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Research Article

Benchmarking docking, density functional theory and molecular dynamics studies to assess the aldose reductase inhibitory potential of *Trigonella foenum-graecum* compounds for managing diabetes-associated complications

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Abstract

Inhibition of aldose reductase (AR) could be a beneficial strategy for managing diabetes-associated complications. *Trigonella foenum-graecum* (TFG) is used around the globe as a traditional medicine for the management of diabetes. Our study aimed to assess the potential of TFG phytocompounds as inhibitors of AR in the context of diabetes-related complications. Our research work employed molecular docking, density functional theory (DFT) and molecular dynamics (MD) to evaluate the efficacy of TFG compounds. The study compared the predictive power of AutoDock and AutoDock Vina docking software and found that AutoDock Vina performs better in ranking and discriminating actives and decoys. The research identified five compounds as potential AR inhibitors from fifty-eight reported TFG phytoconstituents. Tigogenin and Gitogenin stood out as the most promising AR inhibitors. The electronic properties of the compounds were analysed through DFT studies and provided insights into their binding potential. Finally, the results of MD simulations indicated that Tigogenin and Gitogenin bound robustly with AR throughout the simulation period. This study predicted the AR inhibitory potential of TFG compounds for managing diabetes-associated complications and supports further drug development from TFG. The benchmarking approach used in this study improves the accuracy and dependability of bioactivity prediction.

Keywords

Benchmarking docking, aldose reductase, diabetic retinopathy, DFT, *Trigonella foenum-graecum*

Introduction

Diabetes is a hormonal disorder involving deficient insulin secretion or action where glucose levels in the bloodstream are elevated (Teo et al. 2021). The polyol route is an alternate route for glucose metabolism that contributes to the development of diabetes (Yan 2018). Aldose reductase (AR) reduces glucose to sorbitol in the first step of the polyol route and its activation leads to oxidative stress in diabetes (Maccari and Ottanà 2015). This plays a significant role in the development of diabetes and its associated complications, including chronic microvascular complications like diabetic retinopathy, nephropathy and neuropathy. Cardiomyopathy is a diabetes-associated macrovascular disease and recent evidence proves the involvement of the polyol route and AR in its pathogenesis (Jannapureddy 2021). Inhibiting AR could be a beneficial strategy for preventing diabetes-associated complications.

Natural product datasets can be explored using *in silico* techniques like molecular docking and dynamics simulations to discover natural products for diabetes and related complications (Chen and Kirchmair 2020; Rao and Hariprasad 2021). *Trigonella foenum-graecum* (TFG) (Fabaceae) is a herb that has AR-inhibitory activity and is traditionally used in India (Methi) and Saudi Arabia (Hulbah) for diabetes and related complications (Alqethami and Aldhebiani 2021; Visuvanathan et al. 2022). TFG is also known for its anti-cancer, anti-hyperlipidemic, anti-inflammatory, anti-microbial, cardioprotective, neuroprotective and nephron-protective effects (Almatroodi et al. 2021). TFG leaves and seeds are part of the regular diet of Mediterranean and Asian countries (Ahmad et al. 2016; Dhull et al. 2023).

Molecular docking is a computational technique that predicts the binding affinity of a ligand to a protein and the ligand's bioactive conformation. Based on molecular docking and molecular dynamics simulations, aldose reductase inhibitory compounds were identified. Further, it was observed that the phytochemicals of ginger exhibited higher docking scores, binding affinity and protein-ligand interactions than the phytochemicals of turmeric, garlic and TFG (Antony and Vijayan 2015). TFG phytochemicals have been predicted as inhibitors of various enzymes, such as α-glucosidase, α-amylase, dipeptidyl peptidase IV and agonists of peroxisome proliferator accelerator receptor-γ implicated in diabetes (Kumar et al. 2023; Okoh et al. 2023; Sarker et al. 2023). This study uses 58 TFG phytoconstituents from leaves and seeds, including flavonoids, fatty acids, saponins and steroids. Different docking software uses different scoring functions to estimate binding energy and benchmarking molecular docking compares their performance (Thangavel and Albratty 2023).

The study's objective is to assess the AR inhibitory capacity of TFG's chemical components using benchmarking molecular docking, followed by DFT analysis of TFG compounds and stability analysis of the binding of TFG's compounds to AR using molecular dynamics (MD).

Materials and methods

Materials

All the computational studies were carried out on a PC running Windows 7 Ultimate with an Intel Core i3 microprocessor, 4.00 GB of RAM and a 64-bit operating system. AutoDock 4.2.1 (ADock) (https://autodock.scripps.edu/download-autodock4/) and AutoDock Vina 1.1.2 (AVina) (https://vina.scripps.edu/downloads/) were used to benchmark molecular docking. Discovery Studio Visualizer 3.5 (https://discover.3ds.com/discovery-studio-visualizer-download) and PyMol 2.5 (https://pymol.org/2/) were employed to analyse the binding conformations and inter-molecular interactions. OpenBabel 3.1.1 (https://sourceforge.net/projects/openbabel/) was used to create PDBQT files for all test compounds and decoys. To validate and compare the docking results, an online tool called Screening Explorer (http://stats.drugdesign.fr/) was used.

Accession of target protein, TFG compounds and decoys

The 3D crystal structure of AR (PDB accession number ID: 2INE with the best resolution 1.90 Å) was downloaded from the RCSB PDB (http://www.rcsb.org) in PDB format. The native ligand, cofactors, metal ions and water were removed from the protein structure. The residues that constitute the binding pocket of AR were analysed using the Computed Atlas for Surface Topography of Proteins (CASTp), a web-based tool for binding site prediction (http://cast.engr.uic.edu). The 3D structures of 58 TFG compounds and Epalrestat were obtained from the PubChem database (http://pubchem.ncbi.nlm.nih.gov/pccompound). The SDF file of 3D structures of decoys for the AR enzyme was downloaded from the DEKOIS 2.0 database (http://www.dekois.com).

Benchmarking molecular docking

The target protein in PDB format obtained from the above step was further processed by adding polar hydrogens and Gasteiger charges in AVina. This energy-minimised AR enzyme structure was saved as a PDBQT file and was used for docking the ligands in ADock and AVina. All test compounds, including TFG compounds, Epalrestat and decoys in SDF format, were loaded into Open Babel for conversion to PDBQT, during which the molecules are energy minimised by adding polar hydrogen and Gasteiger charges. The binding site was defined by positioning a grid box with a centre of X, Y and Z dimensions 18.61 X -11.39 X 17.71 Å and a size of 60 X 60X 65 Å, respectively, with a spacing value of 0.375 Å. Both ADock and AVina docking were performed using the exact dimensions mentioned above. The genetic algorithm parameter was set at 10 in ADock, while all other parameters were fixed at default values. In AVina docking, ten conformers were generated at a 3 kcal/mol energy gradient, while the rest

of the parameters chosen were at their default numbers. Both software allowed the ligand flexibility; their rotatable bonds could move freely during docking. The energy calculations of protein-ligand complexes were done through the Lamarckian genetic algorithm in both software. The protein-ligand complexes with the least negative binding energy (ΔG) indicate robust binding and favourable conformations were selected for analysing the interaction between the protein-ligand complexes (Alshahrani et al. 2021). The 2D and 3D interactions of docked complexes were studied in Discovery Studio.

Molecular docking validation by individual and comparative scoring

Individual and comparative scoring methods were used to compare the results of ADock and AVina docking software (Thangavel and Albratty 2022). For this purpose, Screening Explorer was employed. The binding energy results obtained from ADock and AVina, along with the ligand ID numbers for TFG compounds, standard drug and decoys, were loaded into Screening Explorer. The description of active compounds was 1, while decoys were 0, which was necessary to compare docking results. These were fed into Screening Explorer in CSV format. Screening Explorer then used the binding energy scores to rank the ligands. The ligand rankings were used to validate the scoring methods used by the docking software. The ligand's binding energy and corresponding rankings were used to construct predictiveness, ROC and enrichment graphs in Screening Explorer. To assess the performance of ADock and AVina, widely-accepted metrics, such as auROC (Area Under Receiver Operating Characteristic Curve), BEDROC (Boltzmann-enhanced discrimination of ROC), EF (Enrichment factor), TG (Total gain) and RIE (Robust initial enhancement), were used. The results of benchmarking docking from individual, comparative scoring and binding interaction analysis were considered to choose the best TFG compound. This compound had the maximum potential to inhibit the AR enzyme and molecular dynamics further assessed the protein-ligand complex to ensure its stability.

Density functional theory calculations

DFT calculations were conducted to investigate the electronic properties of the best-forming TFG compounds. The calculation used Maestro's Jaguar module with the B3LYP functional method and 6-31G* basis set. The electronic properties, such as the Highest Occupied Molecular Orbital (HOMO) energy, Lowest Unoccupied Molecular Orbital (LUMO) energy and its corresponding Band gap energy, were computed.

Molecular dynamics simulations

The GROMACS 4.3.1 package (https://www.gromacs.org/) with GROMOS43al force field was used to perform MD to evaluate the structural stability of protein-ligand complexes. The PRODRG online server (http://prodrg1.dyndns.org/) was utilised to create the ligand topology. The systems were equilibrated using a cubic box with dimensions of $10 \times 10 \times 10$ nm^3 and solvated with the Single Point Charge (SPC) water model. Appropriate counter ions (Na^+ Cl^-) were added to neutralise the systems. The steepest descent algorithm was employed for energy minimisation for 50,000 to eliminate weak van der Waals contacts. The Parrinello-Rahman barostat and modified Berendsen thermostat were applied to maintain constant pressure and temperature at 1 bar and 300 K, respectively, using the NPT and NVT ensemble. The MD simulations were conducted for 50 ns. The g_mmpbsa tool determined the binding free energy of the AR protein-Tigogenin and AR protein-Gitogenin complexes for the last 5 ns of the MD trajectories (Omolo et al. 2018). The expression used to calculate the MM/PBSA energy is:

$$\Delta G_{bind} = G_{docked} - (G_{enzyme} + G_{compound}),$$

wherein, G_{docked} is the binding energy of the docked complexes of TFG compounds with AR, G_{enzyme} and $G_{compound}$ are the energies of AR and TFG compounds in a solvent-filled system.

Results

Active site prediction

The binding pocket of AR protein was analysed using the CASTp web server and the results are depicted in Fig. 1. A single binding site was identified in the AR enzyme. CASTp results indicated that Gly18, Thr19, Trp20, Lys21, Asp43, Val47, Tyr48, Lys77, Trp79, His110, Trp111, Phe122, Ser159, Asn160, Gln183, Tyr209, Ser210, Pro211, Leu212, Ser214, Asp216, Trp219, Leu228, Ala245, Ile260, Pro261, Lys262, Ser263, Val264, Thr265, Arg268, Thr268, Glu271, Asn272, Cys298, Ala299 and Leu300 are the amino acids surrounding the binding site. The predicted binding site residues correlated with the previous reports (Rondeau et al. 1992; El-Kabbani et al. 2003). The predicted binding site residues were used to draw the grid box in Auto Grid for performing docking analysis in AutoDock (ADock) and AutoDock Vina (AVina).

Benchmarking molecular docking

The benchmarking molecular docking protocol for assessing the AR inhibitory potential of TFG compounds utilising ADock and AVina involved 1200 decoys from the DEKOIS database (see Suppl. material 1). These decoys are designed explicitly for AR. These decoys matched the test ligands in less than 500 Da of molecular weight. The test set consisted of 58 TFG compounds and one standard drug, Epalrestat. As a result, 1259 molecules were in the ligand collection overall for benchmarking docking.

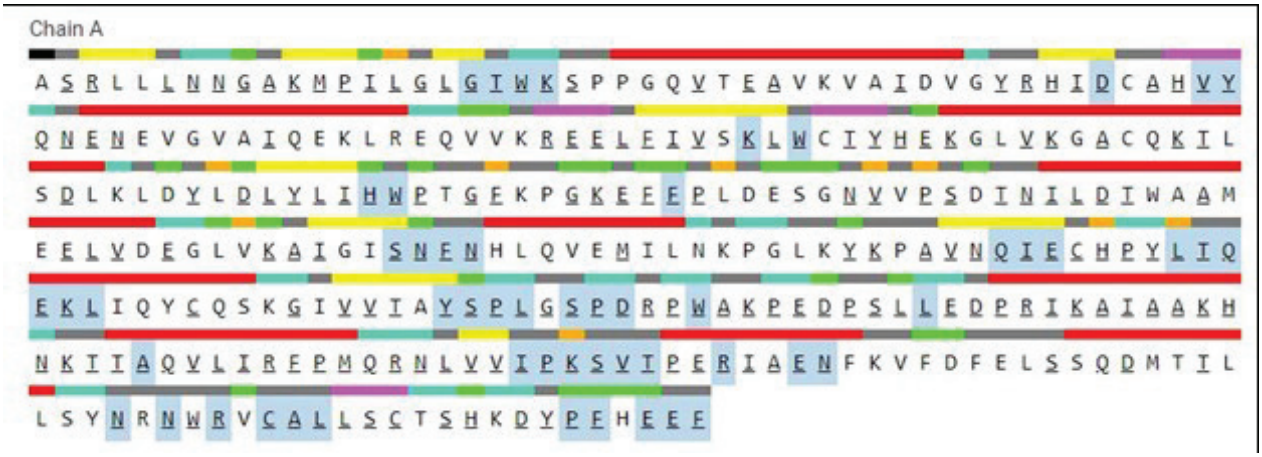


Figure 1. The binding site of aldose reductase was predicted using CASTp. The residues highlighted in blue boxes constitute the binding site.

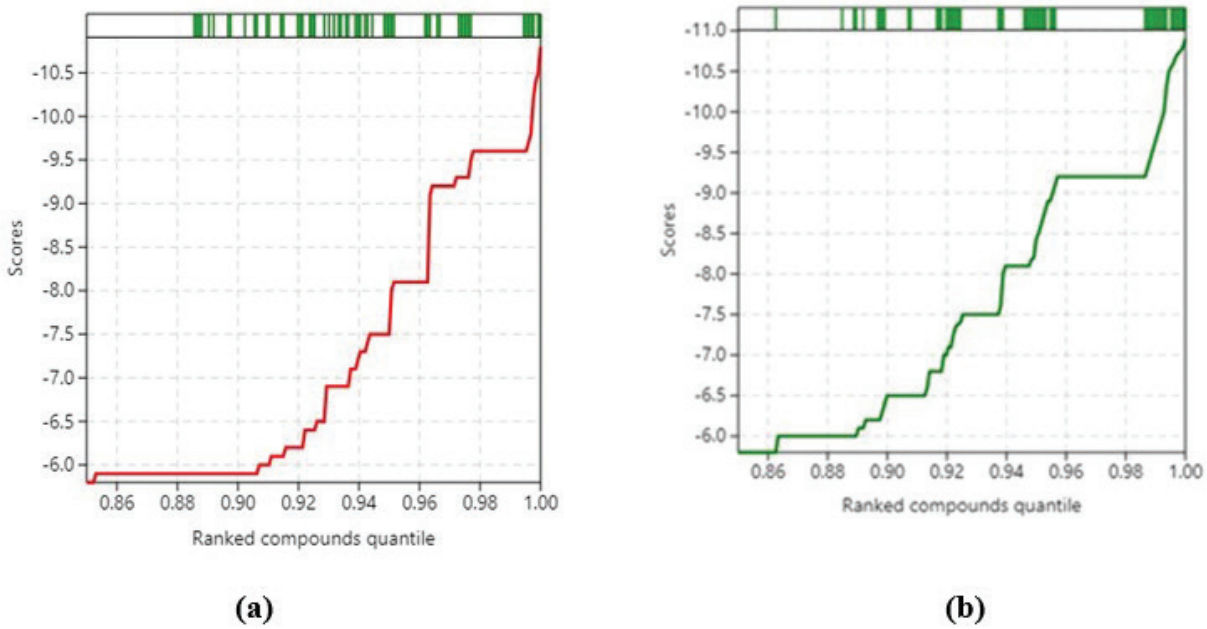


Figure 2. Benchmarking docking binding energy scores distribution: (**a**) AutoDock, (**b**) AutoDock Vina.

The distribution of binding energy scores for TFG compounds, standard drug and decoys predicted by ADock and AVina are shown in Fig. 2. The rankings from Fig. 2 were used to compare the software's predictive power by computing metrics through ROC, predictiveness and enrichment curves. The global and partial metrics computed from these graphs are presented in Table 1. Fig. 3 shows the ROC, predictiveness and enrichment curves derived from the compound rankings.

The performance of docking software in distinguishing active and inactive compounds can be evaluated using the auROC/pauROC. A higher pauROC score at the top 5% retrieval indicates better discrimination by AVina at an early stage. The TG metric reflects the software's ability to prioritise active compounds effectively across various ranked compounds in which AVina performed better. RIE evaluates the efficiency of docking software in terms of information gain per compound. A higher RIE value for prediction by AVina suggests a more robust ranking of compounds at the initial stage. BEDROC emphasises docking software's early recognition ability by quantifying the enrichment of top-ranked compounds. A higher BEDROC score indicates early enrichment and AVina performed better. The TPF and P(Act) scores indicate better predictive power of AVina than ADock for activity prediction.

Table 2 presents information regarding the structures and binding energy scores of 5 TFG compounds that AVina successfully retrieved within the top 5% level. Upon further analysis of the recovered compounds using two different types of software, Tigogenin and Gitogenin were identified as the top two enriched compounds in the top three ranks. Therefore, these two compounds were considered for further molecular interaction, density functional theory (DFT) calculations and molecular dynamic studies.

Fig. 4 shows the intermolecular interactions of Tigogenin, Gitogenin and the standard drug with AR's bind-

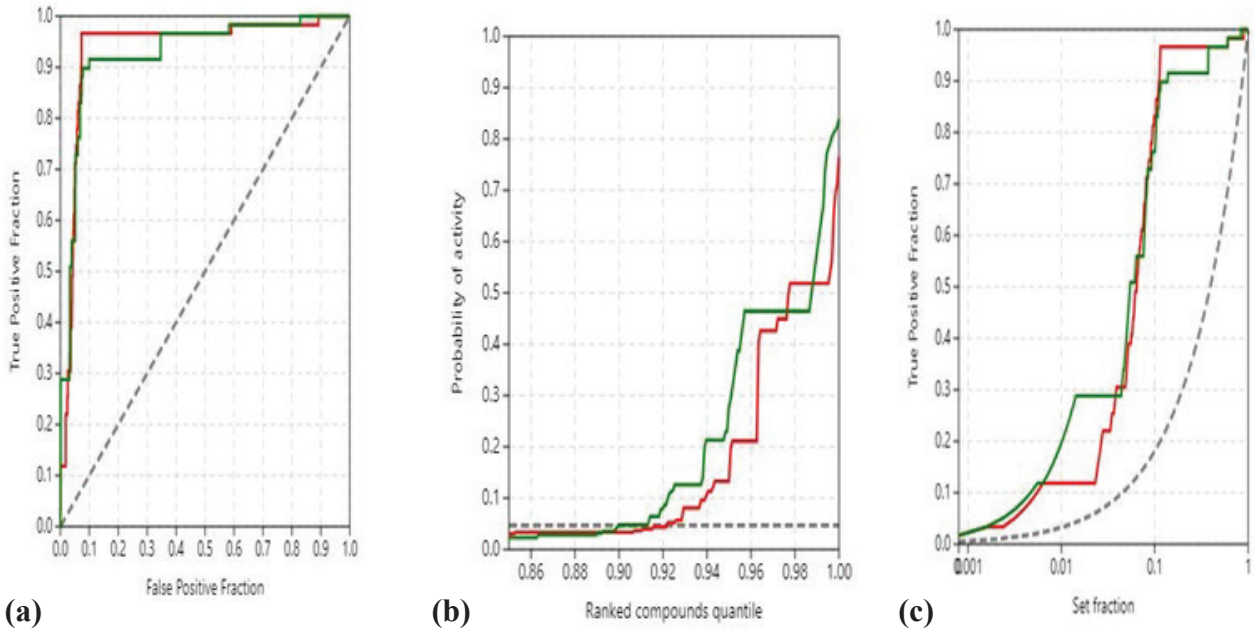


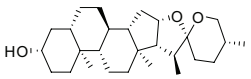
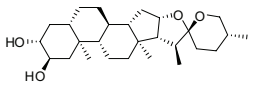
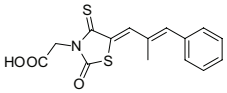
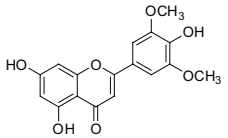
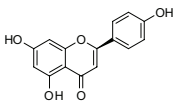
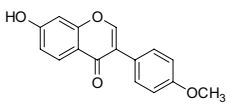
Figure 3. Analysis and comparison of the predictive power of AutoDock and AutoDock Vina: (**a**) Receiver operating characteristic curves, (**b**) Predictiveness curves, (**c**) Enrichment curves, red is ADock and green is AVina.

Table 1. Comparison of the predictive power of AutoDock and AutoDock Vina.

Software	Global metrics (Full threshold)			
	auROC	TG	RIE	BEDROC
AutoDock	0.937	0.459	7.242	0.558
AutoDock Vina	0.927	0.603	8.082	0.622
	Partial metrics (5%)			
	pauROC*	pTG*	Number of Actives	Number of decoys	TPF*	P(Act)*	$EF_{5\%}$
AutoDock	0.034	0.638	5	1	0.102	0.542	17.80
AutoDock Vina	0.102	0.806	6	0	0.119	0.792	21.36

*pauROC: partial area under ROC, pTG: partial total gain, TPF: true positive fraction; P(Act): probability of activity.

Table 2. Structures and binding energy scores of TFG compounds ranked by AutoDock Vina at the top 5% level.

Name	Structure	Binding energy (ΔG, kcal/mol)	RMSD (Å)
Tigogenin		-10.90	1.09
Gitogenin		-10.80	1.26
Epalrestat (Standard drug)		-10.75	0.99
Tricin		-10.72	1.36
Naringenin		-10.68	1.84
Formononetin		-10.60	1.29

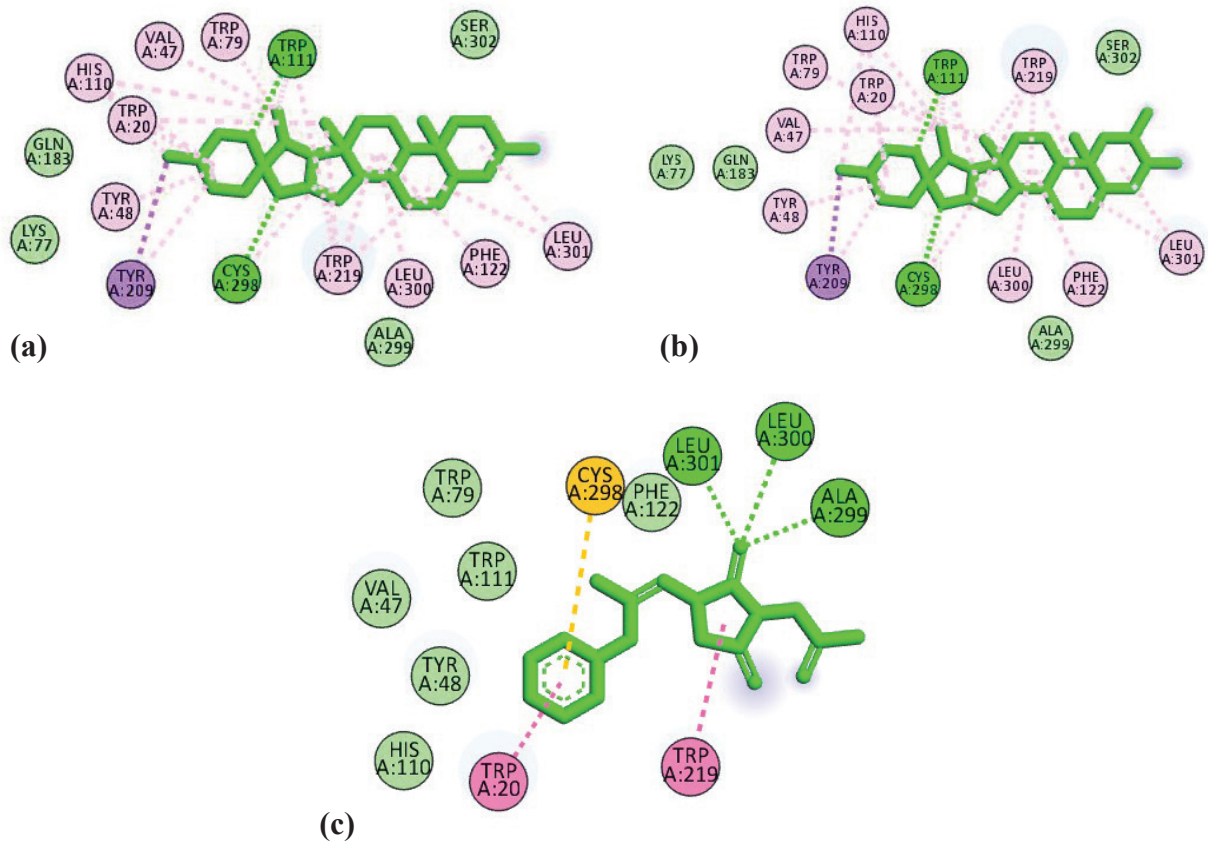


Figure 4. Intermolecular interactions of (**a**) Tigogenin, (**b**) Gitogenin, (**c**) Epalrestat with active-site amino acids of the enzyme aldose reductase. All the ligands are shown in all atoms' green-coloured ball and stick-type representations. The names of amino acids with ID numbers are mentioned under each circle around each ligand. On the 2D figures analysis, green-coloured dotted lines indicate hydrogen bonding interactions involving electronegative elements like nitrogen and oxygen atoms; light purple-coloured dotted lines indicate π-alkyl interactions; violet-coloured dotted lines indicate π-sigma interactions. Light green colour amino acids without bonding represent van der Waals interactions, whereas, orange-red colour amino acids indicate unfavourable interactions. The light-blue halo surrounding the interacting residues represents the solvent-accessible surface that is proportional to its diameter.

ing site residues. Tigogenin forms hydrogen bonds with Trp111 and Cys298, a π-sigma interaction with Tyr209, alkyl interactions with Val47, Cys298, Leu300 and Leu301 and π-alkyl interactions with Trp20, Tyr48, Trp78, His110, Trp111, Phe122 and Trp219. Gitogenin forms hydrogen bonds with Trp111 and Cys298, a π-sigma interaction with Tyr209, alkyl interactions with Val47, Cys298, Leu300 and Leu301 and π-alkyl interactions with Trp20, Tyr48, Trp78, His110, Trp111, Phe122 and Trp219. The TGF compounds also interact through the van der Walls interactions with residues such as Trp20, Ala47, Tyr48, Lys77, Trp111, Gln183, Trp219, Ala299 and Leu301. These amino acid interactions align with earlier literature reports that interactions with Trp20, Tyr48, His110 and Trp111 located in the anion binding site of the AR are critical to its inhibition (Tanawattanasuntorn et al. 2021). The molecular docking study predicted that Tigogenin and Gitogenin can potentially inhibit AR.

Density functional theory calculations

We investigated the electronic and energetic states of the top two TFG compounds, Tigogenin and Gitogenin. Fig. 5 is the HOMO-LUMO distribution plots of Tigogenin and Gitogenin to analyse the atomic contribution of these orbitals. For Tigogenin and Gitogenin, the HOMO energy is -0.254 eV and -0.253 eV, respectively, while the LUMO energy values are -0.077 eV and -0.075 eV. The HOMO-LUMO energy gap (HLG) is -0.197 eV and 0.195 eV for Tigogenin and Gitogenin, respectively. The compounds have smaller HLG, which indicates high chemical reactivity. A higher HLG specifies higher kinetic stability and low chemical reactivity. Since the selected ligands reveal a lower HLG, they are more chemically reactive. The energy value of HOMO is higher than the LUMO energy for the ligands, demonstrating their ability to donate electrons to the binding site of the target.

Thus, the result suggests that orbitals of such ligands are favourable for forming hydrogen bond interactions with binding site amino acid residues of AR.

Molecular dynamic stability studies

The Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF) and hydrogen bond interactions of the docked complexes were analysed and presented (Fig. 6). Fig. 6a shows the RMSD plots of apo-

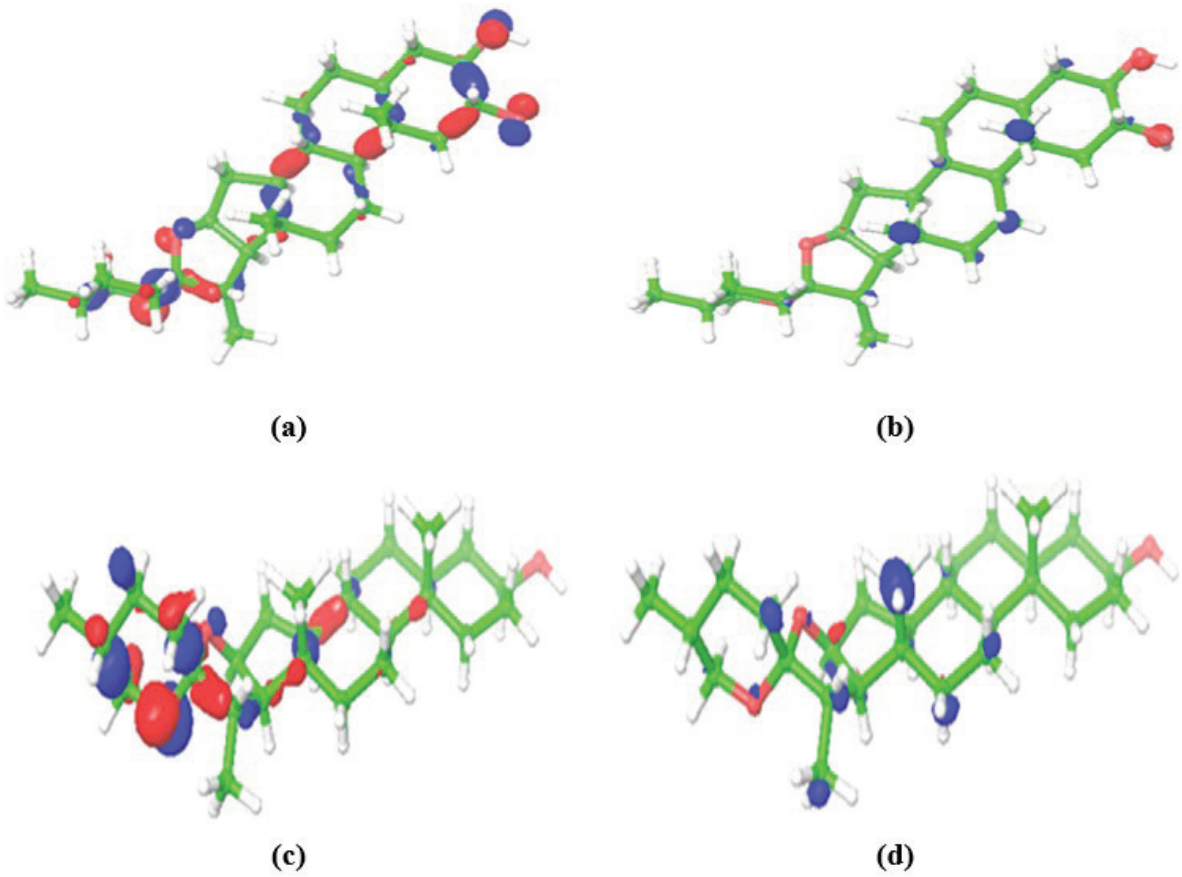


Figure 5. HOMO and LUMO distribution plots: (**a**) HOMO (**b**) LUMO of Tigogenin (**c**) HOMO (**d**) LUMO of Gitogenin.

protein AR, AR with Tigogenin and AR with Gitogenin complexes. The RMSD of the apoprotein achieved stability after 15 ns of equilibration and maintained a stable RMSD value of 0.56 nm. The RMSD plot of AR with Tigogenin and Gitogenin complexes demonstrated stability, with no significant deviations observed during the simulation. The AR with Tigogenin complex maintained an acceptable RMSD value of 0.53 nm throughout the simulation. The AR with Gitogenin equilibrated in 5 ns and had a constant RMSD value of 0.58 nm throughout the simulation.

Fig. 6b shows the RMSF plot of AR with Tigogenin and AR with Gitogenin complexes during a 50 ns simulation period. The average RMSF value is 0.26 nm and most higher fluctuations occur in the loop rather than the structural region. Specifically, the loop region between the residues 110–135 and 217–223 experiences the highest level of fluctuation in each simulation. However, these fluctuations do not affect the structural conformation of the docked complexes (Antony and Vijayan 2015).

It is necessary to analyse the hydrogen bond interaction profile to determine the strength of the hydrogen bond between a ligand and a protein's binding site. As illustrated in Fig. 6c, each of the complex's hydrogen bond interaction profiles was examined during the simulation period. The simulation revealed that the AR with Tigogenin and AR with Gitogenin complexes had a maximum of five and a minimum of four H-bonds. The H-Bond interaction profiles showed that Tigogenin and Gitogenin have firmly occupied the catalytic pockets of the AR protein.

The binding energy of the docked complexes was calculated using the last 10 ns of the MD trajectories with the MM/PBSA method implemented using the g_mmpbsa tool of GROMACS (Kumari et al. 2014). The resulting Table 3 provides the binding energy (ΔG_{bind}), van der Waals energy (E_{vdw}), electrostatic energy (E_{elec}), polar solvation energy (G_{polar}) and non-polar solvation energy ($G_{nonpolar}$) for each complex.

It is important to note that the binding energy is negatively impacted (stabilised) by E_{vdw}, E_{elec} and $G_{nonpolar}$ while positively affected (destabilised) by G_{polar}. With both Tigogenin and Gitogenin, the binding energies with AR are below zero, indicating a strong affinity and interaction. In both complexes studied, van der Waals interactions contribute most to the binding energy.

Table 3. Binding energies of TFG compounds with aldose reductase computed by MM/PBSA method.

Compound	E_{vdw} (kJ/mol)	E_{elec} (kJ/mol)	G_{polar} (kJ/mol)	$G_{nonpolar}$ (kJ/mol)	ΔG_{bind} (kJ/mol)
Tigogenin	−158.321 ± 11.055	−25.831 ± 08.259	95.365 ± 11.705	−15.659 ± 0.257	−104.446 ± 13.954
Gitogenin	−155.361 ± 15.203	−20.033 ± 05.002	84.587 ± 15.385	−10.657 ± 08.325	−101.464 ± 15.662

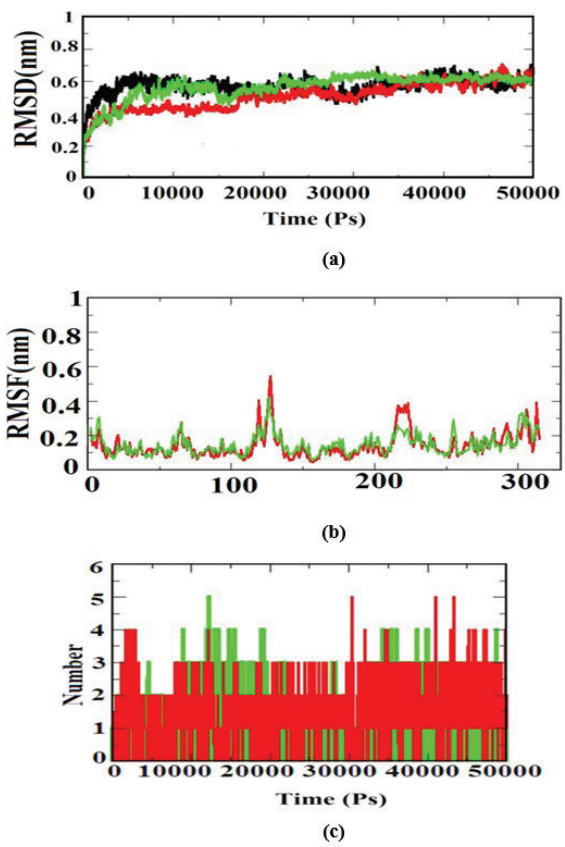


Figure 6. Molecular dynamics of Tigogenin and Gitogenin bound to aldose reductase: (**a**) RMSD, (**b**) RMSF, (**c**) Hydrogen bond profile; green-Tigogenin, red-Gitogenin, black-apoprotein.

Discussion

Targeting AR is a beneficial strategy for the effective management of diabetes-associated complications. Predicting the inhibitory potential by benchmarking docking of compounds of the traditionally used herb TFG to AR is indispensable in drug discovery. The definition of the binding site of AR is the primary step in molecular docking. The predicted AR binding region surrounds Gly18, Thr19, Trp20, Lys21, Asp43, Val47, Tyr48, Lys77, Trp79, His110 and Trp111 which is anionic and the binding specificity aperture is surrounded by Trp111, Phe122, Gln183, Tyr209, Cys298, Ala299 and Leu300 residues.

ADock and AVina are popular types of software for molecular docking (Pagadala et al. 2017). ADock utilises a genetic algorithm to search for optimal ligand poses and a semi-empirical free energy force field to score binding affinity. AVina employs an empirical scoring function, which is an adaptation of the X-Score function comprising five components (Cosconati et al. 2010; Jaghoori et al. 2016). Benchmarking molecular docking compares and evaluates the benefits and drawbacks of various types of docking software and enhances the precision and dependability of docking predictions for drug discovery (Thangavel and Albratty 2022). For this purpose, Screening Explorer, an online tool, was used (Empereur-Mot et al. 2016). Analysing and comparing ADock and AVina's predictive power revealed that both programmes performed comparably, showing agreement in ranking the TFG compounds and differentiating them from decoys. Specifically, in the rank list of AVina, Tigogenin and Gitogenin held the first and second positions, respectively. ADock, on the other hand, ranked the standard drug at the top position, followed by Gitogenin and Tigogenin at the second and third positions, respectively. Examining the interactions of these compounds with the AR binding site and in comparison, with the standard drug Epalrestat, the docking analysis revealed their potential for AR inhibition.

Density functional theory (DFT) calculations provide insights into drug molecules' binding mechanisms and properties. It quantifies electrostatic interactions between drugs and target proteins, helping identify crucial binding interactions (Deghady et al. 2021). Frontier molecular orbitals are essential to charge-transfer interactions with the target binding site. The highest occupied molecular orbital (HOMO) has the highest energy and is electron-rich, which means it can donate electrons and mainly involves nucleophilic reactions. On the other hand, the lowest unoccupied molecular orbital (LUMO) has the lowest energy. It is electron-deficient, which means it can accept electrons and is mainly involved in electrophilic reactions. Predictions of these energies for Tigogenin and Gitogenin suggested they possess favourable orbital energies for interacting with AR.

GROMACS software was utilised to conduct an MD simulation for 50 ns to explore the stability and conformational alterations of the docked complexes. The RMSD analyses revealed that the apoprotein and both complexes were stable and maintained their dynamic nature after achieving equilibrium. The RMSD plot confirmed that the complexes remained stable throughout the simulation. The RMSF plot provides information about how amino acid residues move over time (Antony and Vijayan 2015). The MD results confirm that there are no abnormal residue fluctuations in AR on binding to Tigogenin and Gitogenin. The hydrogen bond interaction analysis during MD showed that AR maintained strong binding stability during interaction with these compounds. In addition, MM/PBSA results strongly correlate with the intermolecular interactions predicted by AVina.

TFG contains 0.6–1.7% of saponins. Tigogenin and Gitogenin, identified as AR inhibitors, are steroidal sapogenins (Visuvanathan et al. 2022). Previous reports show that Tigogenin therapy in diabetic mice resulted in a decrease in lipid accumulation caused by adipogenic induction. It also led to a reduction in visfatin secretion and the expressions of adipocyte fatty acid-binding protein (ap)2 and peroxisome proliferation-activated receptor-γ2 (Zhou et al. 2007). There are no reports about the effect of Gitogenin on diabetes or diabetes-associated complications, to date. This is the first study reporting the AR inhibitory potential of Tigogenin and Gitogenin.

Conclusions

In conclusion, combined benchmarking molecular docking, density functional theory calculations and molecular dynamics stability study have demonstrated the potential of *Trigonella foenum-graecum* compounds as natural alternatives for managing diabetes-associated complications. The study results show that Tigogenin and Gitogenin have strong inhibitory potential against aldose reductase, a key enzyme in the polyol pathway that leads to microvascular complications in diabetes. The docking and molecular dynamics simulations have predicted that both compounds bind well to the aldose reductase enzyme and the interactions exhibit excellent stability. Further research is needed to explore the efficacy of *Trigonella foenum-graecum* compounds in vivo and their long-term safety profile. The study aimed to increase the reliability of bioactivity prediction by utilising molecular docking benchmarking. The results from this approach could enhance the success rate in drug development of *Trigonella foenum-graecum* compounds, turning them into promising drug candidates.

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Supplementary material 1

List of molecular weight matched decoys retrieved from DEKOIS 2.0

Author: Neelaveni Thangavel

Data type: sdf

Explanation note: The list contains information about the ID number and molecular weights of decoys meant for aldose reductase, retrieved from the DEKOIS 2.0 database.

Link: https://doi.org/10.3897/pharmacia.71.e118949.suppl1