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Research Article

Anti-diabetic Inhibitory Effect of Identified Phytochemicals in *Ziziphus spina-christi* on alpha-Amylase: *In silico* Screening Approach

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ABSTRACT

Diabetes Mellitus is a metabolic disorder characterised by persistent high concentration of blood glucose. Its progression results in health complications like neuropathy, retinopathy, nephropathy, pathology of other cell or tissue types of the body and death. *Ziziphus spina-christi* is a recognised plant for its nutritive and medicinal values. The aim of the study was to screen various identified phytochemicals in *Ziziphus spina-christi* on alpha-amylase which is an anti-diabetic drug target through in silico approach. A library of identified phytochemicals of *Ziziphus spina-christi* from literature search was built by downloading the compounds from PubChem. The hits were screened for their drug likeness and pharmacokinetics using the Swiss ADME predictor. The suitable hits from the drug likeness were docked with amylase using Autodock vina and molecular interactions visualized with Discovery studio visualizer. Fifteen compounds in the library were selected based on the Lipinski rule. Jujubogenin-amylase complex had the lowest binding energy of - 8.9 Kcal/mol, followed by maslinic acid, and (+)-Catechin cianidanol complexes with binding energies -8.5 and -8.4 Kcal/mol respectively. All the fifteen phytochemicals that did not violate the Lipinski drug likeness rule had better binding affinity for amylase compared to the clinically approved Acarbose with a binding energy of - 7.3 Kcal/mol. Hence, this investigation on the bioactive compounds from *Ziziphus spina-christi* especially Jujubogenin suggests its potential inhibitory activity on alpha-amylase for diabetes treatment.

Keywords: *Ziziphus spina-christi, Diabetes mellitus, Phytochemicals, in silico, Amylase, Jujubogenin*

INTRODUCTION

Diabetes Mellitus (DM) is a non-infectious disease with debilitating consequence on the wellness of human. DM epidemic and its complications posit a global public health threat (Zheng *et al.*, 2017). Diabetes is a disease underlined by the perturbation in the metabolism of energy biomolecules, principally carbohydrate. There is an alarming prevalence of 537 million adults (20-79 years) living with diabetes, which is 1 in 10 persons, and 6.7 million deaths recorded in 2021, implying 1 every 5 seconds (International Diabetes Federation, 2021).

Notably, α-amylase is an important drug target to reduce or delay intestinal absorption of glucose after a meal. The digestion of food in humans start in the mouth. α-amylase (α -1,4-glucan 4-glucanohydrolase, EC 3.2.1.1) catalyses the breakdown of α-(1-4) glycosidic bonds in glucose polymers such as glycogen and starch comprising of amylose and amylopectin (Kagawa *et al.*, 2003; Li *et al.*, 2005). The product of the amylase reaction is hydrolysed by α-glucosidase to yield glucose that is absorbed and transported into the bloodstream (resulting in postprandial

rise in blood glucose level). In the active site region of the salivary and pancreatic α –amylases, there are 15 non-identical amino acid residues which influence the different cleavage patterns (Brayer *et al.*, 1995).

Acarbose is a natural product of clinical use as an α –amylase inhibitor to manage diabetes and order metabolic disorders. It was isolated from *Streptomyces* species (Li *et al.*, 2005). There is a structural similarity between acarbose and the natural oligosaccharides from starch digestion. Acarbose has a higher affinity for amylases and competitively inhibits the enzyme, thereby making the availability of amylase to catalyse dietary starch markedly reduced. This ultimately brings about a reduction of food-induced postprandial increases in blood glucose and insulin (Rosak & Mertes, 2012).

Plants are a versatile natural source of bioactive compounds. *Ziziphus spina-christi* L. is a tree with thorny branches (Ads *et al.*, 2022). The extracts or isolated compounds of *Ziziphus spina-christi* have demonstrated various biological effects such as antidiabetic, anti-inflammatory, antidepressant, anticancer, antibacterial, hepatoprotective, acetylcholine esterase inhibitors, antidiarrheal, hypotensive, cytotoxic, antipyretic, antinociceptive, and antioxidant activities (Abalaka *et al.*, 2010; Asgarpanah & Haghighat, 2012; Jafarian *et al.*, 2014; Panduraju *et al.*, 2009; Vahedi *et al.*, 2008). *Ziziphus spina-christi* (L.) Willd belongs to the family Rhamnaceae (el Maaiden *et al.*, 2020). The various parts of the plant have been used in folklore medicine to treat ailments including sore throat, chest pain, dysentery, diarrhoea, swollen eyes, snake bites, tooth ache, fever, urinary infection, gynaecological infection, venereal diseases, and wounds (el Maaiden *et al.*, 2020; El-Shahir *et al.*, 2022). Studies on the phytochemical composition screening of the plant revealed the presence of alkaloids, flavonoids, terpenoids, saponins, tannins, sterols, and phenolic compounds (Farmani *et al.*, 2016; Tuenter *et al.*, 2017).

Molecular docking is the most accurate method for predicting the modus of drug-receptor interactions *in silico* (Azam *et al.*, 2012). This technique is routinely used to accelerate the recognition and investigation of novel drug candidates (Sarfaraj *et al.*, 2022). The integration of computer aided drug design (CADD) also referred to as *in silico* has been very useful in exponential reduction of the setbacks in the wet lab approach. This study explores *in silico* approach as a veritable way to screen the identified compounds in *Z. spina-christi* from previous literatures for potential antidiabetic drug leads on α-amylase and their pharmacological properties and potential toxicity. Our research showed that all the fifteen phytochemicals that did not violate the Lipinski drug likeness rule, had better binding affinity for amylase than acarbose. Overall, jujubogenin had the best binding affinity.

MATERIALS AND METHODS

Software and database

Autodock tools (ADT) 1.5.6, PyRx, Autodock vina, Open Babel and BIOVIA discovery studio visualizer 2019, RCSB protein data bank database, PubChem database, and Swiss ADME Server for ADME Predictions.

Preparation of protein

The crystal structure α-amylase (PDB ID: 1XCX) was retrieved from the RCSB protein data bank (Li *et al.*, 2005) (https://www.rcsb.org/) and imported into the ADT where the interacting ligands, and water molecules were removed from the protein. Subsequently, polar hydrogen atoms were added to the proteins followed by addition of Gasteiger charges calculation. The prepared protein was saved as pdbqt file for onward molecular docking.

Preparation of ligands

The identified phytochemical compounds in *Ziziphus spina-christi* from literature search were the selected ligands in this study. The structure data files (SDF) format of the selected ligands were downloaded from PubChem database (Kim *et al.*, 2021) and were converted to protein data bank (PDB) file using the Open Babel software (O'Boyle *et al.*, 2011). Also, energy minimisation of the ligands was done.

Active site

The amino acid residues of α-amylase (1XCX) active site believed to participate directly in catalysis as reported experimentally were retrieved from previous reports. They are D197, E233 and D300 (Li *et al.*, 2005).
The validation of our study protocol was done by re-docking of the co-crystallised ligand into the active site.

Molecular docking studies

The grid dimensions and binding centre were set for x, y, and z coordinate as (10.08, 20.93, and 48.11) respectively around the active site residues. Docking calculations for the binding affinity were then performed by using Autodock Vina (Eberhardt *et al.*, 2021; Trott & Olson, 2010) via the PyRx software (Dallakyan & Olson, 2015). The interactions between the ligand and protein were visualized using the BIOVIA discovery studio 2019.

Drug-likeness and ADME profiling of the identified compounds

Drug likeness based on Lipinski's rule was used to screen the downloaded ligands for further molecular docking analysis. Also, the Pharmacokinetics and pharmacodynamics prediction; absorption, distribution, metabolism, and excretion (ADME) of the ligands was performed using the SwissADME web server tool (Daina *et al.*, 2017). The canonical Simplified Molecular Input Line Entry System

(SMILES) files of the screened compounds obtained from the PubChem database was used for the ADME prediction.

RESULTS

The list of twenty-three compounds of *Ziziphus spina* selected from literatures (Bozicevic *et al.*, 2017; Pawlowska *et al.*, 2009; Sakna *et al.*, 2019)and acarbose which were retrieved from PubChem are shown in table 1. The corresponding chemical identity number (CID), molecular formula and molecular weight are shown alongside. The molecular weight of the phytochemicals ranges from 288.45 to 610.52 g/mol. Quercetin-3-o-robinobioside and Rutin have the highest molecular weight while Eriodictyol has the least molecular weight. All the *Ziziphus spina* compounds retrieved had a lower molecular weight compared to acarbose.

Table 1. List of the PubChem Retrieved Compounds in *Ziziphus spina* Selected from Literature

S/N	Ligand molecule	PubChem CID	Formula	Molecular Weight (g/mol)	Canonical SMILE
1.	(+)-Catechin cianidanol	9064	C15H14O6	290.27	Oc1cc2O[C@H](c3ccc(c(c3)O)O)[C@H](Cc2c(c1)O)O
2.	Procyanidin b2	122738	C30H26O12	578.52	Oc1cc(O)c2c(c1)O[C@@H]([C@@H]([C@H]2c1c(O)cc(c2c1O[C@@H]([C@@H](C2)O)c1ccc(c(c1)O)O)O)O)c1ccc(c(c1)O)O
3.	(-)-Epicatechin	72276	C15H14O6	290.27	Oc1cc(O)c2c(c1)oc(c(c2=O)O[C@@H]1O[C@H](CO[C@@H]2O[C@@H](C)[C@@H]([C@H]([C@H]2O)O)O)[C@@H]([C@@H]([C@H]1O)O)O)c1ccc(c(c1)O)O
4.	Proanthocyanidins	107876	C30H26O13	594.52	Oc1cc(O)c2c(c1)O[C@H]([C@@H](C2)O[C@]1(Oc2cc(O)cc(c2[C@H]([C@H]1O)O)O)c1ccc(c(c1)O)O)c1ccc(c(c1)O)O
5.	Quercetin-3-o-robinobioside	10371536	C27H30O16	610.52	Oc1cc(O)c2c(c1)oc(c(c2=O)O[C@@H]1O[C@H](CO[C@@H]2O[C@@H](C)[C@@H]([C@H]([C@H]2O)O)O)[C@@H]([C@@H]([C@H]1O)O)O)c1ccc(c(c1)O)O
6.	Quercetin-3-o-galactoside hyperoside	5281643	C21H20O12	464.38	OC[C@H]1O[C@@H](Oc2c(oc3c(c2=O)c(O)cc(c3)O)c2ccc(c(c2)O)O)[C@@H]([C@H]([C@H]1O)O)O
7.	Kaempferol-3-o-robinobioside	15944778	C27H30O15	594.52	Oc1ccc(cc1)c1oc2cc(O)cc(c2c(=O)c1O[C@@H]1O[C@H](CO[C@@H]2O[C@@H](C)[C@@H]([C@H]([C@H]2O)O)O)[C@@H]([C@@H]([C@H]1O)O)O)O
8.	Kaempferol-3-o-rutinoside nicotiflorin	5318767	C27H30O15	594.52	Oc1ccc(cc1)c1oc2cc(O)cc(c2c(=O)c1O[C@@H]1O[C@H](CO[C@@H]2O[C@@H](C)[C@@H]([C@H]([C@H]2O)O)O)[C@H]([C@@H]([C@H]1O)O)O)O
9.	Quercetin-3-o-rhamnoside quercetrin	5280459	C21H20O11	448.38	Oc1cc(O)c2c(c1)oc(c(c2=O)O[C@@H]1O[C@@H](C)[C@@H]([C@H]([C@H]1O)O)O)c1ccc(c(c1)O)O
10.	Quercetin	5280343	C15H10O7	302.24	Oc1cc(O)c2c(c1)oc(c(c2=O)O)c1ccc(c(c1)O)O
11.	Eriodictyol	440735	C15H12O6	288.25	Oc1cc2O[C@@H](CC(=O)c2c(c1)O)c1ccc(c(c1)O)O
12.	Sisymbrifolin	101690824	C20H24O8	392.4	OC[C@H]([C@@H](c1cc2c(c(c1)OC)O[C@@H]([C@H]2CO)c1ccc(c(c1)OC)O)O)O
13.	Rutin	5280805	C27H30O16	610.52	Oc1cc(O)c2c(c1)oc(c(c2=O)O[C@@H]1O[C@H](CO[C@@H]2O[C@@H](C)[C@@H]([C@H]([C@H]2O)O)O)[C@H]([C@@H]([C@H]1O)O)O)c1ccc(c(c1)O)O
14.	Jujubogenin	15515703	C30H48O4	472.7	CC(=C[C@@H]1O[C@@]23OC[C@@]4(C2)[C@@H]([C@H]3[C@@](C1)(C)O)CC[C@H]1[C@@]4(C)CC[C@@H]2[C@]1(C)CC[C@@H](C2(C)C)O)C
15.	Christinin iii	14563842	C22H30O7	406.47	CCC(C(=O)OC1C2=C(C)CC(C3C(C2C2(C1O2)C)OC(=O)C3C)OC(=O)C)C
16.	Christinn 1	101324802	C19H24O7	364.39	CC(=O)O[C@@H]1CC(=C2[C@@H]([C@@H]3[C@@H]1[C@@H](C)C(=O)O3)[C@]1([C@@H]([C@H]2OC(=O)C)O1)C)C
17.	Zizyberanalic or colubrinic acid	21672700	C30H46O4	470.68	O=C[C@@H]1[C@@H](O)C([C@H]2[C@@]1(C)[C@H]1CC[C@H]3[C@@]([C@@]1(CC2)C)(C)CC[C@@]1([C@@H]3[C@@H](CC1)C(=C)C)C(=O)O)(C)C
18.	Ceanothenic acid	71451218	C29H42O4	454.64	CC(=C)[C@@H]1CC[C@]2([C@H]1[C@H]1CC[C@H]3[C@@]([C@@]1(CC2)C(=O)O)(C)CC[C@@H]1[C@]3(C)C=CC1(C)C)C(=O)O
19.	Epiceanothic	23631167	C30H46O5	486.68	CC(=C)[C@@H]1CC[C@]2([C@H]1[C@H]1CC[C@H]3[C@@]([C@]1(C)CC2)(C)CC[C@@H]1[C@]3(C)[C@H](C(=O)O)[C@@H](C1(C)C)O)C(=O)O

Table 1. *Continued*

20.	Maslinic acid	73659	C30H48O4	472.7	O[C@@H]1C[C@@]2(C)[C@H](C([C@H]1O)(C)C)CC[C@@]1([C@@H]2CC=C2[C@@]1(C)CC[C@@]1([C@H]2CC(C)(C)CC1)C(=O)O)C
21.	Alphitolic acid	12305768	C30H48O4	472.7	CC(=C)[C@@H]1CC[C@]2([C@H]1[C@H]1CC[C@H]3[C@@]([C@]1(C)CC2)(C)CC[C@@H]1[C@]3(C)C[C@@H](O)[C@@H](C1(C)C)O)C(=O)O
22.	Betulinic acid	64971	C30H48O3	456.7	CC(=C)[C@@H]1CC[C@]2([C@H]1[C@H]1CC[C@H]3[C@@]([C@]1(C)CC2)(C)CC[C@@H]1[C@]3(C)CC[C@@H](C1(C)C)O)C(=O)O
23.	Ceanothic acid	161352	C30H46O5	486.68	CC(=C)[C@@H]1CC[C@]2([C@H]1[C@H]1CC[C@H]3[C@@]([C@]1(C)CC2)(C)CC[C@@H]1[C@]3(C)[C@@H](C(=O)O)[C@@H](C1(C)C)O)C(=O)O
24.	Acarbose	444254	C25H43NO18	645.6	OC[C@H]1O[C@H](O[C@@H]2[C@@H](CO)O[C@@H]([C@@H]([C@H]2O)O)O)[C@@H]([C@H]([C@@H]1O[C@H]1O[C@H](C)[C@H]([C@@H]([C@H]1O)O)N[C@H]1C=C(CO)[C@H]([C@@H]([C@H]1O)O)O)O)O

The fifteen compounds of the plant that did not violate the Lipinski rule of five for druglikeness and acarbose are displayed in table 2. In terms of the Lipinski rule of five parameters, the compounds had 0 or 1 violation. Not more than one of the following criteria was violated: their molecular weight should be less than 500; they should have less than 10 hydrogen bond acceptors or donors; their topological polar surface area (TPSA) should be between 20 and 130; or their lipophilicity (iLogP) should be less than 5. On the other hand, acarbose had three violations.

Table 2. List of the Compounds that did not Violate the Lipinski Rule of Five

S/N	Ligand molecule	Molecular weight	#H-bond acceptors	#H-bond donors	TPSA	iLOGP	Lipinski #violations
1.	(+)-Catechin cianidanol	290.27	6	5	110.38	1.33	0
2.	(-)-Epicatechin	290.27	6	5	110.38	1.47	0
3.	Quercetin	302.24	7	5	131.36	1.63	0
4.	Eriodictyol	288.25	6	4	107.22	1.62	0
5.	Sisymbrifolin	392.4	8	5	128.84	2.27	0
6.	Jujubogenin	472.7	4	2	58.92	4.41	1
7.	Christinin III	406.47	7	0	91.43	3.4	0
8.	Christinn 1	364.39	7	0	91.43	2.58	0
9.	Zizyberanalic or colubrinic acid	470.68	4	2	74.6	3.5	1
10.	Ceanothenic acid	454.64	4	2	74.6	3.37	1
11.	Epiceanothic	486.68	5	3	94.83	3.36	1
12.	Maslinic acid	472.7	4	3	77.76	3.38	1
13.	Alphitolic acid	472.7	4	3	77.76	3.72	1
14.	Betulinic acid	456.7	3	2	57.53	3.81	1
15.	Ceanothic acid	486.68	5	3	94.83	3.11	1
16.	Acarbose	645.6	19	14	321.17	1.43	3

Table 3 describes the pharmacokinetics properties of the compounds. The table depicts the ADME characteristics of the compounds. Epiceanothic, betulinic acid, ceanothic acid, and the standard drug acarbose had low intestinal absorption while the other hit compounds had a high absorption. Quercetin, jujubogenin, christinin III, christinin 1, ceanothic, and the acarbose were not substrates of for P-glycoprotein (P-gp).

Quercetin is the only CYP1A2 inhibitor, and none of the compounds were CYP2C19 inhibitor. Zizyberanalic, ceanothenic acid, epiceanothic, betulinic acid, and ceanothic acid are CYP2C9 inhibitors. Quercetin is the only CYP2D6 inhibitor, and quercetin, eriodictyol, and christinin III were CYP3A4 inhibitors.

Table 3. Pharmacokinetics and Pharmacodynamics Properties of the Phytochemical Compounds from *Ziziphus spina-christi*

		GI absorption	BBB permeant	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Bioavailability Score
1.	(+)-Catechin cianidanol	High	No	Yes	No	No	No	No	No	0.55
2.	(-)-Epicatechin	High	No	Yes	No	No	No	No	No	0.55
3.	Quercetin	High	No	No	Yes	No	No	Yes	Yes	0.55
4.	Eriodictyol	High	No	Yes	No	No	No	No	Yes	0.55
5.	Sisymbrifolin	High	No	Yes	No	No	No	No	No	0.55
6.	Jujubogenin	High	No	No	No	No	No	No	No	0.55
7.	Christinin III	High	No	No	No	No	No	No	Yes	0.55
8.	Christinn 1	High	No	No	No	No	No	No	No	0.55
9.	Zizyberanalic or colubrinic acid	High	No	Yes	No	No	Yes	No	No	0.85
10	Ceanothenic acid	High	No	Yes	No	No	Yes	No	No	0.85
11	Epiceanothic	Low	No	Yes	No	No	Yes	No	No	0.56
12	Maslinic acid	High	No	Yes	No	No	No	No	No	0.56
13	Alphitolic acid	High	No	Yes	No	No	No	No	No	0.56
14	Betulinic acid	Low	No	No	No	No	Yes	No	No	0.85
15	Ceanothic acid	Low	No	Yes	No	No	Yes	No	No	0.56
16	Acarbose	Low	No	Yes	No	No	No	No	No	0.17

Table 4. Toxicity Profile of the Hit Phytochemical Compounds from *Ziziphus spina-christi*

S/N	Phytochemical compounds	Predicted LD50 (mg/kg)	*Predicted Toxicity Class	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
1	(+)-Catechin cianidanol	10000	VI	Inactive	Inactive	Inactive	Inactive	Inactive
2	(-)-Epicatechin	10000	VI	Inactive	Inactive	Inactive	Inactive	Inactive
3	Quercetin	159	III	Inactive	Active	Inactive	Active	Inactive
4	Eriodictyol	2000	IV	Inactive	Active	Inactive	Inactive	Inactive
5	Sisymbrifolin	5000	V	Inactive	Inactive	Inactive	Inactive	Inactive
6	Jujubogenin	5000	V	Inactive	Inactive	Active	Inactive	Inactive
7	Christinin III	7	II	Inactive	Active	Active	Active	Inactive
8	Christinn 1	7	II	Inactive	Active	Active	Active	Inactive
9	Zizyberanalic or colubrinic acid	2610	V	Inactive	Inactive	Active	Inactive	Inactive
10	Ceanothenic acid	11800	VI	Inactive	Inactive	Inactive	Inactive	Inactive
11	Epiceanothic	4820	V	Inactive	Inactive	Active	Inactive	Inactive
12	Maslinic acid	2000	IV	Inactive	Active	Active	Inactive	Inactive
13	Alphitolic acid	2610	V	Inactive	Active	Active	Inactive	Inactive
14	Betulinic acid	2610	V	Inactive	Active	Active	Inactive	Inactive
15	Ceanothic acid	4820	V	Inactive	Inactive	Active	Inactive	Inactive
16	Acarbose	24000	VI	Active	Inactive	Active	Inactive	Inactive

Table 4 shows the toxicity profile for the compounds. (+)-catechin cianidanol, (-)-epicatechin, sisymbrifolin, and ceanothenic acid were non-toxic for all the toxicity parameters studied. The Predicted LD_{50} (mg/kg) in table 4

reveals that Christinin III and I are both fatal if swallowed, quercetin is toxic if swallowed, eriodictyol and maslinic acid are both harmful if swallowed, sisymbrifolin, jujubogenin, colubrinic, and epiceanothic may be harmful if swallowed, while (+)-catechin cianidanol, (-)-epicatechin, ceanothenic acid and acarbose were non-toxic. All the phytochemical compounds are not hepatotoxic whereas acarbose was hepatoxic.

Quercetin, Eriodictyol, Christinin III and I, maslinic acid, alphitolic acid and betulinic acid were carcinogenic compounds. Jujubogenin , Christinin III and I, zizyberanalic or colubrinic acid , epiceanothic, maslinic acid, alphitolic acid, betulinic acid, ceanothic acid and acarbose were immunotoxic . quercetin, Christinin III and I are mutagenic. None of the compounds was cytotoxic. In overall, the toxicity profile of this study reveals that (+)-catechin cianidanol, (-)-epicatechin, sisymbrifolin, and ceanothenic acid were not hepatotoxic, carcinogenic, immunotoxic, mutagenic, and cytotoxic.

The binding affinity from the molecular docking is within the range of - 7.3 and - 8.9 as shown in Table 5. Jujubogenin has the highest binding affinity. of -8.9 kcal/mol, followed by maslinic acid and namely (+)-Catechin cianidanol with binding energies -8.5 and -8.4 Kcal/mol respectively. All of the compounds from *Ziziphus spina-christi* had better affinities compared to the standard drug acarbose.

In table 6, the amino acids residues involved in each of the ligand - amylase complex interactions are itemised.

Table 5. Binding Affinity of the Various Compounds Complex with α-Amylase

S/N	Ligands	Binding Affinity / kcal/mol
1	(+)-Catechin cianidanol	-8.4
2	(-)-Epicatechin	-8.0
3	Quercetin	-8.2
4	Eriodictyol	-8.3
5	Sisymbrifolin	-7.7
6	Jujubogenin	-8.9
7	Christinin III	-8.0
8	Christinn 1	-7.4
9	Zizyberanalic or colubrinic acid	-7.5
10	Ceanothenic acid	-7.5
11	Epiceanothic	-8.0
12	Maslinic acid	-8.5
13	Alphitolic acid	-7.9
14	Betulinic acid	-8.0
15	Ceanothic acid	-7.7
16	Acarbose	-7.3

Table 6. Amino Acids Residues Involved in the Ligand - Amylase Complex Interactions

S/N	Ligands	Amino acids involved in ligand complex Interactions
1	(+)-Catechin cianidanol	*Asp197, *Asp300, *Glu233, His305, Ala198, Arg195, His299, Trp58, Tyr62, His101, Leu165, Gln63, Trp59
2	(-)-Epicatechin	*Asp300, *Glu233, Leu162, Tyr62, Trp59, Thr163
3	Quercetin	*Glu233, *Asp197, His299, *Asp300, Trp59, His305, Gln63, Leu165, His101, Tyr62, Ala198, Leu162, Arg195
4	Eriodictyol	Arg195, *Asp197, *Asp300, Gln63, His305, Trp59, Leu162, Ala198, *Glu233
5	Sisymbrifolin	*Asp300, *Glu233, Ile235, Tyr151, Leu162, Trp59
6	Jujubogenin	His305, *Asp300, Leu165, Tyr62, His101, Ala198, *Asp197, Leu162, *Glu233, Ile235, His201, Gly306, Lys200, Tyr151
7	Christinin III	Gly306, Leu162, Thr163, Trp58, His305, Leu165, Tyr62, Ala198, Tyr151
8	Christinn 1	His305, Leu162, Thr163, Trp59, Leu165, His299, Tyr62, Trp58, Arg195, *Glu233, Ile235,
9	Zizyberanalic or colubrinic acid	Tyr151, His201, *Asp300, Tyr62, His299, Trp58, Thr163, Leu165, His305, Leu162, Ile235,
10	Ceanothenic acid	Trp59, Thr163, *Asp300, Trp58, His305, Gly306, Leu162, Tyr151, Ala198, Tyr62
11	Epiceanothic	His305, Leu162, Ala307, Ile235, Lys200, Tyr151, His201, *Glu233, Tyr62, Trp58, His299, Leu165, Trp59, Thr163,

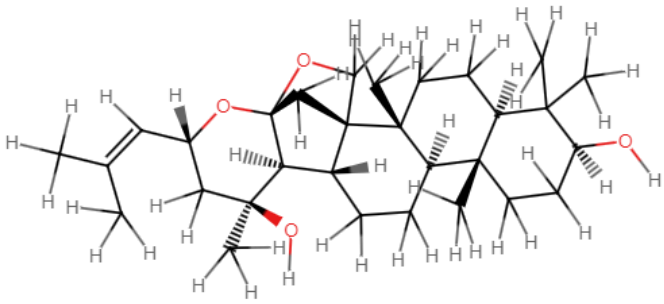
Table 6. *Continued*

12	Maslinic acid	His201, Thr163, Leu162, His101, Leu162, Tyr62, Arg195, *Asp197, His305, Trp58, Ile235, Ala307, Glu240, Tyr151
13	Alphitolic acid	His305, Trp59, Leu165, Thr163, Trp58, Tyr62, Leu162, His101, Ile235, His201, Tyr151, Lys200, Ala307,
14	Betulinic acid	Thr163, Tyr62, His305, Leu162, Tyr151, His201, Ala307, Ile235, His299, His101, Trp58, Trp59, Lys200
15	Ceanothic acid	Gly306, Tyr151, Ile235, Leu162, His101, Tyr62, Trp59, His305, Trp58
16	Acarbose	Tyr62, Leu162, Gln63, Trp59, Thr163, Leu165, His101, Tyr151, Gly306, His305

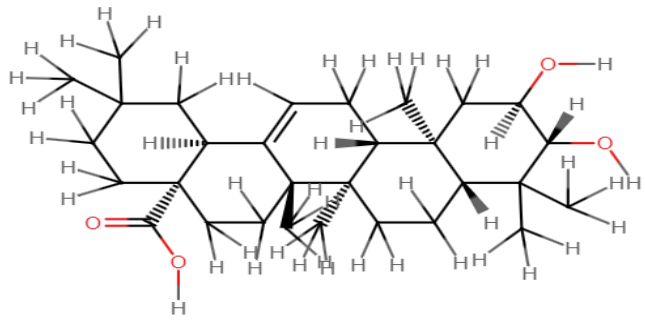
*denote amino acids residues in amylase active site that directly participate in catalysis

Figures 2-5 shows the visualisation of the 3D and corresponding 2D docking poses of the three best hit phytochemicals of *Ziziphus spina-christi* and acarbose in the amylase active site pocket. The interactions comprise of hydrogen, and non-hydrogen bonding. The interactions of jujubogenin with amylase comprise of two hydrogen bonds at His101, and Gly306 as shown in Figure 2. The other interactions include van der Waals, alkyl and pi-alkyl interactions.

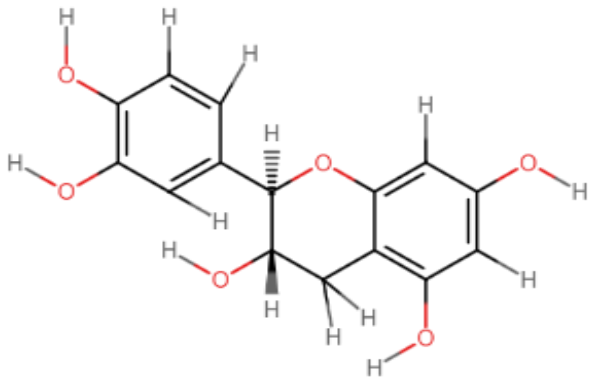
The interactions of maslinic acid with amylase comprise of two hydrogen bonds at Arg195, and Asp197 as shown in Figure 3. The other interactions include van der Waals, pi-sigma, alkyl and pi-alkyl interactions. The interactions of (+)-catechin cianidanol with amylase comprise of three hydrogen bonds at Asp197, asp300, and Glu233 as shown in Figure 4. The other interactions include van der Waals, Pi-anion, Pi-donor hydrogen bond, and pi-pi stacked interaction. The interactions of acarbose with amylase comprise of three hydrogen bonds at Thr163, tyr151, and his305 as shown in Figure 5. The other interactions include van der Waals, carbon hydrogen bond, alkyl and pi-alkyl interactions.



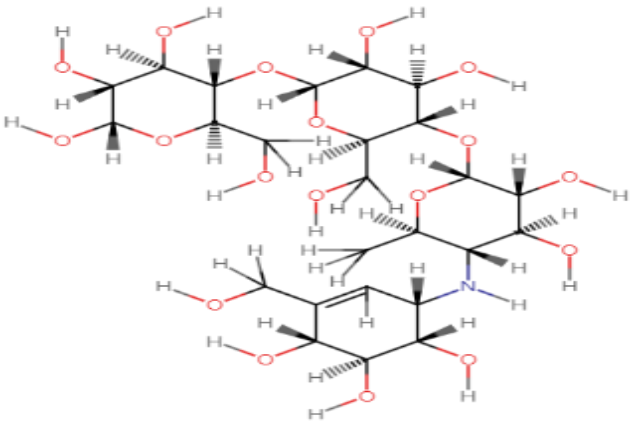
A) Jujubogenin



B) Maslinic acid



C) (+)-Catechin cianidanol



D) Acarbose

Figure 1: Structure of the three best Molecular Docked Phytochemical from *Ziziphus spina-christi*

A) Jujubogenin, B) Maslinic acid, C) (+)-Catechin cianidanol, and D) Acarbose

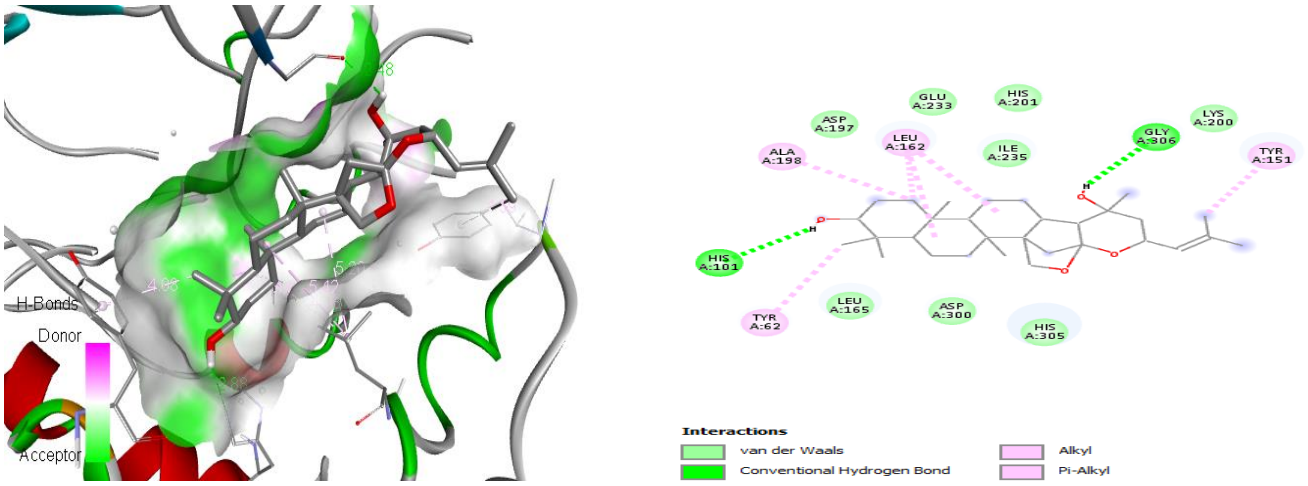


Figure 2. The 3D and Corresponding 2D Docking Pose of Jujubogenin in the Amylase Active Site Pocket

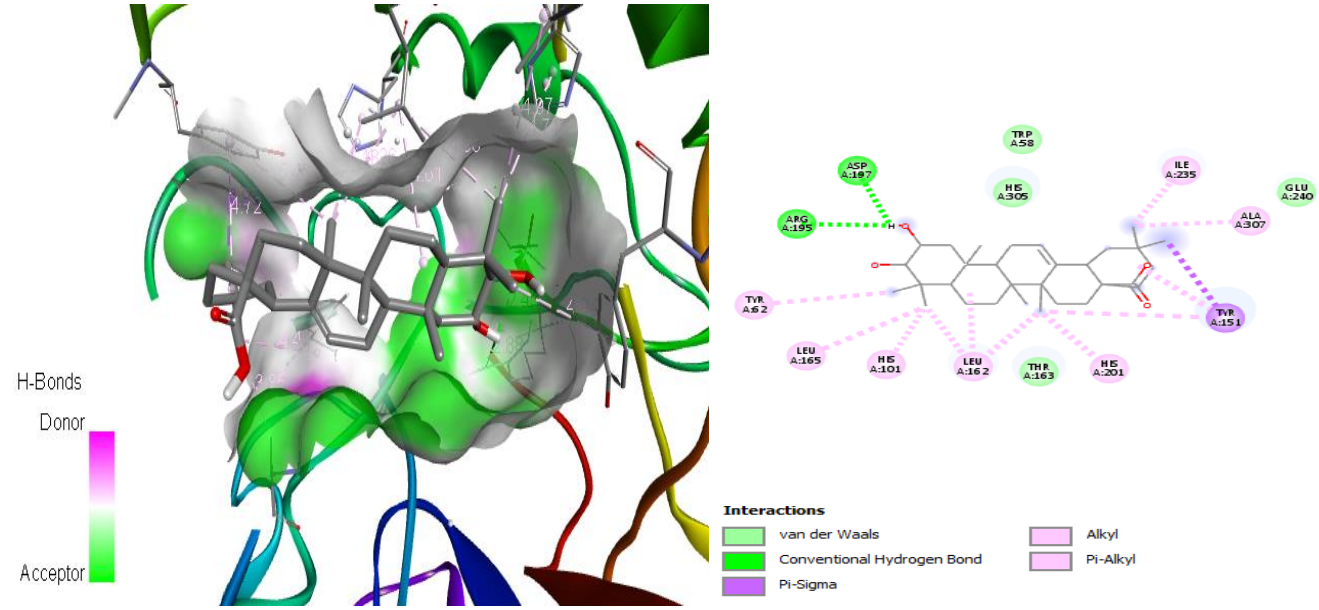


Figure 3. The 3D and Corresponding 2D Docking Pose of Maslinic Acid in the Amylase Active Site Pocket

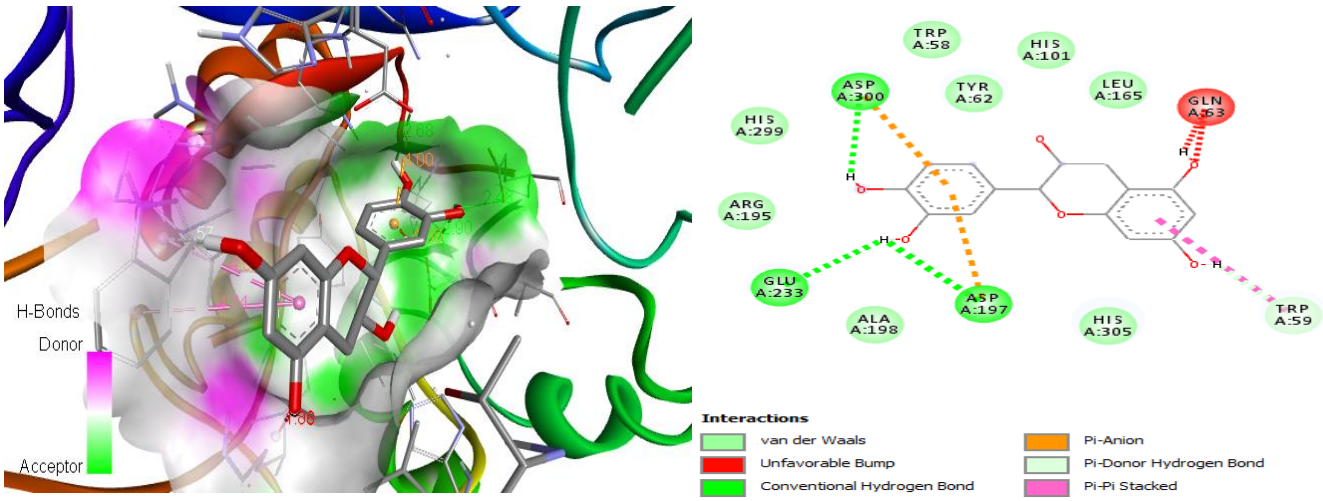


Figure 4. The 3D and Corresponding 2D Docking Pose of (+)-Catechin cianidanol in the Amylase Active Site Pocket

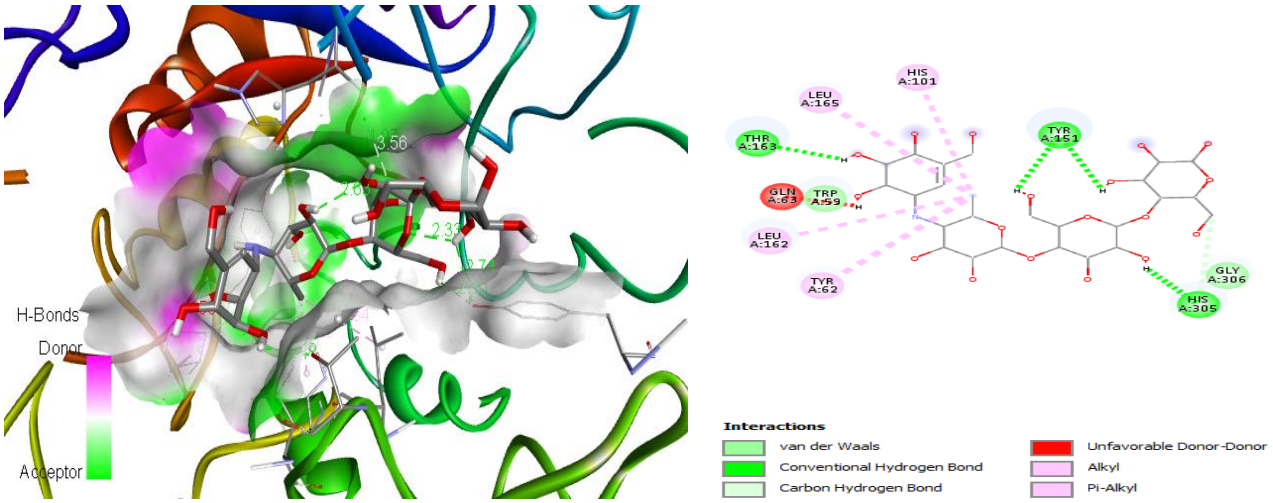


Figure 5. The 3D and Corresponding 2D Docking Pose of Acarbose in the Amylase Active Site Pocket

DISCUSSION

The use of computational (*in silico*) techniques has avail scientist and researchers with a fast and cost-effective approach to screen for potential drug candidates (Hou & Xu, 2004) from the large pool of existing compounds. In order to discover potent antidiabetic drug candidates with high binding affinity for the active site pocket of α-amylase, *Ziziphus spina-christi* ligands library (Table 1) was screened through the Lipinski's rule for drug likeness (Table 2).

Christopher Lipinski proposed the rules of five (ROF) which is employed to determine the druglike potential of compounds via molecular weight < 500, number of HB acceptor < 10, number of HB donor < 5 and octanol/water partition coefficient (log p) <5 and polar surface area ⩽ 140A^0 (Benet *et al.*, 2016; Lipinski *et al.*, 2001). The rule is premised on the non-violation of more than one component of the five components for a compound to be described drug-like. The Lipinski rule was explored in the screening of compounds from the compound library. Fifteen compounds from the library were deemed fit as potential drug like compounds for further analysis. Interestingly, these phytochemical compounds of the *Ziziphus spina-christi* were of a better druglike property compared to the standard drug. Acarbose violated three ROF based on its high molecular weight, hydrogen bond acceptor and donor values.

Poor pharmacokinetic studies have resulted in the enormous attrition of drugs in preclinical and clinical trials (Gurung *et al.*, 2016). Therefore, screening of drug-like molecules can increase the chances of passing through the clinics (Gurung *et al.*, 2020). The ADME test is used to evaluate the pharmacokinetic properties of compounds (Terao & Mukai, 2014). The gastrointestinal intestinal (G.I.) absorption and Pglycoprotein (Pgp) inhibition were used to determine the absorption of the hit compounds (Akinloye *et al.*, 2020). Epiceanothic, Betulinic acid, Ceanothic acid, and the standard drug Acarbose had low intestinal absorption while the other hit compounds had a high absorption as shown in Table 3. Quercetin, Jujubogenin, Christinin III, Christinin 1, Ceanothic, and the reference drug were not substrates of for P-glycoprotein (P-gp) (Table 3). P-gp is a type of membrane transport protein that inhibits the absorption, distribution and bioavailability of drugs that appear to be its substrates and release them out of circulation (Akinloye *et al.*, 2020). With respect to the amylase site of action being in the gastrointestinal tract (GIT) before absorption, it is plausible rational for the effective inhibitor not to be readily absorbable through the GIT. Betulinic acid and ceanothic acid had a low absorption as acarbose. Arguably, the affinity of an inhibitor to amylase is also of immense consideration which this study has revealed that the understudied phytochemical compounds have better affinity (as inhibitors) to acarbose.

Quercetin is the only CYP1A2 inhibitor, none of the compounds was CYP2C19 inhibitor, zizyberanalic or colubrinic acid, ceanothenic acid, epiceanothic, betulinic acid, and ceanothic acid are CYP2C9 inhibitors, Quercetin is the only CYP2D6 inhibitor, Quercetin, Eriodictyol, and christinin III were CYP3A4 inhibitors. The cytochrome P450 enzymes (CYPs) are important proteins in the metabolism and detoxification of xenobiotics (foreign compounds) (Brown *et al.*, 2008). The inhibition of any of the drug-metabolizing CYPs leads to the increase in the concentration of the drug substrate and probable drug overdose (Murray, 2006).

Six toxicity classes were defined based on the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) defined six toxicity classes by making use of the LD_{50} thresholds of 5, 50, 300, 2000 and 5000 mg/kg body weight (Drwal *et al.*, 2014). The LD_{50} is the median lethal dose. This is the dose of a substance which upon administration causes fatality of half or 50% of the test subjects. The Predicted LD_{50} (mg/kg) in table 4 reveals that Christinin III and I are both fatal if swallowed, quercetin is toxic if swallowed, eriodictyol and maslinic acid are both harmful if swallowed, sisymbrifolin, jujubogenin, colubrinic, and epiceanothic may be harmful if swallowed, while (+)-catechin cianidanol, (-)-epicatechin, ceanothenic acid and acarbose were non-toxic. All the phytochemical compounds are not

hepatotoxic whereas acarbose was hepatoxic. Quercetin, eriodictyol, christinin III and I, maslinic acid, alphitolic acid and betulinic acid were carcinogenic compounds. Jujubogenin, christinin III and I, zizyberanalic or colubrinic acid, epiceanothic, maslinic acid, alphitolic acid, betulinic acid, ceanothic acid and acarbose were immunotoxic . quercetin, Christinin III and I are mutagenic. None of the compounds was cytotoxic. In overall, the toxicity profile of this study reveals that (+)-catechin cianidanol, (-)-epicatechin, sisymbrifolin, and ceanothenic acid were not hepatotoxic, carcinogenic, immunotoxic, mutagenic, and cytotoxic.

Molecular docking as a computer-based approach, is used in structure-based drug design comprising of numerous tools for the prediction of binding model and energy of ligands with their orientation or pose on the active site of proteins (Elekofehinti *et al.*, 2021; Kumar *et al.*, 2021). The curated compounds from *Ziziphus spina-christi* namely (+)-Catechin cianidanol, (-)-Epicatechin, Quercetin, Eriodictyol, Sisymbrifolin, Jujubogenin, Christinin III, Zizyberanalic or colubrinic acid, Christinn 1, Ceanothenic acid, Epiceanothic, Maslinic acid, Alphitolic acid, Betulinic acid, and Ceanothic acid demonstrated a better binding affinity and orientation compared to acarbose (the reference drug) that has a binding energy of -7.3 kcal/mol for the selective active site residues of α-amylase as shown in table 5. Generally, the more negative the binding energy the better the affinity of a ligand to a target receptor. Jujubogenin is suggested the best candidate for exhibiting the highest negative binding energy of -8.9 kcal/mol, followed by maslinic acid and (+)-Catechin cianidanol with binding energies -8.5 and -8.4 Kcal/mol respectively. The compounds from *Ziziphus spina-christi* had better affinities compared to the standard drug acarbose. The lower binding energies of these phytochemicals as compared to acarbose is likely due to the greater number of weak non-covalent intermolecular interactions and thermodynamically feasible orientations or pose in the active site, especially the hydrogen bonds interactions and the various hydrophobic interactions with the amino acid residues of the enzyme. Therefore, the relatively higher negative binding energy of these compounds from the docking results shows that they could be better selective inhibitors than the reference drug for α-amylase in the management of postprandial hyperglycaemia.

Jujubogenin is a complex triterpene with nine contiguous stereocenters, six of which are quaternary, including four all-carbon quaternary centers (Karimov *et al.*, 2018). They have been shown to have memory enhancing activity, anti-ageing, anticancer, anticonvulsant, antidepressant, anti-emetic, anti-inflammatory, bronchovasodilatatory, antipyretic, sedative, mast cell stabilizing activities and antibacterial properties in a variety of pre-clinical and clinical studies as reviewed in (Bhandari *et al.*, 2020; Murthy, 2022). Maslinic acid, a pentacyclic triterpene acid, exhibit a broad range of biological activity, for example anti-diabetic, anti-inflammatory, antimicrobial, anticancer, neuroprotective and hepatoprotective activities (Deng *et al.*, 2020). While, catechins are polyphenolic compounds with antioxidative property, and has been linked to the chemo-preventive and anti-inflammatory activities of whole tea (Daussin *et al.*, 2021).

Furthermore, negatively charged (acidic) amino acid residues of D197, E233 and D300 in the active site of α-amylase have been described to play key role in its catalysis. The compounds interactions at the enzyme active site pocket include hydrogen bonding, van der waals, and hydrophobic: pi-pi stacking, pi-anion, alky, pi-alkyl, and pi-sigma interactions as shown in figure 2-5 and table 6. Jujubogenin, maslinic acid, and (+)-catechin cianidanol which are the phytochemicals with the best binding affinity, intermolecularly interacted with Asp197, asp300, or glu233 residues in the active site of the α-amylase. These compounds could comparably inhibit amylase like acarbose since there interactions would inhibit or slow down the catalysis of polysaccharides to oligomers. This inevitably may lead to the postprandial sugar control benefit for type 2 diabetic mellitus patient. Hydrogen bond interactions have strong influence on drug specificity, absorption, and metabolism in drug design. Furthermore, hydrophobic interactions exist between hydrophobic amino acid resides and aromatic or aliphatic group on the compounds (ligands) (Omoboyowa *et al.*, 2021). The molecular docking result of the compounds from *Ziziphus spina* depicts the probable mechanism for the inhibition of α-amylase.

CONCLUSION

The results from this study reveals the potential of phytochemical compounds from *Ziziphus spina-christi* as an inhibitor to α-amylase which is a drug target for the management of diabetes mellitus. Among the compounds that did not violate the Lipinski rule, jujubogenin has the highest negative binding affinity. Also, all the compounds displayed higher negative binding affinity compared to acarbose. Jujubogenin, Maslinic acid, and (+)-Catechin cianidanol which are the best three phytochemicals with α-amylase in terms of binding affinity, intermolecularly interacted with key catalytic residues of Asp197, Asp300, or Glu233 residues in the active site of the α-amylase. *Ziziphus spina-christi* postprandial sugar control benefit for type 2 diabetic mellitus patient and molecular dynamic simulation study on the three best identified compounds is recommended to establish their binding stability with α-amylase.

AUTHORS' CONTRIBUTIONS

Conceptualisation, MAA and AM; investigation- drug likeness, ADME and molecular docking analyses, AH, MAA, EOO, and ALM; writing- original manuscript, MAA, EOO, and ALM; writing- review and editing, AH, MAA and Supervision, AM. All authors read and approved the submission.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

DATA AVAILABILITY

Availability of data that supports the findings of this study is available from the corresponding author, upon reasonable request.

REFERENCES

Abalaka, M., Daniyan, S. and Mann, A. (2010). Evaluation of the antimicrobial activities of two *Ziziphus* species (*Ziziphus mauritiana* L. and *Ziziphus spinachristi* L.) on some microbial pathogens. *African Journal of Pharmacy and Pharmacology*, *4*, 135–139.

Ads, E. N., Hassan, S. I., Rajendrasozhan, S., Hetta, M. H., Aly, S. H. and Ali, M. A. (2022). Isolation, structure elucidation and antimicrobial evaluation of natural pentacyclic triterpenoids and phytochemical investigation of different fractions of *Ziziphus spina-christi* (L.) stem bark using LCHRMS *analysis. Molecules (Basel, Switzerland)*, *27*(6). https://doi.org/10.3390/MOLECULES27061805

Akinloye, O. A., Akinloye, D. I., Onigbinde, S. B. and Metibemu, D. S. (2020). Phytosterols demonstrate selective inhibition of COX-2: *In-vivo* and *in-silico* studies of *Nicotiana tabacum*. *Bioorganic Chemistry*, 102, 104037. https://doi.org/10.1016/J.BIOORG.2020.104037

Asgarpanah, J. and Haghighat, E. (2012). Phytochemistry and pharmacologic properties of *Ziziphus spina christi* (L.) Willd. *Undefined*, 6(31). https://doi.org/10.5897/AJPP12.509

Azam, F., Vijaya Vara Prasad, M., Thangavel, N., Kumar Shrivastava, A. and Mohan, G. (2012). Structure-based design, synthesis and molecular modeling studies of thiazolyl urea derivatives as novel anti-parkinsonian agents. *Medicinal Chemistry*, 8(6), 1057–1068. https://doi.org/10.2174/157340612804075034

Benet, L. Z., Hosey, C. M., Ursu, O. and Oprea, T. I. (2016). BDDCS, the Rule of 5 and drugability. *Advanced Drug Delivery Reviews*, 101, 89–98. https://doi.org/10.1016/J.ADDR.2016.05.007

Bhandari, P., Sendri, N. and Devidas, S. B. (2020). Dammarane triterpenoid glycosides in Bacopa monnieri: A review on chemical diversity and bioactivity. *Phytochemistry*, 172, 112276. https://doi.org/10.1016/J.PHYTOCHEM.2020.112276

Bozicevic, A., De Mieri, M., Di Benedetto, A., Gafner, F. and Hamburger, M. (2017). Dammarane-type saponins from leaves of *Ziziphus spina-christi*. *Phytochemistry*, 138, 134–144. https://doi.org/10.1016/J.PHYTOCHEM.2017.02.028

Brayer, G. D., Luo, Y. and Withers, S. G. (1995). The structure of human pancreatic alpha-amylase at 1.8 A resolution and comparisons with related enzymes. *Protein Science: A Publication of the Protein Society*, 4(9), 1730–1742. https://doi.org/10.1002/PRO.5560040908

Brown, C. M., Reisfeld, B. and Mayeno, A. N. (2008). Cytochromes P450: A Structure-based summary of biotransformations using representative substrates. *40*(1), 1–100. https://doi.org/10.1080/03602530701836662

Daina, A., Michielin, O. and Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules OPEN. *Nature Publishing Group*. https://doi.org/10.1038/srep42717

Dallakyan, S. and Olson, A. J. (2015). Small-molecule library screening by docking with PyRx. *Methods in Molecular Biology*, 1263, 243–250. https://doi.org/10.1007/978-1-4939-2269-7_19/COVER

Daussin, F. N., Heyman, E. and Burelle, Y. (2021). Effects of (−)-epicatechin on mitochondria. *Nutrition Reviews*, *79*(1), 25–41. https://doi.org/10.1093/NUTRIT/NUAA094

Deng, J., Wang, H., Mu, X., He, X., Zhao, F. and Meng, Q. (2020). Advances in research on the preparation and biological activity of maslinic acid. *Mini-Reviews in Medicinal Chemistry*, 21(1), 79–89. https://doi.org/10.2174/1389557520666200722134208

Drwal, M. N., Banerjee, P., Dunkel, M., Wettig, M. R. and Preissner, R. (2014). ProTox: a web server for the *in silico* prediction of rodent oral toxicity. *Nucleic Acids Research*, 42(W1), W53–W58. https://doi.org/10.1093/NAR/GKU401

Eberhardt, J., Santos-Martins, D., Tillack, A. F. and Forli, S. (2021). AutoDock Vina 1.2.0: New docking methods, expanded force field, and python bindings. *Journal of Chemical Information and Modeling*, 61(8), 3891–3898. https://doi.org/10.1021/ACS.JCIM.1C00203

el Maaiden, E., el Kharrassi, Y., Qarah, N. A. S., Essamadi, A. K., Moustaid, K. and Nasser, B. (2020). Genus *Ziziphus*: A comprehensive review on ethnopharmacological, phytochemical and pharmacological properties. *Journal of Ethnopharmacology*, 259. https://doi.org/10.1016/J.JEP.2020.112950

Elekofehinti, O. O., Iwaloye, O., Josiah, S. S., Lawal, A. O., Akinjiyan, M. O. and Ariyo, E. O. (2021). Molecular docking studies, molecular dynamics and ADME/tox reveal therapeutic potentials of STOCK1N-69160 against papain-like protease of SARS-CoV-2. *Molecular Diversity*, 25(3). https://doi.org/10.1007/s11030-020-10151-w

El-Shahir, A. A., El-Wakil, D. A., Latef, A. A. H. A. and Youssef, N. H. (2022). Bioactive Compounds and antifungal activity of leaves and fruits methanolic extracts of *Ziziphus spina-christi* L. *Plants (Basel, Switzerland)*, 11(6). https://doi.org/10.3390/PLANTS11060746

Farmani, F., Moein, M., Amanzadeh, A., Kandelous, H. M., Ehsanpour, Z. and Salimi, M. (2016). Antiproliferative evaluation and apoptosis induction in MCF- 7 cells by *Ziziphus spina christi* leaf extracts. *Asian Pacific Journal of Cancer Prevention : APJCP*, 17(1), 315–321. https://doi.org/10.7314/APJCP.2016.17.1.315

Gurung, A. B., Ali, M. A., Bhattacharjee, A., Abul Farah, M., Al-Hemaid, F., Abou-Tarboush, F. M., Al-Anazi, K. M., Al-Anazi, F. S. M. and Lee, J. (2016). Molecular docking of the anticancer bioactive compound proceraside with macromolecules involved in the cell cycle and DNA replication. *Genetics and Molecular Research: GMR*, 15(2). https://doi.org/10.4238/GMR.15027829

Gurung, A. B., Ali, M. A., Lee, J., Farah, M. A. and Al-Anazi, K. M. (2020). Unravelling lead antiviral phytochemicals for the inhibition of SARS-CoV-2 M pro enzyme through in silico approach. *Life Sciences*, *255*. https://doi.org/10.1016/J.LFS.2020.117831

Hou, T. J. and Xu, X. J. (2004). Erratum: ADME evaluation in drug discovery. 3. Modeling blood-brain barrier partitioning using simple molecular descriptors (J. Chem. Inf. Comput. Sci.

(2003) 43, (2137-2152). In *Journal of Chemical Information and Computer Sciences* 44, (2). https://doi.org/10.1021/ci040020a

International Diabetes Federation. (2021). *IDF Diabetes Atlas, 10th edn.* Brussels, Belgium. Available at: https://www.diabetesatlas.org

Jafarian, A., Zolfaghari, B. and Shirani, K. (2014). Cytotoxicity of different extracts of arial parts of *Ziziphus spina-christi* on Hela and MDA-MB-468 tumor cells. *Advanced Biomedical Research*, 3(1), 38. https://doi.org/10.4103/2277-9175.125727

Kagawa, M., Fujimoto, Z., Momma, M., Takase, K. and Mizuno, H. (2003). Crystal structure of Bacillus subtilis alpha-amylase in complex with acarbose. *Journal of Bacteriology*, *185*(23), 6981–6984. https://doi.org/10.1128/JB.185.23.6981-6984.2003

Karimov, R. R., Tan, D. S. and Gin, D. Y. (2018). Synthesis of the hexacyclic triterpene core of the jujuboside saponins via tandem Wolff rearrangement–intramolecular ketene hetero-Diels–Alder reaction. *Tetrahedron*, *74*(26), 3370–3383. https://doi.org/10.1016/j.tet.2018.04.051

Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., Li, Q., Shoemaker, B. A., Thiessen, P. A., Yu, B., Zaslavsky, L., Zhang, J. and Bolton, E. E. (2021). PubChem in 2021: new data content and improved web interfaces. *Nucleic Acids Research*, *49*(D1), D1388–D1395. https://doi.org/10.1093/NAR/GKAA971

Kumar, H. S. S., Kumar, S. R., Kumar, N. N. and Ajith, S. (2021). Molecular docking studies of gyrase inhibitors: weighing earlier screening bedrock. *In Silico Pharmacology, 9(1).* https://doi.org/10.1007/s40203-020-00064-9

Li, C., Begum, A., Numao, S., Kwan, H. P., Withers, S. G. and Brayer, G. D. (2005). Acarbose rearrangement mechanism implied by the kinetic and structural analysis of human pancreatic alpha-amylase in complex with analogues and their elongated counterparts. *Biochemistry*, *44*(9), 3347–3357. https://doi.org/10.1021/BI048334E

Lipinski, C. A., Lombardo, F., Dominy, B. W. and Feeney, P. J. (2001). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews*, 46(1–3), 3–26. https://doi.org/10.1016/S0169-409X(00)00129-0

Murray, M. (2006). Role of CYP pharmacogenetics and drug-drug interactions in the efficacy and safety of atypical and other antipsychotic agents. *The Journal of Pharmacy and Pharmacology*, 58(7), 871–885. https://doi.org/10.1211/JPP.58.7.0001

Murthy, H. N. (2022). Biotechnological production of bacosides from cell and organ cultures of Bacopa monnieri. *Applied Microbiology and Biotechnology 2022 106:5*, 106(5), 1799–1811. https://doi.org/10.1007/S00253-022-11834-0

O'Boyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T. and Hutchison, G. R. (2011). Open Babel: An Open chemical toolbox. *Journal of Cheminformatics*, 3(10), 1–14. https://doi.org/10.1186/1758-2946-3-33/tables/2

Omoboyowa, D. A., Balogun, T. A., Omomule, O. M. and Saibu, O. A. (2021). Identification of terpenoids from abrus precatorius against Parkinson's disease proteins using *in silico* approach. *Bioinformatics and Biology Insights*, 15. https://doi.org/10.1177/11779322211050757

Panduraju, T., Rao, P. R. S. and Kumar, V. S. (2009). A study on antimicrobial activity of Rumex vesicarius Linn. *International Journal of Pharmacy and Technology*, *1*(1), 21–25.

Pawlowska, A. M., Camangi, F., Bader, A. and Braca, A. (2009). Flavonoids of Zizyphus jujuba L. and Zizyphus spina-christi (L.) Willd (Rhamnaceae) fruits. *Food Chemistry*, *112*(4), 858–862. https://doi.org/10.1016/j.foodchem.2008.06.053

Rosak, C. and Mertes, G. (2012). Critical evaluation of the role of acarbose in the treatment of diabetes: patient considerations. *Diabetes, Metabolic Syndrome and Obesity*, *5*, 357–367. https://doi.org/10.2147/DMSO.S28340

Sakna, S. T., Mocan, A., Sultani, H. N., El-fiky, N. M., Wessjohann, L. A. and Farag, M. A. (2019). Metabolites profiling of *Ziziphus* leaf taxa via UHPLC/PDA/ESI-MS in relation to their biological activities. *Food Chemistry*, *293*, 233–246. https://doi.org/10.1016/j.foodchem.2019.04.097

Sarfaraj, H. M., Azam, F., Ahmed Eldarrat, H., Haque, A., Khalid, M., Zaheen Hassan, M., Ali, M., Arif, M., Ahmad, I., Zaman, G., Alabdallah, N. M. and Saeed, M. (2022). Structural, functional, molecular, and biological evaluation of novel triterpenoids isolated from Helichrysum stoechas (L.) Moench. Collected from Mediterranean Sea bank: Misurata-Libya. *Arabian Journal of Chemistry*, 15(6), 103818. https://doi.org/10.1016/j.arabjc.2022.103818

Terao, J. and Mukai, R. (2014). Prenylation modulates the bioavailability and bioaccumulation of dietary flavonoids. *Archives of Biochemistry and Biophysics*, 559, 12–16. https://doi.org/10.1016/J.ABB.2014.04.002

Trott, O. and Olson, A. J. (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, 31(2), NA-NA. https://doi.org/10.1002/JCC.21334

Tuenter, E., Foubert, K., Staerk, D., Apers, S. and Pieters, L. (2017). Isolation and structure elucidation of cyclopeptide alkaloids from Ziziphus nummularia and *Ziziphus spina-christi* by HPLC-DAD-MS and HPLC-PDA-(HRMS)-SPE-NMR. *Phytochemistry*, *138*, 163–169. https://doi.org/10.1016/j.phytochem.2017.02.029

Vahedi, F., Fathi Najafi, M. and Bozari, K. (2008). Evaluation of inhibitory effect and apoptosis induction of *Zyzyphus jujube* on tumor cell lines, an in vitro preliminary study. *Cytotechnology*, *56*(2), 105. https://doi.org/10.1007/S10616-008-9131-6

Zheng, Y., Ley, S. H. and Hu, F. B. (2017). Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nature Reviews Endocrinology* 2017 14:2, 14(2), 88–98. https://doi.org/10.1038/nrendo.2017.151