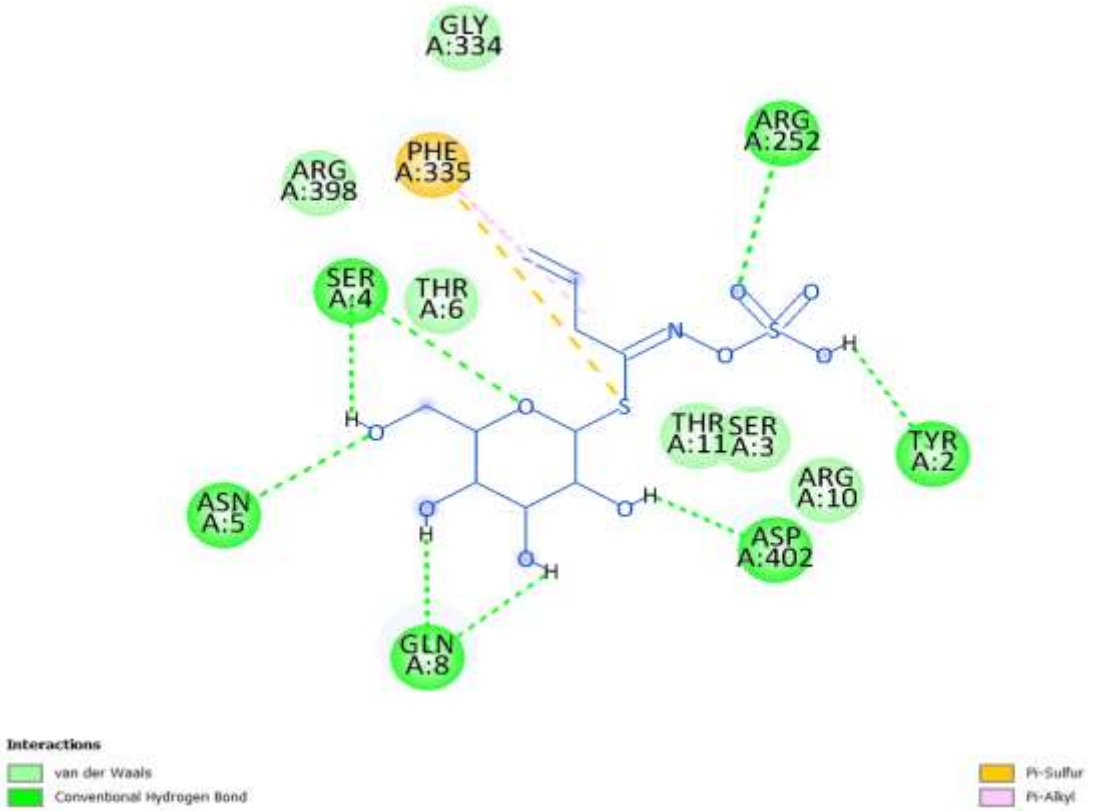


Figure 5: 2D Diagram: Ligand shown in red colour line model and Protein interactions are colored depending on their type: pi-alkyl interactions are colored in light purple. Pi-sigma bond are shown in dark purple color.

Table 2 Binding interactions of key aminoacid residues of alpha amylase with sinigrin

Donor – Acceptor Interaction	Distance (Å)	Category of Bond	Type
A:SER4:OG - :UNL1:O	2.94731	Hydrogen Bond	Conventional Hydrogen

			Bond
A:ASN5:N - :UNL1:O	3.09726	Hydrogen Bond	Conventional Hydrogen Bond
A:ARG252:NH2 - :UNL1:O	3.03329	Hydrogen Bond	Conventional Hydrogen Bond
:UNL1:H - A:TYR2:O	2.05434	Hydrogen Bond	Conventional Hydrogen Bond
:UNL1:H - A:ASP402:OD2	1.92467	Hydrogen Bond	Conventional Hydrogen Bond
:UNL1:H - A:GLN8:OE1:B	2.15977	Hydrogen Bond	Conventional Hydrogen Bond
:UNL1:H - A:SER4:OG	2.10415	Hydrogen Bond	Conventional Hydrogen Bond
:UNL1:H - A:GLN8:OE1:B	1.91392	Hydrogen Bond	Conventional Hydrogen Bond
:UNL1:S - A:PHE335	4.76344	Other	Pi-Sulfur
A:PHE335 - :UNL1	5.08042	Hydrophobic	Pi-Alkyl
A:PHE335 - :UNL1:C	4.28644	Hydrophobic	Pi-Alkyl

Alpha glucosidase

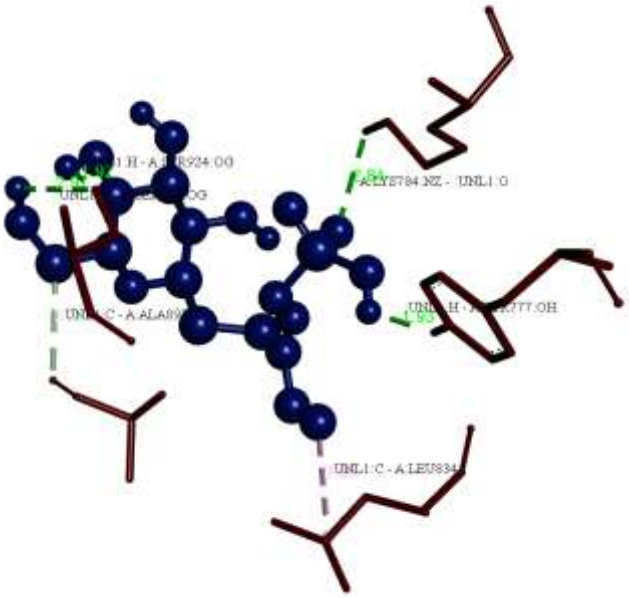


Figure 6: 3D interactions: Alpha glucosidase (protein) –blue colour stick model; and Sinigrin ligand) red colour scaled ball and stick model; green dotted lines – Hydrogen bond interactions

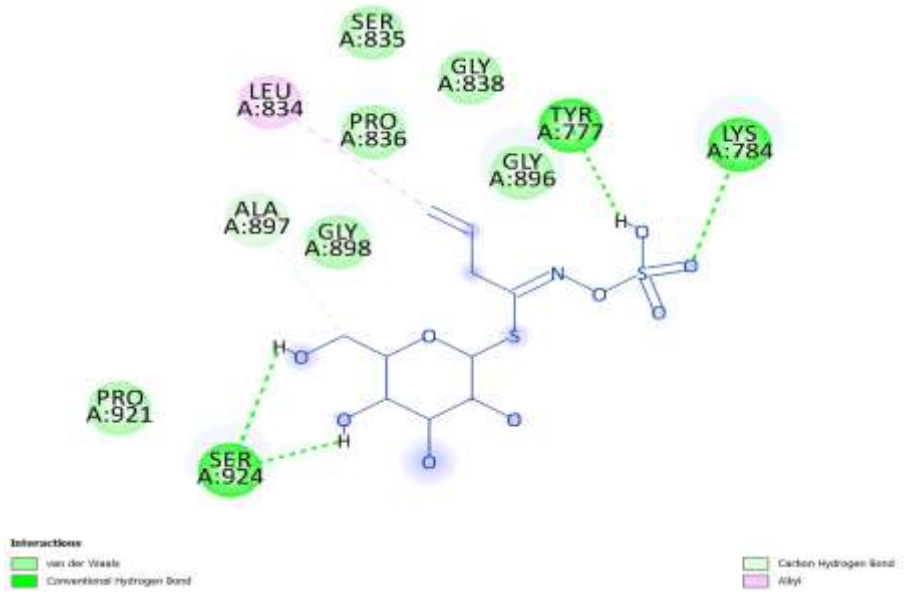


Figure 7: 2D Diagram: Ligand shown in red colour line model and Protein interactions are colored depending on their type: pi-alkyl interactions are colored in light purple. Pi-sigma bond are shown in dark purple color.

Table 3 Binding interactions of key aminoacid residues of alpha glucosidase with sinigrin

Donor – Acceptor Interaction	Distance (Å)	Category of Bond	Type
A:LYS784:NZ - :UNL1:O	2.84038	Hydrogen Bond	Conventional Hydrogen Bond
:UNL1:H - A:TYR777:OH	1.93319	Hydrogen Bond	Conventional Hydrogen Bond
:UNL1:H - A:SER924:OG	2.96579	Hydrogen Bond	Conventional Hydrogen Bond
:UNL1:H - A:SER924:OG	1.91831	Hydrogen Bond	Conventional Hydrogen Bond
:UNL1:C - A:ALA897:O	3.05989	Hydrogen Bond	Carbon Hydrogen Bond
:UNL1:C - A:LEU834	3.95805	Hydrophobic	Alkyl

Table 4 Sinigrin binding energy (kcal/mol)with different targets

Conformation	Binding Energy	Ligand efficiency	Inhibitory constant, K_i (μm)	Intermolecular energy	vdW + H bond + desolv Energy	Electrostatic energy	Torsional energy	Total internal Unbound
Alpha amylase	-6.05	-0.27	36.63	-9.63	-8.82	-0.81	3.58	-3.21
Alpha glucosidase	-2.32	.-0.11	19.97	-5.9	-4.64	-1.26	3.58	-4.69

The molecular docking analysis of alpha-amylase and alpha-glucosidase with Sinigrin provided insights into their binding interactions and inhibitory potential. Sinigrin exhibited a binding energy of -6.05 kcal/mol with alpha-amylase, indicating a moderate binding affinity. Key amino acid residues involved in stabilization included Ser4, Asn5, Arg252, Tyr2, Asp402, Gln8, and Phe335, contributing through conventional hydrogen bonding, Pi-Sulfur, and Pi-Alkyl interactions. These interactions suggest that Sinigrin may effectively interact with alpha-amylase, potentially influencing its enzymatic activity.

In the case of alpha-glucosidase, Sinigrin displayed a weaker binding energy of -2.32 kcal/mol, indicating a lower binding affinity. The key interacting residues included Lys784, Tyr777, Ser924, Ala897, and Leu834, forming hydrogen bonds, carbon hydrogen bonds, and hydrophobic alkyl interactions. The weaker binding suggests that Sinigrin has a comparatively lower inhibitory potential against alpha-glucosidase than alpha-amylase.

Overall, the docking results indicate that Sinigrin interacts more effectively with alpha-amylase than alpha-glucosidase, highlighting its potential as an amylase inhibitor. These findings provide valuable insights into Sinigrin's role in carbohydrate metabolism and its possible therapeutic applications in controlling postprandial glucose levels. Our results are in par with the study conducted by Abbas et al., 2017.

CONCLUSION

The current study highlights the significant anti-diabetic potential of sinigrin. Its ability to inhibit α-glucosidase and α-amylase may contribute to reduction in hyperglycemia. Docking studies demonstrated strong and stable binding affinity with both target proteins. Based on these findings, sinigrin may be used an effective drug for the treatment of hyperglycemia. However, further research is required to explore the molecular mechanisms involved in its inhibition of hyperglycemia.

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