

## Original Research Article

# Molecular interactions of $\alpha$ -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 control by inhibiting the enzyme (e.g.,  $\alpha$ -amylase) involve 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 to explore the molecular interactions of  $\alpha$ -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,  $\Delta G$  (-8, -9.1, -9.5 kcal/mol) and better binding affinity,  $K_i$  ( $7.31 \times 10^5$ ,  $4.68 \times 10^6$ , and  $9.2 \times 10^6 \text{ M}^{-1}$ , respectively) than the acarbose  $\Delta G$  (-7.8 kcal/mol) and  $K_i$  ( $6.18 \times 10^5 \text{ M}^{-1}$ ), 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  $\alpha$ -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.

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*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 receptor 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 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 ( $\alpha$ -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  $\alpha$ -amylase inhibitors such as acarbose, miglitol and voglibose [14]. However, these medications

**Comment [U1]:** Change to «can be»

**Comment [U2]:** Change to «reach»

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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, Prasanthkumar 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  $\alpha$ -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 confirms 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  $\alpha$ -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 only), 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 Lamarckian Genetic algorithm method (Dallakyan and Olson 2015; Trott and Olson 2010). All the natural compounds (ligands) were individually docked with the  $\alpha$ -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 ( $\Delta G$ ) using the following relation [30]:

$$\Delta G = -RT \ln K_d$$

(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)  $\leq 500$ , log P  $\leq 5$ , hydrogen bond donors (HBD)  $\leq 5$ , 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  $\geq 10$  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.**

Co de	Name	Formula	MW	Physicochemical properties					MR	TPS A
				#He avy atom s	#Aroma tic heavy atoms	#Rotat able bonds	#H- bond accept ors	#H- bond donor s		
			194						40.	121.
C1	Methyl inositol 7,10- Epoxytricyclo[4.2.1. 1(2,5)]decane, 1-	C7H14O6	.18	13	0	0	6	6	66	38
C2	trimethylsilyl- 1H-Purin-6-amine, N-((3- fluorophenyl)methyl )-	C13H22O Si	222 .4	15	0	1	1	0	65. 1	9.23
C3	2-[(E)-2-(3,4,5- Trifluorophenyl)viny	C12H10F N5	243 .24	18	15	2	4	2	64. 11	69.7 2
C4		C18H11F 3	284 .28	21	16	2	3	0	79. 19	0

	l]naphthalene									
	13-Docosenamide,	C22H43N	337						110	43.0
C5	(Z)-	O	.58	24	0	19	1	1	.3	9
	Octadecanoic acid,									
	2-hydroxy-1-									
	(hydroxymethyl)eth	C21H42O	358						106	66.7
C6	yl ester	4	.56	25	0	20	4	2	.67	6
	6-(4-Chlorophenyl)-									
	2,5,5-triphenyl-5,8-									
	dihydro-6H-									
	azeto[1,2-									
	a][1,3]thiazolo[4,5-	C31H22Cl	504						155	56.7
C7	d]pyrimidine	N3S	.04	36	29	4	2	0	.6	3
	Cycloheptasiloxane	C14H42O	519						129	64.6
C8	, tetradecamethyl-	7Si7	.08	28	0	0	7	0	.97	1
	1,1,3,3,5,5,7,7,9,9,									
	11,11,13,13,15,15-									
	Hexadecamethyloct	C16H50O	579						150	64.6
C9	asiloxane	7Si8	.25	31	0	14	7	0	.24	1
C1	Cyclooctasiloxane,	C16H48O	593						148	73.8
0	hexadecamethyl-	8Si8	.23	32	0	0	8	0	.54	4
C1	Cyclodecasiloxane,	C20H60O	741						185	
1	eicosamethyl-	10Si10	.54	40	0	0	10	0	.67	92.3
A										
C		C25H43N	645						136	321.
A	Acarbose	O18	.6	44	0	9	19	14	.69	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  $\alpha$ -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 redock and found that redocked acarbose have almost bind 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  $\alpha$ -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  $\alpha$  1,4-glycosidic bond 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  $\alpha$ -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 <sup>-1</sup>	Binding affinity (K <sub>i</sub> ), M <sup>-1</sup>
C1	53645858*	-6.3	4.15 x 10 <sup>4</sup>
C3	6455415*	-8	7.31 x 10 <sup>5</sup>
C4	8740362 <sup>#</sup>	-9.1	4.68 x 10 <sup>6</sup>
C5	4517399 <sup>#</sup>	-5.8	1.78 x 10 <sup>4</sup>
C6	71407 <sup>#</sup>	-5.5	1.08 x 10 <sup>4</sup>
C7	9267510 <sup>#</sup>	-9.5	9.2 x 10 <sup>6</sup>
ACA	41774*	-7.8	6.18 x 10 <sup>5</sup>

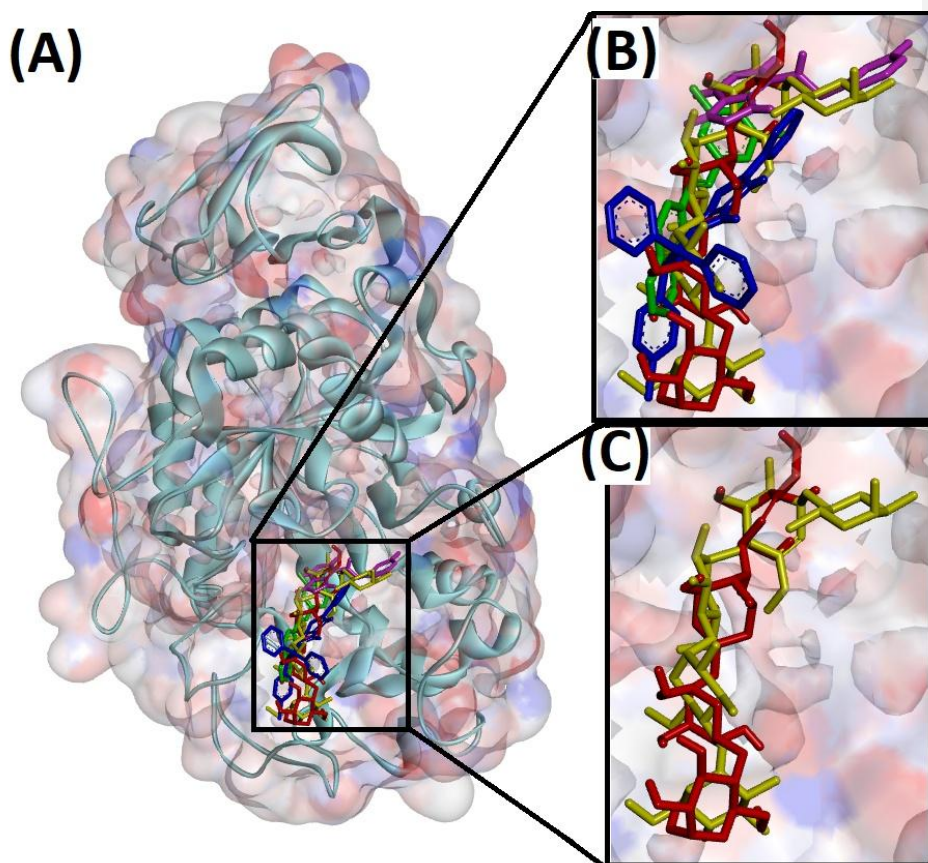
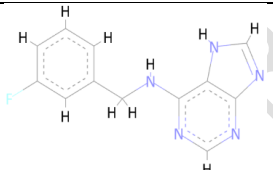
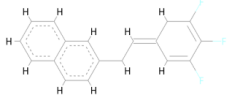
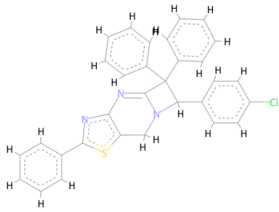


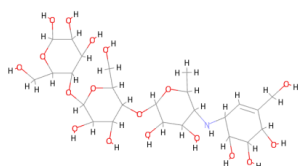
Figure 1. Superimpose image of docked ligands in the active site of  $\alpha$ -amylase (PDB I'd: 2QV4). (A) All the docked ligands (native ligand: yellow color; Redocked Acarbose: Red color; Ligand C3 (Compound I'd-6455415\*): Purple color; Ligand C4 (Compound I'd-8740362<sup>#</sup>): Green color; Ligand C7(Compound I'd-9267510<sup>#</sup>): 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	2D-Structure	2QV4-Ligand Interactions and residues (Distance Å)		
			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

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*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  $7.31 \times 10^5$ ,  $4.68 \times 10^6$ , and  $9.2 \times 10^6$ , respectively than the acarbose binding score (-7.7 Kcal/mol) and binding affinity ( $6.18 \times 10^5$ ). 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



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  $\alpha$ -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  $\alpha$ -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  $\alpha$ -amylase.

In this study the focus was on to analyzing that which compound (metabolite) has better binding affinity to inhibit the  $\alpha$ -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  $\alpha$ -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.

**Comment [U4]:** it does not cure but it can help in management.

#### 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 used 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.

UNDER PEER REVIEW

## References

- [1] Diabetes 2021. <https://www.who.int/westernpacific/health-topics/diabetes> (accessed November 12, 2021).
- [2] Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Res Clin Pract* 2019;157:107843. <https://doi.org/10.1016/j.diabres.2019.107843>.
- [3] Cole JB, Florez JC. Genetics of diabetes mellitus and diabetes complications. *Nat Rev Nephrol* 2020;16:377–90. <https://doi.org/10.1038/s41581-020-0278-5>.
- [4] Ding M, Bhupathiraju SN, Chen M, van Dam RM, Hu FB. Caffeinated and decaffeinated coffee consumption and risk of type 2 diabetes: a systematic review and a dose-response meta-analysis. *Diabetes Care* 2014;37:569–86. <https://doi.org/10.2337/dc13-1203>.
- [5] Guo W, Li M, Dong Y, Zhou H, Zhang Z, Tian C, et al. Diabetes is a risk factor for the progression and prognosis of COVID-19. *Diabetes Metab Res Rev* 2020:e3319. <https://doi.org/10.1002/dmrr.3319>.
- [6] Rangel ÉB, Rodrigues CO, de Sá JR. Micro- and Macrovascular Complications in Diabetes Mellitus: Preclinical and Clinical Studies. *Journal of Diabetes Research* 2019;2019:e2161085. <https://doi.org/10.1155/2019/2161085>.
- [7] Romero-Díaz C, Duarte-Montero D, Gutiérrez-Romero SA, Mendivil CO. Diabetes and Bone Fragility. *Diabetes Ther* 2021;12:71–86. <https://doi.org/10.1007/s13300-020-00964-1>.
- [8] Galicia-García U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, et al. Pathophysiology of Type 2 Diabetes Mellitus. *International Journal of Molecular Sciences* 2020;21:6275. <https://doi.org/10.3390/ijms21176275>.

- [9] Bommer C, Sagalova V, Heesemann E, Manne-Goehler J, Atun R, Bärnighausen T, et al. Global Economic Burden of Diabetes in Adults: Projections From 2015 to 2030. *Diabetes Care* 2018;41:963–70. <https://doi.org/10.2337/dc17-1962>.
- [10] Iqbal D, Burhan IW, Choudhary RK, Alaidarous M, Alshehri BM, Banawas S, et al. Analysis between Cigarette and Shisha Smokers for Early Atherogenesis: A Cardiovascular Disease. *Journal of Pharmaceutical Research International* 2021;175–86. <https://doi.org/10.9734/jpri/2021/v33i41A32316>.
- [11] Niemann B, Rohrbach S, Miller MR, Newby DE, Fuster V, Kovacic JC. Oxidative Stress and Cardiovascular Risk: Obesity, Diabetes, Smoking, and Pollution: Part 3 of a 3-Part Series. *J Am Coll Cardiol* 2017;70:230–51. <https://doi.org/10.1016/j.jacc.2017.05.043>.
- [12] Rehman K, Akash MSH. Mechanism of Generation of Oxidative Stress and Pathophysiology of Type 2 Diabetes Mellitus: How Are They Interlinked? *J Cell Biochem* 2017;118:3577–85. <https://doi.org/10.1002/jcb.26097>.
- [13] Bashary R, Vyas M, Nayak SK, Suttee A, Verma S, Narang R, et al. An Insight of Alpha-amylase Inhibitors as a Valuable Tool in the Management of Type 2 Diabetes Mellitus. *Curr Diabetes Rev* 2020;16:117–36. <https://doi.org/10.2174/1573399815666190618093315>.
- [14] Kaur N, Kumar V, Nayak SK, Wadhwa P, Kaur P, Sahu SK. Alpha-amylase as molecular target for treatment of diabetes mellitus: A comprehensive review. *Chemical Biology & Drug Design* 2021;98:539–60. <https://doi.org/10.1111/cbdd.13909>.
- [15] Haq FU, Siraj A, Ameer MA, Hamid T, Rahman M, Khan S, et al. Comparative Review of Drugs Used in Diabetes Mellitus—New and Old. *JDM* 2021;11:115–31. <https://doi.org/10.4236/jdm.2021.114009>.
- [16] Papoutsis K, Zhang J, Bowyer MC, Brunton N, Gibney ER, Lyng J. Fruit, vegetables, and mushrooms for the preparation of extracts with  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition properties: A review. *Food Chem* 2021;338:128119. <https://doi.org/10.1016/j.foodchem.2020.128119>.
- [17] Patle D, Vyas M, Khatik GL. A Review on Natural Products and Herbs Used in the Management of Diabetes. *Curr Diabetes Rev* 2021;17:186–97. <https://doi.org/10.2174/1573399816666200408090058>.
- [18] Ahmad J. Evaluation of Antioxidant and Antimicrobial Activity of Ficus Carica Leaves: an In Vitro Approach. *J Plant Pathol Microb* 2012;04. <https://doi.org/10.4172/2157-7471.1000157>.
- [19] Ahmad N, Bhatnagar S, Saxena R, Iqbal D, Ghosh AK, Dutta R. Biosynthesis and characterization of gold nanoparticles: Kinetics, in vitro and in vivo study. *Mater Sci Eng C Mater Biol Appl* 2017;78:553–64. <https://doi.org/10.1016/j.msec.2017.03.282>.
- [20] Ahmad P, Alvi SS, Iqbal D, Khan MS. Insights into pharmacological mechanisms of polydatin in targeting risk factors-mediated atherosclerosis. *Life Sci* 2020;254:117756. <https://doi.org/10.1016/j.lfs.2020.117756>.
- [21] Akhter F, Hashim A, Khan MS, Ahmad S, Iqbal D, Srivastava AK, et al. Antioxidant,  $\alpha$ -amylase inhibitory and oxidative DNA damage protective property of Boerhaavia diffusa (Linn.) root. *South African Journal of Botany* 2013;88:265–72. <https://doi.org/10.1016/j.sajb.2013.06.024>.
- [22] Akhter F, Alvi SS, Ahmad P, Iqbal D, Alshehri BM, Khan MS. Therapeutic efficacy of Boerhaavia diffusa (Linn.) root methanolic extract in attenuating streptozotocin-induced diabetes, diabetes-linked hyperlipidemia and oxidative-stress in rats. *Biomedical Research and Therapy* 2019;6:3293–306. <https://doi.org/10.15419/bmrat.v6i7.556>.
- [23] Alvi S, Ahmad P, Ishrat M, Iqbal D, Khan S. Secondary Metabolites from Rosemary (*Rosmarinus officinalis* L.): Structure, Biochemistry and Therapeutic Implications Against Neurodegenerative Diseases, 2019, p. 1–24. [https://doi.org/10.1007/978-981-13-7205-6\\_1](https://doi.org/10.1007/978-981-13-7205-6_1).

- [24] Iqbal D, Dukhyil AB, Khan MS. Geno-Protective, Free Radical Scavenging and Antimicrobial Potential of Hyptis suaveolens Methanolic Fraction: An In-Vitro Study. *Journal of Pharmaceutical Research International* 2021;46–57. <https://doi.org/10.9734/jpri/2021/v33i1131243>.
- [25] Iqbal D, Khan MS, Khan MS, Ahmad S, Hussain MS, Ali M. Bioactivity guided fractionation and hypolipidemic property of a novel HMG-CoA reductase inhibitor from *Ficus virens* Ait. *Lipids in Health and Disease* 2015;14:15. <https://doi.org/10.1186/s12944-015-0013-6>.
- [26] Iqbal D, Khan MS, Khan A, Ahmad S. Extenuating the role of *Ficus virens* Ait and its novel bioactive compound on antioxidant defense system and oxidative damage in cigarette smoke exposed rats. *Biomedical Research and Therapy* 2016;3:723–32.
- [27] Iqbal D, Khan A, Ansari I, Khan MS. Investigating The Role of Novel Bioactive Compound from *Ficus Virens* Ait on Cigarette Smoke Induced Oxidative Stress and Hyperlipidemia in Rats. *Iran J Pharm Res* 2017;16:1089–103.
- [28] Iqbal D, Khan MS, Khan MS, Ahmad S, Srivastava AK. An in vitro and molecular informatics study to evaluate the antioxidative and  $\beta$ -hydroxy- $\beta$ -methylglutaryl-CoA reductase inhibitory property of *Ficus virens* Ait. *Phytother Res* 2014;28:899–908. <https://doi.org/10.1002/ptr.5077>.
- [29] Iqbal D, Khan MS, Khan A, Khan MS, Ahmad S, Srivastava AK, et al. In Vitro Screening for  $\beta$ -Hydroxy- $\beta$ -methylglutaryl-CoA Reductase Inhibitory and Antioxidant Activity of Sequentially Extracted Fractions of *Ficus palmata* Forsk. *BioMed Research International* 2014;2014:e762620. <https://doi.org/10.1155/2014/762620>.
- [30] Iqbal D, Rehman MT, Bin Dukhyil A, Rizvi SMD, Al Ajmi MF, Alshehri BM, et al. High-Throughput Screening and Molecular Dynamics Simulation of Natural Product-like Compounds against Alzheimer's Disease through Multitarget Approach. *Pharmaceuticals* 2021;14:937. <https://doi.org/10.3390/ph14090937>.
- [31] Khatoon A, Khan F, Ahmad N, Shaikh S, Rizvi SMD, Shakil S, et al. Silver nanoparticles from leaf extract of *Mentha piperita*: Eco-friendly synthesis and effect on acetylcholinesterase activity. *Life Sci* 2018;209:430–4. <https://doi.org/10.1016/j.lfs.2018.08.046>.
- [32] Khushtar M, Siddiqui HH, Dixit RK, Khan MS, Iqbal D, Rahman MdA. Amelioration of gastric ulcers using a hydro-alcoholic extract of *Triphala* in indomethacin-induced Wistar rats. *European Journal of Integrative Medicine* 2016;8:546–51. <https://doi.org/10.1016/j.eujim.2016.01.004>.
- [33] Nille GC, Mishra SK, Chaudhary AK, Reddy KRC. Ethnopharmacological, Phytochemical, Pharmacological, and Toxicological Review on *Senna auriculata* (L.) Roxb.: A Special Insight to Antidiabetic Property. *Frontiers in Pharmacology* 2021;12:2180. <https://doi.org/10.3389/fphar.2021.647887>.
- [34] Prasathkumar M, Raja K, Vasanth K, Khusro A, Sadhasivam S, Sahibzada MUK, et al. Phytochemical screening and in vitro antibacterial, antioxidant, anti-inflammatory, anti-diabetic, and wound healing attributes of *Senna auriculata* (L.) Roxb. leaves. *Arabian Journal of Chemistry* 2021;14:103345. <https://doi.org/10.1016/j.arabjc.2021.103345>.
- [35] Burley SK, Bhikadiya C, Bi C, Bittrich S, Chen L, Crichlow GV, et al. RCSB Protein Data Bank: powerful new tools for exploring 3D structures of biological macromolecules for basic and applied research and education in fundamental biology, biomedicine, biotechnology, bioengineering and energy sciences. *Nucleic Acids Res* 2021;49:D437–51. <https://doi.org/10.1093/nar/gkaa1038>.
- [36] Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. *J Comput Chem* 2010;31:455–61. <https://doi.org/10.1002/jcc.21334>.
- [37] BIOVIA Discovery Studio - BIOVIA - Dassault Systèmes® n.d. <https://www.3ds.com/products-services/biovia/products/molecular-modeling-simulation/biovia-discovery-studio/> (accessed October 1, 2021).

- [38] BIOVIA Discovery Studio - BIOVIA - Dassault Systèmes® 2021. <https://www.3ds.com/products-services/biovia/products/molecular-modeling-simulation/biovia-discovery-studio/> (accessed November 12, 2021).
- [39] Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep* 2017;7:42717. <https://doi.org/10.1038/srep42717>.
- [40] Lolok N, Sumiwi SA, Muhtadi A, Susilawati Y, Hendriani R, Ramadhan DSF, et al. Molecular docking and molecular dynamics studies of bioactive compounds contained in noni fruit (*Morinda citrifolia* L.) against human pancreatic  $\alpha$ -amylase. *J Biomol Struct Dyn* 2021:1–8. <https://doi.org/10.1080/07391102.2021.1894981>.
- [41] Maurus R, Begum A, Williams LK, Fredriksen JR, Zhang R, Withers SG, et al. Alternative catalytic anions differentially modulate human alpha-amylase activity and specificity. *Biochemistry* 2008;47:3332–44. <https://doi.org/10.1021/bi701652t>.
- [42] Akshatha JV, SantoshKumar HS, Prakash HS, Nalini MS. In silico docking studies of  $\alpha$ -amylase inhibitors from the anti-diabetic plant *Leucas ciliata* Benth. and an endophyte, *Streptomyces longisporoflavus*. *3 Biotech* 2021;11:51. <https://doi.org/10.1007/s13205-020-02547-0>.
- [43] Lipinski CA. Lead- and drug-like compounds: the rule-of-five revolution. *Drug Discov Today Technol* 2004;1:337–41. <https://doi.org/10.1016/j.ddtec.2004.11.007>.
- [44] Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews* 1997;23:3–25. [https://doi.org/10.1016/S0169-409X\(96\)00423-1](https://doi.org/10.1016/S0169-409X(96)00423-1).
- [45] Veber DF, Johnson SR, Cheng H-Y, Smith BR, Ward KW, Kopple KD. Molecular Properties That Influence the Oral Bioavailability of Drug Candidates. *J Med Chem* 2002;45:2615–23. <https://doi.org/10.1021/jm020017n>.
- [46] Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews* 2012;64:4–17. <https://doi.org/10.1016/j.addr.2012.09.019>.
- [47] Lin X, Li X, Lin X. A Review on Applications of Computational Methods in Drug Screening and Design. *Molecules* 2020;25:E1375. <https://doi.org/10.3390/molecules25061375>.
- [48] Sabe VT, Ntombela T, Jhamba LA, Maguire GEM, Govender T, Naicker T, et al. Current trends in computer aided drug design and a highlight of drugs discovered via computational techniques: A review. *European Journal of Medicinal Chemistry* 2021;224:113705. <https://doi.org/10.1016/j.ejmech.2021.113705>.
- [49] Rahimzadeh M, Jahanshahi S, Moein S, Moein MR. Evaluation of alpha- amylase inhibition by *Urtica dioica* and *Juglans regia* extracts. *Iran J Basic Med Sci* 2014;17:465–9.
- [50] El Bakri Y, Anouar EH, Marmouzi I, Sayah K, Ramli Y, El Abbes Faouzi M, et al. Potential antidiabetic activity and molecular docking studies of novel synthesized 3,6-dimethyl-5-oxo-pyrido[3,4-f][1,2,4]triazepino[2,3-a]benzimidazole and 10-amino-2-methyl-4-oxo pyrimido[1,2-a]benzimidazole derivatives. *J Mol Model* 2018;24:179. <https://doi.org/10.1007/s00894-018-3705-9>.
- [51] Hajlaoui A, Laajimi M, Znati M, Jannet HB, Romdhane A. Novel pyrano-triazolo-pyrimidine derivatives as anti-  $\alpha$ -amylase agents: Synthesis, molecular docking investigations and computational analysis. *Journal of Molecular Structure* 2021;1237:130346. <https://doi.org/10.1016/j.molstruc.2021.130346>.
- [52] Anigboro AA, Avwioroko OJ, Ohwokevwo OA, Pessu B, Tonukari NJ. Phytochemical profile, antioxidant,  $\alpha$ -amylase inhibition, binding interaction and docking studies of *Justicia carnea* bioactive compounds with  $\alpha$ -amylase. *Biophysical Chemistry* 2021;269:106529. <https://doi.org/10.1016/j.bpc.2020.106529>.

- [53] Mor S, Sindhu S. Synthesis, Type II diabetes inhibitory activity, antimicrobial evaluation and docking studies of indeno[1,2-c]pyrazol-4(1H)-ones. *Med Chem Res* 2020;29:46–62. <https://doi.org/10.1007/s00044-019-02457-8>.
- [54] Robyt JF, French D. Multiple attack and polarity of action of porcine pancreatic alpha-amylase. *Arch Biochem Biophys* 1970;138:662–70. [https://doi.org/10.1016/0003-9861\(70\)90394-2](https://doi.org/10.1016/0003-9861(70)90394-2).
- [55] Brayer GD, Sidhu G, Maurus R, Rydberg EH, Braun C, Wang Y, et al. Subsite mapping of the human pancreatic alpha-amylase active site through structural, kinetic, and mutagenesis techniques. *Biochemistry* 2000;39:4778–91. <https://doi.org/10.1021/bi9921182>.
- [56] Falese BA, Kolawole AN, Sarumi OA, Kolawole AO. Probing the interaction of iminium form of sanguinarine with human salivary  $\alpha$ -amylase by multi-spectroscopic techniques and molecular docking. *Journal of Molecular Liquids* 2021;334:116346. <https://doi.org/10.1016/j.molliq.2021.116346>.
- [57] Mehmood H, Haroon M, Akhtar T, Woodward S, Andleeb H. Synthesis and molecular docking studies of 5-acetyl-2-(arylidenehydrazin-1-yl)-4-methyl-1,3-thiazoles as  $\alpha$ -amylase inhibitors. *Journal of Molecular Structure* 2021:131807. <https://doi.org/10.1016/j.molstruc.2021.131807>.
- [58] Siti Halimatul Munawaroh H, Gumilar GG, Nurjanah F, Yuliani G, Aisyah S, Kurnia D, et al. In-vitro molecular docking analysis of microalgae extracted phycocyanin as an anti-diabetic candidate. *Biochemical Engineering Journal* 2020;161:107666. <https://doi.org/10.1016/j.bej.2020.107666>.
- [59] Sujayev A, Tasliimi P, Garibov E, Karaman M, Mahdi Zangeneh M. Novel cyclic thiourea derivatives of aminoalcohols at the presence of  $AlCl_3$  catalyst as potent  $\alpha$ -glycosidase and  $\alpha$ -amylase inhibitors: Synthesis, characterization, bioactivity investigation and molecular docking studies. *Bioorganic Chemistry* 2020;104:104216. <https://doi.org/10.1016/j.bioorg.2020.104216>.