In vivo and *in silico* approaches for exploring the hypoglycemic potential of *Moringa oleifera* Lam. flowers' extract

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ABSTRACT

Moringa oleifera, also known as shajina, is rich in bioactive phytochemicals, including flavonoids, alkaloids, and phenolics, which contribute to its therapeutic potential. Various parts, including leaves, barks, and flowers exhibit antioxidant, anti-inflammatory, and antimicrobial properties. The present study was designed to explore the antidiabetic potential of the flower extract through the in vivo and in silico study. The hypoglycemic activity of the flower extract was evaluated in Wistar rat using an oral glucose tolerance test (OGTT), while molecular docking identified key phytochemicals targeting diabetic pathway proteins. The in vivo study revealed significant glucose-lowering effects of flower extract fractions, particularly n-hexane (NHF) and chloroform. At 200 mg/kg, both n-Hexane and chloroform fractions reduced blood glucose by 30.22% and 33.83%, respectively, increasing to 37.01% and 49.86% at 400 mg/kg, nearing the standard hypoglycemic drug miglitol's 53.92% efficacy. In silico analysis showed strong binding affinity of kaempferol, quercetin, and ar-turmerone to pancreatic alpha-amylase (5E0F) with binding energies of -8.8, -9.0, and -6.9 kcal/mol, respectively. ADMET analysis confirmed their favorable pharmacokinetics, including good solubility, non-toxicity, and non-carcinogenicity. The outcomes of the study assist in concluding the presence of some bioactive substances with promising hypoglycemic activity.

Keywords: Bioactive Phytochemicals, diabetics, hypoglycemic activity, molecular docking, *Moringa oleifera,*

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin action, or both. Type 2 diabetes mellitus accounts for over 90% of all diabetes cases and is associated with severe complications such as cardiovascular diseases, nephropathy, neuropathy, and retinopathy (Faselis *et al.,* 2020). The increasing global prevalence of diabetes necessitates the continuous search for novel therapeutic agents with minimal side effects. Natural products derived from medicinal plants have long been explored as alternative treatments for diabetes due to their bioactive phytoconstituents with hypoglycemic potential (Ríos *et al*., 2015, Jugran *et al.,* 2021). On the other hand, the use of synthetic drugs poses a significant global health risk and increases the likelihood of conditions such as cancer, diabetes, and neurodegenerative diseases. To address this

issue, it is essential to focus on developing medicines derived from natural herbs (Karim *et al*., 2025a).

Moringa oleifera (Family: Moringaceae), commonly known as the drumstick tree, is a nutritionally and medicinally important plant widely used in traditional medicine for its diverse pharmacological activities. Although the leaves and seeds have been extensively studied for their medicinal benefits, the therapeutic potential of the flowers remains underexplored (Karim *et al*.,2025b). Recent phytochemical investigations indicated that *M. oleifera* flowers contain a rich profile of bioactive compounds, including flavonoids, alkaloids, and terpenoids, which may contribute to their pharmacological action like anti-asthmatic, anti-diabetic, hepatoprotective, anti-inflammatory, anti- fertility, anti-cancer, anti-microbial, anti-oxidant, cardiovascular, anti-ulcer, CNS activity, anti-allergic, wound healing, analgesic, and antipyretic activity (Paikra *et al*., 2017).

This study aims to evaluate the hypoglycemic potential of *M. oleifera* flower extract through both *in vivo* and *in silico* approaches. The *in vivo* study investigates the glucose-lowering effects of different solvent fractions of the extract using the oral glucose tolerance test in Wistar rats (Kifle *et al*., 2020, Goyal and Jeyabalan, 2021). The *in silico* study involves molecular docking analysis to identify key phytochemicals responsible for modulating diabetes-related proteins (Ajiboye *et al*., 2022). Additionally, pharmacokinetic and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) predictions were performed to assess the drug-likeness properties of the identified compounds (Bitew *et al.,* 2021, Sucharitha *et al*., 2022).

MATERIALS AND METHODS

Collection and preparation of the plant sample

The fresh flower of *M. oleifera* was collected from Kushtia, Bangladesh, in March 2024, and the sample's authenticity was verified by Jahangirnagar University Herbarium, Bangladesh (JUH- 10271). After being washed, freshly picked flowers were dried for a few days at ambient temperature (25°C-30°C**)** and relative humidity (60%-70%). Then, the dried sample was ground into powder and stored in a closed container.

Extraction and fractionation

The dried powder sample of flower was extracted in methanol using the cold extraction procedure. In a clean beaker, 300 gm of the powder was soaked in about 1L of methanol for 15 days with occasional stirring. The filtrate was collected using a cotton-plugged funnel and then completely evaporated using a rotary evaporator set to a fixed temperature and pressure, condensing the filtrate into a dry crude extract. Around 5.0 gm of the crude extract was dissolved in 10 % aqueous methanol and the fractionation was carried out using n-hexane (NHF), chloroform (CF), ethyl acetate (EAF), and aqueous (AQF) depending upon the polarity applying the modified Kupchan partitioning protocol (Van Wagenen *et al.,* 1993) to separate phytochemicals in different solvents based on their polarity index. The fractionated extracts were collected by evaporation in a Rotary Evaporator in a different quantity; n-hexane soluble fraction (1.7gm), Chloroform soluble fraction (1.4gm), Ethyl acetate soluble fraction (0.8gm), Aqueous fraction AQF (1.1gm).

Drugs and reagents

All reagents involved in the study were of analytical grade. Methanol (100%), n-hexane, chloroform, ethyl acetate, dimethyl sulfoxide (DMSO), Tween-80, and glucose were purchased from BDH Chemicals. Saline

water (from Popular Pharmaceuticals Ltd.) and miglitol (provided by Incepta Pharmaceuticals Ltd.) were also used.

Experimental animal

Wistar rats (standard outbred Wistar strain) were collected from the International Centre for Diarrheal Diseases and Research, Bangladesh (ICDDR'B), and were used to conduct the study. These rats were 8-10 weeks old and weighed 280-340 g. The weight variation may be due to the biological, age, diet or environmental factors. To provide them with a suitable housing duration, the recommended temperature of 24±2°C, relative humidity is 60-70% and other stipulated circumstances were met. The rodent food and water provided to the experimental rats were prepared by ICDDR'B. The Animal Ethics Committee at the Faculty of Biological Science, University of Dhaka conducted a panoramic assessment of the ethical guidelines and protocols of the investigation and generated their systematic review and approval (Ref. No. 270/Biol. Sci.). Then, the investigation was performed according to the ARRIVE guideline 2.0 (Percie du Sert *et al*., 2020).

***In-vivo* study**

Hypoglycemic activity. The hypoglycemic property of the various fractions of the methanolic extract of *M. oleifera* flower was assessed using a slightly modified form of the oral glucose tolerance test (OGTT) (Bogdanet *et al*., 2020). The OGTT is a widely accepted method to evaluate glucose homeostasis, insulin sensitivity, and possible antidiabetic properties of test compounds. Initially, six rats in each group (Negative control, positive control, and test groups) had their blood glucose levels measured using a glucometer by drawing blood from the tail vein (Arifin and Zahiruddin, 2017). In this study, miglitol (10 mg/kg) was used as a positive control, and a 1% Tween 80 saline solution (10 mL/kg) as a negative control, with all rats initially given a 10% glucose solution (2 g/kg) to induce hyperglycemia. The test group received oral doses of plant fractions (200 and 400 mg/kg), and blood glucose levels were measured at 30, 60, 120, and 180 minutes to evaluate the antihyperglycemic effect compared to the synthetic drug, highlighting the impact of miglitol in reducing elevated glucose levels. To evaluate the activity, the test sample's percent reduction in blood glucose level relative to the standard was calculated using the following equation:

$$\% \text{ Reduction} = \frac{(\text{Tn} - \text{Ts})}{\text{Tn}} * 100$$

Where, Tn represents the mean of Blood glucose level in the control group, while Ts represents the mean blood glucose level in the sample treatment groups after 30 minutes of oral administration.

Statistical analysis. The data processing and graph construction from the in vivo data were conducted using MS Excel (version 10.0) and GraphPad software. To accurately represent the results of the in vivo evaluations, the mean ± SEM was used to convey the average values and their corresponding standard errors of the mean. The p-values of the assays were obtained using the student t-test calculator (unpair t-test) and any data with p-values < 0.05 was considered as statistically significant.

***In-silico* molecular modeling studies**

Phytochemicals selection and preparation*:* In this study, 72 compounds from *Moringa oleifera* flowers were identified through literature and the IMPPAT database (Mohanraj *et al.*, 2018). Ligand 3D conformers were retrieved in SDF format from the PubChem database and processed using Open Babel to compile a ligand library. Energy minimization was performed with PyRx 0.8 and Open Babel 2.3.1, employing the MMFF94 force field (Kim *et al*., 2016).

Finally, AutoDock Tools was used to convert the ligands into pdbqt format for further analysis.

Selection and preparation of proteins*:* To perform docking studies, the crystal structure of the human pancreatic Alpha-Amylase complexed with Mini-Montbretin A (PDB ID: 5E0F) was retrieved from the Protein Data Bank. The protein, containing a single chain, was prepared using PyMOL (v2.3) (https://pymol.org/2/) and cleaned by removing unwanted ligands, heteroatoms, and water molecules (Akash *et al.*, 2023). The structure was then imported into AutoDock Tools for conversion into PDBQT format following standard protocols (Chatterjee *et al*., 2018). Energy minimization of the receptor was conducted using Swiss PDB Viewer before saving the refined structure as a PDB file for further analysis (Kaplan and Littlejohn, 2001).

Methods of molecular docking: To ensure proper binding of the drug to the target receptor, a clean receptor site free from interference by water or other molecules is essential. In the docking study, the grid box parameters were set to X=-8.4005, Y=21.6258, and Z=-18.9668, with an exhaustiveness value of 8 to optimize the protein-ligand binding conformation. Using PyRx, ligands were transformed into pdbqt format, and proteins developed as macromolecules. Molecular docking was performed using Auto Dock Vina (Dallakyan and Olson, 2014). The docked complexes were visualized in PyMOL, and further analysis of key amino acid residues and interaction sites was conducted using BIOVIA Discovery Studio Visualizer.

Lipinski rule and drug-likeness properties analysis*:* The pharmacokinetics and Lipinski's Rule of Five for the selected drugs were analyzed using the Swiss ADME online tool (http://www.swissadme.ch) (Azzam, 2023). These parameters evaluate structural and chemical properties to determine a molecule's similarity to existing drugs. Key factors include hydrophobicity, drug-likeness, hydrogen bonding, molecular weight, size, bioavailability, and other relevant characteristics (Ji *et al*., 2020).

Prediction of ADMET profile*:* Insufficient pharmacokinetic and safety profiles are major factors in the failure of drug development. Computational methods can help address these challenges. Among these, pkCSM offers a promising alternative for predicting pharmacokinetic properties and ADMET (absorption, distribution, metabolism, excretion, and toxicity) features. Research indicates that ADMET predictions are valuable for assessing the pharmacokinetics of biomolecules prior to clinical and preclinical trials (Avram *et al*., 2020, Sun *et al*., 2022). The pkCSM web tools (https://biosig.lab.uq.edu.au/pkcsm/) (Azzam, 2023) and http://lmmd.ecust.edu.cn/admetsar2/result/?tid=742441 were used to assess and analyze the ADMET feature.

RESULTS AND DISCUSSIONS

Hypoglycemic Activity

The effects of various extracts on glucose levels were assessed over a period of 180 minutes following oral glucose administration. The data are presented in Table 1**.** In the control group, glucose levels peaked at 30 minutes (10.63±0.28 mmol/L) and declined to 8.33±0.15 mmol/L by 180 minutes. The standard drug (STD) significantly reduced glucose levels at all time points (***$p < 0.001$), confirming the experimental setup. Both NHF-400 and CF-400 extracts showed strong antihyperglycemic effects, significantly lowering glucose at 60, 120, and 180 minutes, with CF-400 being more effective. Lower doses (NHF-200, CF-200) showed moderate activity, while EAF-200 and EAF-400 exhibited no significant

effects. AQF-400 and AQF-200 showed slight, non-significant glucose reductions, indicating weak activity.

The findings of the investigation confirmed that both the dose (200 mg/kg and 400 mg/kg of body weight) of n-hexane fraction (NHF) and chloroform fraction (CF) exhibited statistically significant ($p<0.05$) hypoglycemic effect up to three hours from the administration of glucose solution, compared to the control group. The 200 mg/kg body weight dose of NHF and CF expressed a 30.22% and 33.83% reduction of blood glucose level after 180 minutes, respectively and the 400 mg/kg body weight dose of NHF and CF exhibited 37.01% and 49.86% reduction of blood glucose levels which was very much comparable to the percent reduction value (53.92%) of the positive control drug miglitol (Figure 1).

The *in vivo* findings suggest that *Moringa oleifera* flower extract has notable hypoglycemic effects, likely mimicking the mechanisms of synthetic antidiabetic drugs, such as enhancing insulin release, reducing glucose production, inhibiting glucose absorption, or activating PPARs (Subramoniam, 2016).

In Silico Studies

Molecular docking analysis*:* To justify the *in vivo* results, molecular docking analysis is widely performed for determining the ligand-protein interactions. It offers a detailed understanding of the binding sites of the proteins, binding style, and probable mechanism of action among the existing pathways. H bonding as well as hydrophobic bonding are the main reasons for docking scores because protein-ligand interaction is crucial in structurally oriented drug design. If the docking score is more than -6.00 kcal/mol, the drug is considered standard (Cosconati *et al*., 2010).The identification of the ligand-receptor complex structure is the main objective of molecular docking. This can be achieved in two interrelated steps: first, by sampling ligand arrangements on protein active sites, and second, by organizing the distortions utilizing a score function.

Blind docking analysis revealed that all compounds exhibited strong binding affinities with human pancreatic alpha-amylase (PDB: 5E0F), with binding energies ranging from -6.9 to -9.0 kcal/mol Table 2**.** Notably, kaempferol, quercetin, and ar-turmerone showed the highest affinities, indicating potential hypoglycemic effects of *M. oleifera* phytochemicals against type 2 diabetes (Ponnusamy *et al*., 2015).

Protein-ligand Interaction: Pymol application software and the BIOVIA Discovery Studio were utilized to produce the interaction diagrams for drug-protein configurations, hydrogen bonds, and molecular docking pockets. Protein and ligand interactions have been studied about hydrogen bond donor and donor-acceptor interactions, hydrophobic interactions (such as pi-sigma, alkyl, and pi-alkyl interactions), and hydrogen bond interactions (including conventional and non-conventional H bonds). Hydrophobic and hydrogen bond interactions are important in drug activity. Various involvement and binding activities between the medication and the intended target protein are shown in Figure 2. In hydrogen bonding, the acceptor region is described as red-green, and the receptor region as violet. Furthermore, the two-dimensional image of active amino acid residues shows that A: GLN63 (2.49893), HIS305 (2.42039), ASP197 (2.506.9), ASP300 (2.9675), ASP300 (2.26897), TRP59 (4.83897), TRP59 (4.16462), TRP59 (5.90182), TRP59 (4.33158), and TYR62 (4.94086) are generated for Human pancreatic alpha-amylase (PDB ID 5E0F) with

Lipinski rule analysis for oral medication: The Lipinski rule suggests that orally active drugs should be modest due to

their pharmacological or biological activity, as they possess the necessary molecular and physical properties for oral consumption by mammals. According to the Lipinski Rule, a suitable oral drug must have a topological polar surface area between 17.07 and 131.36 and a molecular weight between 286.24 and 306.24. Additionally, all drugs have greater bioavailability ratings (0.55) (Table 3). The GI absorption rate is another crucial metric that shows how effectively the drugs are absorbed in the digestive system (Table 3).

ADMET profile prediction*:* The computational inspection techniques may be used in drug development to detect ADMET parameters, which have a substantial influence on therapeutic absorption, distribution, metabolism, solubility, and oral bioavailability (Daoud *et al*., 2021). Each of the ADMET features given has a different water solubility value. Since their actual water solubility values vary from -4 to -6, the ligands LM02 and LM03 are extremely soluble in water, with a range of -2.925 to -4.454. It is implied that the remaining substances are highly soluble in fatty substances or lipids since they are only weakly soluble in water. A thorough summary of medication distribution, including volume distribution and blood-brain barrier permeability, is given in Table 4. Another factor that prevents undesirable substances from accessing the brain and CNS is the blood-brain barrier or BBB (Cosconati *et al*., 2010). In our findings, the BBB permeability range was -1.098 to 0.512 (Table 4).

Since highly water-soluble oral medications provide superior oral bioavailability and maximal absorption capacities, water solubility is essential for contemporary drug research. The development of quick, accurate, structure-based strategies for determining an active drug candidate's solubility in water is highly desired (Wang *et al*., 2018). Theoretical results showed that quercetin (LM02) and ar-turmerone (LM03) are highly water-soluble, with low volume of distribution (VD), suggesting higher plasma concentration and limited tissue penetration; most ligands exhibited low VD and BBB permeability ranged from -1.098 to 0.512. All ligands satisfied Lipinski's rule, with suitable topological polar surface area (17.07–131.36 Å²), molecular weight (286.24–306.24 g/mol), and bioavailability scores (0.55), indicating good oral drug potential. (Khan *et al*., 2019).

CONCLUSION

The present study explored the hypoglycemic potential of *Moringa oleifera* flower extract through *in vivo* and *in silico* approaches. The n-hexane and chloroform fractions demonstrated significant glucose-lowering activity in OGTT assays. Molecular docking analysis identified kaempferol, quercetin, and ar-turmerone as potential inhibitors of pancreatic alpha-amylase, with favorable binding affinities and pharmacokinetic properties. These findings suggest that *Moringa oleifera* flowers contain bioactive compounds with antidiabetic potential. Further experimental validation, including studies on diabetic animal models and mechanistic investigations, is necessary to confirm their therapeutic efficacy.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1: Average glucose level (mmol/L) after loading the glucose sample

Group	**Average glucose level (mmol/L) after loading the glucose sample**				
	0 minutes	30 minutes	60 minutes	120 minutes	180 minutes
CTL	5.68±0.08	10.63±0.28	10.02±0.23	9.58±0.11	8.83±0.15
STD	5.45±0.11	10.98±0.24	7.82±0.13***	6.20±0.16***	3.95±0.06***
NHF- 200	7.55±0.20	10.15±0.24	9.45±0.16	8.03±0.16**	7.08±0.15***
NHF -400	6.80±0.14	9.05±0.23	7.20±0.25***	6.32±0.26***	5.70±0.20***
CF -200	5.32±0.17	10.78±0.26	9.33±0.12*	8.95±0.13**	7.13±0.18***
CF -400	6.02±0.16	9.73±0.38	6.17±0.25***	5.46±0.24***	4.88±0.24***
EAF -200	5.48±0.11	11.32±0.26	10.01±0.28	9.58±0.28	8.80±0.21
EAF -400	5.70±0.07	10.69±0.29	9.87±0.23	9.30±0.14	8.80±0.14
AQF-200	5.50±0.07	9.82±0.36	9.43±0.28	9.07±0.13	8.58±0.10
AQF-400	6.15±0.15	10.90±0.21	9.50±0.18	9.18±0.21	8.37±0.16

Note: Data are mentioned as mean ± SEM, n = 6. *p<0.05; **p<0.01; ***p < 0.001 versus negative control.

CTL=Control group; NHF=N-Hexane Fraction; CF=Chloroform Fraction; EAF=Ethyl Acetate Fraction; AQF=Aqueous Fraction

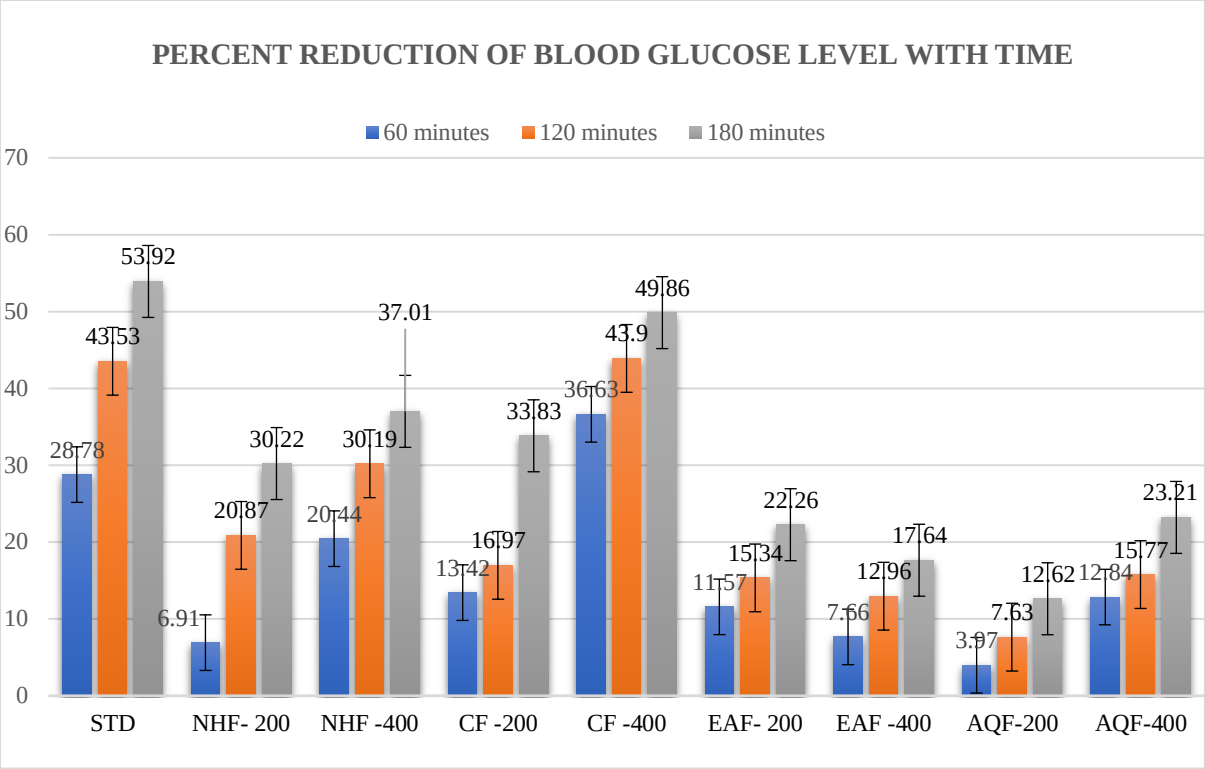
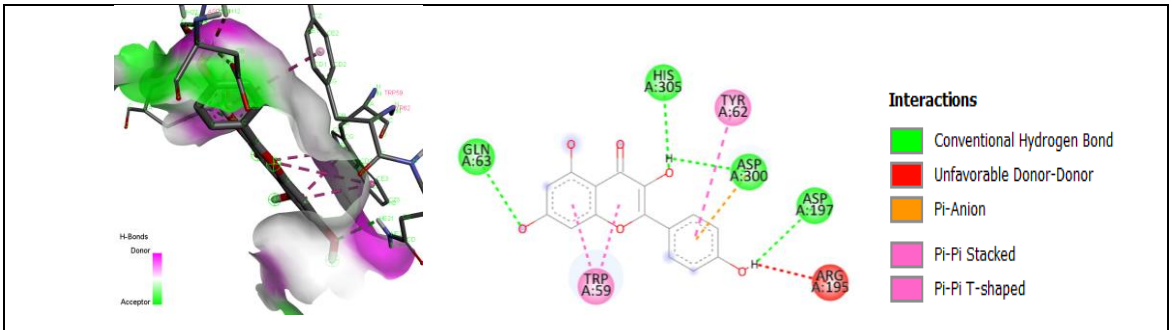


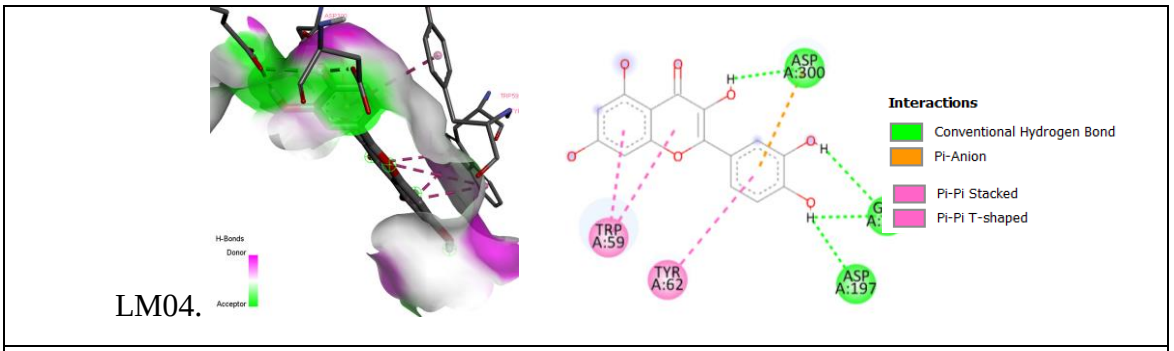
Figure 1. **Percent glucose level reduction (vertical unit) for different fractions *Moringa oleifera* flower with time.**

Table 2: Data on binding energy and the name of the interacted ligand for human pancreatic alpha-amylase (PDB: 5E0F).

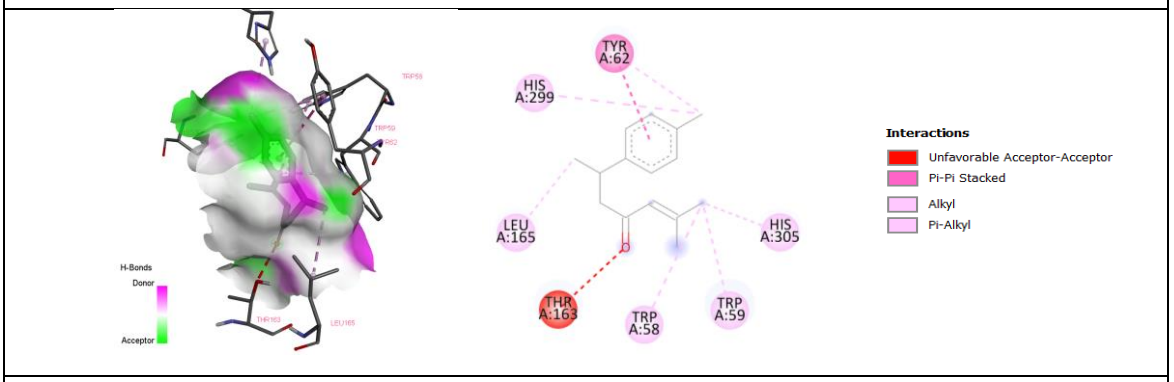
Ligand	Binding Affinity (kcal/mol)	No of H bonds	No of Hydrophobic bonds	Others	Total bonds
Kaempferol	-8.8	5	4	1	10
Quercetin	-9	4	5	1	10
ar-Turmerone	-6.9	0	7	0	7
Standard (miglitol)	-5.9	3	3	0	6



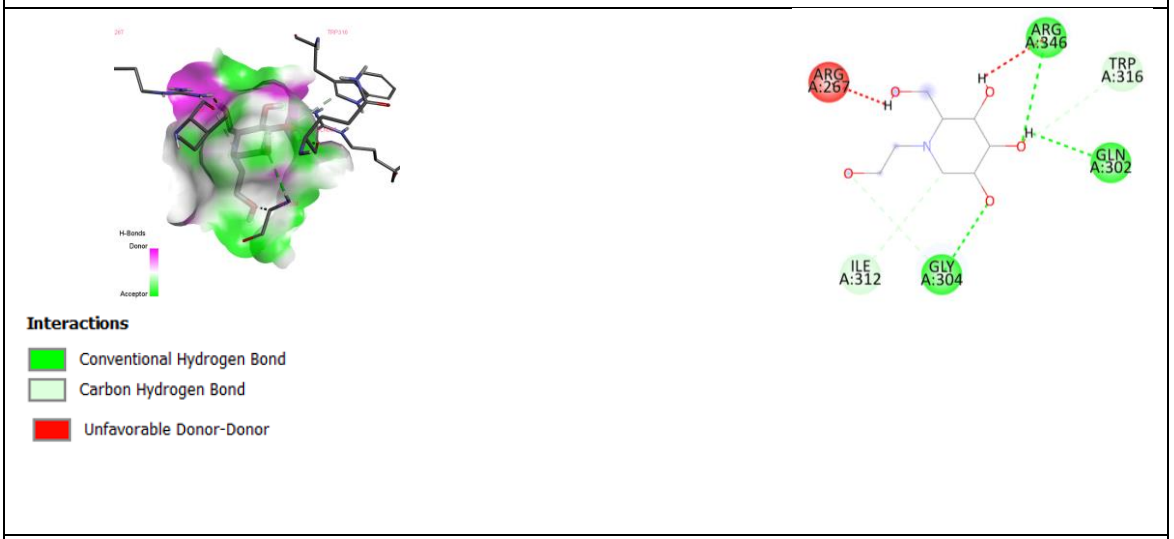
A. Human pancreatic alpha-amylase (PDB ID 5E0F) with LM01(Kaempferol)



B. Human pancreatic alpha-amylase (PDB ID 5E0F) with LM02 (Quercetin)



C. Human pancreatic alpha-amylase (PDB ID 5E0F) with LM03 (ar-Turmerone)



D. Human pancreatic alpha-amylase (PDB ID 5E0F) with Standard (Miglitol)

Figure 2. Molecular docking experiments reveal the interactions between proteins and substances.

Table 3: Data of Lipinski rule, pharmacokinetics, and drug likeness

CID	Molecular weight g/mol	H-bond acceptor	H-bond donor	Molar Refractivity	Topological polar surface area(Å²)	Consensus Log Po/w	Lipinski rule		Bioavailability
							Result	violation	
5280863 (Kaempferol)	286.24	6	4	76.01	111.13	1.58	Yes	0	0.55
5280343 (Quercetin)	302.24	7	5	78.03	131.36	1.23	Yes	0	0.55
160512 (ar-Turmerone)	216.32	1	0	69.75	17.07	3.84	Yes	0	0.55
441314 Miglitol	207.22	6	5	51.08	104.39	-1.94	Yes	0	0.55

Table 4. Computational ADMET Data Prediction

I No	CID	Absorption		Distribution		Metabolism		Excretion		Toxicity			
		Water solubility (Log mol/L)	Human Intestinal Absorption (%)	VDss (log L/kg)	BBB Permeability (log BB)	CYP450 1A2 Inhibitor	CYP450 2D6 Substrate	Total Clearance (log ml/min/kg)	Renal OCT2 substrate	Max. tolerated dose (log mg/kg/day)	Skin Sensitization	Hepatotoxicity	AMES toxicity
01	5280863 (Kaempferol)	-3.04	74.29	1.274	-0.939	Yes	No	0.477	No	0.531	No	No	No
02	5280343 (Quercetin)	-2.925	77.207	1.559	-1.098	Yes	No	0.407	No	0.499	No	No	No
03	160512 (ar-Turmerone)	-4.454	94.489	0.621	0.512	Yes	No	0.295	No	0.846	Yes	N0	No
04	441314	1.229	41.462	-0.607	-1.501	No	No	0.815	No	2.239	No	No	No