

Methanol Extract of *Coccinia grandis* L. Leaves: GC-MS Profiling, Molecular Docking, DFT, and MD Simulations Reveal Phytochemicals as Potential DPP4 Inhibitors

Alomgir Hossain

alamgir199817@gmail.com

University of Rajshahi

Mst. Nushrat

University of Rajshahi

S. M. Shahinul Islam

University of Rajshahi

Sreeramanan Subramaniam

Universiti Sains Malaysia

Md. Borhan Uddin

University of Rajshahi

Md. Shohag Hossain

University of Rajshahi

Md. Shahadat Hossain

University of Rajshahi

Research Article

Keywords: *Coccinia grandis* L., dipeptidyl peptidase 4 (DPP4), Molecular docking, GC-MS Extraction, MD simulations, DFT

Posted Date: December 23rd, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-8248313/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Diabetes mellitus affects approximately 422 million people worldwide, making dipeptidyl peptidase 4 (DPP4) a promising therapeutic target for glucose-lowering drug development. This study investigated bioactive constituents from *Coccinia grandis* leaves as potential inhibitors of dipeptidyl peptidase 4 (DPP4), a validated target in type 2 diabetes therapy. GC–MS analysis identified 33 phytochemicals from the methanolic leaf extract. Molecular docking against DPP4 (PDB: 4A5S) revealed cholesteryl chloroformate (−9.3 kcal/mol), β -sitosterol (−8.6 kcal/mol), and methoxyfenozide (−7.9 kcal/mol) as top ligands forming stable interactions with key catalytic residues. ADME/T predictions indicated acceptable pharmacokinetic behavior and low toxicity. DFT calculations (B3LYP/6-311G(d,p)) confirmed favorable electronic properties supporting ligand reactivity. Molecular dynamics simulations (100 ns) demonstrated structural stability across complexes, with β -sitosterol showing the most consistent behavior. The findings suggest that *C. grandis* phytochemicals represent promising DPP4 inhibitors with potential anti-diabetic properties, necessitating further experimental validation and clinical investigation.

Introduction

Dipeptidyl peptidase 4 (DPP4) inhibitors constitute an important class of glucose-lowering therapeutics used in the treatment of type 2 diabetes mellitus (T2DM). Natural products provide structurally diverse molecules with therapeutic potential. *Coccinia grandis* L. (Cucurbitaceae), traditionally used for diabetes management, contains phytochemicals with reported antioxidant and hypoglycemic activities. However, the DPP4-inhibitory potential of its leaf constituents has not been comprehensively explored using integrated GC–MS and computational strategies. Diabetes mellitus has emerged as one of the most common non-communicable diseases in recent decades. According to the World Health Organization (WHO), diabetes affects approximately 422 million people worldwide, with 1.5 million deaths attributed to diabetes and its complications. Diabetes-associated complications can be severe, and the disease ranks as the third leading cause of death after heart disease and cancer [1, 2]. The International Diabetes Federation estimated that 465 million people (9.3%) globally had diabetes in 2019, a number projected to rise to 700 million (10.9%) by 2045. In Bangladesh, 7.1 million people had diabetes in 2015, of whom 3.7 million were untreated, resulting in over 129,000 deaths [3].

Dipeptidyl peptidase 4 (DPP4) represents a novel class of blood glucose-lowering drugs developed through rational drug design based on the structure of the target enzyme and its mechanism of action [4]. Inspired by these computational results, [5] isolated bioactive compounds from the leaf extract of *Coccinia grandis*. Known as Telakucha in Bengali and as ivy or small pumpkin in English, *C. grandis* belongs to the family Cucurbitaceae [6]. The leaves of this plant are broad, cordate-oval in shape, with a triangular, obtuse, apiculate basal sinus, typically five-lobed [7]. These lobed leaves often have small teeth along their margins, are ivy-like in appearance, and measure 3.5–9 cm in length and 4–9 cm in width [8].

C. grandis exhibits long, creeping or climbing tendrils, including axillary tendrils, that can grow up to 13 meters and form dense layers covering other vegetation [8]. It commonly grows over trees, undergrowth, fences, electricity poles, and other structures in residential and agricultural areas [9]. The roots, leaves, and fruits of *C. grandis* have been traditionally used to treat various ailments, including diabetes, ulcers, wound healing, and jaundice [10].

Coccinia grandis is known to contain phytochemicals such as stigmast-7-en-3-one, lupeol, taraxerol, β -sitosterol, tritriacontane, and cephalandrin A, B, and cephalandrol [6]. It is also commonly consumed as a vegetable crop in Bangladesh and other countries [11]. This study aims to identify phytochemicals from the methanol leaf extract of *C. grandis* through GC–MS profiling and to evaluate their potential as DPP4 inhibitors using molecular docking, DFT calculations, ADME/T screening, and molecular dynamics (MD) simulations.

Materials and Methods

Experimental parts

Plant Materials

Fresh, dust-free leaves of *Coccinia grandis* L. were collected from the campus of the University of Rajshahi, Rajshahi, Bangladesh. The young and mature leaves were carefully harvested, washed under running tap water, and subsequently rinsed with distilled water to remove all dirt particles. The leaves were then shade-dried for three weeks (Fig. 1). After drying, the plant material was processed into a fine powder using a commercial blender, ensuring zero percent moisture content.

Preparation of Plant Extract

A total of 100 g of powdered leaves was weighed using an electronic balance and transferred into clean, sterilized vials. Methanol, as a polar solvent, was added in a 1:4 ratio (powder: solvent), and the vials were sealed with aluminum foil. The mixture was kept at room temperature for 14 days with occasional shaking and stirring. The mixture was then filtered, and the filtrates were passed through Whatman No. 1 filter paper.

Evaporation of Solvent

The methanol filtrate evaporated in the shade at room temperature. The resulting concentration was further dried and powdered using rotary evaporation. The obtained gummy concentrate was labeled as the crude extract and stored in an airtight container at 4°C for further use. Finally, the extract was transferred into Eppendorf tubes for GC-MS analysis to determine the chemical components (Fig. 2).

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS is a comprehensive technique used for identifying bioactive components of *Coccinia grandis* L. The analysis was conducted at the Bangladesh Reference Institute for Chemical Measurements (BRICM), Dr Quadrat-e-Khoda Road, Dhanmondi, Dhaka-1205, Bangladesh. Phytoconstituents of the leaf extract were analyzed using a GC-MS/MS system (Shimadzu, Japan; model GC-MS-TQ8040) with a DB-5ms capillary column (30 m × 0.25 mm ID, film thickness 0.25 µm). One milliliter of the sample was injected in splitless mode at a constant flow rate of 1 mL/min. The injection temperature was maintained at 250°C, and helium was used as carrier gas. The oven temperature was initially set to 50°C for 1 minute, increased to 200°C for 2 minutes, and finally to 300°C for 7 minutes, with a total run time of 40 minutes. Detector parameters were interface temperature 250°C, ion source temperature 230°C, and mass spectrometry Q3 scan with m/z range 50–600, scan speed 2000, and scan time 0.3 s per scan. The relative percentage of each component was determined by peak area. Compounds were identified by comparing their spectra with the NIST mass spectral library [12].

Computational Parts

Retrieval and Preparation of Protein

The three-dimensional (3D) crystal structure of dipeptidyl peptidase 4 (DPP4) at 1.62 Å resolution (PDB ID: 4a5s) was retrieved from the Protein Data Bank (<https://www.rcsb.org/>). Heteroatoms and water molecules were removed using Discovery Studio version 21.1.0. The protein structure was optimized by correcting missing hydrogen bonds, side-chain geometries, and bond orders, followed by energy minimization using the GROMOS 43B1 force field in Swiss-PDB Viewer [13]. The prepared protein was saved in PDBQT format for docking in PyRx (AutoDock Vina).

Preparation of Ligands

The methanol leaf extract of *Coccinia grandis* L., analyzed by GC-MS, revealed 33 phytochemicals, which were selected for molecular docking. The compounds were downloaded in 3D-SDF format from the PubChem database. Ligand energy minimization was performed using the MMFF94 force field and the steepest descent algorithm with 2000 steps in AutoDock Vina.

Molecular Docking Analysis

Molecular docking studies were conducted to reveal binding interactions between the target protein and the ligands. Natural compounds identified by GC-MS [12] were docked to the protein using PyRx (AutoDock Vina). After preparing protein and ligands, a grid box was created with center coordinates X = -26.98, Y = 12.6041, Z = 58.9454, and dimensions X = 50.3333, Y = 66.2746, Z = 60.2586 Å. Binding affinities were expressed in kcal/mol, with more negative values indicating stronger interactions. Docking results were ranked based on these values [14]. The top three compounds with the highest

binding affinity were selected for further analysis. Protein-ligand complexes were visualized using Chimera and Discovery Studio Client.

ADME and Bioactivity Analysis

Pharmacokinetic properties were evaluated by assessing absorption, distribution, metabolism, and excretion (ADME) profiles. Lipinski's rule of five was applied to determine drug-likeness: (a) molecular weight ≤ 500 Da, (b) H-bond acceptors ≤ 10 , (c) H-bond donors ≤ 5 , (d) LogP ≤ 5 , and (e) Lipinski violations ≤ 3 . SwissADME and pKCSM web servers were used to predict various pharmacokinetic and pharmacodynamic properties of the selected molecules.

Assessment of Toxicity

Early toxicity assessment is critical in drug development. Traditional *in vivo* models are time-consuming, expensive, and raise ethical concerns. *In silico* toxicity prediction provides a reliable alternative. The toxicity of selected compounds was predicted using the ProTox-II server, based on canonical SMILES input [15].

Density Functional Theory Calculation

Density functional theory (DFT) was employed to evaluate the functional, structural, and quantum chemical properties of the top three phytocompounds identified through the molecular docking investigation. DFT is based on quantum mechanics and offers a very precise explanation of how electrons are distributed within molecules, allowing the computation of diverse aspects, including molecular energies, geometries, and electronic characteristics. The Gaussian 09 W software package [16] was utilized to calculate various quantum mechanical properties. The electronic characteristics of the molecules were calculated in their singlet ground state, devoid of charge and solvent, using the B3LYP (Becke, 3-parameter, Lee-Yang-Parr) methodology within the framework of density functional theory and a 6-311g(d,p) correlation function basis set [17]; "Performance of B3LYP Density Functional Methods for a Large Set of Organic Molecules", 2025). Gaussian-09 and GaussView-6 parameters [18] were used in this study to calculate the energy of the highest occupied molecular orbital (HOMO), the energy of the lowest unoccupied molecular orbital (LUMO), the energy gap (ΔE), hardness (η), softness (σ), electronegativity (χ), electrophilicity (ω), and other parameters for the three drug molecules linked to the top-ranked complexes. The mathematical definitions (1–8) of these parameters are as follows [19]:

$$1. \Delta E = E_{\text{LUMO}} - E_{\text{HOMO}}$$

$$2. I = -E_{\text{HOMO}}$$

$$3. A = -E_{\text{LUMO}}$$

$$4. \eta = \frac{I - A}{2}$$

$$5. \sigma = \frac{1}{\eta}$$

$$6. \chi = \frac{I + A}{2}$$

$$7. \mu = -\chi$$

$$8. \omega = \frac{\chi^2}{2\eta}$$

Molecular Dynamics Simulation

Molecular dynamics (MD) simulation is a computational method that simulates the relative motion and behavior of atoms and molecules, permitting the analysis of their dynamic interactions within various systems. YASARA software (v25.1.13) was used to perform MD simulations, aiming to explore the stability of the top-ranked protein–ligand complexes [20]. Each complex was investigated for a 100 ns MD run under specific conditions, including pH 7.4, temperature of 298 K, 0.9% NaCl concentration, and an explicit water solvent system [21]. The AMBER14 force field was employed for these simulations [22]. The particle mesh Ewald method was used to compute long-range electrostatic interactions, whereas short-range van der Waals and Coulomb interactions were analyzed within an 8 Å cutoff radius [23, 24]. During the simulations, temperature and pressure were regulated using a Langevin thermostat and a Monte Carlo barostat, respectively [14]. The system was minimized with the steepest descent algorithm. A time step of 1.25 fs was applied during the 100 ns trajectories, with snapshots recorded every 100 ps for analysis. To ensure convergence, multiple parallel trajectories were performed for each complex [15]. Throughout the simulation, several structural and dynamic parameters were examined, including the radius of gyration (Rg), solvent accessible surface area (SASA), number of hydrogen bonds, root mean square deviation (RMSD), and root mean square fluctuation (RMSF) [14, 25, 26]. All plots derived from the MD simulations were generated using the Matplotlib Python package.

Results and Discussion

Gas Chromatography-Mass Spectrometry (GC-MS)

The methanol leaf extract of *Coccinia grandis* L. was analyzed using GC-MS, which revealed a total of 33 bioactive compounds, as summarized in Table 1. The total running time for the analysis was 40 minutes, and the retention times (R. Time) for each compound are listed in Table 1. Figure 3 shows the total ion chromatogram (TIC) of the methanol leaf extract of *Coccinia grandis* L. (ivy gourd), indicating the distribution and relative abundance of the detected phytochemicals (Fig. 3).

Table 1

Phytochemical composition of *Coccinia grandis* L. methanol leaf extract identified by GC-MS analysis. The table shows serial number (Sl.), retention time (R. Time), relative abundance (Area %), and compound names for all 33 identified bioactive compounds.

Sl.	R. Time	Area (%)	Compounds name
1	4.035	0.62	3,3-Dimethoxy-2-butanone
2	4.073	0.60	Phenyl(2-phenyl-1,3-dioxolan-2-yl)methanol, TMS
3	6.085	0.89	6,6,8,8,10,10-Hexamethyl-2,5,7,9,11,14-hexaoxa-6,8,10-trisilapentadecane
4	6.628	0.33	Trimethylsilyl ethaneperoxoate
5	7.993	2.44	2,2'-Bis-trimethylsilylbenzhydryl methyl ether
6	8.514	0.26	Benzothiazole
7	9.509	0.50	Methoxyfenozide
8	9.665	0.51	3-Hydroxyphenylacetic acid, 2TMS derivative
9	10.415	0.61	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-
10	10.983	1.10	2,4,6-Trimethyl-1,3-benzenedimethanol
11	11.101	0.76	Phenol, 3,5-bis(1,1-dimethylethyl)-
12	11.728	0.24	Silane, dimethyl(3-methylbut-3-enyloxy)hexyloxy-
13	12.779	0.30	1,2-Benzenedicarboxylic acid, butyl methyl ester
14	14.864	1.19	Neophytadiene
15	15.328	7.35	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
16	15.496	0.41	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
17	16.103	0.41	6-Octadecenoic acid, methyl ester, (Z)-
18	16.183	8.90	Hexadecanoic acid, methyl ester
19	18.917	2.48	9,12-Octadecadienoic acid, methyl ester
20	19.023	22.18	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-
21	19.173	5.39	Phytol
22	19.428	1.12	Methyl stearate
23	20.36	0.85	Hexadecanamide
24	22.275	0.34	1-Decanol, 2-hexyl-
25	22.79	0.32	Methyl 8-methyl-decanoate

Sl.	R. Time	Area (%)	Compounds name
26	22.901	0.30	Isopropyl tetradecyl ether
27	23.317	0.84	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester
28	23.415	36.58	9-Octadecenamide, (Z)-
29	23.806	0.37	Bis(dodecanamido)methane
30	32.252	0.28	Delta.-Tocopherol
31	34.915	0.28	Vitamin E
32	36.743	0.49	Cholest-5-en-3-ol (3.beta.)-, carbonochloridate
33	38.469	0.76	Beta-Sitosterol

Molecular Docking Analysis

To investigate the binding affinity and molecular interactions between the ligands and the target protein, a molecular docking study was performed. The bioactive compounds were docked to DPP4 to determine their spatial orientation within the binding pocket, yielding binding energies ranging from – 4.5 to – 9.3 kcal/mol.

Methoxyfenozide, one of the newest and most effective drugs in the diacylhydrazine class, can be readily synthesized under standard Schotten–Baumann conditions from tert-butylhydrazine hydrochloride and the corresponding substituted benzoyl chlorides [27]. In the docking study, methoxyfenozide exhibited a binding score of – 8.6 kcal/mol (Table 2) and formed a hydrogen bond with Lys554, as well as two pi-alkyl interactions with Tyr547 and Trp629 within the active site of DPP4. These interactions likely contribute to their strong affinity for the protein.

Cholesteryl chloroformate also demonstrated significant interactions with DPP4. This compound formed a conventional hydrogen bond with Try563 and four pi-alkyl interactions with Tyr547, Trp627, Trp629, and Tyr666, suggesting that its activity is mediated through key residues in the protein's active pocket.

Plant sterols, such as β -sitosterol, exhibit chemical structures similar to cholesterol. Both clinical and experimental studies have reported the anti-diabetic, hypolipidemic, anti-cancer, anti-arthritic, and hepatoprotective properties of β -sitosterol [28]. In docking analysis, β -sitosterol formed three pi-alkyl interactions with Phe357, Tyr547, and Tyr666, indicating strong binding within the active site of DPP4.

Table 2

Non-bond interactions between the DPP4 protein and the top three compounds with highest negative docking scores. The table presents compound names, PubChem CID numbers, binding affinities (kcal/mol), residues in contact, interaction types, and bond distances (Å) for methoxyfenozide, cholesteryl chloroformate, and β -sitosterol.

Compound name	Compound CID	Binding affinity (kcal/mol)	Residue in contact	Interaction type	Bond distance in Å
Methoxyfenozide	105010	-7.9	LYS554	Hydrogen Bond	2.5221
			GLY741		2.8843
			TYR547	Hydrogen Bond	4.8358
			TRP629	Pi-Alkyl	3.7085
			TRP627	Pi-Alkyl	4.7896
Cholesterol chloroformate	111262	-9.3		Pi-Alkyl	
			TRY563	Hydrogen Bond	2.8139
			TYR547		4.5735
			TRP627	Pi-Alkyl	4.5735
			TRP629	Pi-Alkyl	4.5494
Beta-sitosterol	222284	-8.6	TYR666	Pi-Alkyl	4.4058
			LYS554	Hydrogen Bond	2.4683
			PHE357		4.6661
			TYR547	Pi-Alkyl	4.1656
			TYR666	Pi-Alkyl	4.1381
			ASP545	Pi-Alkyl	4.7658

Overall, these results suggest that the phytochemicals interact with key residues of the DPP4 active site in a manner comparable to approved drugs, potentially exerting inhibitory effects through electrostatic and hydrophobic interactions. Figure 4 illustrates the binding interactions of these inhibitors within the active site of DPP4.

ADME/T and Bioactivity Score Assessment

Cholesteryl chloroformate and β -sitosterol showed no violations of the blood-brain barrier, whereas methoxyfenozide exhibited a violation. Methoxyfenozide also had lower synthetic accessibility compared to β -sitosterol and cholesteryl chloroformate. Regarding toxicity parameters, cholesteryl chloroformate was predicted to be active for carcinogenicity and immunogenicity but inactive for

hepatotoxicity, mutagenicity, and cytotoxicity. Methoxyfenozide was predicted to be active only for hepatotoxicity, while all other parameters—including carcinogenicity, immunogenicity, mutagenicity, and cytotoxicity—were inactive. β -Sitosterol was predicted to be active only for immunotoxicity, with the remaining toxicity parameters inactive. Overall, these three compounds—methoxyfenozide, cholesteryl chloroformate, and β -sitosterol—were predicted to have low toxicity, corresponding to toxicity classes 5, 5, and 4, respectively (Table 3). The pharmacological characteristics of these compounds, including physicochemical properties, lipophilicity, aqueous solubility, gastrointestinal absorption, drug-likeness, and synthetic accessibility, were also evaluated to predict their potential as drug candidates.

Table 3

ADME properties and drug-likeness parameters of the three lead compounds (CID: 222284, 111262, and 105010). The table includes physicochemical properties, lipophilicity, water solubility, pharmacokinetics, drug-likeness according to Lipinski's Rule of Five, and synthetic accessibility scores.

Properties		CID: 222284	CID: 111262	CID: 105010
Physico-chemical properties	MW (g/mol)	414.71 g/mol	449.11 g/mol	368.47 g/mol
	Heavy atoms	30	31	27
	Aro. Atoms	0	0	12
	Rotatable bonds	6	7	7
	H-bond acceptors	1	2	3
	H-bond donors	1	0	1
	TPSA ($\text{\AA}^2$)	20.23 $\text{\AA}^2$	26.30 $\text{\AA}^2$	58.64 $\text{\AA}^2$
Lipophilicity	Log Po/w (Con.)	4.79	5.33	3.17
Water solubility	Log S (ESOL)	-7.90	-8.58	-4.32
Pharmacokinetics	GI absorption	Low	Low	High
	BBB permeant	No	No	Yes
	P-GP substrate	No	No	No
Drug likeness	Lipinski	Yes; 1 violation: MLOGP > 4.15	Yes; 1 violation: MLOGP > 4.15	Yes; 0 violation
Medicinal chemistry	Synthetic accessibility	6.30	5.96	2.77

In this study, a total of 33 bioactive compounds were identified from the methanol leaf extract of *Coccinia grandis* L. using GC-MS analysis (Table 1) and docked to the DPP4 protein (PDB ID: 4a5s). Among these, three compounds—methoxyfenozide, cholesteryl chloroformate, and β -sitosterol—showed the highest binding affinities of -8.6 , -9.3 , and -8.5 kcal/mol, respectively (Table 2). These compounds

formed strong hydrogen and hydrophobic interactions within the DPP4 active site, highlighting their potential as therapeutic candidates. The pharmacokinetic properties of these compounds were evaluated to predict their drug-likeness. Methoxyfenozide showed blood-brain barrier (BBB) penetration, whereas β -sitosterol and cholesteryl chloroformate did not. Synthetic accessibility was lower for methoxyfenozide compared to the other two compounds. All three phytochemicals fulfilled Lipinski's Rule of Five, indicating favorable oral bioavailability: molecular weight ≤ 500 Da, ≤ 5 hydrogen bond donors, ≤ 10 hydrogen bond acceptors, and $\log P \leq 5$ (Tijjani et al., 2022) (Table 3).

Renal clearance and toxicity profiles were assessed to determine safety. Organ-specific toxicity, including hepatotoxicity, mutagenicity, immunogenicity, carcinogenicity, and cytotoxicity, was predicted using the ProTox-II server. Methoxyfenozide showed activity only for hepatotoxicity, cholesteryl chloroformate was active for carcinogenicity and immunogenicity, and β -sitosterol was active for immunotoxicity. Overall, the predicted toxicity of all three compounds was low, with toxicity classes of 5, 5, and 4, respectively (Table 4). The estimated lethal doses (LD50) were 5000 mg/kg for methoxyfenozide, 3000 mg/kg for cholesteryl chloroformate, and 390 mg/kg for β -sitosterol, indicating acceptable safety margins.

These findings suggest that methoxyfenozide, cholesteryl chloroformate, and β -sitosterol are potent DPP4 inhibitors with strong binding affinities, favorable pharmacokinetic properties, and low predicted toxicity. Their hydrogen and hydrophobic interactions within the active site support their potential therapeutic roles. Taking together, these results propose these phytochemicals as promising candidates for further experimental validation as anti-diabetic agents targeting DPP4, providing insights into dosage distribution, safety, and drug-likeness profiles essential for drug development.

Table 4

Toxicity predictions for selected compounds including organ toxicity and toxicity endpoints. The table shows hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, cytotoxicity predictions, LD₅₀ values (mg/kg), and toxicity class classifications for methoxyfenozide, cholesteryl chloroformate, and β -sitosterol.

Endpoint	Target	Methoxyfenozide	Cholesterol chloroformate	Beta-Sitosterol
Organ toxicity and toxicity endpoints	Hepatotoxicity	Active	Inactive	Inactive
	Carcinogenicity	Inactive	Active	Inactive
	Immunotoxicity	Inactive	Active	Active
	Mutagenicity	Inactive	Inactive	Inactive
	Cytotoxicity	Inactive	Inactive	Inactive
	LD50 (mg kg ⁻¹)	5000	3000	890
	Toxicity class	5	5	4

Frontier Molecular Orbital Analysis

The frontier molecular orbital analysis is a computational method employed in quantum chemistry that involves calculating and investigating the energies, geometries, and electron distributions of the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) within a molecule. To ascertain the reactivity and delineate the reactive regions within the molecular system of compounds, FMO analysis was used. The molecular orbitals of HOMO and LUMO are illustrated in Fig. 5. The difference in energy between a compound's HOMO and LUMO is a commonly used indicator for evaluating its chemical reactivity. When there is a considerable energy difference between the levels of a molecule's LUMO and HOMO, the molecule exhibits low reactivity and is chemically unstable. This phenomenon is primarily attributed to a significant energy gap between the lowest and highest energy levels, which impedes the electronic transition [29, 30].

A lower HOMO–LUMO energy gap generally signifies greater stability of the compound. Table 5 shows that the HOMO energy levels range from -0.0454 eV to XYZ eV, with compound Cholesteryl chloroformate having the lowest and Beta-Sitosterol having the highest. The LUMO energy levels range from -0.0454 eV to -0.0074 eV, with Methoxyfenozide being the lowest and Cholesteryl chloroformate being the highest. The ΔE gap ranged from 0.1949 eV to 0.2404 eV. Methoxyfenozide has a lower energy gap (0.1949 eV), indicating that it is more reactive than Beta-Sitosterol and Cholesteryl chloroformate, which have higher ΔE values.

Table 5

Computed frontier molecular orbital (FMO) energies and corresponding global reactivity descriptors (eV) for the three lead compounds. The table presents electronic energy, dipole moment, HOMO/LUMO energies, energy gap, ionization potential, electron affinity, chemical hardness, softness, electronegativity, chemical potential, and electrophilicity values.

Parameter	<i>Methoxyfenozide</i>	<i>Beta-Sitosterol</i>	<i>Cholesteryl chloroformate</i>
Electronic energy (a.u.)	-1190.688	-1210.749	-1705.105
Dipole moment, (D)	4.784	1.528	4.441
HOMO energy (E_{HOMO})	-0.2403	-0.2351	-0.2478
LUMO energy (E_{LUMO})	-0.0454	-0.0165	-0.0074
Energy gap ($\Delta E = E_{\text{LUMO}} - E_{\text{HOMO}}$)	0.1949	0.2186	0.2404
Ionization potential (I)	0.2403	0.2351	0.2478
Electron affinity (A)	0.0454	0.0165	0.0074
Chemical hardness (η)	0.0975	0.1093	0.1202
Softness (σ)	10.26	9.149	8.319
Electro-negativity (χ)	0.1429	0.1258	0.1276
Chemical potential (μ)	-0.1429	-0.1258	-0.1276
Electrophilicity (ω)	0.1047	0.0724	0.0677

Global Reactivity Descriptors

Chemical descriptors are particularly applicable to any organic compound or physiologically active molecule. Koopmans' theorem is a theoretical approach for correlating the biological activity of molecular structures with their electronic properties (Fahim & Farag, 2020). A molecule's reactivity can be assessed using quantum chemical descriptors, which include the HOMO and LUMO energy gap (ΔE), ionization potential (I), electronegativity (χ), electron affinity (A), chemical potential (μ), hardness (η), softness (σ), and electrophilicity (ω). A DFT tool was employed to calculate these values. The HOMO and LUMO energies, electrophilic index, chemical hardness, softness, chemical potential, electron affinity, and energy gap (ΔE) of the top-ranked phytocompounds are presented in Table 5.

Electronic energy (E_h) is a fundamental component in quantum chemistry that is used in computational techniques to investigate and comprehend molecular behaviour. Methoxyfenozide exhibited the lowest energy value, at - 1190.688 E_h , whereas Cholesteryl chloroformate demonstrated the highest energy value, at - 1705.105 E_h . In contrast, Methoxyfenozide had the highest dipole moment, at 4.784 D, while Beta-Sitosterol had the lowest value, at 1.528 D. Variations in dipole moment values reflect differences in

charge distribution and polarity. In this case, Methoxyfenozide is highly polar, whereas Beta-Sitosterol is less polar.

Electron affinity (A) refers to the energy produced when a molecule in its ground state acquires an electron. Electron affinity levels provide insights into the activity and chemical reactivity of pharmaceutical compounds. A higher electron affinity value indicates a molecule's capacity to accept electrons, which may influence its interactions with other molecules or biological systems. Methoxyfenozide has a higher A value (0.0689 eV) than Beta-Sitosterol (0.0165 eV) and Cholesteryl chloroformate (0.0074 eV). "Softness" (σ) and "hardness" (η) are crucial factors in determining a molecule's behavior during a chemical process. Electronegativity (χ) quantifies a molecule's capacity to attract electrons (Karton, 2021). The values for chemical hardness and the electrophilicity index are positive (Table 5). This indicates that the substances can influence the binding energy in protein–ligand interactions and are suitable for charge-transfer activities. The higher electrophilicity index (ω) for all compounds suggests they exhibit greater electrophilic character compared to conventional drugs. All compounds can contribute electrons as their chemical potential (μ) values are low.

Electrostatic Potential Map Analysis

The molecular electrostatic potential (MEP) is a widely used approach for describing the reactivity of a broad range of chemical systems in electrophilic and nucleophilic reactions, as well as for examining biological recognition mechanisms and hydrogen-bonding interactions (Debnath et al., 2005). The ESP map arises from interactions between the positively charged nuclei and negatively charged electron distribution, marking zones of electron deficiency and abundance. In an ESP map, red denotes electron-rich regions, blue indicates electron-deficient sites, and yellow/green represents nearly neutral areas [31]. The electrostatic potential surface mapped of the studied molecule is given in Fig. 6. All compounds exhibit similar MEP patterns, with a deep blue region observed around oxygen atoms, indicating strong negative electrostatic potential, while positive potentials appear mainly around hydrogens and peripheral alkyl substituents.

Density Functional Theory (DFT) calculations revealed optimized geometries, frontier molecular orbitals, and electronic properties of the ligands, highlighting their stability and reactivity profiles, which support favorable binding interactions and rationalize their potential as effective therapeutic candidates.

Molecular Dynamics Simulation

The root mean square deviation (RMSD) trajectories reveal distinct stability profiles for the three compounds over a 100-ns simulation. CID: 222284 (red) demonstrates the most stable behavior, maintaining RMSD values between 1.5–2.5 Å throughout the simulation with relatively low fluctuations (Fig. 7). This suggests a well-equilibrated structure with minimal conformational drift. In contrast, CID: 111262 (blue) exhibits higher initial RMSD values around 2.5–3.0 Å with more pronounced fluctuations, indicating greater structural flexibility or potential conformational transitions. CID: 105010 (black) shows

intermediate behavior with RMSD values like CID: 222284 but with occasional spikes reaching ~ 3.0 Å, suggesting periodic conformational sampling of higher-energy states. The convergence patterns indicate that all systems achieved reasonable equilibration, though CID: 111262 required more time to stabilize.

The root mean square fluctuation (RMSF) per residue plot reveals significant differences in local flexibility among the compounds. Most notably, CID: 111262 exhibits two prominent peaks around residues 300 and 550, with RMSF values reaching 8–9 Å, indicating highly flexible regions or loop domains. These peaks suggest specific structural motifs that undergo large-amplitude motions, potentially important for biological function. CID: 105010 shows a single major peak around residue 550 with RMSF ~ 7.5 Å, indicating a localized flexible region. CID: 222284 displays the most uniform flexibility profile with RMSF values generally below 3 Å across all residues, consistent with its stable RMSD behavior. The overall low baseline fluctuations for all compounds (1–2 Å) suggest well-folded structures with defined secondary structure elements, while the specific high-flexibility regions likely correspond to loop regions or binding sites.

The radius of gyration (R_g) measurements provides insights into the overall compactness of the molecular structures. All three compounds maintain relatively stable R_g values around 22.3–22.5 Å throughout the simulation, indicating preserved global fold integrity. CID: 105010 shows a notable trend toward slightly higher R_g values (~ 22.8 –23.0 Å) in the latter half of the simulation, suggesting a gradual expansion or unfolding event. This structural expansion correlates with the occasional RMSD spikes observed for this compound. CID: 222284 and CID: 111262 maintain more consistent R_g values, with CID: 222284 showing the tightest distribution around 22.3 Å, reinforcing its structural stability. The narrow range of R_g variations (± 0.5 Å) for all compounds indicates that while local conformational changes occur, the overall protein architecture remains intact.

The solvent accessible surface area (SASA) trajectories reveal how molecular surface exposure changes over time. All compounds maintain SASA values in the range of 13,500 – 14,500 Å², indicating similar overall surface exposure profiles. CID: 105010 shows the highest average SASA values ($\sim 14,300$ – 14,500 Å²) with increased fluctuations, consistent with its expanded R_g profile and suggesting greater solvent exposure due to structural loosening. CID: 222284 exhibits intermediate SASA values ($\sim 14,000$ –14,200 Å²) with moderate fluctuations, while CID: 111262 shows similar values but with more pronounced periodic variations. The correlation between higher SASA values and increased R_g for CID: 105010 supports the interpretation of gradual structural expansion. The relatively stable SASA profiles for all compounds indicate that major unfolding events did not occur, but subtle conformational rearrangements affecting surface exposure are evident.

The MD analysis reveals that CID: 222284 exhibits the most stable structural behavior across all parameters, making it a potentially suitable candidate for applications requiring structural integrity. CID: 111262 exhibits localized flexibility that may be functionally relevant, while CID: 105010 displays signs of gradual structural relaxation that could impact long-term stability. These differences in dynamic behavior

could have significant implications for binding affinity, catalytic activity, or drug design applications. Overall, the combined GC–MS profiling, molecular docking, molecular dynamics simulations, and DFT analyses revealed that *C. grandis* contains several bioactive phytochemicals with promising stability, favorable binding interactions, and desirable electronic properties. Among these, Methoxyfenozide demonstrated superior reactivity and stability, suggesting its potential as a lead compound. These integrated findings provide mechanistic insights into the behavior of phytochemicals, strengthen the rationale for further experimental validation, and highlight *C. grandis* as a valuable source of therapeutic candidates.

The present study primarily relies on GC–MS profiling and computational analyses, including molecular docking, molecular dynamics, and DFT calculations, without extensive *in vitro* or *in vivo* validation. The identified phytocompounds were evaluated under simulated conditions, which may not fully replicate biological environments. Furthermore, the study focused on a limited set of compounds, and potential synergistic effects within the whole extract were not explored. Future experimental studies are essential to confirm the predicted bioactivity.

Conclusion

This comprehensive study successfully identified bioactive compounds from *Coccinia grandis* L. leaf extract as potential anti-diabetic agents targeting DPP4. GC-MS analysis revealed 33 compounds, with cholesteryl chloroformate, β -sitosterol, and methoxyfenozide showing exceptional binding affinities (-9.3 to -7.9 kcal/mol) and forming stable interactions with critical DPP4 active site residues. ADME/T analysis confirmed favorable pharmacokinetic properties and acceptable safety profiles, while DFT calculations supported observed binding interactions. The 100 ns molecular dynamics simulations validated structural stability, with β -sitosterol demonstrating optimal behavior across all parameters. These findings provide strong computational evidence for *C. grandis*-derived anti-diabetic therapeutics and highlight natural products' potential in addressing the global diabetes burden. Future research should focus on experimental validation through *in vitro* and *in vivo* studies to confirm the anti-diabetic efficacy and safety of these promising phytochemicals.

Declarations

Acknowledgments

The authors wish to acknowledge the Molecular Genetics Laboratory, Dept. of Genetic Engineering and Biotechnology, University of Rajshahi, Bangladesh, for providing the necessary facilities. Thanks, are also extended to the Bangladesh Reference Institute for Chemical Measurements (BRiCM), Dhaka, Bangladesh, for GC-MS analysis.

Authors' Contribution

Alomgir Hossain: Conceptualization, methodology, writing – original draft, formal analysis, Investigation. **Mst. Nushrat:** Methodology, writing – original draft, formal analysis. **Md. Borhan Uddin & Md. Shohag Hossain:** Formal analysis, writing, review & editing. **S. M. Shahinul Islam, Sreeramanan Subramaniam & Md. Shahadat Hossain:** Writing –review & editing, Supervision, Conceptualization.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interests

No potential conflict of interest relevant to this article was reported.

Clinical trial registration: Not applicable

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and materials: All data supporting the findings of this study are available within the paper.

References

1. Shah A, Isath A, Aronow WS. Cardiovascular complications of diabetes. *Expert Rev Endocrinol Metab.* 2022;17:383–8.
2. Tomic D, Shaw JE, Magliano DJ. The burden and risks of emerging complications of diabetes mellitus. *Nat Rev Endocrinol.* 2022;18:525–39.
3. Agarwala M. Comparative evaluation of marketed Linagliptin tablets in Bangladesh. 2024.
4. Deacon CF. Dipeptidyl peptidase-4 inhibitors in the treatment of type 2 diabetes: a comparative review. *Diabetes Obes Metab.* 2011;13:7–18.
5. Srinivasan P, Vijayakumar S, Kothandaraman S, Palani M. Anti-diabetic activity of quercetin extracted from *Phyllanthus emblica* L. fruit: In silico and in vivo approaches. *J Pharm Anal.* 2018;8:109–18.
6. Putra IMWA, Fakhrudin N, Nurrochmad A, Wahyuono S. Antidiabetic activity of *Coccinia grandis* (L.) Voigt: Bioactive constituents, mechanisms of action, and synergistic effects. *J Appl Pharm Sci.* 2021;12:41–54.
7. Varuna V. *Coccinia grandis* (L) Voigt-review. *World J Pharm Res.* 2018;7:188–200.
8. Gautam S, Meshram A, Srivastava N. A brief study on phytochemical compounds present in *Coccinia cordifolia* for their medicinal, pharmacological and industrial applications. *World J Pharm Pharm Sci.* 2014;3:1995–2016.

9. Muniappan R, Reddy GVP, Raman A. *Coccinia grandis* (L.) voigt (cucurbitaceae). New York, USA: Biol Control Trop weeds using arthropods Cambridge Univ; 2009. pp. 175–82.
10. Tamilselvan N, Thirumalai T, Elumalai EK, Balaji R, David E. Pharmacognosy of *Coccinia grandis*: a review. *Asian Pac J Trop Biomed*. 2011;1:S299–302.
11. Nagare S, Deokar GS, Nagare R, Phad N. Review on *Coccinia grandis* (L.) Voigt (ivy gourd). *World J Pharm Res*. 2015;4:728–43.
12. Alam R, Imon RR, Talukder MEK, Akhter S, Hossain MA, Ahammad F, et al. GC-MS analysis of phytoconstituents from *Ruellia prostrata* and *Senna tora* and identification of potential anti-viral activity against SARS-CoV-2. *RSC Adv*. 2021;11:40120–35.
13. Kaplan W, Littlejohn TG. Swiss-PDB viewer (deep view). *Brief Bioinform*. 2001;2:195–7.
14. Hossain A, Rahman ME, Rahman MS, Nasirujjaman K, Matin MN, Faruqe MO, et al. Identification of medicinal plant-based phytochemicals as a potential inhibitor for SARS-CoV-2 main protease (Mpro) using molecular docking and deep learning methods. *Comput Biol Med*. 2023;157:106785.
15. Hossain A, Rahman ME, Faruqe MO, Saif A, Suhi S, Zaman R, et al. Characterization of Plant-Derived Natural Inhibitors of Dipeptidyl Peptidase-4 as Potential Antidiabetic Agents: A Computational Study. *Pharmaceutics*. 2024;16:483.
16. Frisch A. gaussian 09W Reference. Wallingford, USA, 25p. 2009;470.
17. Raghavachari K. Perspective on Density functional thermochemistry. III. The role of exact exchange Becke AD (1993) *J Chem Phys* 98: 5648–52. *Theor Chem Acc*. 2000;103:361–3.
18. Dennington TK, Millam J, GaussView. Version 5, Semichem Inc., Shawnee Mission, KS, 2009. 31Spartan'8, Wavefunction. Inc, Irvine, CA. 2009.
19. El-Shamy NT, Alkaoud AM, Hussein RK, Ibrahim MA, Alhamzani AG, Abou-Krishna MM. DFT, ADMET and Molecular Docking Investigations for the Antimicrobial Activity of 6, 6'-Diamino-1, 1', 3, 3'-tetramethyl-5, 5'-(4-chlorobenzylidene) bis [pyrimidine-2, 4 (1H, 3H)-dione]. *Molecules*. 2022;27:620.
20. Krieger E, Koraimann G, Vriend G. Increasing the precision of comparative models with YASARA NOVA—a self-parameterizing force field. *Proteins Struct Funct Bioinforma*. 2002;47:393–402.
21. Hossain A, Joti FT, Hossain MS, Al-Noman A, Thowing C, Mursona M, et al. Identification of Potential Inhibitors Targeting Non-Structural Proteins NS3 and NS5 of Dengue Virus Using Docking and Deep Learning Approaches. *Pharmaceutics*. 2025;18:566.
22. Case DA, Cheatham TE III, Darden T, Gohlke H, Luo R, Merz KM Jr, et al. The Amber biomolecular simulation programs. *J Comput Chem*. 2005;26:1668–88.
23. Krieger E, Nielsen JE, Spronk CAEM, Vriend G. Fast empirical pKa prediction by Ewald summation. *J Mol Graph Model*. 2006;25:481–6.
24. Sagui C, Darden TA. Molecular dynamics simulations of biomolecules: long-range electrostatic effects. *Annu Rev Biophys Biomol Struct*. 1999;28:155–79.
25. Islam MJ, Parves MR, Mahmud S, Tithi FA, Reza MA. Assessment of structurally and functionally high-risk nsSNPs impacts on human bone morphogenetic protein receptor type IA (BMPRIa) by

- computational approach. *Comput Biol Chem.* 2019;80:31–45.
26. Alam R, Imon RR, Kabir Talukder ME, Akhter S, Hossain MA, Ahammad F, et al. GC-MS analysis of phytoconstituents from *Ruellia prostrata* and *Senna tora* and identification of potential anti-viral activity against SARS-CoV-2. *RSC Adv.* 2021;11:40120–35. <https://doi.org/10.1039/d1ra06842c>.
27. Carlson GR, Dhadialla TS, Hunter R, Jansson RK, Jany CS, Lidert Z, et al. The chemical and biological properties of methoxyfenozide, a new insecticidal ecdysteroid agonist. *Pest Manag Sci Former Pestic Sci.* 2001;57:115–9.
28. Ponnulakshmi R, Shyamaladevi B, Vijayalakshmi P, Selvaraj J. In silico and in vivo analysis to identify the antidiabetic activity of beta sitosterol in adipose tissue of high fat diet and sucrose induced type-2 diabetic experimental rats. *Toxicol Mech Methods.* 2019;29:276–90.
29. Rahman MA, Chakma U, Kumer A, Rahman MR, Matin MM. Uridine-derived 4-aminophenyl 1-thioglucosides: DFT optimized FMO, ADME, and antiviral activities study. *Biointerface Res Appl Chem.* 2022;13:1–15.
30. Tsuneda T, Song J-W, Suzuki S, Hirao K. On Koopmans' theorem in density functional theory. *J Chem Phys.* 2010;133.
31. Pal TK, Mumit MA, Hossen J, Paul S, Alam MA, Islam MA-A-A et al. Computational and experimental insight into antituberculosis agent, (E)-benzyl-2-(4-hydroxy-2-methoxybenzylidene) hydrazinecarbodithioate: ADME analysis. *Heliyon.* 2021;7.

Figures



Figure 1

Leaf of *Coccinia grandis* L. Fresh leaves collected from the University of Rajshahi campus showing the characteristic broad, cordate-oval shape with five-lobed structure typical of ivy gourd.

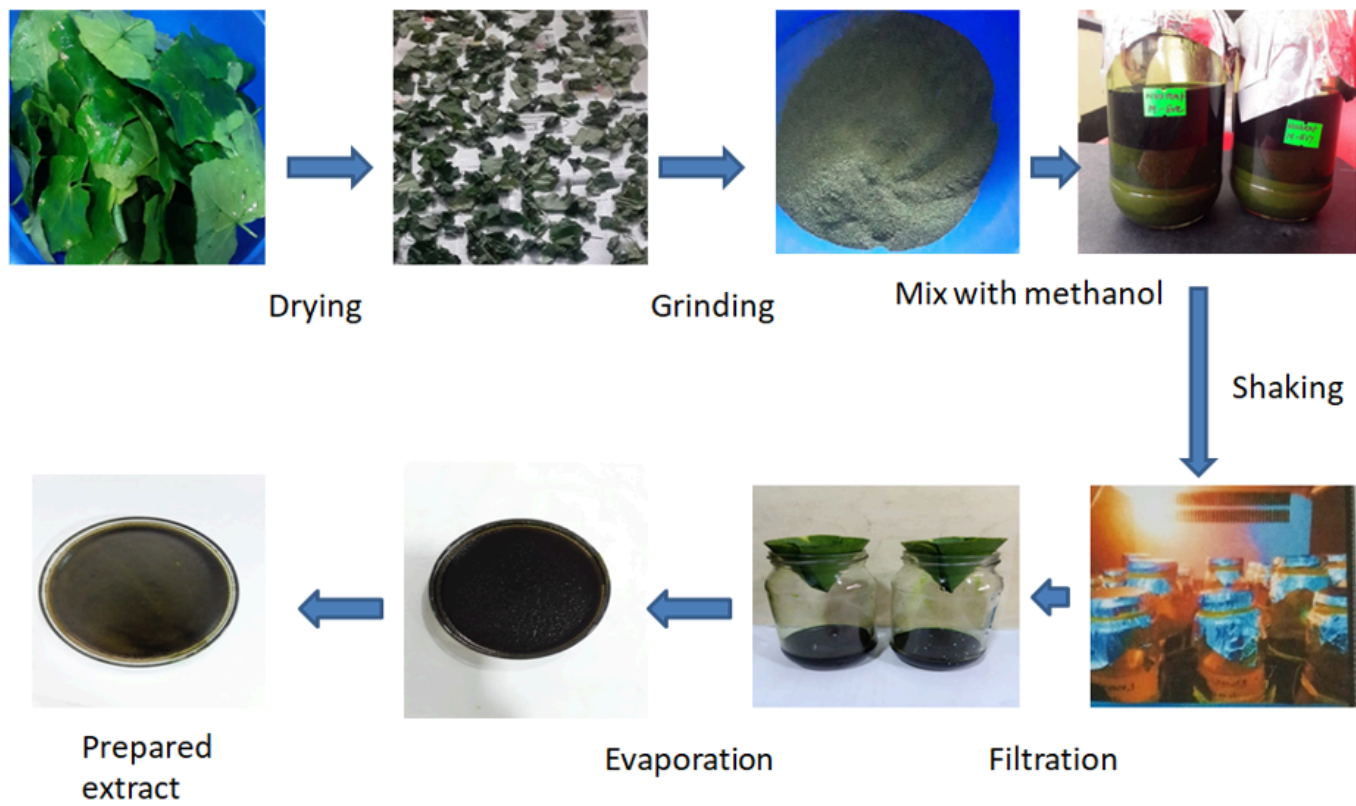


Figure 2

Preparation workflow of *Coccinia grandis* L. leaf extracts. The flowchart illustrates the complete extraction process from fresh leaf collection through drying, powder preparation, methanol extraction, filtration, and final concentrate preparation for GC-MS analysis.

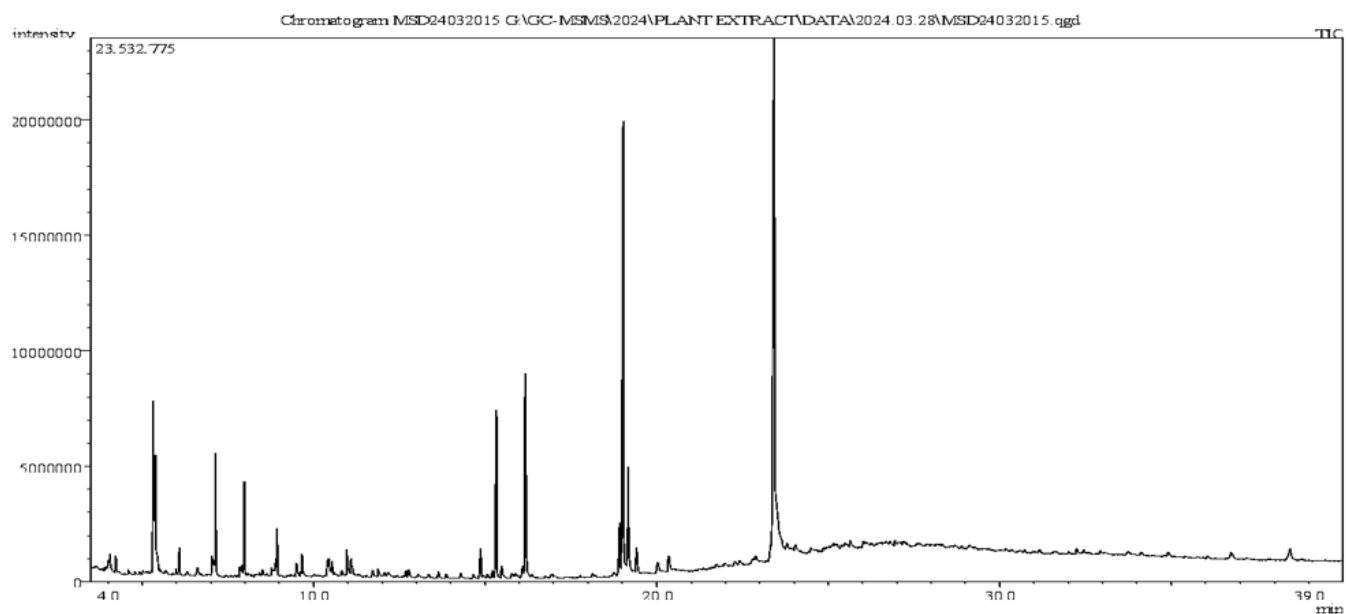


Figure 3

Total ion chromatogram (TIC) of *Coccinia grandis* L. methanol leaf extract obtained by GC-MS analysis. The chromatogram shows the distribution and relative abundance of 33 identified phytochemicals over the 40-minute analysis period, with major peaks corresponding to abundant compounds.

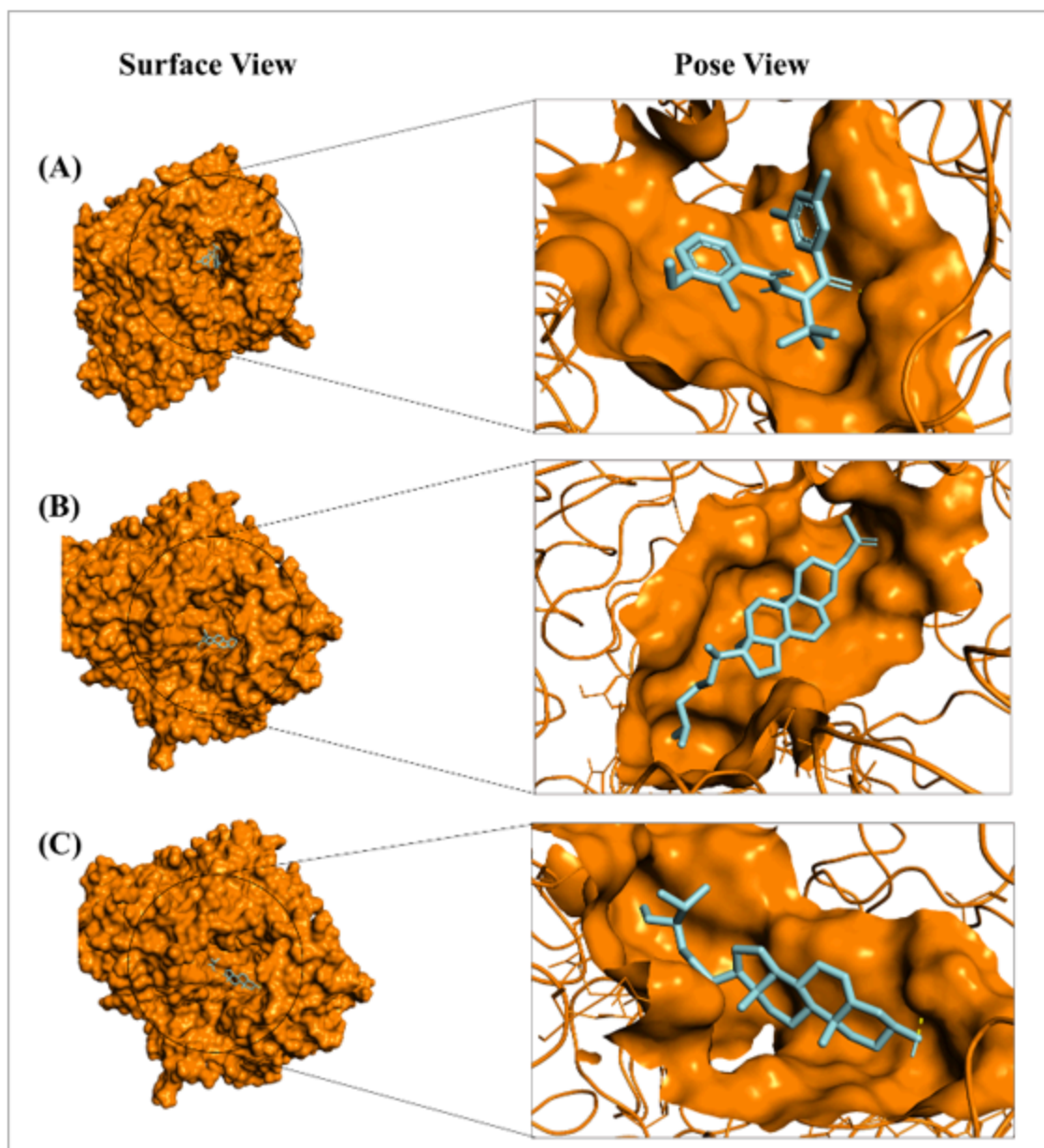


Figure 4

Molecular docking interactions of the top three compounds with DPP4 active site. The figure displays non-bonded interactions between DPP4 protein and (A) Methoxyfenozide, (B) Cholesteryl chloroformate, and (C) β -Sitosterol, showing hydrogen bonds, pi-alkyl interactions, and binding pocket orientations.

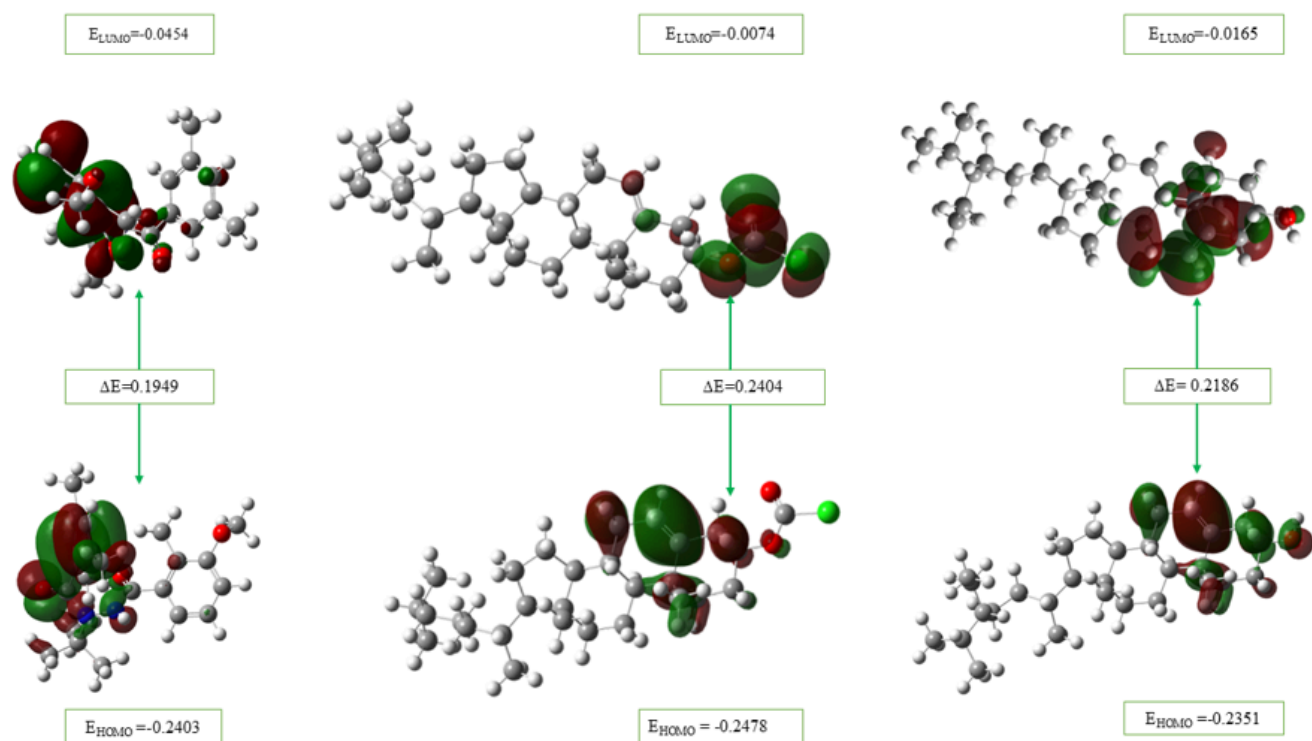


Figure 5

Frontier molecular orbital distribution plots of the three lead compounds. Ground state molecular orbital visualizations show HOMO and LUMO distributions for (A) Methoxyfenozide, (B) Cholesteryl chloroformate, and (C) β -Sitosterol using B3LYP/6-311G(d,p) method, indicating electron density distributions and reactive sites.

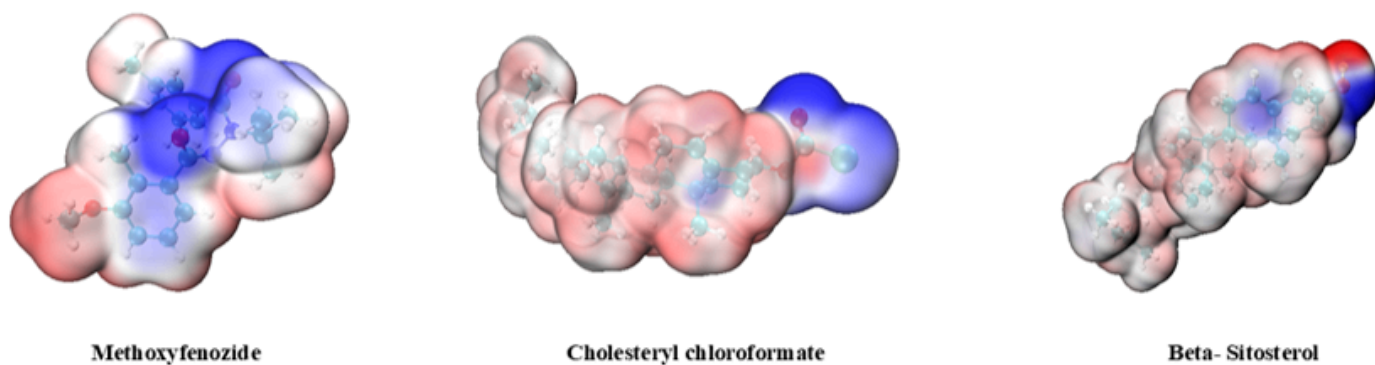


Figure 6

Molecular electrostatic potential (MEP) surface maps of the selected compounds. The ESP surfaces show electron-rich regions (red), electron-deficient sites (blue), and neutral areas (yellow/green) for all three compounds, indicating potential sites for electrophilic and nucleophilic interactions.

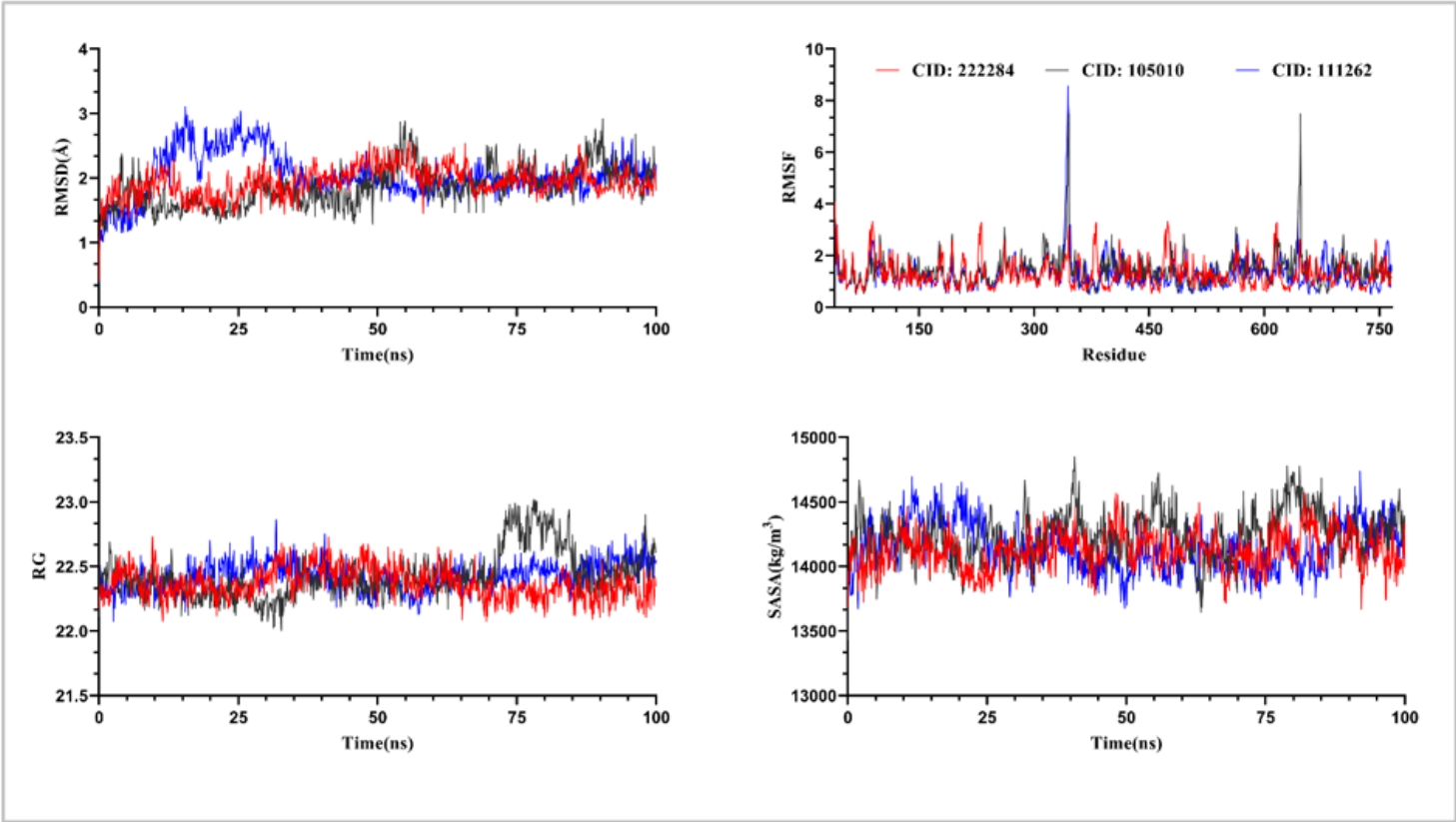


Figure 7

Molecular dynamics simulation analysis of protein-ligand complexes over 100 ns. The figure presents four panels showing: (top left) Root Mean Square Deviation (RMSD) trajectories, (top right) Root Mean Square Fluctuation (RMSF) per residue, (bottom left) Radius of gyration (Rg) measurements, and (bottom right) Solvent Accessible Surface Area (SASA) changes for the three compounds (CID: 222284 - red, CID: 105010 - black, CID: 111262 - blue).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [GraphicalAbstract.png](#)