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RESEARCH ARTICLE

Investigating Anti-Diabetic Potential of Magniferin via Alpha Amylase Inhibitory Activity; *In Silico* Studies and *In Vitro* Validation

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Abstract

Objectives: To determine the affinity of magniferin with alpha amylase enzyme plays a central role in diabetes and diabetes-driven complications. **Methods:** The data for magniferin (mol2) and alpha amylase (pdb) enzyme was retrieved from Pub Chem and the protein data Bank. Molecular docking studies were carried out using Auto Dock Vina. In silico findings were evaluated by alpha Amylase Inhibitory assay and alpha amylase mRNA expression profile. **Findings:** Binding energies (-50.9012 kcal/mol) of the docked structure showed significant affinity that clearly showed alpha amylase inhibitory properties of magniferin. The magniferin affinity with alpha amylase was due to interaction with amino acids (Asp, Glu, and Asp). Further, in vitro studies showed magniferin significantly inhibits alpha amylase enzyme activity (247.63 μg/ml). **Novelty:** The magniferin showed affinity with the amino acid residues present in the enzyme active site. The expression profile of alpha amylase gene was examined using Intestinal epithelial cells treated with magniferin with a control. These findings clearly showed the potential of magniferin as anti-diabetic activity via inhibiting alpha amylase.

Keywords: Magniferin; Natural Products; Docking; Affinity; Anti-Diabetic; Binding Energy and Gene Expression

1 Introduction

Diabetes is the most prevalent non-communicable lifestyle-related disorder worldwide. Diabetes mellitus is a severe metabolic condition with a rising frequency[(1)]. It is characterized by a relative or absolute lack of insulin production and/or activity, which leads to glucose intolerance and compromises the metabolism of lipids, carbohydrates, and proteins. World Health Organization estimates that nearly 300 million people worldwide will have diabetes by 2030[(2)]. Increased blood glucose level (hyperglycemia) with alterations in the metabolism of macromolecules leads to defects in insulin secretion, insulin action, or both characterize diabetes mellitus[(3)]. Alpha-glycosidase inhibitors are promising anti-diabetic medications that govern the sugar levels

that are ordinarily metabolized into monosaccharides before entering into the systemic circulation [2]. Calcium metallo-enzyme alpha amylase triggers catabolism by solubilizing larger sugars into smaller ones like glucose and maltose [4]. The enzyme also raises blood glucose levels and postprandial hyperglycemia. The most significant digestive enzyme for facilitating the breakdown of alpha-1, 4 glycosidic bonds in carbohydrates is alpha-amylase. To pass the gut epithelium, it breaks down the big starch molecules into smaller sugar fragments. Hyperglycemia, on the other hand, can occasionally result from high blood sugar levels brought on by excessive alpha-amylase activity [5]. Inhibiting alpha-amylase activity can thereby lower postprandial hyperglycemia and lower the chance of developing diabetes.

Existing anti-diabetic therapeutics face several limitations and are associated with the drug induced complications (prolong uses) such as gastrointestinal tract dysfunction and impaired renal functions. As an alternative several plant derived natural compounds/products that are an integral part of our diet possess tremendous potential in regulating blood sugar levels via modulating enzymes involved in carbohydrate metabolism [6]. Magniferin (1, 3, 6, 7-tetrahydroxyxanthone-C2-β-D-glucoside) is a bioactive ingredient predominantly isolated from the mango tree, with potent antioxidant activity and multi-factorial pharmacological effects, including anti-diabetic, antitumor, lipometabolism regulating, cardio-protective, anti-hyperuricemic, neuro-protective, antioxidant, anti-inflammatory, antipyretic, analgesic, antibacterial, antiviral and immune-modulatory properties [7]. As a result, it has a number of health-promoting qualities and offers a viable subject for further study and development. However, the development of mangiferin as a clinical therapy is constrained by its low solubility, mucosal permeability, and bioavailability, and alteration in molecules requires broadening its application [8]. Mangiferin has been shown to have a diverse physiological functions, including analgesic, anticancer, antibacterial, antiviral, immunomodulatory, anti-diabetic, anti-oxidative, anti-aging, and anti-tumor properties.

According to a recent study, mangiferin medications helped to produce streptozotocin (STZ) trigger study animals. Following oral administration of 40 mg/kg mangiferin for 30 days, biochemical, toxicological, and hematological parameters were assessed [5]. Magniferin has been shown to have a number of beneficial effects, including anti-inflammatory, anti-microbial, anti-diabetic, anti-allergic, neuroprotective, cardiovascular protective, anti-cancer, and hypocholesterolemic effects. Diabetes-prone mice treated with mangiferin had significantly lower blood sugar, glycosylated hemoglobin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) levels than diabetic control mice. According to BCS categorization, mangiferin is a class IV medication [1]. Low oral bioavailability due to declined affinity with water, low diffusion across the membrane, and intestine metabolic instability restricts its bio efficacy. Mangiferin's inadequate transmembrane absorption can be related to the C-glycosidic bond, with increase persistent than the O-glycosidic bond and difficult to hydrolyze. Despite the tremendous anti-diabetic potential of Mangiferin the clinical profile of the compound is yet to explore. The goal of the current work is to find a substitute medication that can control hyperglycemia by inhibiting alpha-amylase activity using a molecular docking technique. Further, alpha amylase inhibitory activity of magniferin was examined via in vitro studies.

2 Methodology

2.1 Chemical and Consumables

All the chemicals and consumables were purchased from Sigma Aldrich and Hi Media of molecular grades. The enzymes and primers for real-time PCR experimentation were purchased from Sigma Aldrich. The cell lines were purchased from the ATCC and maintained in the prescribed culture media and growth conditions. The molecular docking study was carried out using Auto Doc Vina version 1_1_2. Intestinal epithelial cells (IECs) were used for the mRNA expression under the treatment of magniferin.

2.2 Magniferin affinity with alpha amylase

Magniferin is widely distributed in the higher plant as key plant metabolites and possess several physiological activities. The role of magniferin in the diabetes management is not fully explored however it does possess regulatory activity on different carbohydrate metabolizing enzymes. In the present study, affinity of magniferin with alpha amylase is examined as alpha amylase is key enzyme in metabolizing the carbohydrates.

2.2.1 Ligand

For affinity search and docking study, magniferin structure, smiles and sdf file was retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/). Here, magniferin was refined for docking studies.

2.2.2 Receptor

Alpha amylase enzyme was used for affinity profiling with magniferin where alpha amylase pdb file was retrieved from protein data bank (https://www.rcsb.org/). From protein data bank pdf file for humans (Homo sapiens), magniferin pdb file (1B2Y) was further modified for docking studies.

2.3 Docking

Affinity between magniferin and human alpha amylase was examined via docking study using Auto Dock Vina version 1_1_2 (https://vina.scripps.edu/). Here, alpha amylase and magniferin were modified as per the docking manual. Further, docking simulations using the grid parameter file (Gpf) and a docking parameter file (Dpf) were prepared. For effective affinity, parameters were set using the Lamarckian genetic algorithm (LGA) for 12, 000 run. In the study, the MGL tool 1.5.1 utilized in a setting parameter of protein for docking study (http://mgltools.scripps.edu/). MGL tool 1.5. is composite software providing an interface for docking studies and visualization of protein molecules. Here, MGL tool offer docking interface for AutoDock tool (ADT) and Python Molecule Viewer (PMV).

2.4 Alpha Amylase Inhibitory Assay

Here, 3, 5-dinitrosalicylic acid (DNSA) technique was utilized to perform the alpha amylase inhibition experiment. Molecular grade magniferin was diluted in buffer ((Na_2HPO_4/NaH_2PO_4 (0.02 M), NaCl (0.006 M) at pH 6.9) with a minimum of 10% DMSO to provide concentrations between 10 to 1000 g/ml. The magniferin solution was combined with 200 μl of α amylase solution (2 units/ml), which was then allowed for 10 min at 30°C. Further, 200 μl starch solution (1% in water (w/v)), which was then incubated for 3 min was added in each test tube. Thereafter, 200 ml of the DNSA reagent (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) was poured into the reaction vial to terminate the reaction, and incubated for 10 minutes at 85–90°C in a water bath. Samples vials were diluted by adding 5ml of water and then allowed to reach room temperature. The samples were examined for absorbance at 540nm using a UV-Visible spectrophotometer. 200 μl of buffer was substituted for the magniferin solution to produce a blank with 100% enzyme activity. A blank reaction was created using each concentration in the absence of the enzyme solution. A positive control sample made from acarbose (100 g/ml) was utilized, and the reaction was conducted in the same manner as the reaction with plant extract that was previously described. The graph's IC50 values were computed by plotting the magniferin concentration with the degree of amylase inhibition.

The percentage of the amylase inhibitory activity that was inhibited was calculated using the following equation:

$$\%\ \alpha\ amlyase\ inhibition = 100\frac{Abs100\%\ control - Abs\ sample}{Abs100\%\ control}$$

2.5 Alpha amylase mRNA expression profile

Additionally, Intestinal epithelial cells (IECs) were used to investigate potential of magniferin on the expression of alpha amylase gene expression. The intestinal epithelial cells which were cultured in EMEM (Eagle's modified essential medium) at 37°C with 5% CO_2. The total RNA from Intestinal epithelial cells (IECs) was extracted using Trizol method. Using the microRNAs sequence (Assay Technology), distinct miRNA primers for each of the five miRNAs were designed and synthesized, and then each miRNA's unique annealing temperature was determined. Using an AMV reverse transcriptase kit that is commercially available from GCC Biotech, 500 ng of total RNA were reverse transcribed. Vials containing consumables for rtPCR mix were then incubated for 30 minutes at 16°C, 30 minutes at 42°C, 5 minutes at 85°C, and then for 30 minutes at 4°C. Before usage, the produced cDNA was kept at -20°C. Using the Sybr green matrix kit (Bio-Rad) and 20 ng cDNA per 20 μl reaction, real-time PCR was carried out individually for sera and cell lines with their respective normal controls. The Real-Time PCR cycles included 40 cycles of 95°C for 1 min. and 58^0C for 1 min., followed by pre-denaturation at 1 cycle of 95°C for 3 min. The reverse primerP1 (5'-CAGCAGTCCCATATTCTGGATGG-3') and the forward primer P2 (5'-GCCACATGTGCTTGGAAGCATCA-3') were derived from the common exons 3 and 4 of the alpha amylase gene (AMY] and cover a region of 231 bases and the intervening intron.

3 Results and Discussion

3.1 Profiling Magniferin affinity with alpha amylase

3.1.1 Affinity profiling; docking

The result showed in Table 1 and Figure 1 clearly demonstrates that magniferin possesses a significant affinity with the alpha amylase enzyme. As the results summarized in Table 3.1, four key docking poses with higher docking scores showed acceptable affinity where pose 1 showed the best match. The affinity search of magniferin with alpha amylase also provides interaction details (weak interactions) as mentioned in Table 2. The pose 1 of docked structure is shown in Figure 2 **a**; with grid and **b** without grid. The ligand has shown a smooth docking and higher affinity as most of its residues do interact with the enzyme i.e. alpha amylase. As the data showed in Table 2, ligand ionic interaction with the receptor, where O4 (ligand) showed affinity with the receptor (alpha amylase enzyme) with aspartic acid while in hydrophobic interaction C12, C16 showed affinity with receptor histidine and valine amino acids respectively.

Table 1. Docking profile of magniferin with alpha amylase enzyme (*Homo sapiens*)

Docking pose	Docking score	Binding Energy (kcal/mol)
Pose 1	-8.5	-50.9012
Pose 2	-8.4	-52.6520
Pose 3	-8.3	-53.5534
Pose 4	-8.0	-55.0021

Table 2. Affinity of magniferin with alpha amylase enzyme where ligand showed interaction at different residues

a. Ionic interaction	
Ligand atom	**Receptor**
O4	D300 (A) OD1
b. Hydrophobic Contact	
Ligand atom	**Receptor**
C12	H15 (A) CB
C16	V296 (A) CB
C12	R337 (A) CG
c. Cation-pi Interaction	
Ligand atom	**Receptor**
C6	R337 (A) NE
d. Hydrogen Bonds	
Ligand atom	**Receptor**
O2	H299 (A) ND1
O8	M339 (A) SD
O11	E385 (A) OE1
O5	E337 (A) NH1
O4	E195 (A) NH2
O3	Q41 (A) NE2
O2	H299 (A) N
O2	H299 (A) ND1
e. Weak Hydrogen Bond	
Ligand atom	**Receptor**
C2	H299 (ND1)
C15	R337 (A) O
C9	M339 (A) SD
O2	H15 (A) CD2

Table 3. α **amylase inhibitory activity at the different concentration of magniferin**

Concentration of magniferin (mg/ml)	α amylase inhibitory activity (μg/ml) IC_{50} magniferin	α amylase inhibitory activity (μg/ml) IC_{50} Phaseolamin
10	13.65 $\pm$1.54	19.12 $\pm$1.65
20	18.30 $\pm$2.12	32.65 $\pm$1.89
40	32.55 $\pm$2.14	32.55 $\pm$2.14
80	46.41 $\pm$1.54	59.24 $\pm$2.25
160	68.72 $\pm$1.68	87.36 $\pm$2.52
320	135. 21$\pm$1.98	184.35$\pm$1.64
640	176.24 $\pm$ 1.55	235.41 $\pm$ 2.11
1280	247.63 $\pm$2.20	344.41 $\pm$1.63

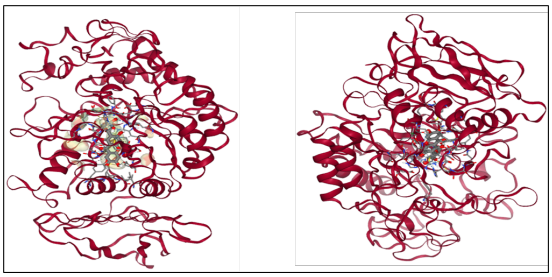

Fig 1. Molecular docking profile of magniferin with alpha amylase enzyme (Homo sapiens)

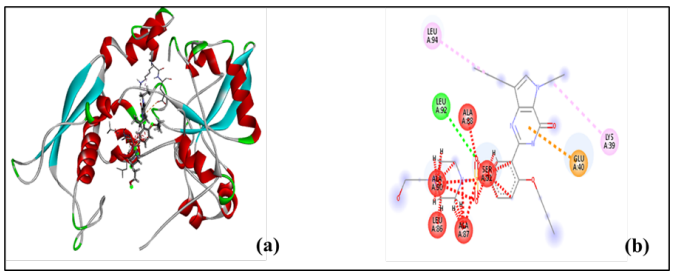

Fig 2. Amino acid interacting residues with themagniferin include Leu (86, 88, 92), Lys (39), Glu (40), Ala (87, 88) and Ser (90, 91)

3.1.2 Ligand receptor interaction

Additionally, C12 (ligand) also possesses affinity with the arginine amino acid of enzyme i.e. alpha amylase. It is important to note that aspartate and glutamate are key residues (aa) present in the catalytic triad of enzyme where these amino acids not only provide space for substrate but also fetch for effective catalysis. Involvement of these amino acids in ionic and hydrophobic interactions with magniferin results in a competitive inhibition for substrate and restricts the enzyme activity. Similarly, other interacting forces as summarized in Table 2 showed the involvement of hydrogen bonding and Cation pi interaction. Ligand C6 showed an affinity with arginine for cation-pi interaction. Additionally, O residues of ligand possess much affinity with different amino acids of alpha amylase. The data showed both carbon and oxygen atoms of magniferin possess affinity with histidine, arginine, and glutamine along with methionine. Kashtoh and Baek, 2023 demonstrated active site of alpha amylase [9]. The active site (substrate-binding) of the alfa amylase is positioned between the carboxyl terminus of the A and B domains. Calcium (Ca^2+) may serve as an allosteric activator and stabilize the three-dimensional structure between the A and B domains. According to the binding of analogs of the substrate, Asp206, Glu230, and Asp297 are involved in catalysis. The catalytic site is located at subsites 3 of the substrate-binding site's 5 subsites [10]. Substrate binding to the first glucose residue in subsites 1 or 2 can result in cleavage between the first and second or second and third glucose residues. In a study, Basnet et al., 2023 showed the association of active site amino acids in establishing affinity with substrate and different inhibitors where reported Asp, Glu, and Asp as key amino acids [11]. In the present study, magniferin showed an affinity with the alpha amylase and involvement of these three amino acids i.e. Asp, Glu, and Asp. In the present study, magniferin showed affinity alpha amylase with oxygen and carbon atoms at different

residues of amino acids.

3.2 In-vitro alpha amylase inhibitory activity

Magniferin has a wide range of physiological qualities, such as antioxidants, that are extremely beneficial for health. These features include antiviral, anticancer, anti-diabetic, anti-oxidative, anti-aging, immune-modulatory, hepato-protective, and analgesic benefits. The phenolic isomangiferin and homomangiferin, which make up 10% of all phenolics, are also found in the mango tree's leaves, mango skin, and twigs. The present investigation emphasizes alpha amylase inhibitory activity was determined in reference with Phaseolamin a well-tested/evaluated alpha amylase inhibitor. As the data showed in Table 3, magniferin showed a significant alpha amylase inhibitory activity in comparison with Phaseolamin where the highest inhibition was reported at 1000 mg/ml concentration of 247.63% (Figure 3). Phaseolamin reported 344.41% at the same concentration. The information also demonstrates a linear link between magniferin concentration and inhibitory alpha amylase activity. Earlier, Benayad et al., 2021 reported plant secondary metabolites to offer a potential inhibition of alpha amylase activity[12]. Similarly, Akshatha et al., 2022 investigated potential secondary metabolites possessing alpha amylase inhibitory activity[4]. There is a grown list of secondary metabolites derived from plant and microbial world that possess alpha amylase inhibitory activity. Actinoplanes utahensis, Streptomyces hygroscopicus-limoneus, Streptomyces calvus, Streptomyces dimorphogenes, have all been reported to produce Acarbos, Voglibose, Valienamine, Adiposin-1, Trestatin-B, and Lipostatin, respectively as potential alpha amylase inhibitory activity[4,13].

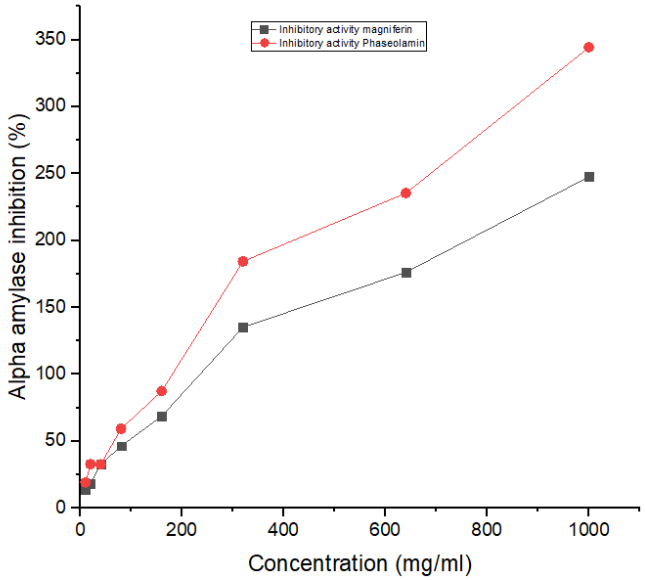

Fig 3. α amylase inhibitory activity at different concentration of magniferin

Magniferin and other foliamangiferosides have been demonstrated to have an anti-diabetic impact by improving insulin sensitivity and decreasing alpha-glucosidase activity. Additionally, it has been shown that magniferin has a pro-hypoglycemic effect by modifying the way that glucose is metabolized, decreasing insulin resistance, lowering cholesterol production, and suppressing the expression of inducible nitric oxide synthase and tumor necrosis factor. Mangiferin (10 and 20 mg/kg) showed anti-diabetic effects when given once daily for 28 days to STZ-induced diabetic rats. It dramatically decreased plasma levels of TG, low-density lipoprotein cholesterol (LDL-C), and total cholesterol while raising HDL-C and decreasing LDL-C levels[14]. Furthermore, magniferin enhanced oral glucose tolerance in normal rats given a glucose overload while the atherogenic index of diabetic rats dropped. Magniferin increased pancreatic cell mass, glucose and insulin absorption, and AMP-activated protein kinase (AMPK) phosphorylation in n 3 T3-L1 cells. It also phosphorylated AMP-activated protein kinase (AMPK) and activated it together with its downstream target, acetyl CoA carboxylase (ACC), in the liver, brain, muscle, and adipose tissue of C57BL/6 mice[14]. After eight weeks of medication, db/db mice with plasma TG and glucose levels considerably decreased. Magniferin consumption in the disease control group significantly reduced the rise in blood sugar two hours before administering glucose solution. By reducing pancreatic damage and boosting endogenous antioxidant enzymes, magniferin also possesses anti-diabetic benefits[15].

3.3 Alpha amylase gene expression profile

The expression of the alpha amylase gene was downregulated by magniferin treatment. In this study, Intestinal epithelial cells (IECs) treated with magniferin expressed less mRNA than the untreated cells. Alpha amylase enzyme is a key regulator that influences carbohydrate metabolism and remains upregulated during hyperglycaemia. With regards to diseases including cancer, diabetes, oxidative stress, autoimmune disorders, and inflammation, it has been discovered that the expression profile of alpha amylase is altered or abnormal. As the result shown in Figure 4, Intestinal epithelial cells (IECs) treated with magniferin showed less copies of alpha amylase gene compared to the untreated. A common pharmacological target for treating diabetes and other metabolic illnesses that cause postprandial hyperglycaemia is alpha-amylase[16]. The findings clearly demonstrate that magniferin has a gene modulation potential where it down-regulates the expression of alpha amylase gene in the Intestinal epithelial cells (IECs). The management of blood glucose levels in type 2 diabetic and borderline patients can benefit from the inhibition of alpha-glucosidase and alpha-amylase, enzymes involved in the digestion of carbohydrates. Alp et al., 2023 demonstrated that the blood glucose levels in type 2 diabetic can significantly reduce the post-prandial increase of blood glucose[17].

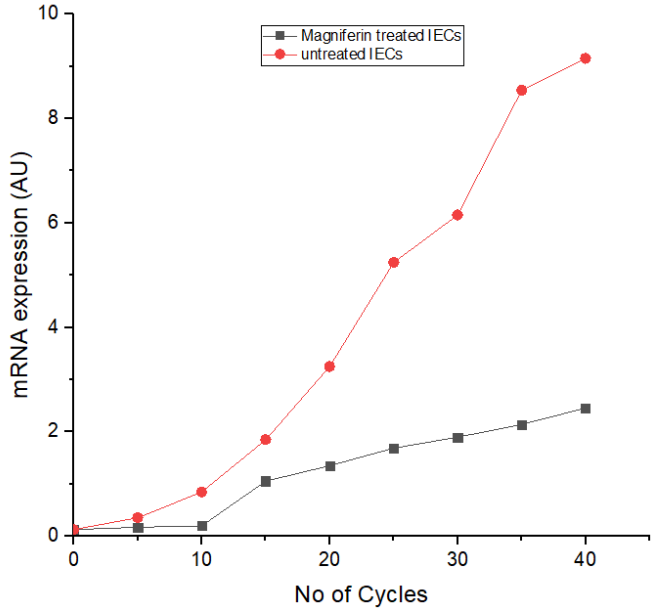

Fig 4. Expression profile of alpha amylase gene under the treatment of magniferin

Alfa amylase is one most promising targets for drug development towards developing anti- hyperglycemic therapeutics for diabetes and other metabolic disorders management. Magniferin has shown tremendous potential towards Alfa amylase activity and hence could be a potential anti-hyperglycemic therapeutic for diabetic management. Alfa amylase active site involved in the catalysis consist of Asp206, Glu230, and Asp297 where magniferin showed a higher affinity with these amino acids in all four poses during docking studies[18]. A complete inhibition via replacing the natural substrate of alfa amylase results in potent anti-hyperglycemic activity. Earlier studies also demonstrated dietary polyphenols are promising Alfa amylase inhibitors that tackle alleviated blood glucose level: hyperglycemia[19]. Similarly, Farazj et al., 2024 demonstrated anti Alfa amylase activity of Anthocleista djalonensis extracts in animal models (rat models) for anti-diabetic activity[20]. Other plant-based metabolites have been reported to deliver anti-diabetic activity primarily anti Alfa amylase activity. Wang et al., 2022 showed Azadirachta indica leaves possess excellent anti-diabetic activity via inhibiting pancreatic Alfa amylase[21]. Zahra et al., 2024 demonstrated α-amylase and α-glucosidase inhibiting activities of bitter gourd (Momordica charantia) in both in vivo and in vitro experiments[22]. Alfa amylase has been a key therapeutic target for diabetes management via inhibiting Alfa amylase where in the present study magniferin had shown a promising affinity with Alfa amylase specific at catalytic site. In the present study, in silico findings are promising however animal and clinical experimental studies are required for the therapeutic efficacy of magniferin.

4 Conclusion

Magniferin has shown the potential to regulate enzyme associated with carbohydrate metabolism and tackle hyperglycemic states where alpha amylase remains a key enzyme. In the present study, using in silico approach, the affinity of magniferin with alpha amylase was examined and validated via in vitro assay. The finding clearly demonstrates that magniferin possesses tremendous affinity with alpha amylase and precisely binds within the active site of the enzyme. As the result shows Asp, Glu, and Asp key amino acids in the active site of alpha amylase possess an affinity with O and C atoms of magniferin. The docking score and binding energies also demonstrated that magniferin effectively binds with catalytic triad of alpha amylase enzyme. The finding of the present study clearly demonstrates that the magniferin exerts a competitive inhibition with the natural substrate of alpha amylase and hence regulate/tackle hyperglycemic state. Additionally, in vitro findings (in vitro inhibitory alpha amylase activity) in comparison with Phaseolamin demonstrate magniferin effectively inhibits the activity of alpha amylase. The gene expression profile of alpha amylase also supports an inhibitory effect of magniferin on the enzyme. These data provide a scientific basis that magniferin can be used as a potential anti-diabetic therapeutic however clinical investigation are required. In the study, in silico findings were validated via laboratory experimentations, however, the promiscuous nature of alfa amylase may be one limitation in identifying outcomes. Ex-vivo and in vivo examination is highly recommended to evaluate the efficacy of the antidiabetic potential of Mangiferin via alfa amylase inhibition.

Author contributions

GNS and SG conceptualized the study, SG carried out experiments and data analysis. PB and SK analyzed the results and interpretation, GNS wrote the manuscript, and communicated with the journal.

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