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Original Article

GCMS fingerprints and phenolic extracts of *Allium sativum* inhibit key enzymes associated with type 2 diabetes

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المخلص

أهداف البحث: تم الإبلاغ عن تثبيط إنزيمات هضم الكربوهيدرات (ألفا أميلاز وألفا غلوكوزيداز) في الدراسات كنهج علاجي تجاه التحكم أو علاج السكري من النمط ٢ بسبب قدرته على خفض ارتفاع السكر في الدم بعد الأكل. أثبتت الدراسات السابقة احتمالية مكافحة السكري باستخدام الثوم ضد السكري. لذلك، في هذه الدراسة، قمنا بتقييم الإمكانات المضادة لمرض السكر للثوم عند مقرن فحص الإنزيم في المختبر واكتشاف الأدوية الحاسوبية.

طرق البحث: استخدمنا في المختبر اختبار تثبيط ألفا أميلاز وألفا غلوكوزيداز لتقييم احتمالية مكافحة السكري وبعد ذلك تم استخدام التحليل الطيفي الكتلي للغاز، لتحديد وقياس المركبات المنشطة حيويا للمستخلص النباتي. بالإضافة إلى ذلك، تم توجيه المركبات المنشطة حيويا المحددة إلى الالتحام وتقييم الحرائك الدوائية.

النتائج: تظهر نتائجنا أن المستخلص الفينولي للثوم له إمكانات مثبطة عالية لألفا أميلاز وألفا غلوكوزيداز ذات دلالة إحصائية معتبرة وبطريقة تعتمد على الجرعة. ومن المثير للاهتمام، أن المستخلص ثبط ألفا غلوكوزيداز إلى نصف التركيز المثبط الأقصى من ٥٣.٧٥ ميكروغرام/مل وهو أعلى مما تم الحصول عليه للأكاربوز القياسي. كشفت محاكاة الالتحام عن موريلينول (قيم تقارب متوسطة تبلغ −٧.٣ كيلو كالوري/مول) وفينتولامين (قيم تقارب متوسطة تبلغ −٧.١ كيلو كالوري/مول) كأفضل روابط ألفا غلوكوزيداز. لم يكن لهذه المركبات تقارب جيد مع بقايا الموقع النشط للإنزيم فحسب، بل لها أيضا خصائص ممتازة تشبه الأدوية والحرائك الدوائية التي يمكن أن تستخدم في العيادة.

الاستنتاجات: يؤكد بحثنا على احتمالية الثوم كغذاء وظيفي للتحكم بالسكري من النمط الثاني ويحث على النظر في موريلينول وفينتولامين لتطوير الأدوية.

الكلمات المفتاحية: السكري من النمط الثاني؛ ألفا غلوكوزيداز؛ ألفا أميلاز؛ الثوم؛ موريلينول؛ فينتولامين

Abstract

Objectives: Inhibition of carbohydrate digestion enzymes (α-amylase and α-glucosidase) has been reported in studies as a therapeutic approach for the management or treatment of type 2 diabetes mellitus, owing to its potential to decrease postprandial hyperglycemia. The anti-diabetic potential of *Allium sativum* (also known as garlic) against diabetes mellitus has been established. Therefore, in this study, we assessed the antidiabetic potential of *A. sativum* using *in vitro* enzyme assays after which we explored computational modelling approach using the quantified GC-MS identities to unravel the key bioactive compounds responsible for the anti-diabetic potential.

Methods: We used *in vitro* enzyme inhibition assays (α-amylase and α-glucosidase) to evaluate antidiabetic

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potential and subsequently performed gas chromatography–mass spectroscopy (GC-MS) to identify and quantify the bioactive compounds of the plant extract. The identified bioactive compounds were subjected to *in silico* docking and pharmacokinetic assessment.

Results: *A. sativum* phenolic extract showed high dose-dependent inhibition of α-amylase and α-glucosidase ($p < 0.05$). Interestingly, the extract inhibited α-glucosidase with a half maximal inhibitory concentration of 53.75 μg/mL, a value higher than that obtained for the standard acarbose. Docking simulation revealed that morellinol and phentolamine were the best binders of α-glucosidase, with mean affinity values of −7.3 and −7.1 kcal/mol, respectively. These compounds had good affinity toward active site residues of the enzyme, and excellent drug-like and pharmacokinetic properties supporting clinical applications.

Conclusions: Our research reveals the potential of *A. sativum* as a functional food for the management of type 2 diabetes, and suggests that morellinol and phentolamine may be the most active compounds responsible for this anti-diabetic prowess. Therefore these compounds require further clinical asessment to demonstrate their potential for drug development.

Keywords: *Allium sativum*; α-Amylase; Garlic; α-Glucosidase; Morellinol; Type 2 diabetes

Introduction

Diabetes, a chronic metabolic disorder characterized by high blood glucose, may progress toward diabetic complications such as retinopathy, nephropathy, or neuropathy over time.[1–3] Diabetes affects all of society across national boundaries, regardless of socio-economic status, and its incidence has reached pandemic proportions. According to the International Diabetes Federation, the number of people with diabetes worldwide increased from 151 million in 2000 to 463 million in 2019, and has been estimated to increase to 700 million by 2045.[4] This forecast has prompted governments and other private stakeholders to take action in preventing future increment. The three most common types of diabetes are type 1, type 2, and gestational diabetes.[5,6] Whereas gestational diabetes is experienced during pregnancy and may compromise the health of both mothers and infants, type 1 diabetes is associated with the massive destruction of pancreatic β cells that produce insulin, Approximately 80–90% of the beta cells are destroyed early in the progression of this type of diabetes, thus leading to low or absent insulin production.[7] Type 1 diabetes is a major cause of death in childhood, and several symptoms such as excessive thirst, bet-wetting, and frequent urination have been associated with the incidence of this metabolic disorder.[7] Type 1 diabetes is usually diagnosed after the first episode of diabetic ketoacidosis, which may lead to death.[8] Type 2 diabetes is commonly seen among older adults and is characterized by the inability of the body cells to respond to insulin, a condition known as insulin resistance,[9] thus resulting in disorders in carbohydrate, lipid, and protein metabolism. The release of insulin prompts insulin-dependent cells to take up glucose; halt hepatic glucose production; inhibit the release of fat from adipose tissue; and mobilize free fatty acids for beta-oxidation.[10–12] Excess nutrients flows within the bloodstream and are regarded as the major causes of microvascular and macrovascular complications in diabetes.[13,14]

Several treatment plans such as lifestyle changes, oral drugs, and surgical interventions have been used to manage diabetes.[15] The advocacy for effective glycemic control and maintaining glycated hemoglobin near normal levels has placed pharmacological interventions at the forefront of therapies. For instance, metformin is the first-line treatment for patients with diabetes; when it is insufficient, other drugs can be added, such as sulfonylureas, glucagon-like peptide-1 (GLP1) analogs, di-peptidyl peptidase 4 inhibitors (DPP-4), SGLT-2 inhibitors, thiazolidinediones, and α-glucosidase inhibitors.[6,16] However, these drugs can have adverse effects; for example, the sulfonylureas potentiate the risk of cardiovascular disease,[16] α-glucosidase inhibitors can cause gastrointestinal tract discomfort,[17] rosiglitazone was recently withdrawn by the European Medicine Agency after careful examination of its risk-to-benefit-relationship, and pioglitazone was withdrawn by the French and German authorities in 2011.[15] Although these drugs have good potency, their adverse effects and high costs have resulted in a redirection of effort toward developing natural products of plant origin18.

The therapeutic potential of plants have evolved greatly, because some contain non-nutritive components that can positively affect human health. Their ability to evoke physiological responses in humans arises from their active components/secondary metabolites, which can act alone or synergistically in inducing therapeutic effects. Liu and coworkers have reported that more than 5000 bioactive compounds have been identified, and more than 25,000 bioactive compounds are believed to be present.[19] Studies from meta-analyses and clinical trials have indicated that plant-based functional foods and their extracts have potent antidiabetic functions.[20,21]

Allium sativum (garlic) is a widely used medicinal herb for the treatment of various human maladies. The activities of this herb are not limited to antioxidant[22] and antimicrobial[23] functions. *A. sativum* has been demonstrated to be a complementary medicine for the management/treatment of disorders such as cardiovascular disease,[24] male infertility,[25] fungal disease,[26] and rheumatoid arthritis.[27] Some researched extracts of this herb include methanol extract[28] and aqueous extract,[29] but limited data are available regarding the antidiabetic potential of the phenolic extract of this herb. To this end, we investigated the antidiabetic potential of *A. sativum* through a combination of *in vitro* wet-lab assays and computational drug discovery. We first generated phenolic extracts of the

plant and evaluated their inhibitory potential against α-amylase and α-glucosidase, the major carbohydrate metabolizing enzymes. We then profiled the bioactive constituents with gas chromatography−mass spectroscopy (GC−MS) for further investigations using computational modeling techniques including molecular docking simulation and *in silico* absorption, distribution, metabolism, excretion, and toxicity (ADMETox). Our overall goal was to critically elucidate the antidiabetic potential of this plant extract.

Materials and Methods

Experimental methods

Sample collection

Garlic bulbs (*A. sativum*) were obtained from Wazo market in March, 2020 in Ogbomosho, Oyo state, Nigeria. The plant was identified and authenticated by Dr. Nalza George, a taxonomist at the University of Lagos. A voucher specimen (LUH/9048) was deposited in the herbarium of the institute.

Chemicals and reagents

Pancreatic α-amylase, phosphate buffer, starch, di-nitro salicylic acid, distilled water, phosphate buffer, maltose, glucose oxidase phenol, 4-aminophenazone, and α-glucosidase were used.

Phenolic extract preparation

For the phenolic extraction, 32 g of ground and air-dried *A. sativum* bulbs was soaked in an extraction medium comprising a mixture of 1 M HCl and methanol (1:1) for 24 h and was subsequently filtered through Whatman filter paper. The filtrate was evaporated to dryness and then stored at room temperature. The resulting air-dried filtrate was dissolved in distilled water.

α-Amylase inhibition assays

The α-amylase inhibitory activity was determined according to a method described by Chatterton.[30] The aqueous extract (50−200 μL) and 500 μL of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing porcine pancreatic α-amylase (EC 3.2.1.1) (0.5 mg/mL) were incubated at 25 °C for 10 min. Subsequently, 50 μL of 1% starch solution in 0.02 M sodium phosphate buffer was added to the reaction mixture. Thereafter, the reaction mixture was incubated in a water bath at 25 °C for 10 min and cooled to room temperature. Subsequently, 200 μL of dinitrosalicylic acid was added. The reaction was stopped by incubation in a water bath at 100 °C for 5 min and was later cooled to room temperature. The reaction mixture was then diluted by addition of 2 mL of distilled water, and the absorbance was measured at 540 nm with a spectrophotometer. The reference sample included all other reagents and the enzyme but not the test sample. The α-amylase inhibitory activity was expressed as percentage inhibition:

$$\text{inhibition}(\%) = \frac{\textit{absorbance of reference} - \textit{absorbance of sample}}{\textit{absorbance of reference}}$$

α-Glucosidase inhibitory assays

The α-glucosidase inhibitory activity was determined according to the standard method of Dahlqvist.[31] Appropriate dilutions of the aqueous extracts (20−100 μL), 20 μL of α-glucosidase solution, 130−250 μL of 0.1 M phosphate buffer at pH 7.0, and 50 μL at 37 mM were incubated for 10 min at room temperature and then boiled at 100 °C for 5 min in a water bath. Subsequently, 20 μL of the mixture was collected from each test tube (sample, buffer, enzyme, and maltose); 2 mL of glucose oxidase-phenol and 4-aminophenazone was added to each test tube; and the reaction was then stopped by incubation in a water bath for 10 min. The absorbance was measured at 450 nm. The reference sample included all other reagents and the enzyme except for the test sample. The α-glucosidase inhibitory activity was expressed as percentage inhibition and was calculated with the formula described for α-amylase.

GC/MS analysis

The qualitative and quantitative analysis of the phenolic compounds of the samples was performed with a method reported by Kelly et al. (1994).[67] The phenolic compounds were extracted from each sample, as described by Kelly et al. (1994).[67] After extraction, the composition of the purified phenolic extracts (1 μL, 10:1 split) was analyzed through comparison with phenolic standards (Aldrich Chemical Co. Milwaukee, WI) and chromatography with standards on a Hewlett-Packard 6890 gas chromatograph (Hewlett-Packard Corp, Palo Alto, CA) equipped with a derivatized, nonpacked injection liner and an anRtx-5MS (5% diphenyl−95% dimethyl polysiloxane) capillary column (30 m length, 0.25 mm column id, and 0.25 μm film thickness), and detected with a flame ionization detector. The following conditions were used: separation; injection temperature, 230 °C; temperature ramping to 80 °C for 5 min and then to 250 °C at 30 °C/min; and detector temperature of 320 °C. The compounds were identified with the aid of the Wiley-9 database combined with the NIST-11 mass spectral database.

Computational methods

Protein and ligand preparation

α-Glucosidase was downloaded from the Protein Data Bank: PDB 2QMJ (Figure 1) with an atomic resolution of 1.9 Å. The native ligand and crystallographic water molecules were removed from the protein. Missing residues were fixed with "Misc residue" function in Autodock software.[32] The residues that composed the active site of the enzyme were noted after the protein coordinates were subjected to Computer Atlas of Surface Topography of proteins (CASTp) analysis.[33]

Ligand preparation

The Simplified Molecular-Input Line-Entry System (SMILES) coordinates of the bioactive compounds detected with GC−MS, including morellinol, estrone, butylated hydroxytoluene, phentolamine, 2-methoxybenzyl ester, *m*-toluic acid, *p*-toluic acid, nonivamide, 1-(4-hydroxy-3-methoxyphenyl)dec-3-en-5-one, gallic acid, 1,2 benzenediol, and 3,4-dihydroxymandelic acid, were obtained from PubChem[34] and converted to 3D PDB format with the Cactus online server (https://cactus.nci.nih.gov/translate/). The resulting structures were retained for subsequent simulations.

Molecular docking and analysis

Molecular docking simulation was performed with three software programs with different scoring functions: Autodock vina 1.5.6,[32] iGEMDOCK,[35] and Software for Adaptive Modeling and Simulation Of Nanosystems (Samson) 2020 R3.[32] The mean scores of the simulation results were determined, the top two compounds were considered hits, and their molecular interactions with the protein were visualized with BIOVIA discovery studio. The method used to dock the compounds was similar to those used in our previous studies.[36–38]

Drug likeness and ADMET profiling

The top three compounds (hits) were subjected to Molinspiration webserver (https://www.molinspiration.com/cgi-bin/properties) analysis to determine their drug-like properties. In addition, their pharmacokinetic and pharmacodynamic properties were predicted with admetSAR (http://lmmd.ecust.edu.cn/admetsar2/).

Results

Inhibition of carbohydrate metabolizing enzymes

The digestion of carbohydrates is a complex phenomenon involving various metabolic enzymes. In the enzyme machinery responsible for this event, α-glucosidase and α-amylase are commonly used for breaking down oligosaccharides into their monomeric glucose units, which are the absorbable form taken up by transporters on the intestinal epithelium and transferred into the blood.[39] Because high blood glucose facilitates susceptability to type 2 diabetes and its subsequent complications, preventing elevations in blood glucose is critical for diabetes therapy. The antidiabetic potential of medicinal plant extracts is tested through assays of their inhibitory activity against α-glucosidase and α-amylase *in vitro*. To that end, using the methods of Chatterton et al.[30] and Dahlqvist,[31] we determined the antidiabetic activity of the phenolic extract of *A. sativum* against the two enzymes (Figure 2). We examined the effects of the extract on the two enzymes at different concentrations, such as 50, 100, 150, 200, and 250 µL. The extract inhibited both enzymes in a dose-dependent manner. Specifically, the extract had a half maximal inhibitory concentration (IC_{50}) of 139.0 µg/mL against α-amylase, whereas that of the standard was 93.65 µg/mL, thus suggesting good inhibitory potential. By contrast, the extract inhibited α-glucosidase with an IC_{50} of 37.68 µg/mL and thus was far more potent than the acarbose 53.75 µg/mL standard. On this basis, acarbose was used as the receptor protein for computational investigations.

GC–MS analysis

Because of the excellent inhibitory potential of *A. sativum* on the carbohydrate metabolizing enzymes α-glucosidase and α-amylase, we subsequently determined the constituents of the extract. The retention times, names, and peak areas of the components of the test extract were ascertained (Table 1), and the spectral signature of the components of the extract is shown in Figure 3. The spectrum showed 29 peaks, but only several pharmacologically active compounds were identified when the mass spectra were compared with the National Institute of Standards Technology (NIST) library. Compounds with high concentrations in the extract included *p*-toluic acid (0.688%) and 1-(4-hydroxy-3-methoxyphenyl)dec-3-en-5-one (0.698%). Other compounds with very low abundance included phentolamine (0.098%) and morellinol (0.139%), among many others.

Molecular docking

Docking of compounds into the active sites of protein targets is a common practice in drug discovery.[40] Information obtained from docking provides a clear pointer as to which compound has the highest affinity for a protein target. The affinity toward a target in the virtual platform provides an indication of the possible potency of the compounds in cellular assays. To identify the compound potentially responsible for the carbohydrate enzyme inhibition of the extract, we docked the 12 virtual compounds into the active site of α-glucosidase. We combined results from three software programs (Table 2) to determine the best binders of the target protein. After the scaling of the results from the iGEMDOCK software by 10 to fit the range of the results obtained from vina and Samson software, we computed a mean value for each compound and the standard. From the mean value, morellinol ranked first, with a binding energy of −7.3 kcal/mol, and was followed by phentolamine, with a mean value of −7.1 kcal/mol. At the individual software level, these compounds ranked highest among all compounds. For instance, morellinol ranked first in vina and Samson software but second in iGEMDOCK. In addition, phentolamine ranked first in iGEMDOCK, third in vina, and fourth in Samson software. The binding energy values of estrone and 3,4-dihydroxymandelic acid were −6.5 kcal/mol in each case. Nonivamide and *p*-toluic acid had a value

Figure 1: 3D structure of α-glucosidase (2QMJ).

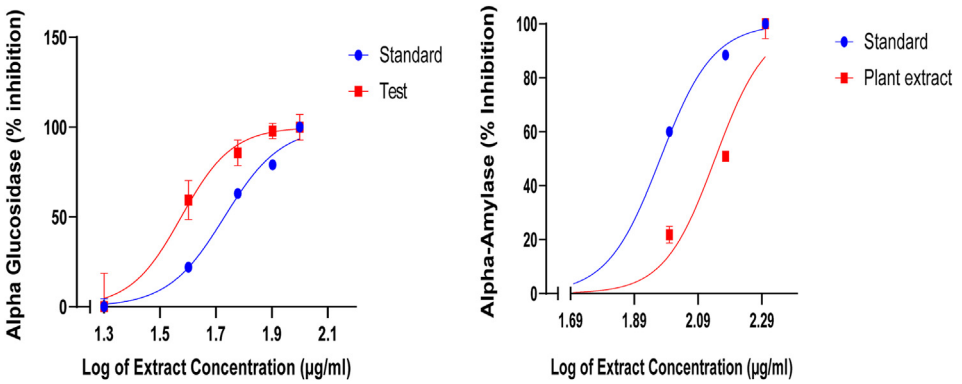

Figure 2: α-Glucosidase and α-amylase inhibition of *A. sativum* extract.

Table 1: Important compounds identified in GC–MS analysis of phenolic extract of *A. sativum*.

Compounds	Retention time	Peak area (%)	PubChemID
Morellinol	13.941	0.139	5364072
Estrone	8.250	0.206	5870
Phentolamine	25.889	0.098	5775
Nonivamide	18.904	0.209	2998
2-Methoxybenzyl ester	20.303	0.147	11135351
Gallic acid	10.341	0.222	370
m-Toluic acid	18.417	0.357	7418
p-Toluic acid	18.947	0.688	7470
Butylated hydroxytoluene	20.250	0.147	31404
1-(4-Hydroxy-3-methoxyphenyl)dec-3-en-5-one	18.064	0.698	11694761
3,4-Dihydroxymandelic acid	13.278	0.219	85782
1,2 Benzenediol	27.708	0.031	289

of −6.0 kcal/mol, whereas the values for 2-methoxybenzyl ester and *m*-toluic acid were −0.2 kcal/mol lower, thus suggesting energy values of −5.8 kcal/mol. Butylated hydroxytoluene and 1-(4-hydroxy-3-methoxyphenyl)dec-3-en-5-one had the lowest score and a value of −5.2 kcal/mol. In molecular docking, compounds with high energy binding values are usually considered favorable for inhibiting target proteins.[38,41] Morellinol and phentolamine, with binding energy values of −7.3 and −7.1 kcal/mol, respectively, were considered the compounds potentially responsible for the extract's inhibition of carbohydrate metabolizing enzymes and were considered in further computational simulations to determine their drug-likeness and pharmacokinetic characteristics.

Phentolamine and morellinol showed excellent binding to α-glucosidase (Figure 4). Specifically, morellinol formed hydrogen bonds with Asp203 and Asp542, an interaction favored by the carboxylic R-group of the amino acid. Other amino acids, such as Trp406, Ile328, Phe575, His600, Tyr299, and Phe450, held morellinol in place with alkyl-type interactions. Similarly, phentolamine interacted with the ligand via an alkyl-type interaction network. The amino acids responsible for this interaction included Tyr299, Phe575, Trp441, Ile364, and Trp406. The interaction networks formed by these two hits were relevant to the inhibition of α-glucosidase, because they were similar to that of acarbose. This finding is important, because a recent study has stressed the importance of appropriate fitting with relevant amino acid residues in virtual screening.[42]

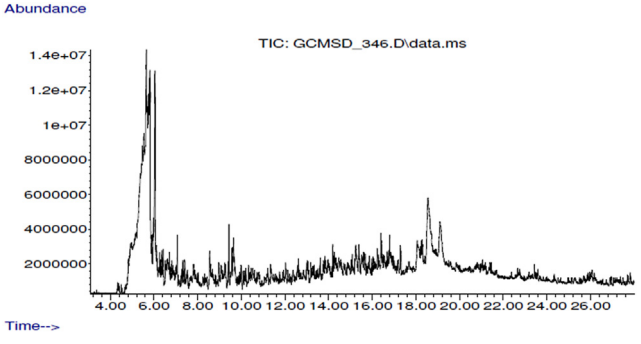

Figure 3: GC–MS analysis of phenolic extract of *A. sativum*.

Table 2: Binding energy values of the compounds, as obtained from Autodock vina, iGEMDOCK, and Samson.

Compounds	Vina (kcal/mol)	iGEMDOCK (kcal/mol)	Samson (kcal/mol)	Mean value
Acarbose	−6.6	−9.7	−7.04	−7.8
Morellinol	−7.2	−7.9	−6.86	−7.3
Estrone	−7.0	−5.6	−6.77	−6.5
Phentolamine	−6.5	−8.3	−6.59	−7.1
3,4-Dihydroxymandelic acid	−6.3	−6.5	−6.67	−6.5
2-Methoxybenzyl ester	−6.0	−5.1	−6.40	−5.8
1-(4-Hydroxy-3-methoxyphenyl)dec-3-en-5-one	−5.9	−7.9	−6.08	−5.2
Butylated hydroxytoluene	−5.3	−5.2	−5.12	−5.2
m-Toluic acid	−5.1	−6.2	−6.04	−5.8
Gallic acid	−5.0	−6.6	−5.96	−5.7
Nonivamide	−4.8	−6.4	−5.96	−6.0
p-Toluic acid	−4.7	−7.7	−5.60	−6.0
1,2 Benzenediol	−4.4	−6.0	−5.64	−5.4

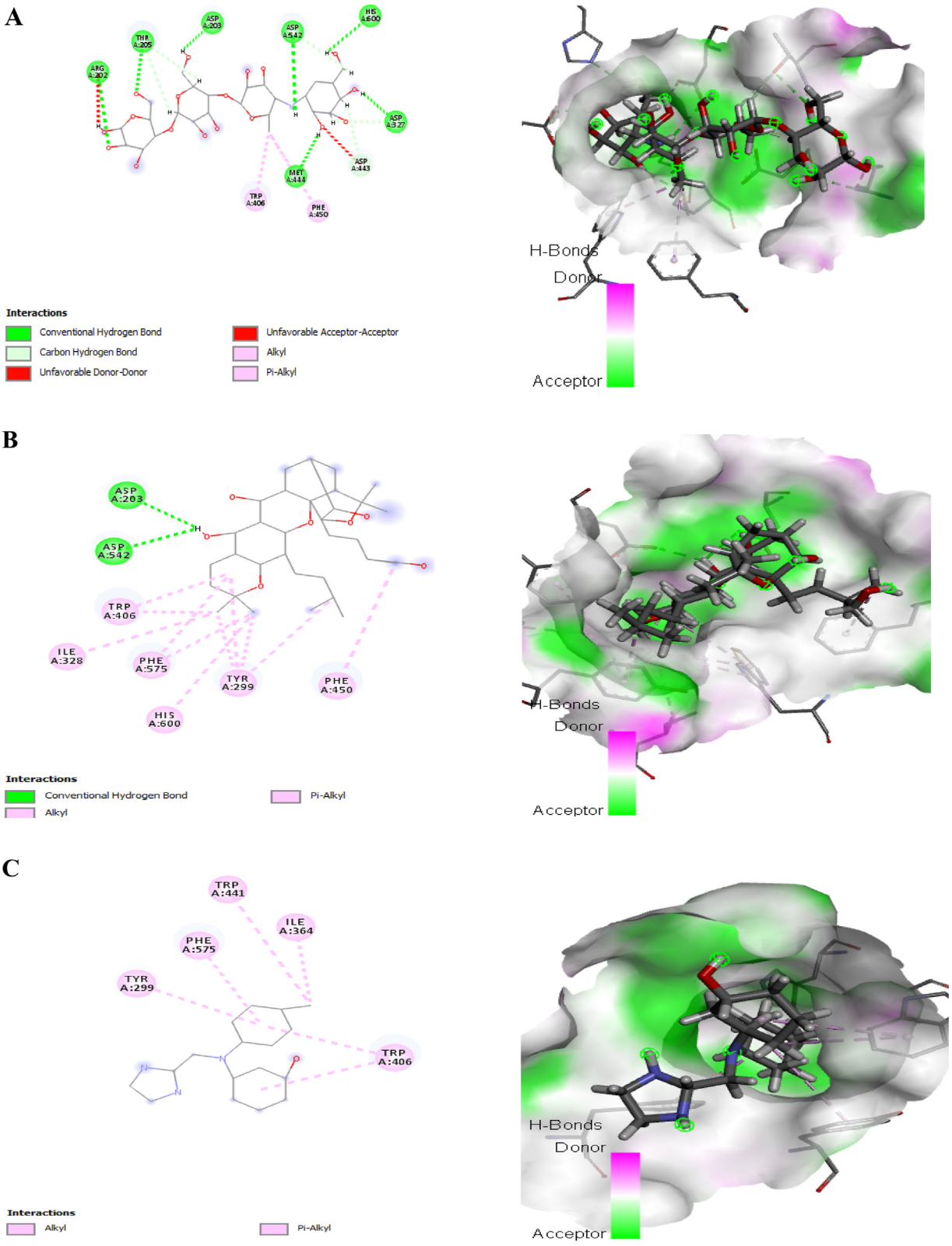

Figure 4: Molecular interactions of the selected bioactive compounds and standard (acarbose) in the active site of α glucosidase. (A) Acarbose; (B) morellinol; (C) phentolamine. Interactions are shown in 2D (left) and 3D within the active pocket of the enzyme (right).

Table 3: ADMETox properties and drug-likeness characteristics of drug candidates.

Parameters	Morellinol	Phentolamine	Acarbose
Human intestinal absorption	+	+	−
Caco-2	−	+	−
Blood−brain barrier	+	+	−
Human oral bioavailability	−	−	−
Carcinogenicity	−	−	−
Ames mutagenicity	−	+	+
Human ether-a-go-go inhibition	−	−	+
Hepatotoxicity	+	−	+
Water solubility (log S)	−4.37	−2.694	−1.383
Lipinski rule of five			
Molecular weight	546.66	281.36	645.61
Number of hydrogen bond donors	2	2	14
Number of hydrogen bond acceptors	7	4	19
Octanol−water partition coefficient	5.84	3.25	−8.56

ADMETox profiling and Lipinski's drug-likeness property

Screening for the pharmacokinetic properties and drug-likeness potential is an important task in drug discovery before drug approval.[43] In early stages of drug discovery, high-throughput screening assays of the different pharmacokinetic parameters are conducted; this labor-intensive and capital-intensive work is now been augmented with computationally based ADMETox predictions. *In silico* ADMETox prediction assesses the potential disposition and pharmacokinetics of drug candidates within just one global model instead of designing assays to test each parameter in experimental settings. The two hits obtained from the GC−MS fingerprints of the phenolic extracts of *A. sativum* were subjected to analysis with the admetSAR algorithm[44] to determine their future potential as drug candidates.

Pharmacokinetically, morellinol and phentolamine have good intestinal absorption and can cross the blood−brain barrier. However, whereas phentolamine can cross a Caco-2 monolayer, no permeability is observed for morellinol. Caco-2 cells and the human intestinal absorption parameters are the same features used to predict the ability of a drug candidate to permeate the human intestinal epithelium.[45] The reason for the contrasting results obtained with these parameters is unclear, but overall, both candidates appear to have good intestinal absorption features. Both candidates have poor oral bioavailability and thus might not appear favorable for development as oral drugs. Nonetheless, examining how they conform to Lipinski's rule provides more insight and enables reasonable conclusions to be drawn regarding their oral bio-accessibility. The propensity of both compounds to cross the blood−brain barrier is a likely undesirable feature, because they might interact with proteins responsible for critical pathways and thus elicit off-target effect. However, this propensity could be fixed during the lead optimization stage.

An important toxicological parameter assayed in drug development is inhibition of human ether-a-go-go (hERG), a potassium channel that facilitates cardiac repolarization.[46] Compounds that interfere with hERG lengthen the QT interval in electrocardiograms and also increase the risk of fatal ventricular arrhythmias.[47] Our toxicological endpoint of morellinol and phentolamine for the inhibition of the hERG indicated no propensity toward the channel; thus, these compounds are good prospective drug candidates. On the one hand, both compounds have not been found to be carcinogenic. On the other hand, whereas morellinol can cause hepatotoxicity, phentolamine is not hepatotoxic. To determine whether a drug candidate will be orally bioavailable, several rules have been suggested, depending on the drug class. For small-molecule inhibitors, a common measure of bioavailability of a compound is Lipinski's rule.[48] On the basis of careful analysis of orally active drugs by Lipinski and colleagues, a prospective drug candidate with prospects for oral administration should not violate more than one of the following: molecular weight less than 500 Da; number of hydrogen bond donors $\leq$5; number of hydrogen bond acceptors $\leq$10; and octanol−water partition co-efficient $\leq$5. As shown in Table 3, phentolamine did not violate any of the rules. However, morellinol violated the molecular weight and the octanol−water partition coefficient rules. Overall, these excellent features suggest that morellinol and phentolamine may be good candidates for drug development.

Discussion

Type 2 diabetes is one of the major causes of death worldwide and was responsible for approximately 1.5 million deaths in 2019. It is characterized by high blood glucose due to the inability of insulin-dependent cells to use glucose as metabolic fuel. This condition, if not treated, can result in microvascular and macrovascular complications, such as nephropathy and neuropathy.[49] The most globally known medical intervention is maintaining normal levels of blood glucose with classes of drugs including biguanides, sulfonylureas, and SGLT-2 inhibitors.[6,16] Most of these drugs work by decreasing glucose in the blood after hyperglycemia. One critical therapeutic approach is regulating glucose entry into the bloodstream. Inhibition of enzymes that convert short-chain oligosaccharides to glucose is a putative approach, and α-glucosidase/α-amylase inhibitors have been effective in that regard.[17,50]Whereas conventional therapies are bound to have adverse effects, medicinal plants provide a good source of potent natural products with glucose-lowering potential. In the past, we have reported the antidiabetic activity of various natural products and plant extracts.[51−55] In this work, we sought to elucidate the antidiabetic potential of the phenolic extract of *A. sativum*. *In vitro* α-amylase and α-glucosidase evaluation revealed a dose-dependent relationship in the inhibitory potential of the extract against the enzymes, with IC_{50} values of 37.68 and 139.0 μg/mL for glucosidase and amylase, respectively. In a previous study, aqueous extract of *A. sativum* has been found to dose-dependently inhibit α-amylase and α-glucosidase, with IC_{50} values of 8.19 and 4.50 μg/mL, respectively.[56] Similarly, *A. sativum* bulbs

inhibited both enzymes with IC_{50} values similar to that of the acarbose standard.[50] Therefore, we reasoned that the phenolic extract of *A. sativum* has antidiabetic potential and that certain bioactive compounds might be responsible for that activity.

To provide verification, we profiled and characterized the bioactive constituents of the extract with GC–MS and obtained 12 active compounds (Figure 1). The compounds *p*-toluic acid and 1-(4-hydroxy-3-methoxyphenyl)dec-3-en-5-one were the most abundant constituents of the extract, because of their relatively high peak area percentages; this abundance may translate to their contribution to the extract's activity. To our knowledge, no data have been published regarding the constituents of the phenolic extract of *A. sativum* on the basis of GC–MS. However, previous results profiling the constituent of this bulb using other extracts have been reported,[57,58] and indicated a composition different from our results in Table 1. Thus, solvent appears to substantially affect the extraction. A GC–MS analysis of ethanol extract of *A. sativum* bulb at 25% concentration has revealed different bioactive constituents.[59] Thus, not only the extraction solvent but also the concentration of the solvent used for the extraction determine the phytochemicals extracted. The resulting active compounds were further subjected to *in silico* docking to determine the putative compound responsible for the antidiabetic activity.

Molecular docking simulation results may be fraught by false-positive results, thus returning mediocre compounds as hits. To minimize this effect, computational chemists often use different docking algorithms in their docking analyses, such that assessment of the docking results is based on the compound's performance across all software programs.[60] We docked the 12 compounds into the active site of α-glucosidase by using different software with distinctive algorithms (Table 2). Morellinol ranked first according to the mean binding energy value and was followed by phentolamine; these compounds were considered potent hits for this enzyme and its activity. These compounds formed a network of interactions with active site residues of the α-glucosidase enzyme in a manner similar to that of the standard acarbose, thus suggesting an ability to prevent the hydrolysis of oligosaccharides and confer overall inhibition of the activity of the enzyme. Importantly, morellinol and phentolamine did not have significant peak area percentages from the GC–MS results. Moreover, *p*-toluic acid and 1-(4-hydroxy-3-methoxyphenyl)dec-3-en-5-one were the most abundant compounds, but their affinity for the α-glucosidase enzyme in the virtual platform was too low. An interpretation of these findings may be that the abundance of a compound in a plant extract does not necessarily affect the therapeutic potential of the extract.

Studies have shown that phentolamine (a phenolic imidazoline) is a potent insulin secretagogue and hypoglycemic agent in fed wild type mice, and its antidiabetic potential has clearly been demonstrated.[61] Although morellinol has not been reported as an antidiabetic compound, the parent compounds from which it is derived (tocopherol or vitamin E) have been reported to have anti-diabetic potential based on their hypoglycemic effects.[62,63] We believe that the nature of the ligand at the active site of the protein is more critical than the concentration to the complementarity and affinity, thus potentially explaining why morellinol and phentolamine, despite low concentrations, showed better interaction with the α-glucosidase active pocket than *p*-toluic acid and 1-(4-hydroxy-3-methoxyphenyl)dec-3-en-5-one, with high concentration.

Drug discovery efforts no longer prioritize potency alone therefore, drug likeness and pharmacokinetic properties are now being pursued in tandem with potency to prevent late-stage attrition. We thus determined the druglike properties, pharmacokinetic attributes, and some toxicological end points of the hit compounds (Table 3). Both compounds possessed outstanding properties that may enable clinical applications for the treatment of type 2 diabetes. Phentolamine is well known in the treatment of various human diseases and conditions such as hypertension,[64] erectile dysfunction,[65] and myocardial injury.[66] However, to our knowledge, we are the first group to report morellinol as a therapeutic agent and a potent inhibitor of α-glucosidase.

Conclusions

A. sativum phenolic extract inhibited pancreatic α-amylase and intestinal α-glucosidase, which are the key enzymes associated with carbohydrate digestion. In addition, our GC–MS analysis and computational simulating methods revealed that morellinol and phentolamine are the key bioactive compounds with strong α-glucosidase inhibitory affinity and pharmacokinetic properties, as compared with acarbose. Thus, our data establish *A. sativum* bulbs as a functional food for the management/treatment of type 2 diabetes. However, more studies is required before this plant source and its bioactive compounds can be clinically applied.

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Conflict of interest

The authors have no conflict of interest to declare..

Ethical approval

Not applicable.

Consent

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Authors contributions

TIA—Conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, visualization, project administration, writing, editing and proofreading; **IDB**—validation, data curation, visualization project administration, writing, reviewing, and editing; **AT**—formal analysis, investigation, data curation, and project administration; **JA**—validation and visualization;

JOF—data curation and visualization; **CDU**—formal analysis, investigation, and project administration; **AKO**—formal analysis, investigation, visualization, and reviewing; **MA**—data curation, visualization, and project administration; **AAT**—formal analysis, investigation, visualization, and project administration. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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