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Ligand-protein docking of phytochemicals in their plausible binding to alpha-amylase and alpha-glucosidase enzymes and ligand bioavailability

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ABSTRACT

Diabetes mellitus is a chronic epidemic disease. Due to the imbalance of both glucose and insulin levels in the body, severe health problems develop, such as retinopathy, nephropathy, and others. The most prominent form of the disease is Type II diabetes, a consequence of insulin resistance. One direct way in alleviating the disease symptoms is by hindering the action of intestinal enzymes that breakdown the polysaccharides into simpler sugar forms that can be absorbed via the intestinal wall to the bloodstream. Two major intestinal enzymes are α-glucosidase and α-amylase. In this study, we elucidate the plausible action of phytochemicals (extracted from *Abelmoschus esculentus, Orthosiphon stamineus,* and *Hypericum triqerdriftium*) in binding to α-glucosidase and α-amylase *in silico*. Our work is based on docking protocols and ADME/Tox (Absorption, distribution, metabolism and elimination) properties for understanding the mechanisms of action of the protein-ligand binding, as well as the prediction of drug likeness and bioavailability. More than a few chemicals presented varying degrees of stable binding, seen in the equilibrium constant of binding as well as the free energy of binding, i.e., beta-sitosterol, gamma-tocopherol, phytol and stigmasterol, 11,14,17-eicosatrienoic acid. The results indicated that the above-mentioned phytochemicals extracted from *Abelmoschus esculentus, Orthosiphon stamineus*, and *Hypericum triqerdriftium* can be starting compounds for launching effective antidiabetic drugs.

1. Introduction

Diabetes mellitus (DM) is an epidemic chronic disease that occurs when the production of insulin is insufficient, or as a result of insulin resistance. Consequently, central metabolism is disrupted, and the levels of carbohydrates, fat and protein are imbalanced. This would result in hyperglycemia and high levels of serum LDL (Duewell et al., 2010; Vandanmagsar et al., 2011). Consequently, several diseases follow as secondary complications, including atherosclerosis, nephropathy, polyphagia, polyuria, polydipsia, and retinopathy (Chaurasia et al., 2023; Gören & Fen, 2008; Kedziora-Kornatowska, 1999; Liu & Daneshgari, 2014; Poznyak et al., 2020; VILLADSEN & PEDERSEN, 1987). The disease has several classifications, but the most focal forms are type I diabetes (T1D) and type II diabetes (T2D) (Antar et al., 2023). In T1D, the immune system induces the pancreatic β-cells destruction, resulting in lower insulin levels than normal (Antar et al., 2023; McCrimmon & Sherwin, 2010). In T2D, which is more common, body cells are resistant to respond to insulin signals, resulting in distorted levels of carbohydrates, fats and proteins (Hameed et al., 2015; Zaid et al., 2018). Hyperglycemia is a serious complication of T2D that results in the destruction of the eyes, heart, kidney and nerve cells (Kitada et al., 2010; Zaid et al., 2008). The levels of fasting serum glucose rise to ≥7 mM; and to ≥11 mM after a meal. Glycated hemoglobin levels exceed 6.5% in T2D pateints (Soliman et al., 2014).

Replacement therapy or transplantation of the pancreatic beta-cells are protocols followed to treat T1D (Pathak et al., 2019; Poradzka et al., 2013). However, strategies to treat T2D focus on alleviating hyperglycemia. Therein, the metabolic pathways of gluconeogenesis and glycogenolysis, which induce glucose production in the hepatic tissue (hepatic glucose output), can be inhibited, e.g., via glucagon receptors antagonists (Ali & Drucker, 2009; Choi et al., 2018; Sorensen et al., 2006; Zhou et al., 2018). An alternative strategy is to increase the uptake

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of serum glucose by specific tissues via augmenting glucose transporter-4 (GLUT4) translocation to the plasma membrane in myocytes and adipocytes (Alam et al., 2016; Shanak et al., 2019; Zaid et al., 2015). Distorted lipolysis causes low insulin secretion, and accordingly hyperglycemia and the buildup of toxic metabolites in several tissues (Dilworth et al., 2021; Gulcelik et al., 2009). Thus, one more strategy deals with reducing inflammation via anti-inflammatory drugs (Deans & Sattar, 2006; Pollack et al., 2016). We shed light in this study on the direct strategy in glycemic control, via inhibiting intestinal enzymes responsible for polysaccharide digestion to simpler sugars that can cross the intestinal walls to the blood; specifically, the α-glucosidase and α-amylase enzymes (Gong et al., 2020; Xie et al., 2021).

The restricted pharmacokinetic effect and hostile side effects are major concerns for antidiabetic synthetic drugs, e.g., biguanides and sulfonylurea (Banerjee et al., 2020; Chaudhury et al., 2017). Thus, the need arises for traditional medicine in introducing medications with fewer side effects and robust pharmacokinetic properties (Gupta et al., 2017; Jacob & Narendhirakannan, 2019). Alternative medicine based on medicinal plants is in use by around 80% of the world's population. Some of the bioactive phytochemicals or their derivatives can be safe and effective in treating many ailments (Shanak et al., 2019). Several medicinal plants showed high levels of efficacy in the treatment of diabetes (Saad et al., 2017).

The anti-diabetic activity was studied in a wide range of medicinal plants (Jacob & Narendhirakannan, 2019; Salleh et al., 2021). We concentrate in this study on three medicinal plants, and selected phytochemicals detected in these plants extracts that might posses anti-diabetic activity. The studied herbs are *Hypericum triqerdriftium, Orthosiphon stamineus*, and *Abelmoschus esculentus. Hypericum triqerdriftium* (HA) is a plant that belongs to the St. John's Wort family and is native to the Mediterranean region and parts of Europe. It has been well-researched for its medicinal properties that include its antidepressant, antibacterial, and antiviral effects(Rouis et al., 2013). In a recent study by Michela Novelli et al., it has been shown that species of the St. John's Wort family could potentially be beneficial in managing diabetes. The study has demonstrated that the extracts were capable of lowering blood glucose levels, suggesting its potential as a natural treatment for diabetes (Novelli et al., 2020).

Orthosiphon stamineus (OS) has been utialized in traditional medicine all across Southeast Asia, specifically in countries like Malaysia, Myanmar, Indonesia, and Thailand. The leaf part of the stamineus is believed to have diuretic properties. Thus, it has been used to treat kidney stones. Other health benefits of *Orthosiphon stamineus* may include alleviating symptoms of health problems like rheumatism, fever, hepatitis, hypertension, gallstones, and diabetes (Almatar et al., 2014). Studies have proven that *Orthosiphon stamineus* has promising anti-diabetic properties, specifically due to its ability to enhance glucose uptake in tissues, and inhibiting its absorption in the intestines, hence reducing glucose quantities in the bloodstream (Mohamed et al., 2013).

Abelmoschus esculentus (AE), or Okra, is a tropical plant that is commonly consumed worldwide and it has been gaining attention for its health benefits. Different parts of the plant are rich in phytochemicals that give it its medicinal properties such as its role in treating cardiovascular diseases, digestive disorders, type-2-diabetes, and other chronic conditions (Elkhalifa et al., 2021). Recent studies showed that ethanol or water extracts of *Abelmoschus esculentus* had antidiabetic potency both *in vitro* and *in vivo*, evident by its effect on the rats' body weight and the histopathology of the pancreas (Anjani et al., 2018; Khatun et al., 2011; Saha et al., 2011).

α-Amylase is an amylolytic enzyme that hydrolyses the alpha-linkage of polysaccharides, such as starch and glycogen, to produce glucose, maltose and dextrins. Two isozymes of α-amylase exist in humans; salivary α-amylase and pancreatic α-amylase. Whereas salivary α-amylase digests starch into maltose and dextrin, the pancreatic α-amylase continues the breakdown of sugars in the small intestine and the consequent uptake in the bloodstream (Butterworth et al., 2011). In addition to directly contribute to the absorption of simple sugars, the pancreatic α-amylase is involved in regulating the levels of serum glucoses through glycoproteins. This mission is accomplished via binding to the glycoproteins at their N-linked oligosaccharides (Asanuma-Date et al., 2012; Matsushita et al., 2002). α-amylases encompasses several domains; The catalytic domain, where the active site is included, with an alpha/beta barrel structure; the calcium-binding domain which projects in the catalytic domain, and a Greek key beta-barrel domain at he carboxyl terminal (Abe et al., 2005; Kadziola et al., 1994; Kadziola et al., 1998; Machius et al., 1995).

α-Glucosidase is a hydrolase that breaks down the terminal nonreducing alpha linkage and releases α-glucose as a product (Chiba, 1997; Krasikov et al., 2001). Few metabolic disorders, such as glycogen storage diseases, are linked to the malfunctioning in α-glucosidase(Gümüs & Özen, 2023). The protein structure of the human lysosomal α-glucosidase reveals Trp-516 and Asp-518 amino acids as two key residues for the enzyme's catalytic functionality (Hermans et al., 1991; Hoefsloot et al., 1990).Knowing that the conformation of the enzyme's active site is less stable than it is in other protein regions introduces the active site as plausible binding site for competitive inhibitors (Wu et al., 2009).

The development in drug discovery protocols that incorporate computational approaches aided in screening hundreds of lead compounds as plausible drugs. One method bases on studying drug likeness and bioavailability via studying the ADME (Absorption, distribution, metabolism and elimination) properties of the drug (Schneckener et al., 2019; Vo et al., 2020). Additionally, the advancement in the isolation of proteins and obtaining their 3D structure resulted in a big databank of resolved protein structures. As a result, modeling for the binding of ligands as plausible drugs to the target proteins helps in understanding the mechanism of action of the drugs under microscope. Docking studies are performed to answer questions regarding protein-ligand binding; including the type of bonds, amino acids included at the binding interface, binding free energy, as well as the equilibrium constant for binding. In this study, we examined the plausible inhibitory effect of twenty-one phytochemicals; extracted from the medicinal plants *Abelmoschus esculentus, Orthosiphon stamineus*, and *Hypericum triqerdriftium*; on α-glucosidase and α-amylase using in silico approaches.

2. Materials and methods

2.1. Docking tests

The 2D structures for the phytochemicals, presented in the supplementary figure S1, were converted to PDB files. Table S1 shows the plant species from which the compounds were previously detected at Zaid's lab; *Hypericum triqerdriftium* (Mahajna et al., 2019) (some data not published), *Orthosiphon stamineus* (Bassalat et al., 2023), and *Abelmoschus esculentus* (Data not published). Based on the systemic IUPAC naming of the compounds, the SMILES structures of the compounds were obtained from the PubChem database (Kim et al., 2023; O'Boyle et al., 2011). Using the OpenBabel server, the SMILES names of the phytochemicals were used to retrieve the 3D PDB structure files (O'Boyle et al., 2011). The *apo* forms of the two enzymes of *α*-glucosidase (PDB: 3TOP (Burley et al., 2019; Ren et al., 2011)) and *α*-amylase (PDB: 4W93 (Burley et al., 2019; Williams et al., 2015)) enzymes were retrieved using the RCSB/PDB database (Burley et al., 2019). AutoDock tools were used to add polar hydrogens and prepare ligand-protein grid and docking parameter files (Morris et al., 2009; Park et al., 2006). Then, the AutoDock program was used to perform the docking regime (Trott & Olson, 2010). Docking protocol was performed by introducing flexibility to the ligands and keeping the proteins rigid (Morris et al., 2009). Rectangular grid boxes were prepared. In preparing the grid point file for both alpha-amylase and alpha glucosidase proteins, the number of grid points in x-, y- and z- dimensions were set to 60 points. Spacing was set to 0.5 Å. The center of protein made as the center of mass for the docking procedure. The grid center in the X-, Y- and Z-

coordinates was -10.525 8.241 -17.354, and -32.594 30.986 27.823; for alpha-amylase and alpha glucosidase, respectively. Docking was initiated with the following parameters: genetic algorithm was applied, with the maximum initial energy was equal to zero, and the maximum number of retries equal to 1000. The genetic algorithm population size was set to 150, and the maximum number of evaluation times was 2500, 000. The genetic algorithm number of generations was 27,000. The mutation rate was 0.02, and the crossover rate was 0.8. The genetic algorithm window size was 10. The iterations of Solis & Wets local search were set to 300. The consecutive successes/failures before changing rho were both set to 4. The alpha parameter of Cauchy distribution was set to 0.0, and the beta parameter for the distribution was set to 1.0. Finally, the PDB files were extracted and assessed for the best-ranked fit of the enzyme-ligand interaction for each of the ligands. The best 20 structures were retrieved. Ranking was performed for the resultant binding free energies (lowest values being on top of files), and the inhibition constants (where lowest values for the equilibrium constants indicating the best affinities). For the analysis, DiscoveryStudio software was used (BIOVIA, 2023). Therein, amino acid residues at the binding interface of any of the two proteins to the several phytochemicals were detected (Seeliger & de Groot, 2010). Additionally, the different types of electrostatic interactions within 5 Å of the ligand at the protein interface were detected, including polar, hydrophobic, and Van der Waals force interactions (Monteiro et al., 2018; Zheng et al., 2019).

2.2. Ligand ADME/Tox properties- prediction

SwissADME is a free web tool that enables users to evaluate the pharmacokinetics, drug-likeness, and medicinal chemistry friendliness of small molecules. It provides access to a pool of fast yet powerful predictive models for physicochemical properties, pharmacokinetics, drug-likeness and medicinal chemistry parameters. The tool has a user-friendly interface where molecules can be input by drawing them using a molecular sketcher or by providing SMILES strings (Daina et al., 2017). SwissADME then computes various properties and generates an output panel with the results. The tool is intended for use in drug discovery and medicinal chemistry contexts, providing a balance between accuracy and speed to enable analysis of large numbers of molecules. SwissADME results should be interpreted with care given their predictive na-ture. The SwissADME website allows users to evaluate the pharmacokinetics, drug-likeness, and medicinal chemistry friendliness of molecules (Daina et al., 2017). To use the tool, molecules can be imported from external files or databases using the molecular sketcher based on ChemAxon's Marvin JS. After that, the imported molecules are converted into SMILES notation. Consequently, SwissADME then calculates various physicochemical properties, lipophilicity, pharmacokinetics, drug-likeness and medicinal chemistry parameters for each molecule (Daina et al., 2017).

The Lipinski rule of five (RO5) is used to evaluate drug-likeness and predict oral bioavailability (Karami et al., 2022; Lipinski et al., 2001). The rule states that poor absorption or permeation is more likely when there are more than 5 H-bond donors, the molecular weight is over 500 daltons, the log P (octanol/water partition coefficient) is over 5, there are more than 10 H-bond acceptors, and there are more than 10 rotatable bonds. Molecules that violate more than three these rules are less likely to be orally active drugs. However, many orally active drugs were scanned for their bioavailability and found to violate two to three rules (Benet et al., 2016). SwissADME provides a bioavailability radar plot to visualize how a molecule scores on these parameters (lipophilicity, molecular weight, solubility, and flexibility), see figure S3 (Daina et al., 2017). Figure S2 shows biological activity of the reported phytochemicals, where the expected binding to the several target classes is revealed.

3. Results

3.1. Docking experiments

Phytochemicals, extracted from the medicinal plants AE, OS, and HT, were screened for their binding affinities, inhibition constants, and the root mean square deviation (RMSD) values of the ligand after docking to the reference structure for both α-glucosidase and α-amylase. Table 1 shows the results for ligand binding to alpha-amylase. Among the twenty-one tested phytochemicals, we picked the results that do not exceed -5 kcal/mol and have up to 100 μM values for the inhibition constant. For the docking results of alpha-amylase, five phytochemicals displayed strong binding affinities and introduced stable structures, measured via the results of binding free energies. The phytochemicals are beta-sitosterol, 11,14,17-Eicosatrienoic acid, gamma-tocopherol, phytol and stigmasterol. Four out of the five phytochemicals, with 11,14,17-eicosatrienoic acid being excluded, showed robust results in the docking experiments with alpha-glucosidase; see Table 2 for the best ranked phytochemicals. Yet, results for 11,14,17-eicosatrienoic acid in binding to alpha-glucosidase showed fine binding. Most of the five mentioned phytochemicals with robust interactions are extracted from the three plants. On the other hand, 11,14,17-eicosatrienoic acid and gamma-tocopherol are only water-extracted from AE. Results for the phytochemicals were compared to the results reference co-crystallized positive control, acarbose (with -11.86 kcal/mol binding free energy, and 2.02 nM inhibition constant for alpha-amylase; -7.89 kcal/mol binding free energy and 1.66 μM inhibition constant for alpha-glucosidase). Table S2 shows the additional predicted targets for all phytochemicals usind SWISSPredict.

The 3D structures for the most stable protein-ligand contacts were analyzed via Discovery Studio (BIOVIA, 2023) (see Fig.s 1 and 2 for ligand contacts to alpha-amylase and alpha-glucosidase, respectively). At the alpha-amylase binding interface (see Fig. 1), and in most protein-ligand contacts, weak nonpolar interactions predominated. Only few polar contacts (mainly via H-bonds, e.g., Glu-233 with stigmasterol) happened at the binding interface between the amino acids and the target ligands. Interestingly, the five phytochemicals were bonded to the same binding site of the inhibitor of the amylase enzyme, montbretin A (Williams et al., 2015). The catalytic residues, Asp-197 and Glu-233 that interact with the montbretin A inhibitor, were found at the binding interfaces for majority of the strongly bonded phytochemicals to the alpha-amylase catalytic site.

Indeed, analyzing the alpha-glucosidase binding interface for the most robust phytochemical binding showed prevailing weak nonpolar interactions (see Fig. 2). A limited number of polar contacts (primarily via H-bonds, e.g., Gln-1372 with stigmasterol and Gln-1286 with gamma-tocopherol) occurred at the binding interface between the protein and the target ligands. Interestingly, the four phytochemicals were equivalently bonded to the maltase-glucosylamylase binding site (MGAM-C) of the inhibitor, acarbose, of the glucosidase enzyme (Ren et al., 2011). The catalytic 'ring-containing residues' were emphasized at the binding interface of the protein to acarbose. These include His-1584, Phe-1559, Phe-1427 in beta-sitosterol; Phe-1427, Phe-1559, Phe-1560, Tyr-1251, Trp-1355, Trp-1369 in gamma-tocopherol; His-1584, Phe-1559, Phe-1560, Tyr-1251, Trp-1355, Trp-1369 in phytol; and His-1584, Trp-1418, Trp-1523, Trp-1355, Phe-1427, Phe-1559, Trp-1369 in stigmasterol. Additionally, similar to acarbose, the binding interface of the four phytochemicals to the alpha-glucosidase enzyme was enriched in aspartic acid residues. The following aspartate residues were found at the binding interface: Asp-1157, Asp-1526 in beta-sitosterol; Asp-1279, Asp-1357 in tocopherol; Asp-1157; Asp-1279, Asp-1317 in phytol; Asp-1157, Asp-1279, Asp-1420 in stigmasterol.

3.2. Drug bioavailability and likeness properties of bioactive compounds

To determine whether the bioactive phytochemicals have

Table 1
Binding free energies, inhibition constants, and the RMSD values calculated by AutoDock for ligand binding to alpha-amylase.

	RMSD from reference structure	Estimated Free Energy of Binding	Estimated Inhibition Constant, Ki	No. Of H-bond (Ligand/ 4W93)	No. Of Other Bonds (Ligand/4W93)
Acarbose (control)	38.535 Å	-11.86 kcal/mol	2.02 nM	14	1
9-12-Octadecadienoic acid	13.591 Å	-4.89 kcal/mol	259.47 μM	3	39
Alpha-linolenic acid	29.285 Å	-5.14 kcal/mol	170.60 μM	3	43
Beta-sitosterol	11.349 Å	-10.17 kcal/mol	34.83 nM	1	51
11,14,17-Eicosatrienoic acid	32.946 Å	-5.47 kcal/mol	98.28 μM	4	40
gamma-Tocopherol	24.846 Å	-8.77 kcal/mol	371.67 nM	3	61
Phytol	22.396 Å	-5.60 kcal/mol	79.08 μM	1	60
Stigmasterol	11.552 Å	-9.89 kcal/mol	56.01 nM	2	89

Table 2
Binding free energies, inhibition constants, and the RMSD values calculated by AutoDock for ligand binding to alpha-Glucosidase.

	RMSD from reference structure	Estimated Free Energy of Binding	Estimated Inhibition Constant, Ki	No. Of H-bond (Ligand/ 3TOP)	No. Of Other Bonds (Ligand/3TOP)
Acarbose (control)	69.50 Å	-7.89 kcal/mol	1.66 μM	8	18
Alpha-linolenic acid	47.606 Å	-5.17 kcal/mol	163.50 μM	2	35
Beta-sitosterol	53.779 Å	-10.03 kcal/mol	44.10 nM	1	46
Diethanolamine	55.376 Å	-5.24 kcal/mol	144.04 μM	4	0
11,14,17-Eicosatrienoic acid	46.546 Å	-4.94 kcal/mol	241.21 μM	0	43
gamma-tocopherol	54.062 Å	-7.83 kcal/mol	1.83 μM	2	34
N-Acetyl-D-glucosamine	54.691 Å	-4.94 kcal/mol	238.29 μM	10	3
Stigmasterol	53.818 Å	-10.03 kcal/mol	44.52 nM	1	53

advantageous ADME (absorption, distribution, metabolism, and excretion) qualities, drug-likeness was examined. These outcomes are anticipated from a good drug: it should be specific in nature, it follows the Lipinski RO5, it has a good solubility, it has no excretion issues, and it does not block CYP-enzymes (Jorgensen & Duffy, 2002). If three or more of the Lipinski Five Rules—that is, that a molecule's molecular weight (MW) not to exceed 500 g/mol, its number of rotatable bonds not to exceed 10, its hydrogen bond donors not to exceed 5, and its hydrogen bond acceptors not to exceed 10—are broken, the molecule is considered non-orally active (Karami et al., 2022; Lipinski et al., 2001).

Our calculations show that each ligand of the twenty-one compounds obeys the Lipinski's rule of five (Lipinski et al., 2001) using SwissADME online web tool (Daina et al., 2017), see Table 3, with some exceptions; myo-inositol had a number of H-bond donor equal to 6; the logP value of gamma-tocopherol is slightly above the threshold (logP=5.76), docosanoic acid and 11,14,17-eicosatrienoic acid had a number of rotatable bond more than 10 (20 and 15, respectively). Thus, all compounds are orally active; as they do follow Lipinski RO5 (None of them has a violation of two or more rules. Table 4 lists additional drug-likeness characteristics for bioactive substances along with their respective parameters.

The bioavailability score shows the fraction of an orally administered compound that reaches the systemic circulation. The bioavailability score ranges from 0 to 1, and a score closer to 1 suggests a better bioavailability. In shedding the light on the five phytochemicals that showed the best docking results (beta-sitosterol, 11,14,17-eicosatrienoic acid, stigmasterol, gamma-tocopherol, and phytol), the one phytochemical that shows best bioavailability (0.85 score) is 11,14,17-eicosatrienoic acid. On the other hand, beta-sitosterol, phytol, stigmasterol, and gamma-tocopherol, show the intermediary bioavailability (0.55). However, compounds with drug-likeness should have a good aqueous solubility which is predicted by three methods: ESOL, (ALI) logS, and (SILICOS- IT) logS but all of them showed moderate to poor solubility (Daina et al., 2017).

Cytochrome P450 enzymes are responsible for metabolising a wide variety of drugs during their first pass in the liver (Furge & Guengerich, 2006). In screening CYP450 inhibitors, beta-sitosterol and gamma-tocopherol are non-inhibitors of CYP enzymes. 11,14,17-Eicosatrienoic acid is an inhibitor of CYP1A2 and CYP2C9 enzymes. Stigmasterol and phytol are both inhibitors of the CYP2C9 enzyme.

4. Discussion

Alternative medicine is nowadays used for primary health care by nearly 80% of the world's population. Safety and efficacy are major factors in scanning compounds for their drug likeness. Thus, several phytochemicals are being subjected to critical examination as potential drugs (Shanak et al., 2019). Specifically, several medicinal plant extracts were safe and effective in the management of diabetes (Saad et al., 2017; Shanak et al., 2019). One direct route in alleviating hyperglycemia and the consequent T2D is via hindering intestinal digestive enzymes of polysaccharides, present in the intestinal lumen. The simple sugars, which are the products of the digestion process, can pass via the intestinal walls to the blood (Gong et al., 2020; Xie et al., 2021).

Extracts of the medicinal plants *Abelmoschus esculentus, Orthosiphon stamineus*, and *Hypericum triqerdriftium* have been reported for their antidiabetic effects (Anjani et al., 2018; Mohamed et al., 2013; Novelli et al., 2020). Yet the route of glycemic control, and the exact action mechanisms of the several phytochemical components were not yet discussed. Herein, we test drug likeness of twenty-one selected phytochemicals, and whether alpha-amylase and alpha-glucosidase are druggable targets to the ligands under the microscope. Indeed, structural virtual screening that bases on docking protocols, and drug bioavailability using ADME/Tox properties and the bioavailability radar were undertaken.

In performing the docking protocol and filtering the phytochemicals, the binding free energy and the equilibrium constant for binding were retrieved. According to literature, the best docking is expected toresult in a binding free energy lower than -5 kcal/mol and an inhibition constant less than 100 μM(Shivanika et al., 2022). Thus, we obtained the phytochemicals that fulfilled the above criteria for structural analysis. In binding to alpha-amylase, five phytochemicals displayed strong

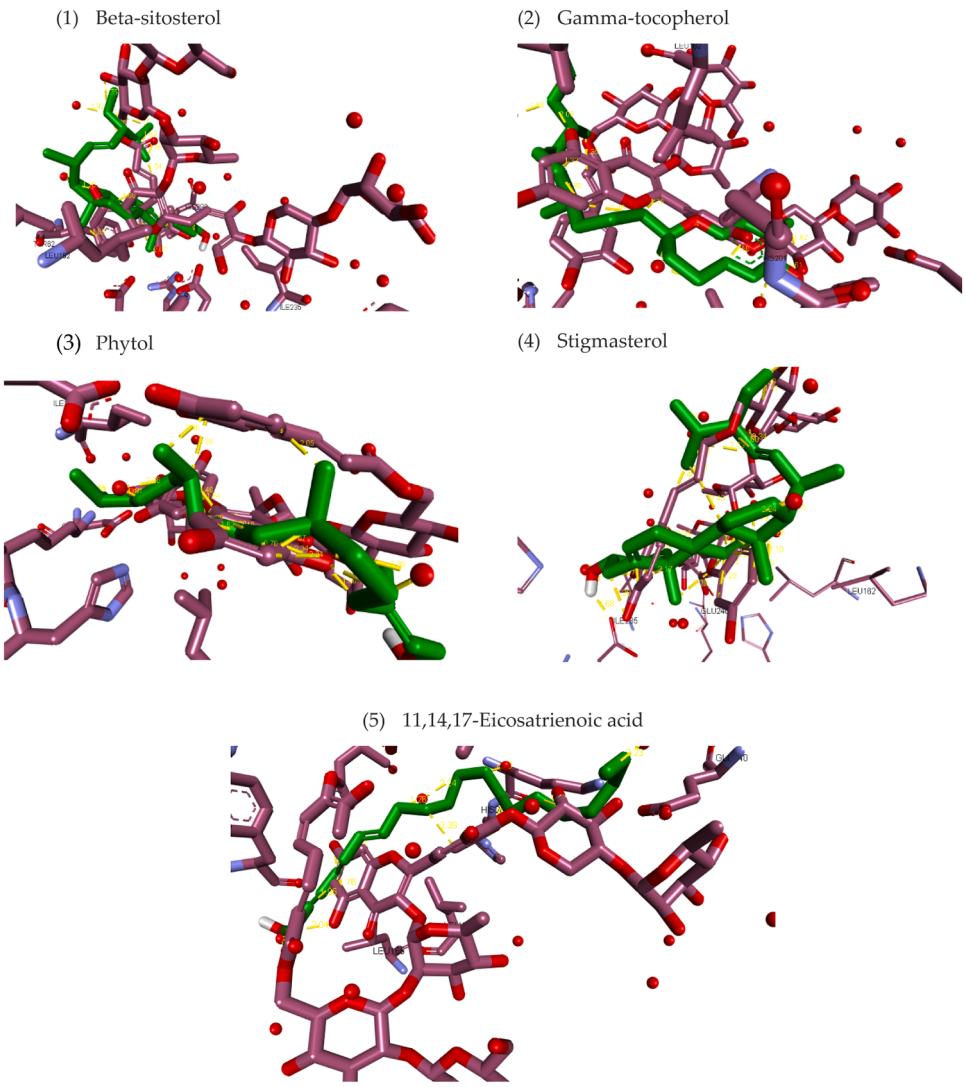

Fig. 1. Binding interface between plausible inhibitors and the α-amylase enzyme: (1) beta-sitosterol, (2) gamma-tocopherol, (3) phytol, (4) stigmasterol, and (5) eisosatrienoic acid. All amino acids that are within 5 Å from the ligand are shown as sticks. For rest of the amino acids in the protein, an 80% transparent cartoon model is used. Polar bonds are presented in yellow. Other bonds are emphasized in blue.

affinities and introduced stable structures. The phytochemicals in an ascending order with respect to the binding free energy and concomitantly with the inhibition constant are; beta-sitosterol (binding free energy= -10.17 kcal/mol; equilibrium inhibition constant= 34.83 nM), stigmasterol (binding free energy= -9.89 kcal/mol; equilibrium inhibition constant= 56.01 nM), gamma-tocopherol (binding free energy= -8.77 kcal/mol; equilibrium inhibition constant= 371.67 nM), phytol (binding free energy= -5.60 kcal/mol; equilibrium inhibition constant= 79.08 μM), and 11,14,17-eicosatrienoic acid (binding free energy= -5.47 kcal/mol; equilibrium inhibition constant= 98.28 μM). Of these five phytochemicals, and in binding to alpha-glucosidase, four were committed to the aforementioned golden rules with respect to the binding free energy and inhibition equilibrium constants. The phytochemicals followed the same ascending order with respect to the binding free energy and concurrently with the inhibition constant in both binding to alpha-glucosidase and alpha-amylase. The values for their fee energy and inhibition constant in binding to alpha-glucosidase were; for beta-sitosterol (binding free energy= -10.03 kcal/mol; equilibrium inhibition constant= 44.10 nM), for stigmasterol (binding free energy= -10.03 kcal/mol; equilibrium inhibition constant= 44.52 nM), for gamma-tocopherol (binding free energy= -7.83 kcal/mol; equilibrium inhibition constant= 1.83 μM), and for phytol (binding free energy= -7.16 kcal/mol; equilibrium inhibition constant= 5.64 μM). The least stringent phytochemical to the said rule in binding to alpha-amylase, 11, 14,17-eicosatrienoic acid, is excluded from the list of phytochemicals showing robust results in the docking experiments with alpha-glucosidase. However, results for 11,14,17-eicosatrienoic acid in binding to alpha-glucosidase showed acceptable values for binding free energy (=- 4.94 kcal/mol) and inhibition constant (= 241.21 μM). Three out of the five mentioned phytochemicals with robust interactions are extracted from all of the three plants (beta-sitosterol, stigmasterol, and phytol). In screening the experimental solvent used in the extraction procedure of the three phytochemicals, methanol, hexane and water were the solvents of use. Beta-sitosterol and stigmasterol were methanol-extracted, whereas phytol was found in both methanol and hexane extracts. However, 11,14,17-eicosatrienoic acid and gamma-tocopherol are only hexane-extracted from AE. This goes well with the hydrophobic nature of the five phytochemicals seen in our bioavailability test on solubility (logS ranging between 0.55 and 0.85). Additionally, the non-polar bonding of the phytochemicals at the binding interfaces of both alpha-amylase and alpha-glucosidase matches with experimental extraction of the phytochemicals on one hand (the non-polar nature of the solvent), and our ADME/Tox and bioavailability studies on the other hand.

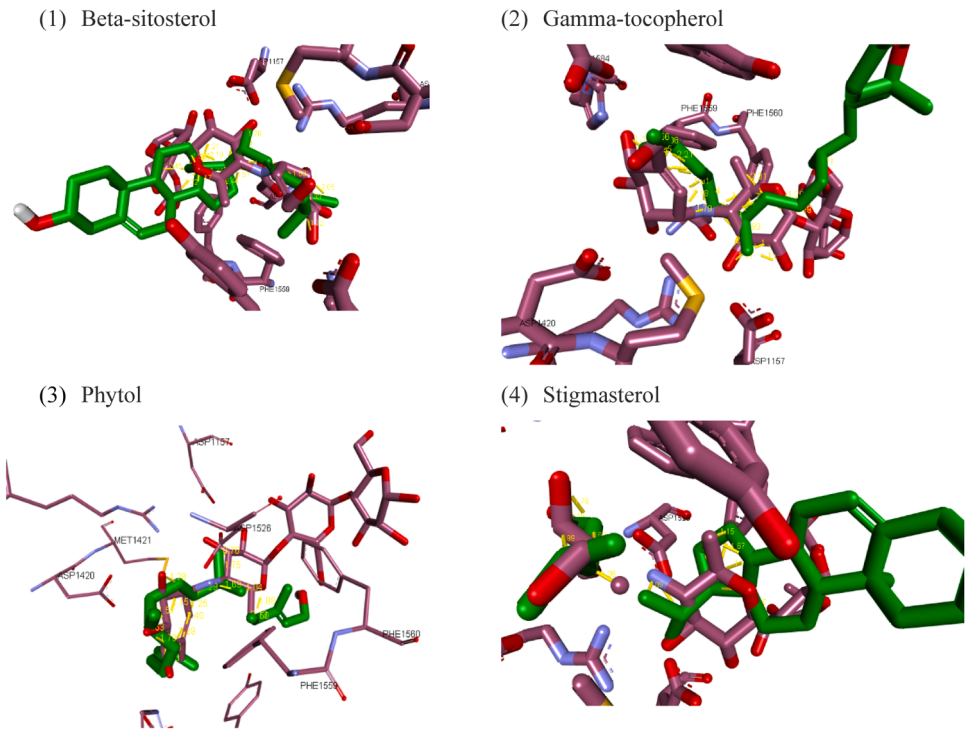

Fig. 2. Binding interface between plausible inhibitors and the α-glucosidase enzyme: (1) beta-sitosterol, (2) gamma-tocopherol, (3) phytol, and (4) stigmasterol. All amino acids that are within 5 Å from the ligand are shown as sticks. For rest of the amino acids in the protein, an 80% transparent cartoon model is used. Polar bonds are presented in yellow. Other bonds are emphasized in blue.

Table 3
ADME analysis -Physicochemical properties (Lipinski rule of five of ligands).

No.	Phytochemical	Physicochemical properties (Lipinski rule of five)				
		Molecular weight (g/mol)	H-bond acceptor	H-bond donor	Log P	No of rotatable bond
1.	1-Monopalmitin	330.50	4	2	3.93	18
2.	2-Butenedioic acid	116.07	4	2	0.32	2
3.	9,12-Octadecadienoic acid	280.45	2	1	4.14	14
4.	Alpha-Linolenic acid	278.43	2	1	3.36	13
5.	Beta-sitosterol	414.71	1	1	4.79	6
6.	Diethanolamine	105.14	3	3	1.11	4
7.	Docosanoic acid	340.58	2	1	5.26	20
8.	11,14,17-Eicosatrienoic acid	306.48	2	1	4.50	15
9.	Stigmasterol	412.69	1	1	5.01	5
10.	gamma-Tocopherol	416.68	2	1	5.76	12
11.	Glyceric acid	106.08	4	3	0.54-	2
12.	10-Heptadecenoic acid	268.43	2	1	3.90	14
13.	Malic acid	134.09	5	3	0.01-	3
14.	Myo-Inositol	180.16	6	6	0.31	0
15.	N-Acetyl-D-glucosamine	221.21	6	5	0.54	3
16.	Pentadecanoic acid	242.40	2	1	3.66	13
17.	Phytol	296.53	1	1	4.71	13
18.	Propane-1,2-diol	76.09	2	2	1.00	1
19.	Pyroglutamic acid	129.11	3	2	0.51	1
20.	Pyrrolidinone	85.10	1	1	1.06	0
21.	Trigonelline	137.14	2	0	3.11-	1

We additionally studied the drug likeness for the twenty-one under study. The twenty-one studied phytochemicals follow Lipinski's rule of five to a great extent (Table 3). A slight deviation from the LO5 was found for myo-inositol in having a number of H-bond donors equal to 6; gamma-tocopherol, with a logP value somewhat above the threshold (logP=5.76), docosanoic acid and 11,14,17-eicosatrienoic acid in having a number of rotatable bond more than 10 (20 and 15, respectively). Nicely, none of the phytochemicals violated more than two rules. In previous studies, orally active drugs were scanned for their bioavailability and found to violate two to three rules (Benet et al., 2016).

Bioavailability was measured for the twenty-one phytochemicals, and the results were depicted in Table 4 and figure S3. In concentrating on the five ligands showing the best structural screening via docking, the best bioavailability is found in 11,14,17-eicosatrienoic acid. The remaining ligands; beta-sitosterol, 11,14,17-eicosatrienoic acid, stigmasterol, gamma-tocopherol, and phytol have intermediate scores. Although these five ligands (beta-sitosterol, 11,14,17-eicosatrienoic acid, stigmasterol, gamma-tocopherol,and phytol) have the highest binding affinities and obey the Lipinski RO5 properties, all of them have moderate to poor solubility in nature which makes them unstable in GI absorption. Still, a major obstacle to any drug delivery system (DDS) is to keep sufficient drug concentration at the target location in body, and

Table 4
Drug-likeness analysis of bioactive compounds.

No.	Phytochemical	Bioavailability score	Solubility			Pharmacokinetics	
			Log S (ESOL)	Log S (Ali)	Log S (SILICOS-IT)	GI absorption	CYP enzymes inhibitors
1.	1-Monopalmitin	0.55	moderately soluble	Poorly soluble	Poorly soluble	high	CYP2D6
2.	2-Butenedioic acid	0.85	Very soluble	Very soluble	Soluble	high	No
3.	9,12-Octadecadienoic acid	0.85	moderately soluble	Poorly soluble	moderately soluble	high	CYP1A2, CYP2C9
4.	Alpha-Linolenic acid	0.85	moderately soluble	Poorly soluble	Soluble	high	CYP1A2, CYP2C9
5.	Beta-sitosterol	0.55	Poorly soluble	Poorly soluble	Poorly soluble	low	No
6.	Diethanolamine	0.55	highly soluble	highly soluble	Soluble	high	No
7.	Docosanoic acid	0.85	Poorly soluble	insoluble	Poorly soluble	low	CYP1A2
8.	11,14,17-Eicosatrienoic acid	0.85	moderately soluble	Poorly soluble	moderately soluble	high	CYP1A2, CYP2C9
9.	Stigmasterol	0.55	Poorly soluble	Poorly soluble	moderately soluble	low	Only CYP2C9
10.	gamma-Tocopherol	0.55	Poorly soluble	insoluble	Poorly soluble	low	NO
11.	Glyceric acid	0.56	highly soluble	highly soluble	Soluble	high	No
12.	10-Heptadecenoic acid	0.85	moderately soluble	Poorly soluble	moderately soluble	high	CYP1A2, CYP2C9
13.	Malic acid	0.56	highly soluble	very soluble	Soluble	high	NO
14.	Myo-Inositol	0.55	highly soluble	highly soluble	Soluble	low	No
15.	N-Acetyl-D-glucosamine	0.55	very soluble	very soluble	Soluble	low	No
16.	Pentadecanoic_acid	0.85	moderately soluble	Poorly soluble	moderately soluble	high	CYP1A2, CYP2C9
17.	Phytol	0.55	moderately soluble	Poorly soluble	moderately soluble	low	CYP2C9
18.	Propane-1,2-diol	0.55	highly soluble	highly soluble	Soluble	high	No
19.	Pyroglutamic acid	0.85	very soluble	very soluble	Soluble	high	No
20.	Pyrrolidinone	0.55	highly soluble	highly soluble	Soluble	low	No
21.	Trigonelline	0.55	very soluble	very soluble	Soluble	high	No

to solve problems related to low bioavailability, solubility and blood distribution. The solution to this obstacle is via modifying the drugs to more superior forms that overcome bioavailability complications (Li et al., 2019). In addition, some of the five phytochemicals are inhibitors of CYP enzymes. Thus, drug-drug interaction needs to be taken into account in designing modified drugs of the five phytochemicals.

We additionally compared the results of bioavailability and drug likeness of the most potent five chemicals in our study with our positive controls, acarbose and montbretin A. The latter two compounds serve as potent inhibitors to alpha-glucosidase and alpha-amylase, respectively. The evidence to their mechanism of action was retrieved by them being co-crystallized in the 3D structures with either of the two proteins (Ren et al., 2011; Williams et al., 2015). The two positive controls both violate three rules of Lipinski; both inhibitors have large molecular weight (exceeding 500 g/mol), and exceed the threshold of H-bond donors and H-bond acceptors. Thy also have very low bioavailability score (0.17) and low absorption in the gastrointestinal tract. Figure S1 contrasts the structures for the five phytochemicals (a-e) to the two positive controls (f-g). The figure shows high similarity in terms of the aliphatic hydrocarbon skeleton as well as the benzene ring, present in most structures of the five plausible inhibitors investigated in this study.

Previous studies have looked into the potential role of the five phytochemical compounds (beta-sitosterol, gamma-tocopherol, phytol and stigmasterol, 11,14,17-eicosatrienoic acid) in the managing diabetes. *In silico* and *in vitro* analysis of β-sitosterol, a plant sterol, on diabetic rats has highlighted that it can improve glucose metabolism and insulin sensitivity while also modulating lipid profiles (Babu & Jayaraman, 2020; Ponnulakshmi et al., 2019). Docking experiments of b-sitosterol and stigmasterol with α-amylase and α-glucosidase have shown that b-sitosterol might be able to become an anti-T2D drug candidate by inhibiting α-amylase and α-glucosidase (Lolok et al., 2022). Stigmasterol has a structural similarity to β-sitosterol and has demonstrated anti-diabetic activity by targeting the glucose transporter, GLUT4, enhancing glucose uptake into tissues and improving insulin sensitivity (JL Wang et al., 2017). Research indicates that gamma-tocopherol may be a beneficial nutrient for controlling oxidative stress and inflammation caused by hyperglycemia-induced acute kidney inflammation. Taking into consideration gamma-tocopherol's ability to modulate inflammation, it could further develop as a therapeutic to prevent diabetes-induced delayed wound healing (Shin et al., 2016; Shin et al., 2017). Phytol is a diterpene alcohol and a naturally occurring precursor of phytanic acid. Previous studies have demonstrated that administering phytol to mice fed a high-fat and high-fructose diet (HFFD) prompted hyperplasia of adipose tissue, enhancing adipogenesis and glucose uptake via activation of the PI3K/Akt pathway. These data suggest that phytol may serve as a nutritional agent to potentially combat obesity and type 2 diabetes (JB Wang et al., 2017a). However, specific studies looking into 11,14,17-eicosatrienoic acid in the context of DM were not evident in the investigated literature, which suggests that its effect on diabetes management is not yet discovered (Figure S3 – S4).

5. Conclusions

Alpha-glucosidase and alpha-amylase inhibitors aid in glycemic control and consequently in alleviating diabetes via the route of decreasing glucose absorption in the intestinal walls. This study was conducted by running docking protocols and analyzing ADME/Tox (Absorption, distribution, metabolism, elimination and toxicity) properties. We aimed at structurally screening phytochemicals for their plausible contribution to lessening the effect of diabetes by targeting the intestinal wall and decreasing the absorption of simple sugars. Thus, the action mechanisms for the protein-ligand binding, and drug likeness were studied. A number of chemicals showed robust binding, i.e., beta-sitosterol, gamma-tocopherol, phytol, stigmasterol, and 11,14,17-eicosatrienoic acid and simulated the binding of the positive controls that were co-crystallized with the proteins under the microscope. The results indicated that the above-mentioned phytochemicals extracted from *Abelmoschus esculentus, Orthosiphon stamineus*, and *Hypericum triqerdriftium* can be "lead" compounds to conduct further *in vitro, in vivo* and clinical investigations leading to the launch of safe and efficient anti-diabetic drugs.

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CRediT authorship contribution statement

Siba Shanak: Supervision, Formal analysis, Writing – original draft, Software, Data curation. **Shahd Abu Naim:** Validation, Methodology, Writing – original draft, Software. **Beesan AlArdah:** Software, Writing – original draft. **Najlaa Bassalat:** Data curation, Writing – review & editing. **Hilal Zaid:** Supervision, Project administration, Funding

acquisition, Resources, Investigation.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Siba Shanak and Hilal Zaid reports financial support was provided by Arab American University. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.focha.2025.101051.

Data availability

Data will be made available on request.

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