Original Research Article

Molecular interactions of α-amylase with compounds and their physicochemical properties for antidiabetic potentials reported in Senna auriculata leaves methanolic extract

ABSTRACT

Aims: Diabetes mellitus (DM) is chronic disorder well known for increased glucose level in blood. This disease can be controlled by inhibiting the enzyme (e.g., α-amylase) involved in carbohydrate hydrolysis. Senna auriculata leaves methanolic extract (SALME) have potential antidiabetic properties and it was also found to be safe in preclinical studies. In this study the aim was to explore the molecular interactions of α-amylase and bioactive compounds in SALME and their physicochemical properties.

Methodology: Computational approach such as molecular docking and physicochemical analysis prediction was applied to understand the antidiabetic potential of natural compounds present in SALME.

Results: The results showed from physicochemical analysis that out of 11 only 7 compounds are having drug likeness properties which are orally and intestinally better bioavailable. Furthermore, molecular docking analysis explained that three compounds (C3, C4, and C7) have lower binding energy, ΔG (-8, -9.1, -9.5 kcal/mol) and better binding affinity, Ki (7.31 x 10^5, 4.68 x 10^6, and 9.2 x 10^6 M^{-1}, respectively) than the acarbose ΔG (-7.8 kcal/mol) and Ki (6.18 x 10^5 M^{-1}), a well-known FDA approved medication for DM. The study also explained the binding pattern that the catalytic residue such as Asp197, Glu233 and Asp300 are involved in stabilizing the natural compounds with in the catalytic active site of target enzyme.

Conclusions: From the results it has been concluded that these three compounds found in SALME have better inhibitory potential for α-amylase in comparison with acarbose. Further validation of the findings is required through molecular dynamics simulation, ADME-T study, and in-vitro enzyme inhibition by the purified compounds.

Keywords: Diabetes Mellitus, Senna Auriculata, A-amylase, molecular docking, Enzyme inhibition.

1. INTRODUCTION

Diabetes is a non-communicable chronic disorder which effects nearly 422 million people globally, responsible for 1.5 million deaths annually and predicted to negatively effects around 700 million people in 2045 [1,2]. Type 2 Diabetes mellitus (T2DM) occurs due to imbalance in carbohydrate metabolism which decreases the cellular concentration of glucose and negatively effects several other metabolic processes related to nephropathy, retinopathy, heart, fracture, Covid-19,

neuro-disorder [3–7]. The common causes for T2DM are destruction of beta cell in pancreas, insulin deficiency, and/or non-responsive insulin receptors which leaves the high level of glucose in blood and is the primary diagnostic parameter for hyperglycemia [8]. Oxidative stress and other environmental factors (cigarette smoking) also play a major role for the imbalance of several metabolic activities and can be interlinked with diabetes, inflammation, and cardiovascular diseases [9–12]. There is an increased economic burden of diabetes management and presumed to reached up to USD 2.5 million in 2030, which indicates an urgent need of cost-effective management and control of T2DM [9].

Previous studies focused on to control the increased level of glucose in blood by inhibiting the enzyme (α-amylase) responsible for the catabolism of polysaccharides into smaller molecules such as monosaccharides [13]. Several therapeutic medications for the management of T2DM are well-proven α-amylase inhibitors such as acarbose, miglitol and voglibose [14]. However, these medications have several side effects like diarrhea, gastrointestinal discomfort, hepatotoxicity, and pancreatitis [15]. Therefore, efforts made to develop novel inhibitors, of natural origin to minimize the side effects and economic burden [16,17].

Plants are well-known for good source of medicinal metabolites which can cure various human disease complications such as oxidative stress, gastric ulcer, microbial infection, inflammation, hyperglycemia, hyperlipidemia, neuro-disorders, including cancer by inhibiting the key regulatory enzymes [18–32]. Moreover, various medicinal properties (antioxidant, antidiabetic, anti-inflammatory, anticancer) have been reported in leaves, flowers, roots, seeds, and stem Cassia auriculata also known as Senna auriculata [33]. Recently it has been confirmed from invitro approach that methanolic extracts of Senna auriculata leaves (SALME) have great anti-diabetic potential including anti-inflammatory and antioxidant properties [34]. Beside invitro medicinal properties of SALME, Prasanth kumar et al., also evaluated their metabolites through GC-MS analysis and reported twenty-one compounds which might be responsible for its medicinal properties [34]. Some of these compounds are available in chemical database such as Pubchem and ChemSpider. Therefore, it has been hypothesized in this study to evaluate the physicochemical properties, medicinal chemistry and to find that which of these metabolites are highly effective for antidiabetic properties by inhibiting α-amylase enzyme through several computational tools. Moreover, the molecular interactions of best bioactive metabolite have also been evaluated. This study will give an idea of best bioactive metabolite of SALME for antidiabetic potential which can be further confirmeds through in-vitro and in-vivo approaches.

2. METHODOLOGY

2.1. Hardware and Software Used

The 3-D crystallographic structure of the target protein (2QV4) was downloaded from the protein data bank database (http://www.rcsb.org/pdb/) [35]. The molecular docking was performed using PyRx-Python prescription 0.8 using Autodock-Vina with the Lamarckian genetic algorithm as a scoring function [30,36]. The interactions of molecules between best scoring ligand and protein were individually visualized and analyzed through Discovery Studio visualizer 2021 (BIOVIA) software tool [37,38]. The system used for computational study was Intel(R) Core (TM) i7-8550U CPU @ 1.80GHz, having 2.0 GHz processor including 16 GB RAM. The graphic card used in this workstation was Intel® UHD Graphics 620.

2.2. Prediction of physicochemical properties

The physicochemical properties such as MW (molecular weight), MR (molar refractivity), PSA (polar surface area), nHBD (number of hydrogen bond donors), nHBA (number of hydrogen bond acceptor), RB (number of rotatable bond), HA (number of heavy atoms), AHA (number of aromatic heavy atoms) were predicted through SwissADME (http://www.swissadme.ch) web based tool [39].

2.3. Preparation of Ligands

The ligands (natural compounds) predicted through GC-MS analysis of SALME were used in this study [34]. The ".sdf" and/or ".mol" file for 3-D structure of ligands were downloaded from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and ChemSpider database (http://www.chemspider.com/), respectively. These ligands were then energy minimized using universal force field (UFF) and density function theory (DFT and further converted to Autodock suitable ".pdbqt" file format through inbuilt OpenBabel tool in PyRx software.

2.4. Preparation of target protein

The co-crystallized 3-D structure of human pancreatic α-amylase enzyme (PDB Id: 2QV4) at 1.97 Å resolution with its native ligand (acarbose) was extracted from Protein data bank (PDB) database (http://www.rcsb.org/pdb/) [40,41]. The target protein was prepared for molecular docking study by removing the all heteroatom such as native ligands, and non-essential water molecules, adding hydrogens (polar olnly), calculating Gasteiger charges, and converting ".pdb" file format to ".pdbqt" format. The energy minimization and ensuring that no residues carry the non-integral charges of protein structure was performed using a built-in tool in PyRx.

2.5. Molecular docking Study

Virtual screening was performed through the PyRx-Python 0.8 software. PyRx uses the AutoDock 4.2 and AutoDock Vina docking engine with Lamarkian Genetic algorithm method (Dallakyan and Olson 2015; Trott and Olson 2010). All the natural compounds (ligands) were individually docked with the α-amylase protein (2QV4). The grid box dimensions for target protein were selected through discovery studio visualizer and was set to 25x25x25 Å, the coordinates of grid box were centered at 12.33x48.02x25.63 Å, which was similar as discussed in previous report [42]. The docking was performed with the "exhaustiveness" set to 8. All other docking parameters were set to the default values of the software. The binding affinity (Kd) of ligands for the target protein was calculated from the Gibb's free binding energy (ΔG) using the following relation [30]:

$$\Delta G = -RT\ lnK_d \quad (1)$$

In this equation universal gas constant is denoted as "R" whereas temperature is defined as "T".

The ligands with the minimum Gibb's free binding energy were selected for further analysis. The best pose of each "protein–ligand complex" was generated and analyzed using Discovery Studio 2021 (BIOVIA).

3. RESULTS AND DISCUSSION

3.1. Physicochemical properties of SALME compounds

The reason behind most of the drugs failed during clinical trials and drug development process are now well understood that the druglike compounds have certain criteria to be followed and it is well documented in several reports [43,44]. Ninety percent of orally active medications that have accomplished the clinical phase 2 trial represents the four physicochemical parameters in specific range such as molecular weight (MW) ≤ 500, log P ≤ 5, hydrogen bond donors (HBD) ≤ 5, and hydrogen bond acceptor (HBA) less than 10. Compounds having more than 10 rotatable bonds usually have poor oral bioavailability [45]. Some of the SALME compounds reported in this current study follow the criteria of these physicochemical properties (Table 1). Our results depicted that out of 11 compounds (C1-C11) 5 compounds (C7, C8, C9, C10, and C11) have more than 500 of molecular weights. Three (C5, C6, and C9) out of eleven compounds (C1-C11) selected for this study have more than 9 rotatable bonds, one compound (C11) and standard drug acarbose (ACA) represents ≥10 HBA, acarbose also showed more than 5 HBD. Molar refractivity (MR) range considered to be from 40 to 130 for better intestinal and oral absorption [46]. The results showed that four SALME compounds (C7, C9, C10, and C11) have higher than 130 value for MR. Those compounds follow the three

properties out of five properties of Lipinski rule can be acceptable for the drug-likeness [44]. Our results showed that C9, and C11 are not suitable as drug candidate.

Table 1: Physicochemical properties of compounds present in SALME.

			Physicochemical properties							
Code	Name	Formula	MW	#Heavy atoms	#Aromatic heavy atoms	#Rotatable bonds	#H-bond acceptors	#H-bond donors	MR	TPSA
C1	Methyl inositol	C7H14O6	194.18	13	0	0	6	6	40.66	121.38
C2	7,10-Epoxytricyclo[4.2.1.1(2,5)]decane, 1-trimethylsilyl-	C13H22OSi	222.4	15	0	1	1	0	65.1	9.23
C3	1H-Purin-6-amine, N-((3-fluorophenyl)methyl)-2-[(E)-2-(3,4,5-Trifluorophenyl)vinyl]	C12H10FN5	243.24	18	15	2	4	2	64.11	69.72
C4	naphthalene	C18H11F3	284.28	21	16	2	3	0	79.19	0
C5	13-Docosenamide, (Z)-	C22H43NO	337.58	24	0	19	1	1	110.3	43.09
C6	Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C21H42O4	358.56	25	0	20	4	2	106.67	66.76
C7	6-(4-Chlorophenyl)-2,5,5-triphenyl-5,8-dihydro-6H-azeto[1,2-a][1,3]thiazolo[4,5-d]pyrimidine	C31H22ClN3S	504.04	36	29	4	2	0	155.6	56.73
C8	Cycloheptasiloxane, tetradecamethyl-	C14H42O7Si7	519.08	28	0	0	7	0	129.97	64.61
C9	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyloctasiloxane	C16H50O7Si8	579.25	31	0	14	7	0	150.24	64.61
C10	Cyclooctasiloxane, hexadecamethyl-	C16H48O8Si8	593.23	32	0	0	8	0	148.54	73.84
C11	Cyclodecasiloxane, eicosamethyl-	C20H60O10Si10	741.54	40	0	0	10	0	185.67	92.32
ACA	Acarbose	C25H43NO18	645.6	44	0	9	19	14	136.69	321.17

3.2. Molecular Docking study

Computational study of molecular docking is widely used to identify the inhibitory property of various small molecules which can decrease the efforts and time of wet lab work [47,48]. In this study six compounds (C1, C3, C4, C5, C6, C7) were selected for molecular docking study to analyze the binding score (delta G) and binding affinity (Kd) for the target protein α-amylase (PDB I'd: 2QV4). We have excluded the compounds out of natural compounds identified in GC-MS analysis of SALME which are having higher molecular mass than 510 kD for molecular docking study. Before the docking of natural compounds with protein the native ligand (acarbose) was redocked and found that the redocked acarbose has almost bound to the similar residues where native ligand interacted, which validate the accuracy of results. The natural compounds were individually docked on the active site residues by removing the acarbose which generally binds as a competitive inhibitor [14,49]. The 2-D structure of α-amylase (2QV4) depicted through discovery studio visualizer and previous reports reveals that acarbose binds in the active site of protein with Trp59, Tyr62, Gln63, His101, Asn105, Ala106, Thr163, Arg195, Glu233, His299 and Asp300 by hydrogen bond. Moreover, several residues of protein such as Asp197, Ile235, His299, Leu165, Gln63, Ala198, were interacted with acarbose via van der Waals forces [50,51]. Similar amino acids such as Asp197, Glu233 and Asp300 were previously reported as key catalytic residues [42,52,53]. These catalytic residues having carboxyl group in it play a major role in catalyzing the α 1,4-glycosidic bondg of polysaccharides and help in carbohydrate digestion [54]. Brayer et al. [55] analyzed that there was 10^6-fold decline in catalytic activity of human pancreatic α-amylase enzyme by substituting Asp197 and 10^3-fold decrease in activity by substituting Glu233 and Asp300. This showed the importance of these residues for catalytic activity of enzyme.

Table 2: Molecular docking binding score of selected natural compounds of SALME.
(Acarbose (reference standard) = 41774; Superscript: * = PubChem-ID, # =ChemDraw-ID**)**

3.3. Binding score analysis

The results showed in Table 2, that binding energy of redocked acarbose to target protein is -7.7 Kcal/mol which is similar to the recent reports, where it was depicted as -7.8 Kcal/mol by Falese et al., -7.7 Kcal/mol by Munawaroh et al., -7.3 Kcal/mol by Mehmood et al., and -7.4 Kcal/mol by Sujayev et al. [56–59]. The results confirms that redocked acarbose mimic the binding pattern of reference inhibitor that is acarbose co-crystallized with target protein (Table 3, Figure 1A & 1C).

2QV4-Ligands binding score			
Code	**Compound name**	**Binding energy, kcal mol M^{-1}**	**Binding affinity (Ki), M^{-1}**
C1	53645858*	-6.3	4.15×10^4
C3	6455415*	-8	7.31×10^5
C4	8740362#	-9.1	4.68×10^6
C5	4517399#	-5.8	1.78×10^4
C6	71407#	-5.5	1.08×10^4
C7	9267510#	-9.5	9.2×10^6
ACA	41774*	-7.8	6.18×10^5

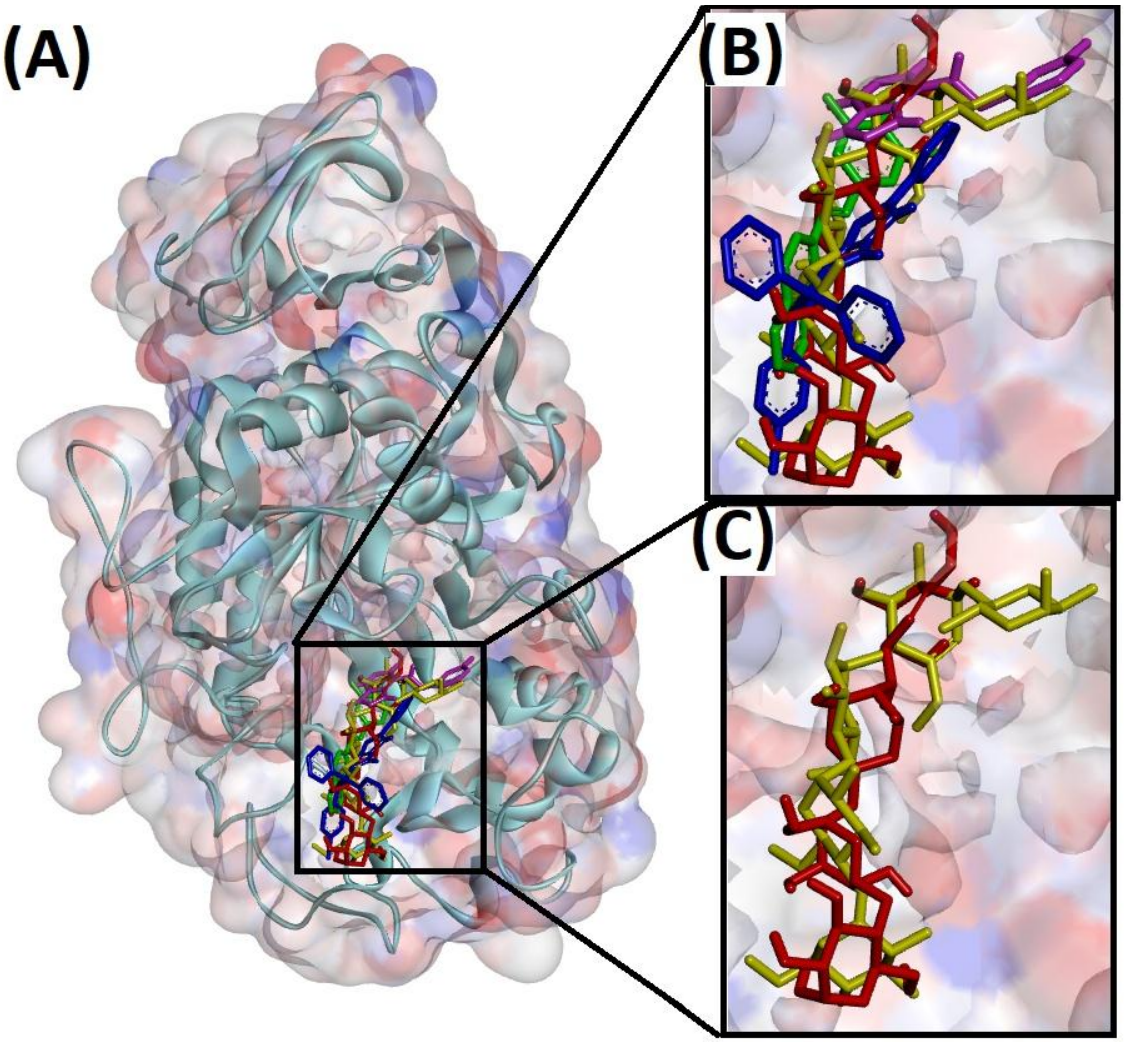
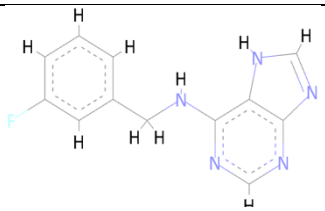
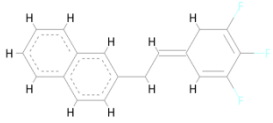
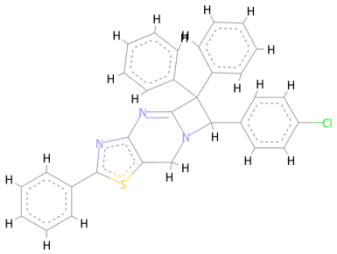
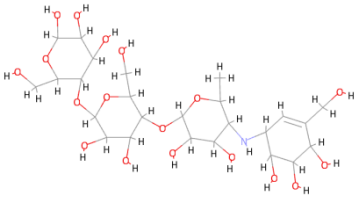


Figure 1. Superimpose image of docked ligands in the active site of α-amylase (PDB I'd: 2QV4). (A) All the docked ligands (native ligand: yellow color; Redocked Acarbose: Red color; Ligand C3 (Compound I'd-6455415*): Purple color; Ligand C4 (Compound I'd-8740362[#]): Green color; Ligand C7(Compound I'd-9267510[#]): Blue color) in catalytic active site (B) Zoom in image of all the docked ligands (C) Superimpose zoom in image of native ligand (acarbose) and redocked acarbose.

Table 3: Molecular interactions analysis and 2-D structure of best docked SALME metabolites.

Code	Compound-ID	2QV4-Ligand Interactions and residues (Distance Å)			
		2D-Structure	Hydrogen bond	Hydrophobic	Others [Halogen (Fluorine)]
C3	6455415		ILE235 (3.1), ASP197 (2.91), GLU233 (2.63), VAL234 (3.38)	ILE235 (3.52), HIS201 (4.45), LYS200 (5.02)	ILE235 (3.1), GLU233 (3.29)
C4	8740362			TRP59 (4.31, 5.68, 3.82, 4.23, 4.43)	ASP197 (2.71, 3.31, 3.17), HIS299 (3.14), ASP300 (2.88, 3.41)
C7	9267510		THR163 (2.99, 2.79)	LEU163 (3.48), THR163 (3.82), LEU165 (3.68), TRP59 (3.89, 4.33, 4.13), ALA106 (4.27), VAL107 (4.5), ALA198 (4.86)	NR
ACA	41774		GLN63 (3.53), ASN105 (3.14), ALA106 (3.09), THR163 (2.85), ASP300 (2.1), GLU233 (2.6), GLU233 (2.66), THR163 (2.4)	NR	NR

NR- Not reported

The redocked acarbose binds in the active site of protein with Asn105, Ala106, Thr163, Glu233 and Asp300 by conventional hydrogen bond and with Gln63 by carbon hydrogen bond. Moreover, several residues of protein such as Trp59, Leu165, Arg195, Asp197, Ala198, Ile235, and His299 were interacted with acarbose via van der Waals forces (Figure 2A). These residues are similar to those which present for the interactions of the co-crystallized acarbose in native structure of protein. The acarbose demonstrate the similar interactions with protein as reported earlier [59]. All the compounds reported in SALME depicted the binding energy from -5.5 to -9.5 Kcal/mol (Table 2). Three compounds in SALME coded as C3 (1H-Purin-6-amine, N-((3-fluorophenyl)methyl)-), C4 (2-[(E)-2-(3,4,5-Trifluorophenyl)vinyl]naphthalene), and C7 (6-(4-Chlorophenyl)-2,5,5-triphenyl-5,8-dihydro-6H-azeto[1,2-a][1,3]thiazolo[4,5-d]pyrimidine) exhibited better binding score such as -8, -

9.1, and -9.5 Kcal/mol, respectively and better binding affinity (Ki) such as 7.31 x 10^5, 4.68 x 10^6, and 9.2 x 10^6, respectively than the acarbose binding score (-7.7 Kcal/mol) and binding affinity (6.18 x 10^5). From the results it has been noted that the compounds who have more rings in their structure have better binding affinity and they are in relation with acarbose which also have 4 rings in its structure. The results of this study are in accordance with previous reports where natural compounds exhibited better activity than the standard drug against α-amylase [21,58].

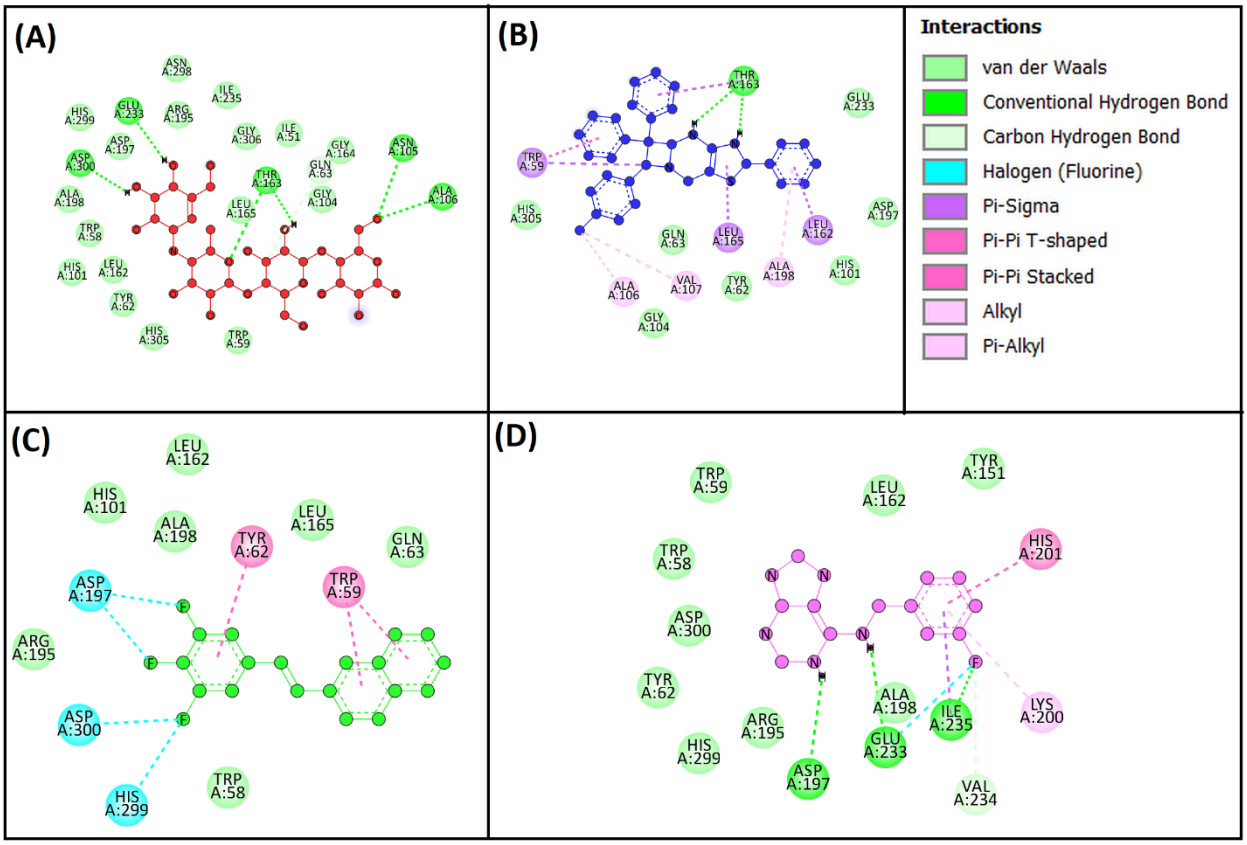


Figure 2. Molecular interactions analysis of best docked ligands with α-amylase (2QV4) enzyme. (A) Redocked acarbose; (B) C7 compound; (C) C4 compound; (D) C3 compound.

3.4. Molecular interactions analysis

All the compounds of SALME interacted with the same catalytic active site pocket of α-amylase where acarbose get binds (Figure 1A & 1B). The interaction pattern of best three natural compounds (C3, C4, and C7) with target protein are represented in Figure 2. The binding interactions of these three compounds were further compared with the binding pattern of control inhibitor (acarbose) and briefed in Table 3. It was observed that C3 and α-amylase complex was stabilized by

three halogen (Fluorine) interaction between ILE235:N - Ligand C3:F, VAL234:CA - Ligand C3:F, and GLU233:O - Ligand C3:F. Whereas three conventional hydrogen bond interactions were formed between ILE235:N - Ligand C3:F, Ligand C3:HN - ASP197:OD1, and Ligand C3:H - GLU233:OE2. Moreover, three hydrophobic interactions were observed between ILE235:CD1 - Ligand C3 (Pi-Sigma), HIS201 - Ligand C3 (Pi-Pi T-shaped) and Ligand C3 - LYS200 (Pi-Alkyl) to stabilize the complex (Figure 2D). The complex of amylase and C4 was stabilized by six halogen (fluorine) interactions between ASP197:OD1 - Ligand C4:F, ASP197:OD2 - Ligand C4:F, ASP197:OD3 - Ligand C4:F, HIS299:NE2 - Ligand C4:F, ASP300:OD1 -Ligand C4:F, and ASP300:OD2 - Ligand C4:F. Moreover, this complex was also stabilized by five hydrophobic Pi-Pi Stacked interactions between Ligand C4 and amino acid residues such as TRP59 and TYR62 of target protein (Figure 2C). The Ligand C7 and target protein (amylase) was stabilized by two conventional hydrogen bond between Ligand C7:HN - THR163:OG1 and Ligand C7:N - THR163:OG1, whereas nine hydrophobic interactions was observed in stabilizing the complex (amylase-Ligand C7) at amino acid residues LEU162, THR163, LEU165, TRP59, ALA106, VAL107, and ALA198. It was noticed that hydrophobic interactions were prominent in C7 and amylase complex (Figure 2B).

Interestingly, the amino acid residues of α-amylase commonly engaged in the interaction with natural compounds (C3, C4, and C7) as well as acarbose in the catalytic active site gorge including ASP197, GLU233, whereas ASP300 was observed in C3, C4 and acarbose when interacted with protein which show that natural compounds interacted with all the specific residues for acarbose [42,52,53]. These docking results confirms that natural compounds are competitive inhibitor of α-amylase.

In this study the focus was on to analyzing that which compound (metabolite) has better binding affinity to inhibit the α-amylase by using molecular docking software tools and can be a better therapeutic cure for Diabetes mellitus Type 2. However, there are some limitations in this study that the further explanation of interactions through molecular dynamics simulation analysis such as RMSD, RMSF, Rg, SASA, MolSA, PSA etc. was not analyzed and there was no in-vitro protocol used to understand the enzyme inhibition by these metabolites.

4. CONCLUSIONS

From the current study it has been concluded that some natural metabolites present in SALME such as C3, C4, and C7 exhibited better inhibitory potential of α-amylase than the standard approved drug acarbose. These compounds can be a better cure for non-insulin dependent diabetes. Further validation of this docking study required through molecular dynamics simulations and in-vitro enzyme inhibition by the purified compounds present in SALME.

CONSENT

"Author declares that this section is not applicable to this study."

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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