Auli et al (2023)
Vol 11(2): 11704
https://doi.org/10.20884/1.api.2023.11.2.11704

Acta Pharmaciae Indonesia:
Acta Pharm Indo
E-ISSN 2621-4520

RESEARCH ARTICLE Open Access

In silico screening of pomegranate (*Punica granatum*) and celery (*Apium graveolens*) metabolites for potential anti-type 2 diabetes activity

Winni Nur Auli*, Syaikhul Aziz, Dewi Damayanti Abdul Karim, Arnanda Evanggeulista, Aryn Fatkhul Jannah, Alexander Yoel Harazachi Mendrofa

ABSTRACT

Background: Alpha (α)-amylase is a popular pharmacological target for controlling postprandial blood glucose levels. The exploration of natural ingredients for drug development is particularly promising. Notably, pomegranate (*Punica granatum*) and celery (*Apium graveolens*) are rich in phenols and flavonoids, making them potential candidates for anti-type 2 diabetes treatments.

Objective: This study aims to identify the most promising derivatives from pomegranate and celery using a combined metabolomic and in silico approach.

Methods: The study began by identifying metabolites from the KnapSack database, selecting based on primary and secondary metabolites also selecting them based on their pharmacokinetic profile. The selected metabolites were then docked with alpha-amylase (PDB ID: 2QV4). Furthermore, the interactions were analyzed using Discovery Studio, and toxicity profiles were assessed in silico using ECOSAR and Toxtree software.

Results: The analysis identified punicaflavone and 2-deoxybrassinolide as the compounds with the highest binding affinity, at -10.06 kcal/mol and -10.89 kcal/mol respectively, both surpassing acarbose's -9.33 kcal/mol. These compounds interacted with 11 common residues in alpha-amylase, mirroring acarbose's interactions. In silico toxicity analysis revealed that punicaflavone might pose risks to aquatic organisms but does not exhibit potential as a genotoxic or non-genotoxic carcinogen. Conversely, 2-deoxybrassinolide displayed moderate toxicity to aquatic organisms but was also free from genotoxic and non-genotoxic carcinogenic potential.

Conclusion: Punicaflavone and 2-deoxybrassinolide emerged as the most promising compounds, demonstrating strong binding affinities and similar interaction patterns with α-amylase as acarbose. Although both compounds may pose risks to aquatic environments, they do not show potential as genotoxic or non-genotoxic carcinogens, supporting their further exploration as anti-diabetic agents.

Keywords: in silico, *Punica granatum*, *Apium graveolens*, alpha amylase

Introduction

Diabetes mellitus is a chronic metabolic disease indicated by raised blood glucose levels, which causes severe damage to the heart, blood vessels, kidneys, nerves, and even eyes. Type 2 diabetes becomes the most common diabetes, usually occurring in adults, which happens when the body is resistant to insulin or cannot produce enough insulin as needed. Over the past three decades, type 2 diabetes has become far more common in every country. This led to the development of a globally recognized goal to slow the rise in diabetes and obesity rates by 2025 [1]. An estimated 422 million individuals globally have diabetes, with the majority residing in low- and middle-

Department of Pharmacy of Faculty Science of Institut Teknologi Sumatera, Terusan Ryacudu Street, Way Hui, Jati Agung District, South Lampung, Lampung, Indonesia 35365

*Corresponding author: Terusan Ryacudu Street Way Hui, Jati Agung District, South Lampung, Lampung, Indonesia 35365. E-mail: winni.auli@fa.itera.ac.id

income nations. The disease is directly responsible for 1.5 million fatalities annually. Indonesia is ranked seventh out of ten countries with the highest number of people living with diabetes [1-2].

Type 2 diabetes is the most common metabolic disorder caused by a combination of two primary factors: impaired insulin secretion by pancreatic β-cells and the tissues' inability to respond appropriately to insulin [3]. A calcium metalloenzyme called alpha (α)-amylase helps the digestive process by breaking polysaccharide molecules into glucose, maltose, or other smaller molecules. The enzyme raises blood glucose levels and induces postprandial hyperglycemia. Alpha-amylases (α-1,4-glucan-4-glucanohydrolases), which are the main secretory products of the pancreas and salivary glands, are responsible for the initial step of hydrolysis of complex carbohydrates into a mixture of oligosaccharides and disaccharides in the intestinal mucosa. Management of type 2 DM by inhibiting α-amylase aims to reduce glucose reabsorption in the intestine [4]. Because of the success of several enzymatic inhibitors in inhibiting this particular enzyme, including acarbose, miglitol, and voglibose, scientists are now interested in creating potent alpha-amylase inhibitors. However, these drugs are known to have various side effects, for example, bloating, intestinal cramps, and stomach pain. Therefore, new enzyme inhibitors need to be found to plan and develop new antidiabetic medication [4-5].

The potential of natural ingredients as alpha-amylase inhibitors can be seen from the structure of the substrate that may bind to the alpha-amylase enzyme. The natural ligands of alpha amylase are maltooligosaccharides, phenyl α-maltoside, nigerose, soluble starch, amylose, amylopectin, and β-limit dextrins [6]. The approach to finding traditional herbal medicine candidates is seen from the possibility of bioactive plant contents that can directly and targeted work to reduce blood glucose levels. Red pomegranate skin (*Punica granatum*) from the Punicaceae family contains a total phenol content of not less than 10.2%, calculated as gallic acid; besides celery leaves (*Apium graveolens*), the Apiaceae family contains total flavonoid levels of not less than 11.76%, calculated as apigenin [7].

The selection of these two plants was based on the content of phenol and flavonoid derivatives, which could be potential candidates that interact with alpha-amylase enzyme as an inhibitor. The high phenol and flavonoid content in these two plants is the basis for developing antidiabetic herbal medicines. Several studies have shown that these two plants have diabetes activity, but the development has not been standardized [7-12]. Research also shows a link between the phenol and flavonoid content in plants and antidiabetic activity. The combination of these two extracts has the potential to develop standardized herbal medicines that have much better antidiabetic activity.

The research began with a metabolomics study to determine the metabolite profile of red pomegranate peel and celery leaves using a metabolomics website. The metabolites on the list were selected based on their primary and secondary metabolites. Using Lipinski's rule of five, secondary metabolites were selected again based on the pharmacokinetic profile. In silico analysis was carried out to predict antidiabetic activity against specific receptors. In silico analysis is also used to analyze the toxicity of plant bioactive compounds.

Methods

Software and database

This study utilized various software tools, including the Windows 10 operating system, GaussView 6.0, Gaussian 09, AutoDock Tools 1.5.7, Avogadro 1.2, and Biovia Discovery Studio Visualizer 2021 Client. Additionally, online resources such as SwissADME (https://swissadme.ch/index.php), Protein Data Bank (https://rcsb.org.pdb/), the Knapsack database (http://www.knapsackfamily.com), ECOSAR 2.2, and Toxtree v3.1.0 were integral to our research processes.

Metabolite selection

Compounds from red pomegranate (*Punica granatum*) and celery (*Apium graveolens*) were identified using the Knapsack database. The metabolites listed were analyzed to differentiate primary metabolite derivatives from secondary metabolites. Secondary metabolite data were then screened using Lipinski's rule of five to select compounds with favorable pharmacokinetic profiles. SMILES codes for each compound were entered into SwissADME to evaluate their pharmacokinetic characteristics, aligning these findings with those from in silico ligand tests.

In silico study

The enzyme target, human pancreatic alpha-amylase (PDB ID: 2QV4), was obtained from the Protein Data Bank. This protein structure, crystallized with

Table 1. Binding energy of secondary metabolites of *Punica granatum*

No	Compound	Binding energy (kcal/mol)	No	Compound	Binding energy (kcal/mol)
1	3-O-Methylellagic acid	-6.8	40	Cyanidin	-6.99
2	6-Methyl-gamma-ionone	-5.82	41	Pomegranatate	-6.81
3	7-Hydroxymatairesinol	-5.3	42	Pregnenolone	-8.29
4	Apigenin	-6.67	43	**Punicaflavone**	**-10.06**
5	6-Methyl-5-hepten-2-one	-3.79	44	Punicanyl benzoate	-5.52
6	alpha-Terpineol	-4.94	45	Punicin	-9.14
7	alpha-terpinolene	-4.2	46	Quercetin	-6.77
8	3,3'-di-O-Methylellagic acid	-6.46	47	Quinic acid	-3.41
9	alpha-Zearalanol	-6.63	48	Resveratrol	-6.46
10	alpha-Zearalenol	-6.55	49	Urolithin A	-6.32
11	Arctigenin	-7	50	Urolithin A 3-glucuronide	-7.47
12	Ascorbic Acid	-3.53	51	Urolithin B	-6.17
13	3,3',4'-tri-O-methylellagic acid	-6.73	52	Urolithin B glucuronide	-6.74
14	4-Terpineol	-4.33	53	Epicatechin	-6.74
15	4,4'-Di-O-methylellagic acid	-6.35	54	Matairesinol	-6.9
16	alpha-Estradiol	-7.49	55	Menthol	-5.18
17	Daidzein	-6.58	56	Nortrachelogenin	-7.25
18	Daidzein 6''-O-acetate	-7.53	57	Secoisolariciresinol	-6.63
19	Isourolithin A	-6.64	58	Catechin	-6.68
20	Isourolithin B	-5.95	59	Dihydrokaempferol	-6.53
21	Kaempferol	-6.42	60	Lariciresinol	-7.57
22	Asiatic acid	-8.5	61	Pinoresinol	-7.75
23	beta-Myrcene	-3.84	62	linalool	-4.47
24	beta-Zearalenol	-7.38	63	Lirioresinol B	-7.46
25	Biochanin A	-7.74	64	Luteolin	-6.82
26	Brevifolin	-4.7	65	Medioresinol	-7.39
27	Brevifolincarboxylic acid	-4.95	66	Melatonin	-6.21
28	Butanoic acid	-2.58	67	Naringenin	-6.49
29	Caffeic acid	-4.47	68	Naringenin chalcone	-6.58
30	Caffeine	-4.14	69	(-)-Epicatechin	-7.27
31	Capric acid	-2.96	70	(-)-Matairesinol	-6.49
32	Caproic acid	-2.3	71	(-)-Menthol	-4.69
33	Chrysin	-6.91	72	(-)-Nortrachelogenin	-7.25
34	cis-p-Coumaric acid	-4.22	73	(-)-Secoisolariciresinol	-6.63
35	Citric acid	-1.15	74	(+)-Catechin	-4.63
36	Citronellol	-4.31	75	(+)-Dihydrokaempferol	-6.53
37	Coumaric acid	-4.03	76	(+)-Lariciresinol	-7.57
38	Coumestrol	-6.88	77	(+)-Pinoresinol	-7.75
39	Coutaric acid	-3.85			

Table 2. Binding energy of secondary metabolites of *Apium graveolens*

No	Compound	Binding energy (kcal/mol)	No	Compound	Binding energy (kcal/mol)
1	Quercetin	-6.77	20	(2E)-2-Nonenal	-3.76
2	(+)-Camphor	-4.92	21	(3E,5Z)-1,3,5-Undecatriene	-4.05
3	Celephthalide C	-5.2	22	(E)-2-Decenal	-3.33
4	(-)-alpha-Cadinol	-6.16	23	(E)-Ferulic acid	-3.47
5	Celereoin	-6.85	24	(R)-(+)-Verbenone	-5.22
6	4-Terpineol	-4.33	25	1-Octen-3-yl acetate	-3.77
7	Celereoside	-7.46	26	1-Tetradecene	-3.18
8	Celerin	-5.88	27	Osthenol	-6.1
9	Celerioside A	-8.12	28	Angelicin	-5.3
10	Celephthalide A	-6.42	29	Apigenin	-6.67
11	Celerioside B	-7.35	30	Apigravin	-5.03
12	Quinic acid	-3.41	31	Apiol	-4.22
13	Sapienic acid	-3.95	32	Aspidinol	-5.26
14	Scopoletin	-4.95	33	beta-Eudesmol	-6.97
15	**2-Deoxybrassinolide**	**-10.89**	34	beta-Ionone	-5.97
16	(-)-2,3-Dihydro-9-O-beta-glucosyloxy-2-isopropenyl-7H-furo[3,2-g][1]benzopyran-7-one	-7.29	35	beta-Myrcene	-3.84
			36	beta-Phellandrene	-4.68
			37	Senkyunolide A	-5.11
17	(-)-Elemol	-6.09	38	Senkyunolide J	-5.3
18	(-)-Epicatechin	-7.27	39	Senkyunolide N	-5.65
19	(+)-Catechin	-4.63	40	Xanthotoxin	-5.49

acarbose and resolved via X-ray diffraction to 1.97 Å, showed no mutations. Protein preparation involved using AutoDock Tools 1.5.7, with acarbose re-docked to validate the procedure.

Ligand preparation

Three-dimension structures of the compounds were optimized using GaussView 6.0 and the Density Functional Theory (DFT) method with the B3LYP/6-31G basis set. Optimization outputs, saved in .log format, were converted to PDB files using Avogadro software. Ligands were then docked with the target protein, setting the docking grid dimensions at x = 12.942, y = 47.17, z = 26.2, and grid sizes of x = 42.0, y = 34.0, z = 32.0. A total of 100 poses were analyzed for each ligand-protein interaction, visualized using Discovery Studio 2021 Client.

Toxicity analysis

To predict potential toxicity in aquatic organisms (fish, daphnids, and green algae), ECOSAR software was employed. Additionally, Toxtree v3.1.1 was used to assess the carcinogenicity and mutagenicity of the compounds.

Results

Metabolite selection

This study collected 455 and 311 metabolites from the KnapSack database for *P. granatum* and *A. graveolens*, respectively.

Molecular docking

The model validation analysis with acarbose showed a binding energy of -9.33 kcal/mol. The molecular docking study revealed that the compound punicaflavone from *Punica granatum* and 2-deoxybrassinolide from *Apium graveolens* were the most promising metabolites, as indicated by their lowest binding energy value of -10.06 kcal/mol and -10.89 kcal/mol respectively lower than acarbose. The binding energy values for each ligand from two species are presented in Table 1 and Table 2.

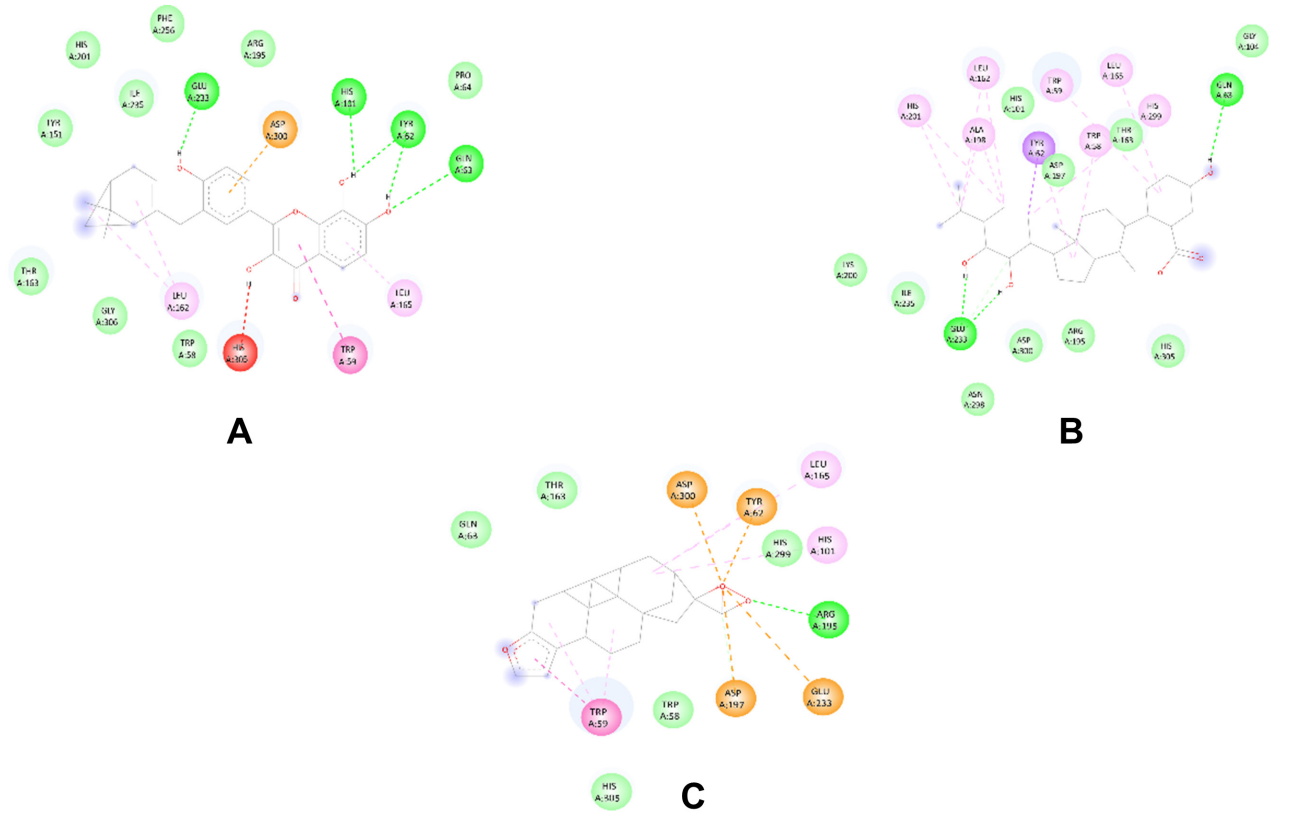

Figure 1. Visualization interaction of compound sand protein target. (A) Interaction between punicaflavone and 2qv4, (B) interaction between 2-deoxybrassinolide and 2qv4, (C) interaction between native ligand (acarbose) with 2qv4

Punicaflavone bind to protein by Van der Waals bonds on amino acid residues Phe256, His201, Tyr151, Ile235, Arg195, Pro64, Trp58, Gly306, and Thr163 (Figure 1A). Other interactions are known, such as conventional hydrogen bond (Glu233, His101, Tyr62, and Gln63), unfavorable donor-donor (His305), Pi-anion (Asp300), Pi-Pi stacked (Trp59), alkyl (Leu162), and Pi-alkyl (Leu165). 2-deoxy brassinolide binds to protein by Van der Waals bonds on amino acid residues Lys200, Ile235, Asn298, Asp300, Arg195, His305, Gly104, Thr163, Asp197, and His101 (Figure 1B). Other interactions are known, such as conventional hydrogen bond (Glu233 and Gln63), carbon-hydrogen bond, Pi-sigma (Tyr62), alkyl (His201, Ala198, Leu162, and His299), and Pi-alkyl (Trp59, Leu165, Trp58, and Tyr62). Acarbose binds to protein by Van Der Waals bonds with Thr163, Gln63, His299, Trp58, and His305. It interacted with conventional hydrogend bond with Arg195, Pi-alkyl (Leu165), Pi-Pi stacked (Trp59), alkyl (His101), and Pi-anion (Glu233, Asp197, Asp300, Tyr62).

There are many amino acid residues in common in punicaflavone, 2-deoxy brassinolide, and acarbose in interactions with alpha-amylase such as Glu233, Asp300, Tyr62, Gln63, His101, Arg195, Trp59, Leu165, Trp58, Thr163, and His305. Although some of the types of interactions are different, these results show similarities in the important amino acids for interaction. The most likely interactions are Van der Waals, conventional hydrogen bond, and pi-anion interaction. It has been shown that Glu233 and Asp300 function as catalytic residues in alpha-amylase hydrolytic processes. Tyr62 has also been demonstrated to be a crucial residue for binding some chalcones to the active site of alpha-amylase. Based on the interaction in Figure 1, ether and alcohol groups are important for interaction with Glu233, Asp300, and Tyr62.

Environmental and organism toxicity prediction

Acarbose is predicted to fall into the category of low acute toxicity because all LC_{50} and EC_{50} values are more than 100 mg/L and low chronic toxicity because all ChV values are more than 10 mg/L (Table 3). So, acarbose is safe for the aquatic environment in fish, daphnids, and green algae. Punicaflavone, potential compound of *Punica granatum* for antidiabetic, has high

Table 3. Environment and organism toxicity prediction (using ECOSAR)

Compound	Fish LC_{50} 96 h	Daphnid LC_{50} 48 h	Green algae EC_{50} 96 h	Fish ChV	Daphnid ChV	Green algae ChV
Acarbose (standard)	5.99E+11	1.34E+11	2.13E+09	1.95E+10	9.81E+08	7.02E+07
Punicaflavone	0.4960046	0.37724	0.940896	0.06844	0.082981	0.472402
2-Deoxybrassinolide	5.258984	3.615673	5.94055	6.44E-01	0.600516	2.382078

Table 4. Human body toxicity prediction (using Toxtree)

Compound	Cramel rules	Kroes TTC	*Benigni/Bossa Rulebase*			
			Genotoxic	Non-genotoxic	Mutagenic	Carcinogen potential
Acarbose (standard)	Class 3	Class 2	-	-	-	-
Punicaflavone	Class 3	Class 1	-	-	-	-
2-Deoxybrassinolide	Class 3	Class 2	-	-	-	-

Information:
(+) = Positive change / toxic
(-) = Negative chane / nontoxic
Mutagenic = Potential *S. typhymurium* TA100 mutagen based on QSAR
Carcinogen potential = Potential carcinogen based on QSAR

acute and chronic toxicity in the aquatic environment because all LC_{50} and EC_{50} values are less than 100 mg/L dan all ChV values are less than 10 mg/L. Meanwhile, 2- Deoxybrassinolide, potential compound of *Apium graveolens*, categorized as moderate acute and chronic toxicity.

Human body toxicity prediction

Punicaflavone was predicted not to be included in class 2, while 2-Deoxybrassinolide was predicted to be included in class 2, same as standard acarbose (Table 4).

Discussion

KnapSack is a database that describes the interactions between species and their metabolites. It is important for metabolomics research since it systematically examines massive quantities of organic molecules with known or unknown structures [13]. Due to a decline in the number of new drug approvals and skyrocketing costs, new drug innovation is experiencing significant difficulties. Combinatorial chemistry's introduction raised hopes for improved new chemical entities (NCEs) success rates; however, despite this advancement in science, the rate of new drug discovery success has remained unchanged [14].

SwissADME was utilized to conduct pharmacokinetic parameter testing on plant secondary metabolite chemicals based on Lipinski's rule of five. Lipinski's rule includes a molecular mass of less than 500 Da, a maximum of 5 hydrogen bond donors, a maximum of 10 hydrogen bond acceptors, and an octanol-water partition coefficient log P of no higher than 5[15]. Lipinski's rule can ascertain a ligand's physicochemical characteristics and a compound's hydrophobic/ hydrophilic characteristics when it diffuses passively through cell membranes [16]. Based on selection for secondary metabolites only and selection based on pharmacokinetic profile, 77 compounds from pomegranate and 40 compounds from celery were analyzed using molecular docking.

In drug discovery, molecular docking analysis is a basic and important method for predicting the interactions that stabilize a protein-ligand complex in its bound state [17]. This technique is unique in its ability to achieve accurate predictions of activity and to model specific structural interactions [18]. In addition, molecular docking is useful for examining how inhibitors interact with a protein's active site [19]. The primary metric evaluated in molecular docking is the free binding energy (ΔG). This value is calculated by summing the final intermolecular energy, total internal energy, and torsional free energy. From this total, the energy of the unbound system is subtracted to derive the free energy (G) of the system. A more negative ΔG value indicates stronger bond stability, enhancing the

likelihood of a significant binding interaction between the ligand and its receptor [20]. Furthermore, variations in ΔG can arise due to different interactions between the ligand and the amino acids in the target protein's active site [21].

Toxicity prediction using ECOSAR v2.2 to predict acute and chronic toxic properties of compounds contained in pomegranate and celery. Prediction of acute and chronic toxicity is important in the environment, especially to identify the impact of chemical compounds or pollutants on freshwater environments and the organisms that exist in them. Acute toxicity is the toxic effect that occurs in organisms after exposure to compounds in a short period. Acute toxicity can be assessed based on LC_{50} and EC_{50} values, which indicate the compound concentration required to kill 50% of a given population of organisms. Meanwhile, chronic toxicity is a toxic effect that occurs in organisms after exposure to compounds over a long period. Chronic toxicity can be assessed based on the ChV (chronic value), which indicates the level of exposure to a compound that does not cause significant harmful effects on certain organisms over a longer period [22].

The term "carcinogenicity" describes a substance's capacity to cause cancer in experimental animals and humans, either genotoxic or non-genotoxic. In this study, we used the Benigni/Bossa rules, Caramel rules, and Kroes TTC as the foundation for the Toxtree rule-based paradigm. Toxtree shows the structural alerts for genotoxic and non-genotoxic carcinogenicity if present in the input compound [23].

In Cramel rules parameters, compound toxicity is classified into three classes, namely low (class 1), medium (class 2), and high (class 3) toxicity. The acarbose as a standard was predicted to belong to class 3, while the two potential compounds, both punicaflavone and 2-deoxybrassinolide, were predicted to belong to class 3 [22]. There are two categories in Kroes TCC decision parameter: class 1 for compounds that are negligible risk (low chance of lifetime cancer risk more than 1 in 1,000,000), and class 2 for compounds that would not be expected to be a safety concern. Punicaflavone was predicted not to be included in class 2, while 2-Deoxybrassinolide was predicted to be included in class 2, the same as standard acarbose.

Based on carcinogenicity (genotoxicity and non-genotoxicity) and based on mutagenicity by ISS, the test compounds were classified into seven classes: structure alert for genotoxic carcinogens (class 1), structure alert for nongenotoxic carcinogens (class 2), potential mutagen of Salmonella typhimurium TA100 (QSAR or Quantitative Structure Activity Relationship) (class 3), not potential mutagen of *Salmonella typhimurium* TA100 based on QSAR (class 4), potential carcinogen based on QSAR (class 5), not potential carcinogen (QSAR) (class 6), negative genotoxic carcinogen (class 7), negative non-genotoxic carcinogen (class 9). Both punicaflavone and 2-deoxybrassinolide compounds do not have structures that can increase the potential of genotoxic and non-genotoxic carcinogens.

This in silico screening shows the potential for developing pomegranate or celery isolate compounds as new lead compounds for alpha-amylase inhibitors. These two plants are still being analyzed in extract form, and punicaflavone and 2-deoxybrassinolide have not been analyzed. Therefore, it is still essential to continue developing isolation of compounds to be analyzed in vitro and in vivo for their activity and toxicity.

Conclusion

The results showed that punicaflavone and 2-deoxybrassinolide were the most promising compounds, with the highest binding affinity values of -10.06 kcal/mol and -10.89 kcal/mol, respectively, compared to -9.33 kcal/mol for acarbose. The interactions showed that there are 11 common residues and the same interaction between punicaflavone, 2-deoxybrassinolide, and acarbose with α-amylase. Toxicological analysis revealed that punicaflavone may be toxic to aquatic organisms but is not a potential genotoxic and non-genotoxic carcinogen. 2-Deoxybrassinolide is moderately toxic to aquatic organisms and has no genotoxic and non-genotoxic carcinogenic potential. These two compounds are potential for further development as candidates for alpha-amylase inhibitors.

Acknowledgment

This research is supported by funding from the 2023 Ministry of Education, Culture, Research, and Technology Grant No.113/E5/PG.02.00.PL/2023.

Conflict of interest

There is no conflict of interest in this research

Author contributions

WNA, SA, DDAK, AE, APJ, and AYHM wrote the initial manuscript; and all authors contributed to data interpretation and final approval of the manuscript.

Received: March 27, 2024
Revised: April 27, 2024
Accepted: April 29, 2024
Published online: April 30, 2024

References

1 WHO. (2022). Diabetes. https://www.who.int/healthtopics/diabetes? gclid=Cj0KCQiA 1rSsBhDHARIsANB4EJY0_Y6ecKccRdbcbZaTyfTVXY6WEvAkmD0Ltd_vGEhwZ40kuKzBH-EaAqsWEALw_wcB#tab=tab_1

2 Infodhtin Republik Indonesia. (2020). *Tetap Produktif, Cegah, dan Atasi Diabetes Melitus*. Kementerian Kesehatan Republik Indonesia.

3 Galicia-Garcia, U., Benito-Vicente, A., Jebari, S., Larrea-Sebal, A., Siddiqi, H., Uribe, K. B., Ostolaza, H., & Martín, C. (2020). Pathophysiology of Type 2 Diabetes Mellitus. *International Journal of Molecular Sciences*, 21(17), 6275.

4 Kaur, N., Kumar, V., Nayak, S. K., Wadhwa, P., Kaur, P., & Sahu, S. K. (2021). Alpha-Amylase as Molecular target for Treatment of Diabetes Mellitus: A Comprehensive Review. *Chemical Biology & Drug Design*, 98(4), 539–560.

5 Yuan, H., Wan, H., Hu, Y.-K., Ayeni, E. A., Chang, Q., Ma, C., & Liao, X. (2021). Fishing of α-Glucosidase's Ligands from Aloe vera by α-Glucosidase Functionalized Magnetic Nanoparticles. *Molecules*, 26(19), 5840.

6 Dahlén, A. D., Dashi, G., Maslov, I., Attwood, M. M., Jonsson, J., Trukhan, V., & Schiöth, H. B. (2022). Trends in Antidiabetic Drug Discovery: FDA Approved Drugs, New Drugs in Clinical Trials and Global Sales. *Frontiers in Pharmacology*, 12, 807548.

7 Kementerian Kesehatan Republik Indonesia. (2017). Farmakope Herbal Indonesia (II). Kementerian Kesehatan RI.

8 Abozid, M., El-Rahman, H., & Mohamed, M. (2018). Evaluation of the Potential Anti-diabetic Effect of *Apium graveolens* dan *Brassica oleracea* Extract in Alloxan induced Diabetic Rats. *Int. J. Pharm*, 49(2), 39–44.

9 Gutierrez, R. M. P., Juarez, V. A., Sauceda, J. V., & Sosa, I. A. (2014). In Vitro and In Vivo Antidiabetic and Antiglycation Properties of Apium graveolens in Type 1 and 2 Diabetic Rats. *International Journal of Pharmacology*, 10(7), 368–379.

10 Rahmani, A. H., Alsahli, M. A., & Almatroodi, S. A. (2017). Active Constituents of Pomegranates (Punica granatum) as Potential Candidates in The Management of Health Through Modulation of Biological Activities. *Pharmacognosy Journal*, 9(5).

11 Syarifahnur, F., Roslizawaty, R., Amiruddin, A., Hasan, M., Karmil, T. F., & Budiman, H. (2018). The Effect of Celery Leaves Infusa (*Apium graveolens* L) on Reducing Level of Blood Glucose on Rat (*Rattus norvegicus*) Induced by Alloxan. *Jurnal Medika Veterinaria*, 12(1), 36–39.

12 Wasfi, R. M. A. H., & AL-kabi, Y. S. A.-J. (2019). Studying the Hypoglycemic Activity of Celery Herb Extract *Apium graveolens* in Blood Glucose Level of Laboratory Rats (*Sprague Dawely*). *Journal of Pure and Applied Microbiology*, 13(4), 2389–2395.

13 Afendi, F. M., Okada, T., Yamazaki, M., Hirai-Morita, A., Nakamura, Y., Nakamura, K., Ikeda, S., Takahashi, H., Altaf-Ul-Amin, M., & Darusman, L. K. (2012). KNApSAcK Family Databases: Integrated Metabolite–Plant Species Databases for Multifaceted Plant Research. *Plant and Cell Physiology*, 53(2), e1–e1.

14 Katiyar, C., Gupta, A., Kanjilal, S., & Katiyar, S. (2012). Drug Discovery From Plant Sources: An Integrated Approach. *Ayu*, 33(1), 10.

15 Goodwin, R. J. A., Bunch, J., & McGinnity, D. F. (2017). Mass Spectrometry Imaging in Oncology Drug Discovery. *Advances in Cancer Research*, 134, 133–171.

16 Syahputra, G. (2014). Simulasi Docking Kurkumin enol, bisdemetoksikurkumin dan Analognya sebagai Inhibitor Enzim 1,2-lipoksigenase. *Jurnal Biofisika*, 10(1).

17 Silakari, O., & Singh, P. K. (2020). *Concepts and Experimental Protocols of Modelling and Informatics in Drug Design*. Academic Press

18 Maurus, R., Begum, A., Williams, L. K., Fredriksen, J. R., Zhang, R., Withers, S. G., & Brayer, G. D. (2008). Alternative Catalytic Anions Differentially Modulate Human α-Amylase Activity and Specificity. *Biochemistry*, 47(11), 3332–3344.

19 Kumar, P., Duhan, M., Kadyan, K., Sindhu, J., Kumar, S., & Sharma, H. (2017). Synthesis of Novel Inhibitors of α-Amylase Based on The Thiazolidine-4-one Skeleton Containing a Pyrazole Moiety and Their Configurational Studies. *MedChemComm*, 8(7), 1468–1476.

20 Bender, B. J., Gahbauer, S., Luttens, A., Lyu, J., Webb, C. M., Stein, R. M., Fink, E. A., Balius, T. E., Carlsson, J., & Irwin, J. J. (2021). A Practical Guide to Large-Scale Docking. Nature Protocols, 16(10), 4799–4832.

21 Malmstrom, R. D., & Watowich, S. J. (2011). Using Free Energy of Binding Calculations to Improve the Accuracy of Virtual Screening Predictions. *Journal of Chemical Information and Modeling*, 51(7), 1648–1655.

22 Damayanti, S., Khonsa, K., & Amelia, T. (2021). Antiviral Activity and Toxicity Prediction of Compounds Contained in Figs (*Ficus carica* L.) by In Silico Method. In Indonesian *Journal of Pharmaceutical Science and Technology Journal Homepage (Issue 1)*. http://jurnal.unpad.ac.id/ijpst/UNPAD21.

23 Bhat, V., & Chatterjee, J. (2021). The Use of In Silico Tools for The Toxicity Prediction of Potential Inhibitors of SARS-CoV-2. *Alternatives to Laboratory Animals*, 49(1–2), 22–32.