Kastamonu Uni., Orman Fakültesi Dergisi, 2022, 22(3): 202-216 Research Article
Kastamonu Univ., Journal of Forestry Faculty
Doi:10.17475/kastorman.1215281

In Silico Proof of the Effect of Quercetin and Umbelliferone as Alpha-Amylase Inhibitors, Which Can Be Used in the Treatment of Diabetes

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Received Date: 16.03.2021 Accepted Date: 27.07.2022

Abstract

Aim of study: The aim of this study is to show the *in silico* evidences about the potential use of quercetin and umbelliferone as α-amylase inhibitors, which is important for the treatment of diabetes.

Material and methods: The possible conformations and orientations of quercetin, umbelliferone, and acarbose, in binding to the active sites of alpha-amylase, were analysed by CASTp server. The molecular dockings of these compounds to the potential active site were performed by AutoDock Tools to obtain 3D interactions and binding energies. In addition, the interaction scores were calculated by iGEMDOCK. The 2D enzyme-inhibitor interactions, which clearly show the interactions at the active sites, were analysed by LigPlot$^+$. The drug-likeness properties of quercetin and umbelliferone were compared to acarbose by DruLiTo software and SWISSADME server. The absorption, distribution, metabolism, excretion, and toxicity (ADMET) scores, which present the pharmacokinetic properties of the compounds were analysed by ADMETLab, admetSAR, and PreADMET servers

Main results: As a result, the α-amylase inhibitor activity and the potential use of quercetin and umbelliferone were proved *in silico*.

Highlights: The results of the study clearly put forward that quercetin and umbelliferone could have possible medicinal use in the treatment of diabetes.

Keywords: Quercetin, Umbelliferone, Acarbose, Alpha-Amylase, Inhibitor, Molecular Docking, ADMET

Diyabet Hastalığının Tedavisinde Kullanılabilecek Alfa-Amilaz İnhibitörü Olarak Kuersetin ve Umbelliferonun Etkisinin *In Silico* Kanıtı

Öz

Çalışmanın amacı: Bu çalışmanın amacı, diyabet tedavisinde önemli α-amilaz inhibitörü olarak, kuersetin ve umbelliferonun potansiyel kullanımına ilişkin *in silico* kanıtları ortaya koymaktır.

Materyal ve yöntem: Kuersetin, umbelliferon ve ticari bir α-amilaz inhibitörü olan akarboz'un alfa -amilaza bağlanması sırasındaki olası konformasyonları ve yönelimleri taranmadan önce, enzimin potansiyel aktif bölgeleri CASTp 3.0 sunucusu tarafından tahmin ve analiz edilmiştir. Bu bileşiklerin potansiyel aktif bölgelerle 3D etkileşimleri ve bağlanma enerjilerini elde etmek için moleküler kenetlenme (docking) analizi Auto Dock Tools v.1.5.6 kullanılarak yapılmıştır. Ayrıca etkileşim skorları iGEMDOCK v.2.1 ile hesaplanmıştır. Aktif bölgelerdeki etkileşimleri açıkça gösteren 2D enzim-inhibitör etkileşimleri LigPlot$^+$ v.2.2 ile analiz edilmiştir. Kuersetin ve umbelliferonun ilaç benzerliği özellikleri DruLiTo yazılımı ve SWISSADME sunucusu tarafından akarboz ile karşılaştırılmıştır. Bileşiklerin farmakokinetik özelliklerini gösteren absorpsiyon, dağılım, metabolizma, boşaltım ve toksisite (ADMET) skorları ADMETLab, admetSAR ve PreADMET sunucuları kullanılarak analiz edilmiştir.

Temel sonuçlar: Sonuç olarak, kuersetin ve umbelliferonun α-amilaz inhibitör aktivitesi ve bunların kullanım potansiyelleri *in silico* kanıtlanmıştır.

Araştırma vurguları: Çalışmanın sonuçları, kuersetin ve umbelliferonun diyabet tedavisinde olası bir tıbbi kullanıma sahip olabileceğini açıkça ortaya koymaktadır.

Anahtar Kelimeler: Kuersetin, Umbelliferon, Akarboz, Alfa Amilaz, İnhibitör, Moleküler Kenetlenme, ADMET

Citation (Atıf): Altuner, E. M. (2022). *In Silico* Proof of the Effect of Quercetin and Umbelliferone as Alpha-Amylase Inhibitors, Which Can Be Used in the Treatment of Diabetes. *Kastamonu University Journal of Forestry Faculty*, 22 (3), 202-216.

Introduction

Diabetes is a combination of some metabolic problems, which triggers chronic lifelong complications that cause an increase in glucose levels in the blood. (Elmiar et al., 2018). Having elevated glucose levels will not only cause short-term but also long-term complications as well (White, 2015). Short-term complications include psychosocial problems, hypoglycaemia, and diabetic ketoacidosis, whereas long-term complications can be grouped under two main categories, such as microvascular (retinopathy, nephropathy, and neuropathy) and macrovascular (coronary artery disease, cerebrovascular disease, and peripheral vascular disease) complications (White, 2015; Elmiar et al., 2018)

Unfortunately, the prevalence of diabetes is increased tremendously all around the world (Nyenwe et al., 2011). There are several strategies in the treatment of patients with diabetes, such as monitoring glucose levels, medical nutritional therapy, exercise, bariatric surgery, and pharmacotherapy as well (Nyenwe et al., 2011).

One of the strategies to manage the glucose levels in the blood is using inhibitors for α-glucosidase and α-amylase enzymes, which take a role in digesting carbohydrates in the body. It is known that using such inhibitors reduces the glucose level of blood significantly after consuming food, thus it is an effective strategy for managing blood glucose levels (Tundis et al., 2010).

Today, there is a remarkable increase in the research about natural compounds, which modulate the physiological effects both in curing and preventing diabetes. Most researchers are working on the plant-derived α-amylase and α-glucosidase inhibitors, which offer a successful strategy for controlling hyperglycaemia (Kwon et al., 2008; Tundis et al., 2010).

Some plant-derived compounds have already been screened for their α-amylase inhibitory activities. In one of these studies cinnamic acid, umbelliferone, quercetin, naringenin, and phloridzin were tested for their α-amylase inhibition activity, and observed that quercetin and umbelliferone were effective α-amylase inhibitors (Menshaz & Altuner, 2020).

Quercetin is a flavonol, one of the plant flavonoids, which is classified under polyphenols. It has a bitter taste and can be extracted from many types of fruits, vegetables, seeds, and grains (Petrus et al., 2011). Quercetin is known to have antioxidant activity and also can activate or inhibit several enzymes (Murakami et al., 2008; Russo et al., 2014).

Umbelliferone is a 7-hydroxycoumarin, which can be extracted from plants classified under Rutaceae and Apiaceae (Umbelliferae) families such as coriander and carrot (Mazimba, 2017). Umbelliferone is also known to inhibit some enzymes (Poirier, 2003).

The aim of this study is to support the inhibition activity of quercetin and umbelliferone against α-amylase, which was previously proved experimentally with some *in silico* tests.

Materials and Method

Target Enzyme Preparation

The human salivary amylase X-ray crystal structure (PDB ID: 1SMD) (Ramasubbu et al., 1996) was downloaded from the Protein Data Bank (http://www.rcsb.org/structure/1SMD) (URL-1, 1996). This protein structure contains a Cl^- ion, which is bound to Arg195, Asn298, and Arg337, and a Ca^{2+} ion bound to Asnl00, Arg158, Asp167, and His201. Thus, before screening and molecular docking, ions and H_2O molecules were deleted from the structure of the enzyme by Discovery Studio Visualizer v.20.1.0.19295 (Biovia, Dassault Systèmes, 2019).

Then the refined 3D structure of α-amylase was edited by Auto Dock Tools v.1.5.6, in which polar H atoms and charges were added.

Compound Preparation

The 3D structure of quercetin, umbelliferone, and acarbose were downloaded from PubChem (National Institute of Health). These compounds were processed by Open Babel v.3.1.1 (O'Boyle et al., 2011) in order to protonate at pH 7.4, assign Gasteiger charges and generate 3D coordinates (Joshi et al., 2020).

Prediction and Analysis of Active Sites

To predict the pockets of α-amylase and amino acids, which are crucial for enzyme-substrate/inhibitor interaction, CASTp v.3.0 was used (Tian et al., 2018). The pockets were visualised by UCSF Chimera v.1.14 software (Pettersen et al., 2004).

The binding pocket, which is responsible for binding α-amylase and its substrate, obtained from CASTp was used in molecular docking analysis. It is previously published that α-amylase contains three acidic groups at its active site, which are Asp 197, Glu 233, and Asp 300 (Qian et al., 1994).

It is known that during interaction H bonds form between the NH group of the acarviosine group and the carboxylic oxygens of Glu233 and Asp300. The inhibitors bind to Asp197, which is present on the other side of the active site. As inhibitors bind to the enzyme, they induce structural changes at the active site and also they cause a rotation in the side chain of Asp300, which leads to forming a strong Van der Waals interaction with the imidazole ring of His299. Also, His101, His201, His299, and His305 are important in forming hydrogen bonds with inhibitors (Qian et al., 1994). As a reason for these, Asp197, Glu233, Asp300, and their surroundings were accepted as reference amino acids, which could affect the interaction between enzymes and inhibitors, and used in molecular docking studies.

Virtual Screening by Molecular Docking

To obtain possible conformations and orientations of quercetin, umbelliferone, and acarbose, when binding to α-amylase, virtual screening by molecular docking was performed by using AutoDock Vina developed for Auto Dock Tools v.1.5.6 (Trott & Olson, 2010)

The best binding location and position of the compounds were determined by their binding affinities.

In this process, firstly a virtual screening for compounds was performed by rigid molecular docking with a grid box covering the location of previously mentioned amino acids. In the application of molecular docking, acarbose, quercetin, and umbelliferone were kept flexible, while α-amylase was rigid. As a final point, the lowest binding energies of all possible conformations and orientations were chosen for further analysis.

In addition to Auto Dock Tools, the interaction scores of quercetin, umbelliferone, and acarbose were determined by iGEMDOCK v.2.1 (Graphical Drug Design system for Docking, Screening, and Post-analysis) (Hsu et al., 2011).

Validation of Docking Protocol

Acarbose, a co-crystallized ligand present in the two other proteins' structures (PDB ID: 1XD0 and 1XD1), was obtained from the Protein Data Bank and extracted from the protein's active site. The docking protocol was validated by redocking the ligand (acarbose) back into the binding site of alpha-amylase and comparing the predicted pose to the experimental or crystallographic pose.

Visualisation

The 2D interactions of the compounds with α-amylase were analysed by using LigPlot$^+$ v.2.2 software (Wallace et al., 1995). It gives better visualization to understand the nature of interactions between inhibitor and enzyme in the docking, indicating the hydrogen bonds, and hydrophobic bonds with the length of bonds.

Drug-likeness Evaluation

The drug-likenesses of the compounds were evaluated by five rules of Lipinski according to their structural and physicochemical properties (Leeson, 2012). The drug-likeness properties of quercetin and umbelliferone were compared to acarbose by DruLiTo software (Drug Likeness Tool, 2018) and SWISSADME server (Daina et al., 2017).

ADMET Analysis

The absorption, distribution, metabolism, excretion, and toxicity (ADMET) scores, which present the pharmacokinetic properties of the compounds were predicted by the ADMETLab server (Dong et al., 2018), admetSAR (Cheng et al., 2012; Yang et al., 2019) and PreADMET (Lee et al., 2003; Lee

et al., 2004). The data were also supported by SWISSADME.

By using the above-mentioned tools, some parameters like AMES toxicity, carcinogenicity, human Ether-a-go-go-Related Gene (hERG) inhibition, cytochrome p450 (CYP450) substrate/inhibitor, P-glycoprotein substrate/inhibitor, human colon adenocarcinoma cells (CaCo-2) permeability, human intestinal absorption (HIA), blood-brain barrier (BBB) and MDCK (Madin-Darby canine kidney cells) permeability were predicted.

Results and Discussion

Target Enzyme Preparation

As it was mentioned before performing any screening and molecular docking analysis ions and water molecules in the X-ray crystal structure of the enzyme were removed (Figure 1A).

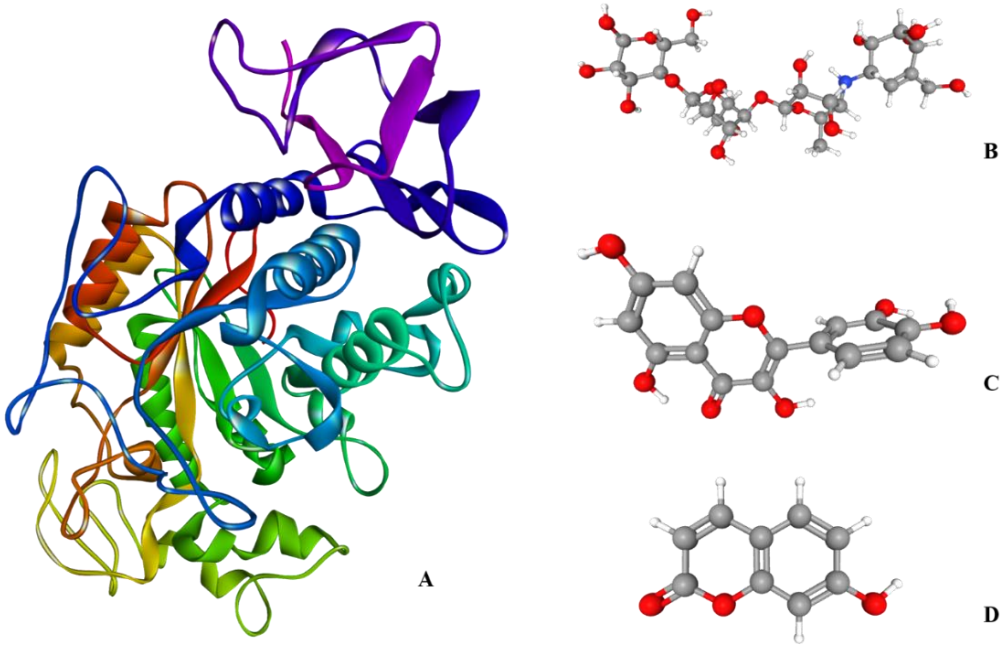

Figure 1. 3D Structure of A. α-amylase, B. acarbose, C. quercetin, D. umbelliferone

Compound Preparation

The 3D structures of acarbose, quercetin, and umbelliferone, which were downloaded from PubChem (National Institute of Health) are given in Figures 1B, C, and D respectively.

Prediction and Analysis of Active Sites

The pockets, where acarbose, quercetin, and umbelliferon can bind were predicted by CASTp v.3.0 (Tian et al., 2018). According to the results 86 possible pockets are present in α-amylase, which have solvent accessible (SA) area ranging between 159.662 and 0.000 $Å^2$ and solvent accessible (SA) volume between 177.930 and 0.000 $Å^3$.

But 7 of them can be accepted as major pockets having volume (SA) higher than 10.000 $Å^3$. The locations of these seven major pockets are shown in Figure 2A and the data regarding these pockets are given in Table 1.

The pocket, where substrate binds were visualised by UCSF Chimera v.1.14 software (Pettersen et al., 2004) (Figure 2B), and this pocket was used for further analysis.

The pocket chosen for further analysis has the following amino acid residues; Trp58, Trp59, Tyr62, Gln63, His101, Leu162, Ser163, Leu165, Arg195, **Asp197**, Ala198, **Glu233**, His299, **Asp300**, and His305, where the bold amino acid residues are involving in active interaction with the natural substrate.

Table 1. Data about major pockets.

ID	MS Volume	SA Volume	Pocket MS Area	Pocket SA Area	# openings	Mouth MS Area	Mouth SA Area
1	457.9	177.9	241.0	159.7	1	130.3	61.2
2	226.9	97.4	111.6	80.0	1	138.7	68.1
3	343.4	93.9	234.8	136.8	1	110.2	43.7
4	220.1	43.2	177.4	89.9	3	112.9	33.1
5	106.2	18.7	91.1	38.5	1	50.2	15.2
6	72.6	12.8	61.7	25.9	1	54.1	14.3
7	81.0	11.2	75.9	26.8	1	32.0	7.9

MS volume: pocket volume based on the molecular surface; SA volume: pocket volume based on the solvent-accessible surface; pocket MS area: pocket molecular surface area; pocket SA area: pocket solvent-accessible surface area; # openings: number of mouths, or openings to the external molecular surface; mouth MS area: total area of mouth opening(s) based on the molecular surface; mouth SA area: total area of mouth opening(s) based on the solvent-accessible surface

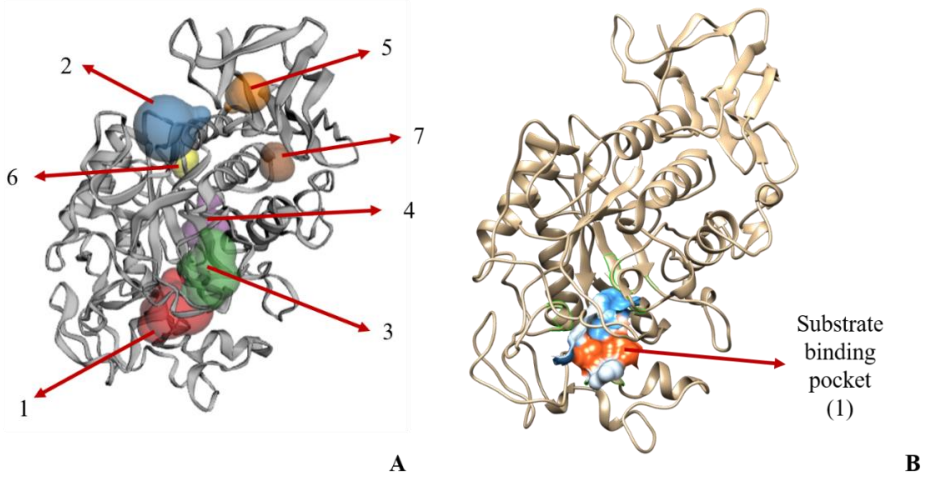

Figure 2. A Major pockets of α-amylase (Numbers are showing pocket IDs), B. Substrate binding pocket

The positions of these amino acids are shown in Figure 3. The molecular surface area of this pocket was observed as 241.0, the solvent-accessible surface area as 159.7, the volume based on the molecular surface as 457.9, and the volume based on the solvent-accessible surface as 177.9 (Table 1).

Molecular Docking Analysis

Firstly, the molecular docking protocol ran for a reference inhibitor (acarbose) to find interaction with the active site of α-amylase. After processing a molecular docking analysis, the interaction between acarbose and α-amylase was observed as it was previously proposed by Qian et al. (1994). The interaction between acarbose and α-amylase at the active site is given in Figure 4A and the 2D interaction of acarbose with α-amylase is given in Figure 4B.

Secondly, quercetin and umbelliferone were docked in the active site of α-amylase to predict the best possible binding pose of these compounds for higher binding scoring. The interaction between quercetin and α-amylase at the active site is given in Figure 5A and the 2D interaction of quercetin with α-amylase is given in Figure 5B, where the interaction between umbelliferone and α-amylase at the active site is given in Figure 6A and the 2D interaction of umbelliferone with α-amylase is given in Figure 6B.

Figure 4 shows the interaction between acarbose and α-amylase, and the amino acid residues taking a role in this interaction is; Trp58, **Trp59**, Tyr62, Leu162, Asp197, Ala198, Glu233, Ile235, **His299**, **Asp300**, **His305**, Gly306, Asp356 and Trp357, where the bold amino acid residues are involving in active interaction with acarbose.

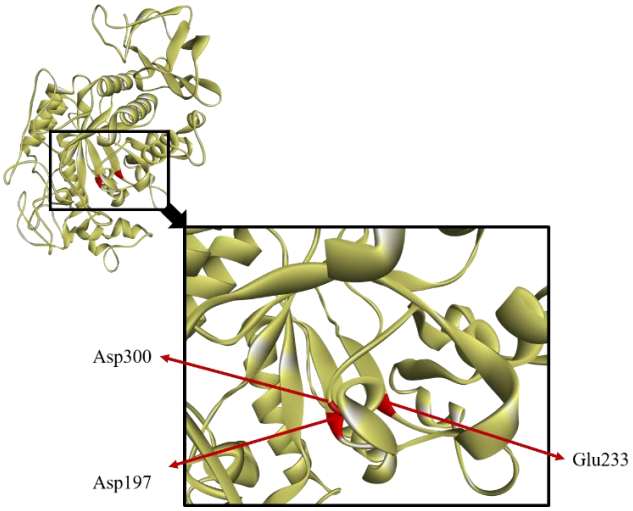

Figure 3. Amino acid residues involving in active interaction with the natural substrate

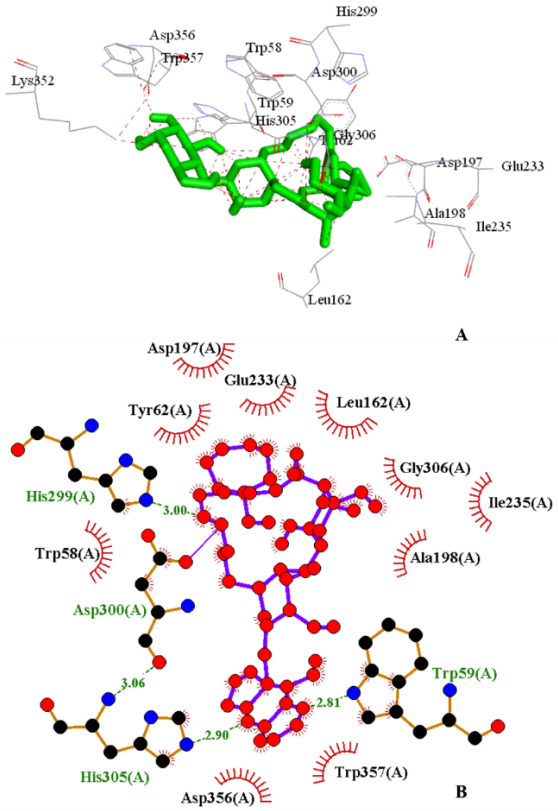

Figure 4. The interaction between acarbose and α-amylase at the active site of α-amylase A. 3D, B. 2D

Jhong et al. (2015) previously showed a similar binding location for acarbose. They also observed that quercetin binds α-amylase successfully. But the results showed that there are slight differences between the amino acid residues involving the interaction in this current study and the study conducted by Jhong et al. (2015). The reason for this difference is the X-ray crystal structures of α-amylase used in these two studies were different. In this present study, the human salivary amylase (PDB ID: 1SMD) was used, whereas Jhong et al (2015) used human pancreatic α-amylase (PDB ID: 1HNY).

Figure 5 shows the interaction between quercetin and α-amylase, and the amino acid

residues taking a role in this interaction is; Gln302, Arg303, Gly304, His305, Ala310, Ile312, Thr314, Trp316, Trp344, Arg346, Lys352, Asp353, and Asp356. The amino acid residues involved in the interaction between quercetin and α-amylase are different than the amino acid residues involved in the interaction between acarbose and α-amylase. As Qian et al. (1994) mentioned before that His305 is one of the amino acid residues, which is important in forming hydrogen bonds with inhibitors and the analysis showed that quercetin binds to His305. This probably causes a change in the active site of the enzyme, thus substrate cannot bind effectively. Kim et al. (2010) previously performed molecular docking for quercetin and quercetin glycosides but observed different amino acid residues involved in the interaction between quercetin and α-amylase. The reason for this difference is the X-ray crystal structures of α-amylase used in these two studies were different. In this present study as was mentioned before that the human salivary amylase (PDB ID: 1SMD) was used, whereas Kim et al. (2010) used alpha-amylase from *Bacillus subtilis* (PDB ID: 1UA7).

Figure 6 shows the interaction between umbelliferone and α-amylase, and the amino acid residues taking a role in this interaction are Gln302, His305, Arg303, Trp344, **Arg346**, Phe348, **Lys352**, **Asp353,** and Asp356, where the bold amino acid residues are involving in active interaction with umbelliferone. Umbelliferone also interacts with His305, thus it probably has a similar mode of activity as quercetin has. But in the literature, there are not many detailed studies about the interaction between umbelliferone and α-amylase.

The molecular docking results are mainly based on the binding energies of inhibitors to α-amylase for all possible interactions. The binding energy of acarbose was found as -9.84 kcal/mol, where this value was -10.92 kcal/mol for quercetin and -7.56 kcal/mol for umbelliferone. Quercetin showed slightly less binding energy, which means better binding affinity than the commercial inhibitor acarbose and other compound umbelliferone (Table 2).

The results showed that quercetin and umbelliferone can bind the same active site as both acarbose and substrate, but by different amino acid residues. Although they also inhibit the enzyme, the inhibition mechanism of action is different than the commercial inhibitor acarbose.

The post-analysis was performed for acarbose, quercetin, and umbelliferone by iGEMDOCK, and these compounds were again checked for docking to α-amylase.

Tested compounds were ranked by using both scores based on energies and pharmacological interactions. If a negative value for binding energy was observed, it means this interaction will be spontaneous.

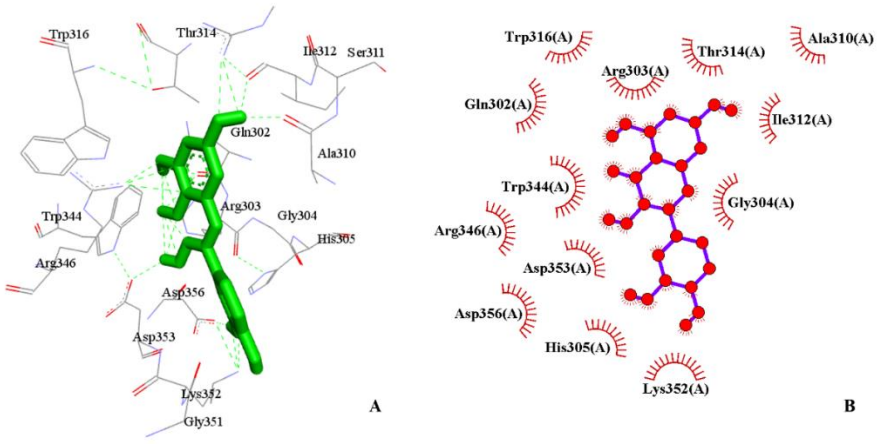

Figure 5. The interaction between quercetin and α-amylase at the active site of α-amylase A. 3D, B. 2D

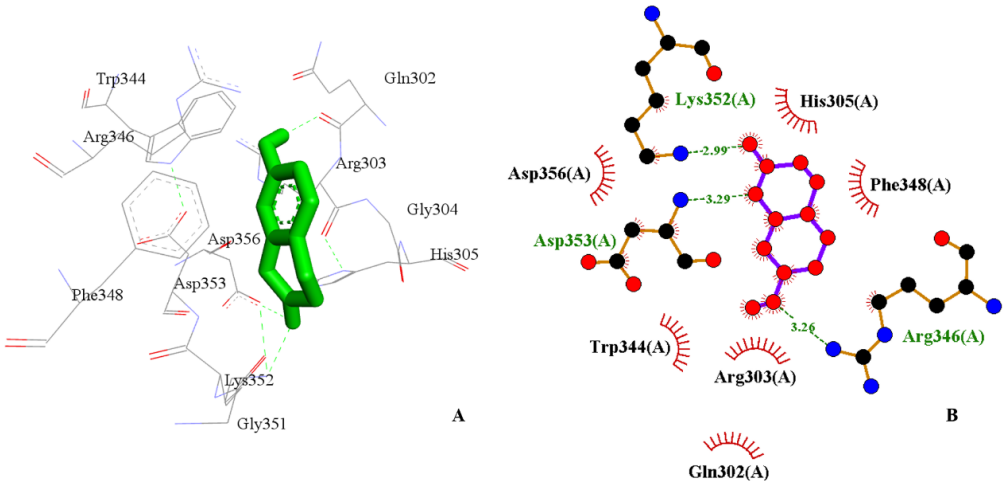

Figure 6. The interaction between umbelliferone and α-amylase at the active site of α-amylase A. 3D, B. 2D

Table 2. Binding energies of acarbose, quercetin, and umbelliferone according to Auto Dock Tools and iGEMDOCK.

		Acarbose	Quercetin	Umbelliferone
Auto Dock Tools	Estimated Free Energy of Binding (kcal/mol)*	-9.84	-10.92	-7.56
	Final Intermolecular Energy (kcal/mol)	-16.40	-12.71	-7.86
	vdW + Hbond + desolv Energy (kcal/mol)	-16.40	-12.71	-7.86
	Electrostatic Energy (kcal/mol)	0.00	0.00	0.00
	Final Total Internal Energy (kcal/mol)	-15.93	-1.74	0.00
	Torsional Free Energy (kcal/mol)	+6.56	+1.79	+0.30
	Unbound System's Energy (kcal/mol)	-15.93	-1.74	0.00
	Estimated Inhibition Constant	61.25 nM	9.97 nM	2.89 µM
iGEMD OCK	Total Energy (kcal/mol)	-93.20	-75.28	-64.09
	vdW (kcal/mol)	-93.20	0.00	-51.44
	Hbond (kcal/mol)	0.00	0.00	-12.65
	Electrostatic Energy (kcal/mol)	0.00	0.00	0.00

*Estimated Free Energy of Binding = Final Intermolecular Energy + Final Total Internal Energy + Torsional Free Energy-Unbound System's Energy; nM: nanomolar, µM: micromolar; vdW: Van der Waals, Hbond: Hydrogen Bond

In addition, if this negative value is higher, the chance of being accepted as a drug candidate will be higher too (Balavignesh et al., 2013).

The lowest binding energies in an enzyme-inhibitor interaction present that the inhibitor is fitting to the target enzyme.

Among the screened compounds, quercetin (-10.92 kcal/mol) has the lowest binding energy and umbelliferone (-7.56 kcal/mol) has the highest binding energy (Table 2).

This means quercetin can bind easier than acarbose, but umbelliferone has the lowest binding energy, which shows that acarbose can bind to the enzyme better than umbelliferone.

Patil et al. (2021) analysed the inhibitor potentials of quercetin and catechin on *Saccharomyces cerevisiae* isomaltase (PDB ID: 3AXH) that have 72% identical and 84% similar sequence to that of α-glucosidase, which has similar activity with α-amylase. They observed that the binding affinity of quercetin was -8.4 kcal/mol and -8.5 kcal/mol for acarbose. Since *S. cerevisiae* isomaltase and human salivary amylase have similar but not the same amino acid sequence, the difference in binding affinities can be acceptable.

Drug-likeness Evaluation

The evaluation of drug-likeness is an important step in pre-clinic drug development since any failure at the following steps will be extremely costly. It is known that some molecular properties have great importance in evaluating the drug-

likeness of any compound. In analysing the drug-likeness of a compound some filters are used and the Lipinski rule of five is one of them. It is accepted as a rule for evaluating the drug ability of a compound (Leeson, 2012). Lipinski's rule proposes that most of the compounds, which have the potential of being a drug have several properties, such as LogP≤5, mw≤500, number of HBA≤10, and the number of HBD≤5.

Table 3 clearly shows that when quercetin and umbelliferone were compared with acarbose, these two molecules satisfy the basic drug-likeness rule better than acarbose, the commercially used α-amylase inhibitor. Thus, both quercetin and umbelliferone can be accepted to have drug-like nature.

SWISSADME is also used for evaluating drug-likeness of acarbose, quercetin, and umbelliferone. The oral bioavailability of acarbose, quercetin, and umbelliferone are given in Figure 7A, Figure 7B, and Figure 7C respectively. The coloured zone in Figure 7 shows an appropriate physicochemical area of oral bioavailability, which shows lipophility as LIPO, flexibility as FLEX, insaturation as INSATU, insolubility as INSOLU, polarity as POLAR, and size as SIZE. As the reference limits for these parameters, XLOGP3 value should be between -0.7 and 5.0 for lipophility, the number of rotatable bonds should be lower than 9 for flexibility, the fraction of carbons in the sp3 hybridization should be between 0.25 and 1 for insaturation, logS should be between 0 and 6 for insolubility, TPSA value should be between 20 $Å^2$ and 130 $Å^2$ for polarity and lastly, molecular weight should be between 150 and 500 g/mol for size.

Figure 7 clearly shows that none of the compounds are directly in the coloured zone. Acarbose is out of the zone both for polarity and size, but quercetin and umbelliferone are only out for insaturation.

The evaluation of drug-likeness in SWISSADME reveals the results not only for Lipinski's (Pfizer) filter but also for Ghose (Amgen), Veber (GSK), Egan (Pharmacia), and Muegge (Bayer) filters as well (Ghose et al., 1999; Veber et al., 2002; Egan et al., 2000; Muegge et al., 2001). The analyses according to these filters are given in Table 4.

According to the results in Table 4, acarbose did not satisfy the fundamental drug-likeness rules for all filters, but quercetin satisfied all, whereas umbelliferone satisfied 3 of 5 filters.

Table 3. Some properties of screened compounds were obtained from DruLiTo.

	Acarbose	Quercetin	Umbelliferone
Molecular weight (mw)	645.25	302.04	162.03
LogP	-5.53	1.834	0.73
H-Bond Acceptor (HBA)	19	7	3
H-Bond Donor (HBD)	14	5	1
Total Polar Surface Area (TPSA)	321.17	127.45	46.53
Atom Molar Refractivity (AMR)	137.77	83.44	47.2
Number of Rotable Bond (nRB)	9	1	0
Number of Atom	87	32	18
Number of Rigid Bond (nRigidB)	38	23	13
Number of Aromatic Ring	0	2	1
Violations of Lipinskies Rule of Five	1	0	0

Table 4. The drug-likeness evaluation of acarbose, quercetin, and umbelliferone by SWISSADME.

	Acarbose	Quercetin	Umbelliferone
Lipinski's (Pfizer)	No; 3 violations	Yes	Yes
Ghose (Amgen)	No; 4 violations	Yes	No; 1 violation
Veber (GSK)	No; 1 violation	Yes	Yes
Egan (Pharmacia)	No; 1 violation	Yes	Yes
Muegge (Bayer)	No; 5 violations	Yes	No; 1 violation

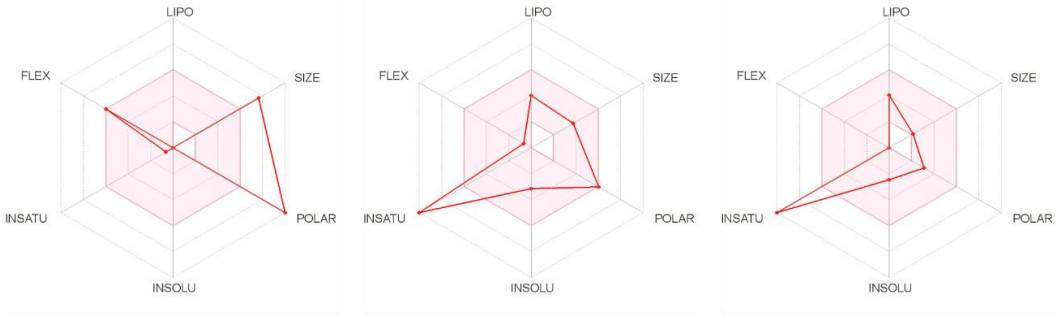

Figure 7. The oral bioavailability of A. acarbose, B. quercetin, C. umbelliferone.

ADMET Analysis

ADMET profiles for acarbose, quercetin, and umbelliferone were evaluated using ADMETLab, admetSAR, and SWISSADME, which generate the pharmacokinetic properties of compounds under different criteria. The results of three different analyses are combined and given in Table 5.

Table 5. ADMET profiles for acarbose, quercetin, and umbelliferone.

	Acarbose	Quercetin	Umbelliferone
1. Absorption			
Water solubility (logS)	-1.383	-2.999	-2.746
Human Intestinal Absorption	HIA- (0.97)	HIA+ (0.98)	HIA+ (0.99)
Blood Brain Barrier (log BB)	BBB- (0.32)	BBB- (0.46)	BBB- (0.76)
CaCo-2 Permeability	Caco2- (0.90)	Caco2- (0.64)	Caco2+ (0.93)
2. Distribution			
Renal Organic Cation Transporter	No	No	No
P-glycoprotein Substrate	No	No	No
P-glycoprotein Inhibitor	No	No	No
3. Metabolism			
CYP450 2D6 Substrate	No	No	No
CYP450 3A4 Substrate	Yes	Yes	No
CYP450 1A2 Inhibitor	No	Yes	Yes
CYP450 2C9 Inhibitor	No	No	No
CYP450 2D6 Inhibitor	No	No	No
CYP450 2C19 Inhibitor	No	No	No
CYP450 3A4 Inhibitor	No	Yes	No
4. Excretion			
MDCK	0.52	13.35	55.93
5. Toxicity Assays			
hERG Inhibition	ambiguous	medium_risk	medium_risk
AMES Toxicity	+ (0.52)	+ (0.90)	- (0.71)
Carcinogenicity	- (1.00)	- (1.00)	- (1.00)
Acute Oral Toxicity (kg/mol)	2.334	2.559	1.759

As it was given in Table 5 the ADMET profiles can be grouped as Absorption, Distribution, Metabolism, Excretion, and Toxicity (Cheng et al., 2012).

Although there are other absorption parameters, water-solubility, human intestinal absorption (HIA), blood brain barrier (BBB), and colorectal carcinoma (CaCo-2) permeability parameters were selected according to Joshi et al. (2020).

In terms of absorption, both quercetin and umbelliferone are in an acceptable range, which shows that they are efficient drug candidates.

Log S indicates the water solubility of the compound of interest and the reference limits are accepted to be between -6.5 and 0.5.

Table 5 clearly presents that acarbose, quercetin, and umbelliferone have logS values in the reference limits. The results revealed that the lowest logS value was observed in quercetin (-2.999) and the highest one was in acarbose (-1.383).

It is known that the drugs are mainly absorbed in the intestines. The results presented that acarbose, which is used as a commercial α-amylase inhibitor has a negative HIA value, which means this compound has some problems in the absorption through the intestines. On the other hand, both quercetin and umbelliferone are observed to have a positive HIA value, which shows that these compounds can easily be absorbed or assimilated through the human intestines.

In this study, the two parameters selected to understand membrane permeability were BBB and CaCo-2.

BBB is an important parameter for drugs, which will be used against neurodegenerative disorders, but it was previously proven that about 98% of therapeutic agents fail inadequate BBB permeability in clinical trials (Pardridge, 2007; Fong, 2015). Therapeutic compounds, which target the central nervous system (CNS) should be able to be BBB permeable (Nielsen et al., 2011; Muehlbacher et al., 2011). On the other hand, low BBB permeability decreases the probability of unwanted side effects associated with CNS (Muster et al., 2008; Lacombe et al., 2010; Muehlbacher et al., 2011). Acarbose, quercetin and umbelliferone have negative BBB permeability. Acarbose is readily in use, and quercetin and umbelliferone are proposed to be used as α-amylase inhibitors, and they don't need to target CNS. Having low BBB permeability for these compounds is important in decreasing unwanted side effects associated with CNS.

CaCo-2 is one of the human cell lines, which stands for colon epithelial cancer. It is one of the model systems used to understand human intestinal absorption of drugs. It is known that CaCo-2 is suitable to predict oral absorption potentials and understand the intestinal permeability of test compounds (Castillo-Garit et al., 2014). In order to act successfully, most therapeutic compounds, and all oral administrated drugs, should essentially be permeable at least for one cell membrane (Kell & Oliver, 2014), and CaCo-2 is accepted as a successful test to predict oral absorption potential of therapeutic compounds (Castillo-Garit et al., 2014).

According to the results in Table 5, only umbelliferone has a positive CaCo-2 permeability.

Renal organic cationic transporter (OCT2) and P-glycoprotein substrate/inhibitor parameters were selected according to Joshi et al. (2020) to understand the distribution of the compounds.

The OCT2 analysis is important to understand the possibility of drug-drug interactions. It is known that an inhibitor drug decreases the renal organic cationic transporter-dependent clearance of the compound affected by the inhibitor. ADMET analysis presented that none of the compounds may act as a renal organic cationic transporter inhibitor (Ivanyuk et al., 2017).

P-glycoprotein is one of the efflux transporters, which affects the absorption, distribution, and elimination of therapeutic agents. P-glycoprotein has several functions, such as limiting the absorption of orally administered drugs from the intestines, limiting drug penetration through the BBB (Fromm, 2004; Elmeliegy et al., 2020), and facilitating hepatobiliary and renal drug efflux. Thus, the systemic exposure of the substrates of P-glycoprotein is mainly limited by the enzyme itself (Lin & Yamazki, 2003; Elmeliegy et al., 2020). Any compound, which can either inhibit or induce P-glycoprotein activity, has the capacity of increasing or decreasing the systemic exposure of P-gp substrates respectively (Lund et al., 2017; Elmeliegy et al., 2020).

Any P-glycoprotein substrate has the potential of acting as either inhibitor or inducer for the enzyme itself. If P-glycoprotein is inhibited, the bioavailability of the susceptible therapeutic compound will increase, but the induction of it reduces the bioavailability of the compound. According to the analysis given in Table 5, neither of

the compounds are substrate or inhibitors for P-glycoprotein.

Cytochromes P450 (CYP) has great importance to understand the pharmacokinetics of drugs. CYP enzymes, which belong to three families (CYP1, 2, and 3) have responsibilities in the biotransformation of most of the compounds with clinical use and also in fatty acid metabolism (Guengerich, 2003; Zanger & Schwab, 2013).

According to the data given in Table 5 acarbose and quercetin is a substrate for CYP450 3A4. On the other hand, quercetin is an inhibitor for CYP450 1A2 and CYP450 3A4, whereas umbelliferone is an inhibitor for only CYP450 1A2.

MDCK (Madin - Darby Canine Kidney) cells are model cells commonly used to study cell growth regulation, metabolism, and transport mechanisms in distal renal epithelia, which are also used as an excretion parameter to predict the renal clearance of drugs (Horster & Stopp, 1986; Horio et al., 1989; Brandsch et al., 1995; Ganapathy et al., 1995; Irvine et al., 1999).

Acarbose, a commercial inhibitor was observed to have a 0.52 MDCK value, but the MDCK values for quercetin and umbelliferone were observed much better values, such as 13.35 and 55.93 respectively.

The toxicity properties of compounds were determined by the human ether-a-go-go-related gene (hERG), AMES toxicity, carcinogenicity, and acute oral toxicity.

The inhibition of hERG will inhibit the potassium channel encoded by the hERG gene and inhibition of this channel will cause severe cardiac problems (Wang et al., 2012). The results showed that acarbose has an ambiguous hERG inhibition risk, where this risk was at a medium level for both quercetin and umbelliferone.

The AMES test is a commonly used test to present probable mutagenesis and carcinogenicity at the early stages. The result for the AMES test was positive both for acarbose and quercetin, but this result was negative for umbelliferone.

Additionally, carcinogenicity and acute oral toxicity were two tests related to toxicity, which are probably the highest concern for human health. Results given in Table 5 show that carcinogenicities for all compounds were negative and acute oral toxicity (kg/mol) values were 2.334 for acarbose, 2.559 for quercetin, and 1.759 for umbelliferone.

Conclusion

The aim of this study was to prove the α-amylase inhibition activity of quercetin and umbelliferone by *in silico* tests. For this purpose quercetin and umbelliferone were analysed by molecular docking techniques, and the results were compared with a commercial α-amylase inhibitor, acarbose. The detailed tests showed that both quercetin and umbelliferone bind and inhibit α-amylase. In addition, both drug-likeness and ADMET tests proved that these two compounds are inhibitors, which are as good as acarbose, even better in some points.

Ethics Committee Approval

N/A

Peer-review

Externally peer-reviewed.

Author Contributions

Conceptualization: E.M.A.; Investigation: E.M.A.; Material and Methodology: E.M.A.; Supervision: E.M.A.; Visualization: E.M.A.; Writing-Original Draft: E.M.A.; Writing-review & Editing: E.M.A.; Other: The author have read and agreed to the published version of manuscript.

Conflict of Interest

The authors have no conflicts of interest to declare.

Funding

The authors declared that this study has received no financial support.

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