

Petasiphenol and Spathulenol as Promising Natural DPP-4 Inhibitors: An *In Silico* Multiple Ligand-Based Study Against Type 2 Diabetes Mellitus

Nazmun Nahar¹, Joy Datta Ripa¹, Rubaiya Hossain Tansura¹,
Kangkan Sharma¹, Abul Kalam Azad^{2*}, Ashok Kumar Balaraman³

¹Department of Pharmacy, University of Science and Technology Chittagong,
4202 Foy's Lake, Khulshi, Chittagong, Bangladesh

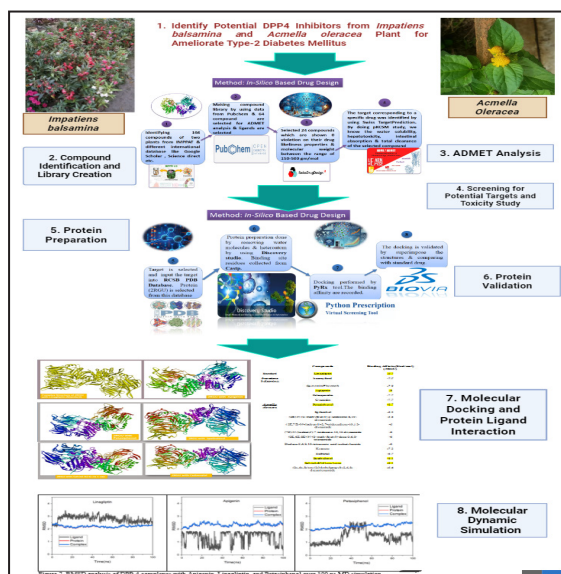
²Department of Pharmaceutical Technology, Faculty of Pharmacy, MAIWP International University,
68100 Batu Muda, Kula Lumpur, Malaysia.

³Faculty of Medicine and Health Sciences, Hormuud University, Mogadishu, Somalia

⁴Research Management Department, University of Cyberjaya, Persiaran Bestari, Cyber 11, 63000
Cyberjaya, Selangor, Malaysia

*Corresponding author: drazad@unimaiwp.edu.my

Graphical Abstract



Abstract

Type 2 Diabetes Mellitus (T2DM) is a prevalent metabolic disorder with significant global morbidity and mortality. Dipeptidyl peptidase-4 (DPP-4) is a key enzyme regulating glucose metabolism, and its inhibition represents a promising therapeutic strategy. This

study evaluated the binding affinity and interaction mechanisms of natural compounds from *Impatiens balsamina* and *Acmella oleracea* against DPP-4 (PDB ID: 2RGU) using molecular docking, with Linagliptin as a reference inhibitor. Docking results revealed that Linagliptin exhibited the strongest interactions, forming multiple hydrogen bonds, π - π interactions, and hydrophobic contacts with catalytic residues. Among the phytochemicals, Petasiphenol demonstrated the highest affinity, stabilized by hydrogen bonds and aromatic interactions, while Spathulenol showed favorable binding through a combination of polar and hydrophobic contacts. Apigenin exhibited moderate interactions, whereas sesquiterpenoids Cedrenol and Salvia-1(14)-en-1-one showed limited binding due to their predominantly hydrophobic nature. These findings indicate that natural compounds containing hydroxyl groups and aromatic scaffolds are more effective DPP-4 binders. Petasiphenol and Spathulenol emerge as promising lead compounds for further molecular dynamics, pharmacokinetic studies, and experimental validation, supporting their potential development as plant-derived antidiabetic agents.

Keywords: DPP-4, molecular docking, *impa-*

Petasiphenol and spathulenol as promising natural DPP-4 inhibitors: An *in silico* multiple ligand based study against type 2 diabetes mellitus

tiens balsamina, *acmella oleracea*, apigenin, petasiphenol, spathulenol.

Introduction

A complex metabolic disorder known as diabetes mellitus is characterized by chronic hyperglycemia, caused by impaired insulin secretion, insulin action, or both (1). Type 1 diabetes occurs due to a complete deficiency of insulin, in contrast, type 2 diabetes is caused by the body's inability to effectively use insulin even when it is produced in adequate or elevated amounts (2). Type 2 diabetes is significantly more prevalent and largely preventable, impacting approximately 32 million individuals globally with an equal incidence among men and women (3). Type 2 diabetes-related persistent hyperglycemia results in numerous complications, including cardiovascular disease, nephropathy, neuropathy, and various metabolic dysfunctions. The increasing worldwide occurrence of type 2 diabetes highlights the demand for safe, effective, and affordable treatments (4).

Dipeptidyl peptidase-4 (DPP-4) inhibitors, also known as gliptins, have become a significant group of oral medications used to manage diabetes, especially when lifestyle changes are not enough to achieve good blood sugar control. Inhibitors of this type work by raising the levels of active incretin hormones that are circulating in the body, like glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), which are rapidly broken down by the DPP-4 enzyme (5). DPP-4 inhibitors enhance incretin-mediated pathways to promote glucose-dependent insulin secretion, reduce glucagon release, and improve glycemic stability. Agents commonly used in this class include sitagliptin, saxagliptin, linagliptin, and alogliptin, which can be used individually or in combination with other antidiabetic medications such as metformin (6). Linagliptin has shown long-lasting inhibition of DPP-4, achieving a suppression of the enzyme of more than 80% over a 24-hour period (6).

Medicinal plants continue to be a crucial source of bioactive compounds for the

development of new medicines (7). *Impatiens balsamina*, a herbaceous plant native to tropical and subtropical areas, has been traditionally used in medicine for its properties as a cathartic, diuretic, anti-inflammatory agent, and wound healer (8). Leaves, stems, and extracts from it are used to ease joint pain, treat warts, and control skin irritations. The toothache plant, *Acmella oleracea*, is a medicinal plant that is known for containing numerous bioactive compounds and exhibiting analgesic, sialagogue, anti-inflammatory, and antioxidant effects (9). Chemical analysis of *A. oleracea* has shown a diverse combination of fatty acids, terpenoids, sterols, alkylamides, and antioxidants containing DL- α -tocopherol, β -caryophyllene, phytol acetate, and N-isobutyl-dodecatetraenamide. Many of these compounds exhibit therapeutic properties relevant to metabolic conditions (10). The majority of substances found in the plant comprise 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (14%), 3,7,11,15-Tetramethylhexadec-2-En-1-Ol (16%), Cedrene (10.65%), Pentadecanoic acid (8%), 1-Butanol, 3-methyl-, acetate (5.65%), Stigmast-5-en-3-Ol, (3- β)—(1.79%), Ethyl (9Z,12Z)-9,12-Octadecadienoate (1.35%), Phytol, acetate (2.67%), 1,3-Bis(Cinnamoyloxy-methyl) adamantane (2.13%), Beta-elementol (1.90%), DL- α -tocopherol (1.84%), N-Isobutyl-(2E,4Z,8Z,10E)-dodeca-tetraenamide (1.29%), Trans (Beta)-Caryophyllene (3.28%), and Ethyl linoleate (2.85%) (11).

The process of drug discovery is frequently hindered by challenges, leading to only a small amount of candidates progressing from initial compounds to marketable products, mainly because of factors including inadequate binding affinity, unforeseen off-target effects, or properties like solubility and stability (12). Research and development incur substantial costs and time commitments, thereby complicating this journey. Drug targets can be identified through methods such as data mining, phenotypic screening, and bioinformatics, using techniques that involve epigenetics, genomics, transcriptomics, and proteomics (13). Once these targets have been identified, their importance in

disease progression must be confirmed through validation. After validation, a compound screening process is conducted to identify potential drug candidates through techniques like mass spectrometry, NMR, fragment-based screening, high-throughput screening (HTS), and virtual screening (VS) (14). Following the discovery of potential hits, elements such as ADME (absorption, distribution, metabolism, excretion) and toxicity are refined to minimize the likelihood of failure in clinical trials. In the drug discovery process, computer-aided drug design (CADD) assists both physical and computational techniques in lowering the time and resources required to identify new drug candidates (15).

Advanced molecular simulation techniques were employed in this study, and in recent years, the use of MD simulations has risen remarkably in drug discovery and molecular biology(16). This is because such simulations provide deep insights into the behavior of proteins and other biomolecules with exceptional temporal resolution and atomic-level detail. Consequently, the increasing interest among experimental scientists in biomolecular modeling has been driven by noteworthy improvements in simulation speed, accuracy, accessibility, and the availability of extensive structural data, a trend especially evident in neurological research but influential across multiple scientific fields(17). As a result, MD-based approaches have greatly supported the design, optimization, and mechanistic investigation of small molecules, peptides, and proteins(18). In alignment with these advancements, the present study integrates a comprehensive *in silico* workflow to explore the therapeutic potential of natural compounds derived from *Impatiens balsamina* and *Acmella oleracea*, particularly in response to the global need for novel DPP-4 inhibitors and the growing promise of plant-based antidiabetic agents. Within this framework, the research focuses on identifying key phytochemical constituents linked to antidiabetic properties, followed by predicting their binding affinity, interaction patterns, and inhibitory potential against the DPP-4 enzyme using ligand-based virtual

screening and molecular docking methods. Subsequently, molecular dynamics simulations are applied to evaluate the stability, conformational flexibility, and dynamic behavior of the ligand DPP -4 complexes at the atomic scale. Furthermore, the study includes an ADME/Tox assessment to determine drug-likeness and pharmacokinetic suitability. Altogether, this integrated computational strategy aims to pinpoint the most promising plant-derived compounds capable of serving as safe and effective lead molecules for the development of new antidiabetic therapeutics.

Materials and Methods

Compound identification and library creation

Around 63 compounds associated with diabetes-related effects were chosen from the IMPPAT database (<https://cb.imsc.res.in/imp-pat/>) and various international sources including Google Scholar, Science Direct, and others. A compound library was set up with data from PubChem, which was accessed in SDF format at (<https://pubchem.ncbi.nlm.nih.gov/>). Using this approach, ligands were selected (19).

ADMET analysis

The Swiss ADME database (<http://www.swissadme.ch/>) was used to evaluate the ADMET properties of 19 compounds from *Acmella oleracea* and 5 compounds from *Impatiens balsamina*, which were selected after screening the plants. Records were made of parameters including pharmacokinetics, water solubility, physicochemical properties, lipophilicity, drug-likeness, and medicinal chemistry. Compounds with molecular weights between 150 and 500 gm/mol were found to adhere to their respective drug-likeness criteria without any exceptions (20).

Screening for potential targets and toxicity study

The Swiss Target Prediction website (<http://www.swisstargetprediction.ch/>) was used to identify potential targets. We assessed the compound's solubility in water, hepatotoxicity,

Petasiphenol and spathulenol as promising natural DPP-4 inhibitors: An *in silico* multiple ligand based study against type 2 diabetes mellitus

intestinal absorption, and overall clearance by conducting research with pKCSM (<https://bio-sig.lab.uq.edu.au/pkcsm/>) (21).

Protein preparation

The three-dimensional arrangement of human DPP4 complexes with an inhibitor protein, identified by the PDB ID: 2RGU, was obtained from the RCSB protein databank at <https://www.rcsb.org/>. This structure was created by removing co-factors using BIOVIA Discovery Studio 2024 and then further optimised with the SWISS PDB viewer. Multiple binding sites were compiled from CASTp (<http://sts.bioe.uic.edu/castp/index.html?3trg>). The three-dimensional arrangement of the human DPP4 complexes with an inhibitor protein, identified by the PDB ID: 2RGU, was retrieved from the RCSB protein databank at <https://www.rcsb.org/>. This structure was developed using BIOVIA Discovery Studio 2024 by removing co-factors and was then further refined with the SWISS PDB viewer. Several binding sites were compiled from the CASTp website at <http://sts.bioe.uic.edu/castp/index.html?3trg>. This structure was developed with BIOVIA Discovery Studio 2024 by eliminating co-factors and was further refined using the SWISS PDB viewer. Several binding sites were compiled from CASTp (<http://sts.bioe.uic.edu/castp/index.html?3trg>) (22)).

Protein validation

The Ramachandran Plot is commonly employed for protein validation. The Ramachandran Plot was introduced by Dr. G.N. Ramachandran. A space-filling model of peptides is used to visualise energetically possible (i.e. sterically permitted) values for dihedral angles Ψ against Φ for a polypeptide chain. High-quality protein structures should have the majority of their residues in the most favorable region of the plot. Variations from this region may signal issues with the structure, such as steric collisions or improper backbone geometry. The results of the selected protein structure validation by The Ramachandran Plot revealed that the proteins were at their most stable position and there were no issues with the protein structures,

as 90% were located in the most favored region (23).

Molecular docking and protein ligand interaction

The PyRx tool, available at <https://pyrx.sourceforge.io/>, was used to determine the optimal ligand-target protein binding configuration. Observations of binding affinity were made. Docking was confirmed by superimposing the structures and comparing them to a reference drug. The Ramachandran Plot is a widely employed method for validating proteins. The tool is primarily employed for examining the conformation of amino acids and the structure of proteins. The interaction between 2RGU and the chosen ligands was investigated using the BIOVIA Discovery Studio 2024 to explore the connection between the ligands and the targeted protein (24).

Molecular dynamic simulation

Molecular dynamics (MD) simulations were carried out using AMBER20 to evaluate the stability of the protein–ligand complexes. The protein was described using the ff14SB force field, while ligand parameters were generated using the General AMBER Force Field (GAFF) with AM1-BCC partial charges. Each system was solvated in an explicit TIP3P water model with a minimum buffer distance of 10 Å, neutralized, and supplemented with Na^+/Cl^- ions to achieve a physiological salt concentration of 0.15 M. Energy minimization was performed in two stages, followed by gradual heating from 0 to 300 K under the NVT ensemble and subsequent equilibration under the NPT ensemble at 300 K and 1 atm.

Production MD simulations were performed for 100 ns under periodic boundary conditions using a 2 fs time step, with the SHAKE algorithm applied to constrain bonds involving hydrogen atoms. Long-range electrostatic interactions were treated using the Particle Mesh Ewald (PME) method with a 10 Å non-bonded cutoff, while temperature and pressure were controlled using a Langevin thermostat and

Berendsen barostat, respectively. Trajectory analyses were conducted using CPPTRAJ, including root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), and solvent-accessible surface area (SASA) to assess the conformational stability, compactness, and dynamic behavior of the complexes throughout the simulation.

Results and Discussion

Compound library of *Impatiens balsamina* and *Acmella oleracea*

A comprehensive phytochemical library comprising 30 bioactive compounds from *Impatiens balsamina* was successfully compiled. The library includes a structurally diverse range of biomolecules representing flavonoids, anthraquinones, naphthoquinones, sterols, glycosides, fatty acid esters, and triterpenoids, reflecting the plant's rich secondary metabolite

profile. Flavonoids such as kaempferol, quercetin, apigenin, pelargonidin, delphinidin, cyanidin, and their glycosides predominantly contributed to the antioxidant and anti-inflammatory potential of the library. Quinone derivatives, including 1, 4-benzoquinone, 1, 4-naphthoquinone, 2-methoxy-1,4-naphthoquinone, anthraquinone, Balsaminone A and B, formed a major class of bioactive compounds known for antimicrobial, cytotoxic, and enzyme-modulating activities. A series of complex glycosides such as Hosenkoside A, D, E, F, G, K, and M added structural diversity, while fatty acid esters ethyl stearate and ethyl palmitate supported the lipid-soluble fraction. Phytosterols including β -sitosterol and α -spinasterol, along with the triterpenoid β -amyirin, further represented pharmacologically relevant metabolites with potential antidiabetic and anti-inflammatory activity (Table 1).

Table1: Compound Library of *Impatiens balsamina*.

S.No	Compound name	Canonical smile
1	kaempferol (Flavonol)	<chem>C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O</chem>
2	Quercetin(Flavonol)	<chem>C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O</chem>
3	kaempferol 3-rutinoside	<chem>CC1C(C(C(C(O1)OCC2C(C(C(C(O2)OC3=C(OC4=CC(=CC(=C4C3=O)O)O)C5=CC=C(C=C5)O)O)O)O)O)O</chem>
4	1,4-Benzoquinone	<chem>C1=CC(=O)C=CC1=O</chem>
5	Apigenin	<chem>C1=CC(=CC=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O</chem>
6	2-Methoxy-1,4-naphthoquinone	<chem>COC1=CC(=O)C2=CC=CC=C2C1=O</chem>
7	Anthraquinone	<chem>C1=CC=C2C(=C1)C(=O)C3=CC=CC=C3C2=O</chem>
8	Ethyl stearate	<chem>CCCCCCCCCCCCCCCC(=O)OCC</chem>
9	Ethyl acetate	<chem>CCOC(=O)C</chem>
10	Hosenkoside A	<chem>CC(CO)C1CCC2(CCC3(C(C2O)CCC4C3(CCC5C4(CCC(C5(C)COC6(C(C(C(C(O6)CO)O)O)OC7C(C(C(C(O7)CO)O)O)OC8C(C(C(C(O8)CO)O)O)C)C)C)CO1</chem>
11	Ethyl palmitate	<chem>CCCCCCCCCCCCCCCC(=O)OCC</chem>

Petasiphenol and spathulenol as promising natural DPP-4 inhibitors: An *in silico* multiple ligand based study against type 2 diabetes mellitus

12	Hosenkoside D	<chem>CC(CO)C1CCC2(CCC3(C(C2O)CCC4C3(CCC5C4(CCC(C5(C)COC6C(-C(C(C(O6)CO)O)O)OC7C(C(C(C(O7)CO)O)O)O)C)C)C)CO1</chem>
13	Hosenkoside E	<chem>CC(CO)C1CCC2(CCC3(C(C2O)CCC4C3(CCC5C4(CCC(C5(C)CO)OC6C(C(C(C(O6)CO)O)O)OC7C(C(C(C(O7)CO)O)O)O)C)C)C)CO1</chem>
14	Hosenkoside M	<chem>CC(COC1C(C(C(C(O1)CO)O)O)O)C2CCC3(CCC4(C(C3O)CCC5C4(CCC6C5(CCC(C6(C)COC7C(C(C(C(O7)CO)O)O)O)OC8C(C(C(C(O8)CO)O)O)OC9C(C(C(C(O9)O)O)O)C)C)C)CO2</chem>
15	Hosenkoside K	<chem>CC(COC1C(C(C(C(O1)CO)O)O)O)C2CCC3(CCC4(C(C3O)CCC5C4(CCC6C5(CCC(C6(C)COC7C(C(C(C(O7)CO)O)O)O)OC8C(C(C(C(O8)CO)O)O)OC9C(C(C(C(O9)CO)O)O)O)C)C)C)CO2</chem>
16	Hosenkoside F	<chem>CC(COC1C(C(C(C(O1)CO)O)O)O)C2CCC3(CCC4(C(C3O)CCC5C4(CCC6C5(CCC(C6(C)CO)OC7C(C(C(C(O7)CO)O)O)OC8C(C(C(C(O8)O)O)O)C)C)C)CO2</chem>
17	Hosenkoside G	<chem>CC(=CCCC1(CCC2(C(C1O)CCC3C2(CCC4C3(CCC(C4(C)COC5C(C(C(C(O5)CO)O)O)O)OC6C(C(C(C(O6)CO)O)O)OC7C(C(C(C(O7)O)O)O)C)C)C)CO)CO</chem>
18	1,3-Dihydroxypropan-2-yl formate	<chem>C(C(CO)OC=O)O</chem>
19	beta-Sitosterol	<chem>CCC(CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C</chem>
20	1,4-Naphthoquinone	<chem>C1=CC=C2C(=O)C=CC(=O)C2=C1</chem>
21	Balsaminone B	<chem>COC1=C(C2=CC=CC=C2C3=C1C4=C(O3)C(=O)C5=CC=CC=C5C4=O)OC6C(C(C(C(O6)CO)O)O)O</chem>
22	Pelargonidin	<chem>C1=CC(=CC=C1C2=(O+)C3=CC(=CC(=C3C=C2O)O)O)O.(Cl-)</chem>
23	Planteose	<chem>C(C1C(C(C(C(O1)OCC2C(C(C(O2)(CO)OC3C(C(C(C(O3)CO)O)O)O)O)O)O)O)O</chem>
24	Delphinidin	<chem>C1=C(C=C(C(=C1O)O)O)C2=(O+)C3=CC(=CC(=C3C=C2O)O)O.(Cl-)</chem>
25	Balsaminone A	<chem>COC1=C(C2=CC=CC=C2C3=C1C4=C(O3)C(=O)C5=CC=CC=C5C4=O)O</chem>
26	Cyanidin	<chem>C1=CC(=C(C=C1C2=(O+)C3=CC(=CC(=C3C=C2O)O)O)O)O</chem>
27	alpha-Spinasterol	<chem>CCC(C=CC(C)C1CCC2C1(CCC3C2=CCC4C3(CCC(C4)O)C)C)C(C)C</chem>
28	Leucocianidol	<chem>C1=CC(=C(C=C1C2C(C(C3=C(C=C(C=C3O2)O)O)O)O)O)O</chem>
29	beta-Amyrin	<chem>CC1(CCC2(CCC3(C(=CCC4C3(CCC5C4(CCC(C5(C)C)O)C)C)C2C1)C)C)C</chem>
30	Myricetin 3-rhamnoside	<chem>CC1C(C(C(C(O1)OC2=C(OC3=CC(=CC(=C3C2=O)O)O)C4=CC(=C(C(=C4O)O)O)O)O)O</chem>

A total of 33 phytochemicals were identified and compiled into the *Acmella oleracea* compound library, reflecting the plant's rich reservoir of phenolic, alkaloids, flavonoids, sesquiterpenes, and aromatic compounds. The compounds exhibit substantial structural diversity, supporting their known broad-spectrum biological activi-

ties. Phenolic derivatives such as cis-clovamide, trans-clovamide, petasiphenol, miquelianin, and glycosylated flavonoids including quercetin-3-glucoside, cosmosiin, and rutin contribute to antioxidant, anti-inflammatory, and cytoprotective activities. These polar compounds form the hydrophilic component of the library.

A major portion of the library consists of alkaloids, including spilanthol, dodeca-tetraenoic acid isobutylamide, and multiple polyacetylenic and polyunsaturated alkamide derivatives such as (2E)-N-(2-methylbutyl)-2-undecene-8, 10-dynamide, (2E, 7Z)-N-(isobutyl)-2, 7-tridecadiene-10, 12-dynamide, and (Z)-non-2-en-6, 8-dynoic acid isobutylamide. These represent the key bioactive constituents responsible for the plant's analgesic, immunomodulatory, and

pharmacological effects, particularly via TRPV1 and neural pathways. The library also includes a diverse range of sesquiterpenes and terpenoid derivatives, such as bicyclogermacrene, β -copaene, spathulenol, kessane, terpinolene, humulene epoxide II, δ -cadinene, caryophyllene oxide, and 14-hydroxy-9-epi-(E)-caryophyllene. These nonpolar constituents contribute strongly to antimicrobial, anti-inflammatory, and metabolic regulatory activities (Table 2).

Table 2: Compound Library of *Acmella oleracea*

S. No	Compound name	Canonical smile
1.	cis-clovamide	<chem>C1=CC(=C(C=C1CC(C(=O)O)NC(=O)C=CC2=CC(=C(C=C2)O)O)O)O</chem>
2.	trans-clovamide	<chem>C1=CC(=C(C=C1C(C@@H)(C(=O)O)NC(=O)C=C/C2=C(C=C(C=C2)O)O)O)O</chem>
3.	miquelianin	<chem>C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)OC4C(C(C(C(O4)C(=O)O)O)O)O)O)O</chem>
4.	petasiphenol	<chem>C1=CC(=C(C=C1CC(=O)COC(=O)C=CC2=CC(=C(C=C2)O)O)O)O</chem>
5.	spilanthol	<chem>CC=CC=CCCC=CC(=O)NCC(C)C</chem>
6.	(2E)-N-(2-methylbutyl)-2-undecene-8,10-dynamide	<chem>CCC(C)CNC(=O)C=CCCCC#CC#C</chem>
7.	(2E,7Z)-N-(isobutyl)-2,7-tridecadiene-10,12-dynamide	<chem>CC(C)CNC(=O)C=CCCCC=CCC#CC#C</chem>
8.	(7Z)-N-(isobutyl)-7-tridecene-10,-12-dynamide	<chem>CC(C)CNC(=O)CCCCC=CCC#CC#C</chem>
9.	(2E,6Z,8E)-N-(2-methylbutyl)-deca-2,6,8-trienamide	<chem>CCC(C)CNC(=O)C=CCCC=CC=CC</chem>
10.	dodeca-2,4,8,10-tetraenoic acid isobutylamide	<chem>CC=CC=CCCC=CC=CC(=O)NCC(C)C</chem>
11.	Quercetin-3-glucoside	<chem>C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)OC4C(C(C(C(O4)CO)O)O)O)O)O</chem>
12.	Cosmosiin	<chem>C1=CC(=CC=C1C2=CC(=O)C3=C(C=C(C=C3O2)OC4C(C(C(C(O4)CO)O)O)O)O)O</chem>
13.	Rutin	<chem>CC1C(C(C(C(O1)OCC2C(C(C(C(O2)OC3=C(OC4=C(C(=CC(=C4C3=O)O)O)C5=CC(=C(C=C5)O)O)O)O)O)O)O)O</chem>
14.	Kessane	<chem>CC1CCC2C1CC3CCC2(OC3(C)C)C</chem>
15.	Bicyclogermacrene	<chem>CC1=CCCC(=CC2C(C2(C)C)CC1)C</chem>
16.	beta-Copaene	<chem>CC(C)C1CCC2(C3C1C2C(=C)CC3)C</chem>
17.	Safranal	<chem>CC1=C(C(C=C1)(C)C)C=O</chem>
18.	Spathulenol	<chem>CC1(C2C1C3C(CCC3(C)O)C(=C)CC2)C</chem>

Petasiphenol and spathulenol as promising natural DPP-4 inhibitors: An *in silico* multiple ligand based study against type 2 diabetes mellitus

19	(1aR,7S,7aS,7bR)-1,1,4,7-tetramethyl-2,3,5,6,7a,7b-hexahydro-1aH-cyclopropa(h)azulen-7-ol	<chem>CC1=C2CCC(C2C3C(C3(C)C)CC1)(C)O</chem>
20	Terpinolene	<chem>CC1=CCC(=C(C)C)CC1</chem>
21	Humulene epoxide II	<chem>CC1=CCC(C=CCC2(C(O2)CC1)C)(C)C</chem>
22	(+)-delta-Cadinene	<chem>CC1=CC2C(CCC(=C2CC1)C)C(C)C</chem>
23	Caryophyllene oxide	<chem>CC1(CC2C1CCC3(C(O3)CCC2=C)C)C</chem>
24	alpha-Calacorene	<chem>CC1=CCC(C2=C1C=CC(=C2)C)C(C)C</chem>
25	delta-Elementene	<chem>CC(C)C1=CC(C(CC1)C)C=C(C)C</chem>
26	14-Hydroxy-9-epi-(E)-caryophyllene	<chem>CC1=CCCC(=C)C2CC(C2CC1)(C)CO</chem>
27	Cedrelanol	<chem>CC1=CC2C(CCC(C2CC1)(C)O)C(C)C</chem>
28	alpha-Copaene	<chem>CC1=CCC2C3C1C2(CCC3C(C)C)C</chem>
29	3-Methylbenzaldehyde	<chem>CC1=CC(=CC=C1)C=O</chem>
30	(2e,6e,8e)-n-(2-Methylpropyl)-2,6,8-decatrienamide	<chem>CC=CC=CCCC=CC(=O)NCC(C)C</chem>
31	(2E)-N-(2-Methylbutyl)-2-undecene-8,10-diynamide	<chem>CCC(C)CNC(=O)C=CCCCC#CC#C</chem>
32	(z)-Non-2-en-6,8-diynoic acid isobutylamide	<chem>CC(C)CNC(=O)C=CCCC#CC#C</chem>
33	(z)-Dec-2-en-6,8-diynoic acid isobutylamide	<chem>CC#CC#CCCC=CC(=O)NCC(C)C</chem>

ADMET analysis, toxicity, other pharmacokinetic analysis of the *Impatiens balsamina* and *Acmella oleracea* selected compound by swiss ADME of the compounds

Table 3 summarizes the ADMET profiles of five key phytochemicals from *Impatiens balsamina*, highlighting their drug-likeness, pharmacokinetic behavior, and oral bioavailability. All compounds kaempferol, quercetin, apigenin, pelargonidin, and cyaniding satisfied standard drug-likeness parameters with zero Lipinski rule violations, indicating favorable physicochemical properties for oral drug development. In terms of lipophilicity, apigenin (Log Po/w = 1.89), kaempferol (1.70), and quercetin (1.63) displayed

moderate hydrophobicity, suggesting good membrane permeability. Conversely, pelargonidin (-4.50) and cyanidin (-2.62) were highly hydrophilic, which may reduce passive diffusion across lipid membranes. Despite differences in lipophilicity, all compounds showed high gastrointestinal (GI) absorption, indicating their potential suitability for oral administration. None of the compounds demonstrated blood brain barrier (BBB) permeability, which is advantageous for avoiding central nervous system side effects. The bioavailability score for all molecules was 0.55, suggesting a moderate probability of achieving adequate systemic exposure when administered orally (Table 3).

Table 3. ADMET Analysis of *Impatiens balsamina* compound

	Compounds	Lipophilicity (Log P _{ow})	Druglikeness	Pharmacokinetics	Bioavailability
1.	kaempferol	1.7	Yes; 0 violation	GI abs: High; BBB: No	0.55

2.	Quercetin	1.63	Yes; 0 violation	GI abs: High; BBB: No	0.55
3.	Apigenin	1.89	Yes; 0 violation	GI abs: High; BBB: No	0.55
4.	Pelargonidin	-4.5	Yes; 0 violation	GI abs: High; BBB: No	0.55
5.	Cyanidin	-2.62	Yes; 0 violation	GI abs: High; BBB: No	0.55

Table 4 provides a comprehensive evaluation of the ADMET characteristics of nineteen bioactive compounds identified from *Acmella oleracea*. All compounds demonstrated excellent drug-likeness, with zero Lipinski violations, indicating strong compatibility with fundamental drug-design criteria. Most compounds exhibited moderate-to-high lipophilicity (Log Po/w = 1.58–3.88), suggesting good membrane permeability. Compounds such as spilanthol, various alkamides, and sesquiterpenes displayed particularly higher lipophilicity (Log Po/w > 3.0), which is generally associated with enhanced absorption and intracellular penetration. Across all molecules, gastrointestinal (GI) absorption was uniformly high, reflecting strong potential for oral bioavailability. A notable proportion of compounds including spilanthol, alkamide derivatives, humulene epoxide II, caryophyllene oxide, and cedrelanol showed predicted blood–

brain barrier (BBB) permeability, which could contribute to central nervous system activity or neuromodulatory effects associated with *A. oleracea*. However, a few compounds such as petasiphenol and salvial-4(14)-en-1-one lacked BBB penetration. Despite structural diversity, all compounds demonstrated a consistent bioavailability score of 0.55, indicating a moderate likelihood of achieving effective systemic exposure upon oral administration. Overall, the ADMET profile suggests that *Acmella oleracea* contains a wide range of drug-like molecules particularly alkamides and sesquiterpenes with favorable pharmacokinetic properties, strong GI absorption, and significant BBB permeability. These characteristics support their potential roles in biological activity and suitability for further drug development targeting DPP4 and related metabolic pathways.

Table 4. ADMET Analysis of *Acmella oleracea* compound

	Compounds	Lipophilicity (Log P _{o/w})	Druglikness	Pharmacokinetics	Bioavailability
1.	Petasiphenol	1.94	Yes; 0 violation	GI abs: High; BBB: No	0.55
2.	Spilanthol	3.55	Yes; 0 violation	GI abs: High; BBB: Yes	0.55
3.	(2E)-N-(2-methylbutyl)-2-undecene-8,10-diynamide	3.69	Yes; 0 violation	GI abs: High; BBB: Yes	0.55
4.	(2E,7Z)-N-(isobutyl)-2,7-tridecadiene-10,12-diynamide	3.88	Yes; 0 violation	GI abs: High; BBB: Yes	0.55
5.	(7Z)-N-(isobutyl)-7-tridecene-10,12-diynamide	3.78	Yes; 0 violation	GI abs: High; BBB: Yes	0.55
6.	(2E,6Z,8E)-N-(2-methylbutyl)-deca-2,6,8-trienamide	3.74	Yes; 0 violation	GI abs: High; BBB: Yes	0.55
7.	Dodeca-2,4,8,10-tetraenoic acid isobutylamide	3.77	Yes; 0 violation	GI abs: High; BBB: Yes	0.55
8.	Kessane	1.58	Yes; 0 violation	GI abs: High; BBB: Yes	0.55
9.	Safranal	2.13	Yes; 0 violation	GI abs: High; BBB: Yes	0.55
10.	Spathulenol	2.88	Yes; 0 violation	GI abs: High; BBB: Yes	0.55

Petasiphenol and spathulenol as promising natural DPP-4 inhibitors: An *in silico* multiple ligand based study against type 2 diabetes mellitus

11.	Salvial-4(14)-en-1-one	2.87	Yes; 0 violation	GI abs: High; BBB: No	0.55
12.	(2e,6e,8e)-n-(2-Methylpropyl)-2,6,8-decatrienamide	3.55	Yes; 0 violation	GI abs: High; BBB: Yes	0.55
13.	(1aR,7S,7aS,7bR)-1,1,4,7-tetramethyl-2,3,5,6,7a,7b-hexahydro-1aH-cyclopropa(h)azulen-7-ol	2.89	Yes; 0 violation	GI abs: High; BBB: Yes	0.55
14.	Humulene epoxide II	3.11	Yes; 0 violation	GI abs: High; BBB: Yes	0.55
15.	Caryophyllene oxide	3.15	Yes; 0 violation	GI abs: High; BBB: Yes	0.55
16.	14-Hydroxy-9-epi-(E)-caryophyllene	2.96	Yes; 0 violation	GI abs: High; BBB: Yes	0.55
17.	Cedrelanol	3.15	Yes; 0 violation	GI abs: High; BBB: Yes	0.55
18.	(z)-Non-2-en-6,8-dienoic acid isobutylamide	2.98	Yes; 0 violation	GI abs: High; BBB: Yes	0.55
19.	(z)-Dec-2-en-6,8-dienoic acid isobutylamide	3.47	Yes; 0 violation	GI abs: High; BBB: Yes	0.55

Table 5 presents the toxicity-related pharmacokinetic parameters of key *Impatiens balsamina* phytochemicals, focusing on water solubility, intestinal permeability, systemic clearance, and hepatotoxicity. All five compounds kaempferol, quercetin, apigenin, pelargonidin, and cyanidin showed no predicted hepatotoxicity, demonstrating a strong safety profile for potential therapeutic use. Water solubility values ranged from -3.32 to -2.93, indicating moderate aqueous solubility, typical for polyphenolic compounds. Caco-2 permeability values varied among the molecules: apigenin exhibited the highest permeability (1.007), suggesting favorable intestinal

transport, whereas quercetin, cyanidin, and pelargonidin showed lower or negative permeability values, indicating more limited passive membrane diffusion. Despite this variation, all compounds displayed high human intestinal absorption, with values between 71.83% and 93.25%. Apigenin showed the highest absorption (93.25%), while pelargonidin had the lowest but still acceptable absorption at 71.83%. Total clearance values ranged from 0.407 to 0.569 mL/min/kg, suggesting moderate elimination rates and the potential for sustained systemic presence without rapid clearance.

Table 5. Toxicity Analysis of *Impatiens balsamina* compound

Compounds	Water solubility	Caco2 permeability	Intestinal absorption (human)	Total Clearance	Hepatotoxicity
kaempferol	-3.04	0.032	74.29	0.477	No
Quercetin(Flavonol)	-2.925	-0.229	77.207	0.407	No
Apigenin	-3.329	1.007	93.25	0.566	No
Pelargonidin	-3.07	-0.076	71.828	0.569	No
Cyanidin	-2.935	-0.35	87.303	0.532	No

Table 6 summarizes the *in silico* toxicity profiles of various phytochemicals identified from *Acmella oleracea*. The parameters evaluated include water solubility, Caco-2 permeability, human intestinal absorption, total clearance, and hepatotoxicity. Most compounds exhibit low water solubility (ranging from -2.49 to -5.20), which is typical for hydrophobic phytochemicals. However, they show high Caco-2 permeability (mostly above 1.2), indicating good potential for intestinal epithelial transport. Correspondingly,

the human intestinal absorption (HIA) values are consistently high, ranging between 68% and 96%, suggesting strong absorption efficiency. The total clearance values vary moderately among the compounds, with some metabolites (e.g., spilanthol, kessane, humulene epoxide II) showing higher clearance, indicating efficient systemic elimination. Importantly, none of the compounds exhibited hepatotoxicity, confirming the liver-safe nature of these phytoconstituents. Table 6. Toxicity Analysis of *Acmella oleracea*

Compounds	Water solubility	Caco2 permeability	Intestinal absorption (human)	Total Clearance	Hepatotoxicity
petasiphenol	-3.127	-0.565	68.157	-0.012	No
spilanthol	-4.374	1.368	92.81	0.583	No
(2E)-N-(2-methylbutyl)-2-undecene-8,10-diynamide	-3.797	1.233	95.026	1.825	No
(2E,7Z)-N-(isobutyl)-2,7-tridecadiene-10,12-diynamide	-4.412	1.253	95.755	0.767	No
(7Z)-N-(isobutyl)-7-tridecene-10,-12-diynamide	-4.55	1.246	95.248	1.768	No
(2E,6Z,8E)-N-(2-methylbutyl)-deca-2,6,8-trienamide	-3.917	1.227	92.849	0.629	No
dodeca-2,4,8,10-tetraenoic acid isobutylamide	-5.2	1.373	92.629	0.698	No
Kessane	-4.566	1.502	95.145	0.879	No
Safranal	-2.495	1.501	96.298	0.226	No
Spathulenol	-3.866	1.388	93.235	0.895	No
Salvial-4(14)-en-1-one	-5.094	1.527	95.994	1.141	No
(2e,6e,8e)-n-(2-Methylpropyl)-2,6,-8-decatrienamide	-4.374	1.368	92.81	0.583	No
(1aR,7S,7aS,7bR)-1,1,4,7-tetramethyl-2,3,5,6,7a,7b-hexahydro-1aH-cyclopropa(h)azulen-7-ol	-4	1.492	94.495	0.881	No
Humulene epoxide II	-4.162	1.515	95.737	1.065	No
Caryophyllene oxide	-4.321	1.414	95.669	0.905	No
14-Hydroxy-9-epi-(E)-caryophyllene	-4.058	1.512	93.603	1.172	No
Cedrelanol	-4.073	1.479	94.296	1.085	No
(z)-Non-2-en-6,8-diynoic acid isobutylamide	-4.374	1.368	92.81	0.583	No
(z)-Dec-2-en-6,8-diynoic acid isobutylamide	-3.797	1.233	95.026	1.825	No

Petasiphenol and spathulenol as promising natural DPP-4 inhibitors: An *in silico* multiple ligand based study against type 2 diabetes mellitus

Three-dimensional structure of human Dipeptidyl Peptidase-4 (DPP4, PDB ID: 2RGU) The three-dimensional structure of human Dipeptidyl Peptidase-4 (DPP4, PDB ID: 2RGU) was analyzed using a multi-colored ribbon model to highlight its structural domains. The receptor was prepared for molecular docking by removing water and heteroatoms and defining the AutoDock Vina grid box to cover all potential ligand-binding sites (center_x = 56.1663, center_y = 61.3494, center_z = 52.5903; size_x = 90.1273, size_y = 89.9198, size_z = 125.3163). Energy minimization using Swiss PDB Viewer yielded a stable, energetically favorable structure (E = -81,005.648 kJ/mol). Binding site residues were identified via CASTp to ensure accurate docking simulations (Figure 1).

Binding sites of protein

The binding site of human DPP4 (PDB

ID: 2RGU) is a surface pocket where ligands interact via hydrogen bonds, electrostatic forces, ionic interactions, and Van der Waals forces, inducing conformational changes that modulate protein function. Key residues forming this binding site include PHE357, TYR666, TYR631, TYR547, SER630, TRP629, GLU205, GLU117, PRO159, SER158, TRP157, TRP154, VAL155, ASN103, GLU91, ILE102, LYS71, THR94, ARG669, LYS554, ARG358, PHE240, PHE95, and ASN74. Protein binding site is the cavity or pocket on the surface of the target protein/nucleic acid to which the ligand/drug molecule binds through intermolecular forces (e.g., hydrogen bonds, electrostatic, ionic bonds, Van der Waals forces, etc.), resulting in conformational change of the target protein and leading to its functional activation/inhibition.

Protein	Protein ID	Total Energy Minimized
Crystal structure of complex of human DPP4 and inhibitor	2RGU	E = -81005.648 KJ/mol

Table 7. Binding sites of the selected protein (2RGU)

Protein name	Binding Sites
2RGU	PHE357, TYR666, TYR631, TYR547, SER630, TRP629, GLU205, GLU206, GLU117, PRO159, SER158, TRP157, TRP154, VAL155, ASN103, GLU91, ILE102, LYS71, THR94, PHE357, ARG669, LYS554, ARG358, PHE240, PHE95, ASN74

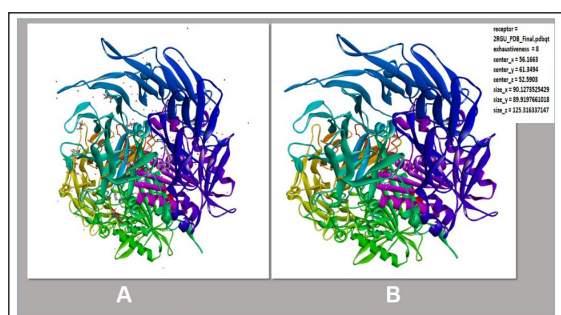


Figure 1. 2RGU Protein Before (A) and After Cleaning (B)

Selected protein structure validation by Ramachandran Plot.

The Ramachandran plot analysis of the DPP4 (PDB ID: 2RGU) structure indicates that the majority of amino acid residues occupy favored regions, reflecting a sterically favorable and well-modeled protein backbone. A small fraction of residues fall within allowed regions, suggesting flexible loop regions, while very few residues appear in disallowed regions, which may correspond to unusual conformations or potential modeling artifacts. Overall, these results confirm the structural reliability of the DPP4 model for subsequent computational and docking studies (Figure 2).

Ligands library of *Impatiens balsamina* and *Acmella oleracea*

The image displays six compounds studied for potential DPP4 inhibition. The top row shows Linagliptin (standard inhibitor), Apigenin (flavonoid), and Petasiphenoln (polyphenol), highlighting their relevance in binding

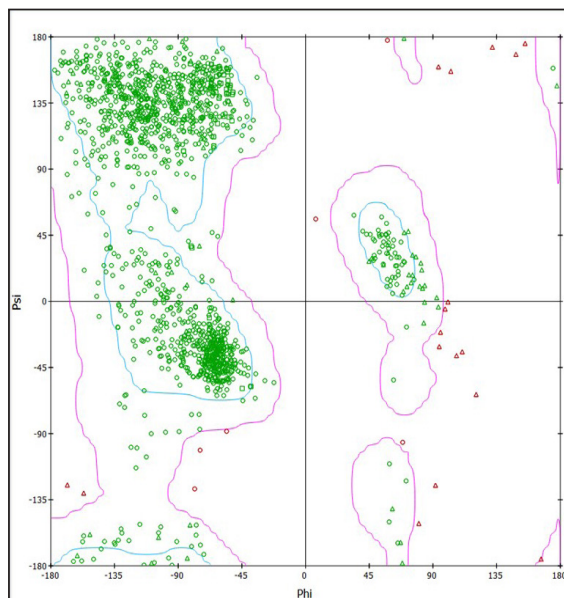


Figure 2. Ramachandran Plot Analysis for Protein Validation. 2RGU Protein after cleaning.

studies. The bottom row presents Spathulenol, Salvial-4(14)-en-1-one, and Cedrenolal, all bicyclic compounds with various functional groups such as ketones and hydroxyls. The 3D representations emphasize molecular geometry, stereochemistry, and functional groups, which are crucial for understanding binding affinity and inhibitory potential (Figure 3).

Table 8. Ligands Library of *Impatiens balsamina*

Compounds	Formula	M/W (g/mol)	PubChem CID
kaempferol	C ₁₅ H ₁₀ O ₆	286.24 g/mol	5280863
Quercetin (Flavonol)	C ₁₅ H ₁₀ O ₇	302.24 g/mol	5280343
Apigenin	C ₁₅ H ₁₀ O ₅	270.24 g/mol	5280443
Pelargonidin	C ₁₅ H ₁₁ ClO ₅	306.70 g/mol	67249
Cyanidin	C ₁₅ H ₁₁ O ₆ ⁺	287.24 g/mol	128861

Table 9. Ligands Library of *Acmella oleracea*

Compounds	Formula	M/W (g/mol)	PubChem CID
Petasiphenol	C ₁₈ H ₁₆ O ₇	344.32 g/mol	6438779
Spilanthol	C ₁₄ H ₂₃ NO	221.34 g/mol	5353001
(2E)-N-(2-methylbutyl)-2-undecene-8,10-diynamide	C ₁₆ H ₂₃ NO	245.36 g/mol	14259836
(2E,7Z)-N-(isobutyl)-2,7-tridecadiene-10,12-diynamide	C ₁₇ H ₂₃ NO	257.37 g/mol	15609892
(7Z)-N-(isobutyl)-7-tridecene-10,12-diynamide	C ₁₇ H ₂₅ NO	259.39 g/mol	129848092
(2E,6Z,8E)-N-(2-methylbutyl)-deca-2,6,8-trienamide	C ₁₅ H ₂₅ NO	235.37 g/mol	5352999
Dodeca-2,4,8,10-tetraenoic acid isobutylamide	C ₁₆ H ₂₅ NO	247.38 g/mol	156638
Kessane	C ₁₅ H ₂₆ O	222.37 g/mol	11310616
Safranal	C ₁₀ H ₁₄ O	150.22g/mol	61041
Spathulenol	C ₁₅ H ₂₄ O	220.35 g/mol	92231
Salvial-4(14)-en-1-one	C ₁₅ H ₂₄ O	220.35 g/mol	42608172
(2e,6e,8e)-n-(2-Methylpropyl)-2,6,8-decatrienamide	C ₁₄ H ₂₃ NO	220.35 g/mol	11506817

Petasiphenol and spathulenol as promising natural DPP-4 inhibitors: An *in silico* multiple ligand based study against type 2 diabetes mellitus

(1aR,7S,7aS,7bR)-1,1,4,7-tetramethyl-2,3,5,6,7a,7b-hexahydro-1aH-cyclopropa(h)azulen-7-ol	C ₁₅ H ₂₄ O	220.35 g/mol	102303030
Humulene epoxide II	C ₁₅ H ₂₄ O	220.35 g/mol	10704181
Caryophyllene oxide	C ₁₅ H ₂₄ O	220.35 g/mol	1742210
14-Hydroxy-9-epi-(E)-caryophyllene	C ₁₅ H ₂₄ O	220.35 g/mol	5352484
Cedrelanol	C ₁₅ H ₂₆ O	222.37 g/mol	160799
(z)-Non-2-en-6,8-diynoic acid isobutylamide	C ₁₃ H ₁₇ NO	203.28 g/mol	92033736
(z)-Dec-2-en-6,8-diynoic acid isobutylamide	C ₁₄ H ₁₉ NO	217.31 g/mol	129706500

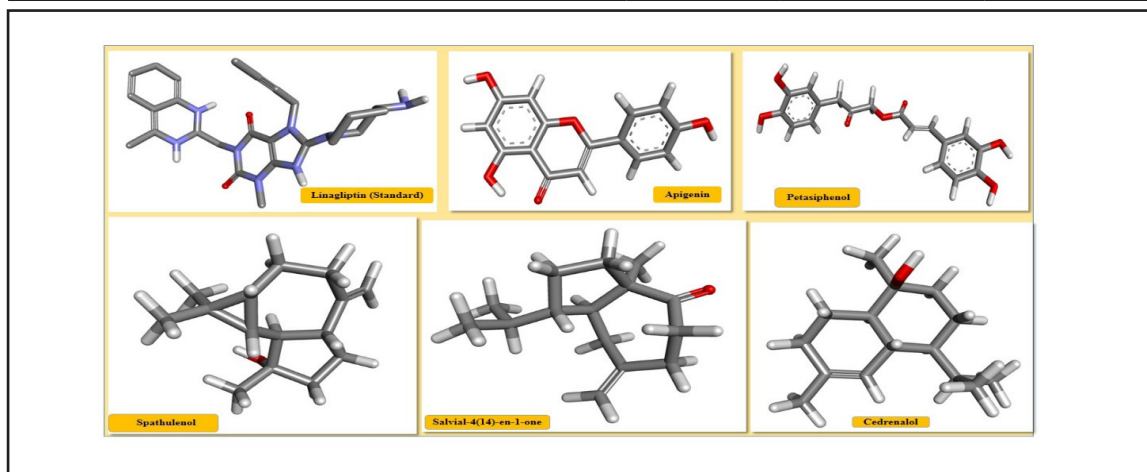


Figure 3. Ligand structure of Linagliptin, and compound observed in *Impatiens balsamina* and *Acmella oleracea*.

Molecular docking analysis

The docking study evaluated the binding affinities of standard and plant-derived compounds against DPP4. Linagliptin, the reference inhibitor, exhibited the strongest binding with a value of -9.7 kcal/mol, serving as a benchmark for comparison. Among the *Impatiens balsamina* compounds, Apigenin (-8.0 kcal/mol) and Quercetin (-7.9 kcal/mol) showed relatively strong interactions, while Kaempferol, Pelargonidin, and Cyanidin displayed moderate binding (-7.7 kcal/mol). These results suggest that flavonoids from *I. balsamina* can interact favorably with the DPP4 active site. For *Acmella oleracea* compounds, Petasiphenol (-8.7 kcal/mol) demonstrated the highest affinity, comparable to the standard inhibitor. Other bicyclic and ter-

penoid-like molecules, such as Cedrenanol (-8.2 kcal/mol), Spathulenol (-8.1 kcal/mol), and Salvial-4(14)-en-1-one (-8.1 kcal/mol), also showed strong binding potential, highlighting the importance of their structural features in DPP4 interaction. Compounds like Spilanthal and various diynamides exhibited lower affinities (-5.6 to -6.4 kcal/mol), indicating weaker interactions. Several bioactive compounds from *Acmella oleracea* and *Impatiens balsamina*, particularly polyphenols and terpenoid derivatives, have promising binding affinities with DPP4 and may serve as potential natural inhibitors for type-2 diabetes management (Figure 4; Figure 5; Table 10).

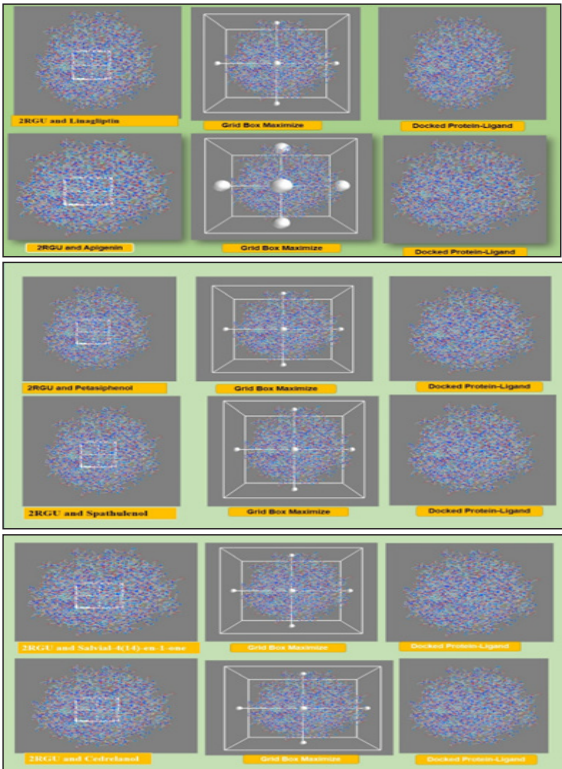


Figure 4. Molecular Docking 2RGU protein with multiple ligands by PyRX tool.

Table 10. Docking score of Linagliptin (Standard), compound from *Impatiens balsamina* and *Acemella oleracea*.

	Compounds	Binding Affinity(Kcal/mol) (2RGU)
Standard	Linagliptin	-9.7
Impatiens balsamina	kaempferol	-7.7
	Quercetin(Flavonol)	-7.9
	Apigenin	-8
	Pelargonidin	-7.7
	Cyanidin	-7.7
Acemella oleracea	Petasiphenol	-8.7
	Spilanthol	-6.2
	(2E)-N-(2-methylbutyl)-2-undecene-8,10-diynamide	-5.8
	(2E,7Z)-N-(isobutyl)-2,7-tridecadiene-10,12-diynamide	-6
	(7Z)-N-(isobutyl)-7-tridecene-10,12-diynamide	-6

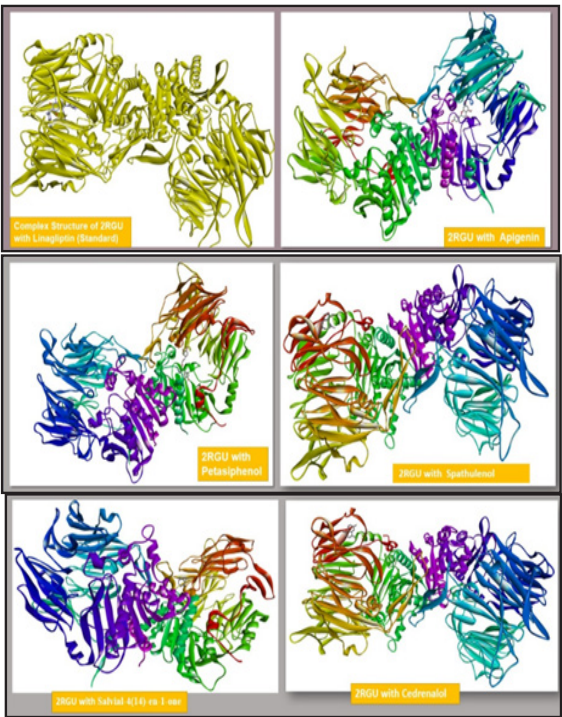


Figure 5. Protein-Ligand binding interaction of linagliptin and top five compounds based on binding score.

Petasiphenol and spathulenol as promising natural DPP-4 inhibitors: An *in silico* multiple ligand based study against type 2 diabetes mellitus

	(2E,6Z,8E)-N-(2-methylbutyl)-deca-2,6,8-trienamide	-6
	Dodeca-2,4,8,10-tetraenoic acid isobutylamide	-6
	Kessane	-7.2
	Safranal	-6.7
	Spathulenol	-8.1
	Salvia-4(14)-en-1-one	-8.1
	(2e,6e,8e)-n-(2-Methylpropyl)-2,6,8-decatrienamide	-6.4
	(1aR,7S,7aS,7bR)-1,1,4,7-tetramethyl-2,3,5,6,7a,7b-hexahydro-1aH-cyclopropa(h)azulen-7-ol	-7.2
	Humulene epoxide II	-7
	Caryophyllene oxide	-6.7
	14-Hydroxy-9-epi-(E)-caryophyllene	-7.9
	Cedrelanol	-8.2
	(z)-Non-2-en-6,8-dienoic acid isobutylamide	-6.2
	(z)-Dec-2-en-6,8-dienoic acid isobutylamide	-5.6

Binding Mechanism of selected compound of *Impatiens balsamina* and *Acmella oleracea* with PDB ID: 2 RGU. Molecular docking analyses were conducted to investigate the binding mechanisms of several natural compounds and a reference inhibitor within the active site of the DPP-4 protein (PDB ID: 2RGU). The results revealed distinct interaction patterns across the evaluated ligands, providing insights into their potential inhibitory properties and structure–activity relationships.

Linagliptin (standard drug control)

Linagliptin binds deeply within the active site of DPP-4 and establishes a wide range of stabilizing contacts. The ligand interacts with key residues including Leu154, Val155, Ser158, Trp193, Pro159, and Glu117, as confirmed in the 3D binding pose. The 2D interaction map highlights conventional hydrogen bonds with Tyr158 and Glu117, along with π – π and π –alkyl interactions involving Trp154, Pro159, and Ser158, complemented by carbon–hydrogen bonds. This rich interaction network explains the high inhibitory potency of Linagliptin and validates the docking protocol.

Apigenin

Apigenin binds within the DPP-4 binding pocket through a combination of hydrogen bonding and hydrophobic contacts. The 3D visualization shows its stable accommodation among residues such as Tyr, Ser, and Lys, while the 2D interaction diagram identifies specific contacts with Tyr631, Tyr669, and Tyr630. Dashed lines highlight conventional hydrogen bonds and additional non-covalent interactions, indicating moderate stabilizing forces that support Apigenin's binding affinity.

Petasiphenol

Petasiphenol demonstrates strong and specific binding within the active site of 2RGU. The ligand forms multiple conventional hydrogen bonds with Arg-669, Glu-206, Ser-630, Trp-629, and Lys-554, alongside π – π stacking interactions with the aromatic residues Tyr-547 and Phe-357. These combined polar and aromatic interactions firmly position Petasiphenol within the pocket, suggesting a high binding affinity and supporting its potential as a potent DPP-4 inhibitor.

Cedrenol

Cedrenol binds primarily through hydrophobic forces and does not engage in polar interactions. The ligand forms multiple alkyl interactions exclusively with Ile-102, indicating insertion into a non-polar region of the active site. Its interaction profile suggests a weaker and less specific binding mode relative to ligands that form hydrogen bonds.

Salvia-1(14)-en-1-one

Similar to Cedrenol, Salvia-1(14)-en-1-one interacts only through hydrophobic stabilization. Its binding is dominated by a single π -alkyl interaction with Phe-240, with no observed hydrogen bonds or additional contacts. This

simplified interaction pattern indicates relatively low binding specificity.

Spathulenol

Spathulenol displays the most diverse interaction profile among the sesquiterpenoids. A key conventional hydrogen bond forms between its hydroxyl group and Asn-74, serving as a strong polar anchor. Additional stabilization is provided by π -alkyl interactions with Phe-95 and alkyl contacts with Ile-102. The presence of the hydroxyl group enables dual polar-hydrophobic stabilization, contributing to a more favorable binding conformation than the purely hydrophobic sesquiterpenoids.

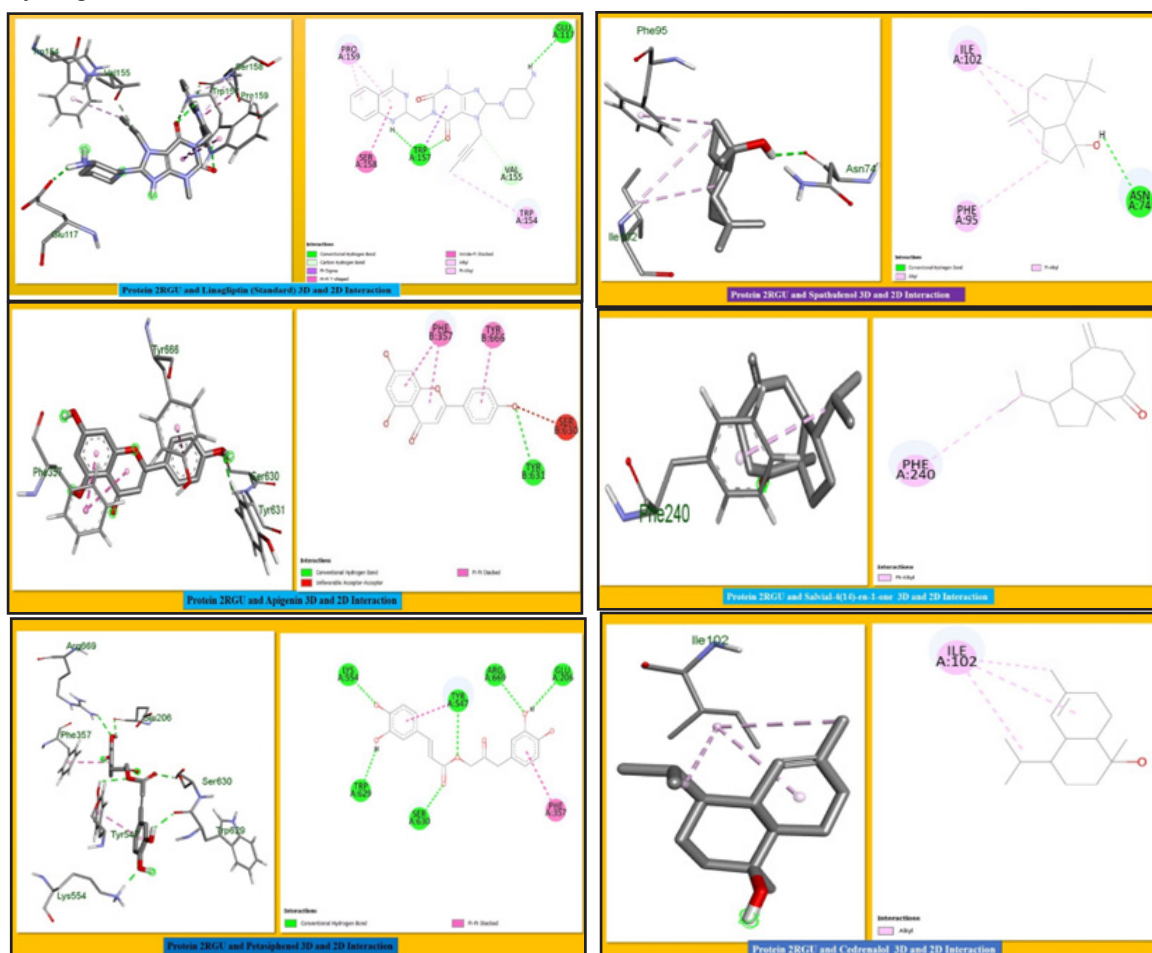


Figure 6. Molecular docking validation: interaction

Petasiphenol and spathulenol as promising natural DPP-4 inhibitors: An *in silico* multiple ligand based study against type 2 diabetes mellitus

Molecular dynamic simulation analysis

Based on the molecular docking results, Apigenin and Petasiphenol were selected for molecular dynamics (MD) simulations along with Linagliptin as the standard inhibitor, as these compounds exhibited the most favorable binding affinities toward DPP-4 (PDB ID: 2RGU). Other docked compounds were excluded from MD analysis due to comparatively lower docking scores and weaker interaction profiles. The MD simulations were therefore performed to further validate the dynamic stability and binding behavior of these top-ranked complexes under physiological conditions.

Root mean square deviation (RMSD) analysis

The root mean square deviation (RMSD) analysis was employed to assess the structural stability of DPP-4 in complex with the standard inhibitor Linagliptin and the two selected compounds Apigenin and Petasiphenol over the 100 ns molecular dynamics simulation (Figure 7). RMSD profiles of the protein backbone, ligand, and protein–ligand complexes provide insights into the equilibration behavior and dynamic stability of each system. For the Linagliptin–DPP-4 complex, the protein and complex RMSD values stabilized rapidly within

the first few nanoseconds and remained relatively constant around ~ 2.1 – 2.3 Å throughout the simulation, indicating a well-equilibrated and highly stable complex. Although the ligand RMSD showed moderate fluctuations, these did not propagate to the protein or complex RMSD, confirming that Linagliptin maintained a stable binding conformation within the active site.

In the Apigenin–DPP-4 complex, the protein and complex RMSD values also remained stable after initial equilibration, with fluctuations comparable to those observed for the standard ligand, and the complex RMSD remained within ~ 2.2 – 2.5 Å throughout the simulation. The ligand RMSD exhibited higher fluctuations, reflecting conformational flexibility within the binding pocket; however, the absence of a continuous upward drift in the complex RMSD indicates that these movements did not compromise complex stability. For the Petasiphenol–DPP-4 complex, a gradual increase in RMSD was observed during the early simulation stage, followed by stabilization after approximately 40 ns, with the complex RMSD plateauing around ~ 2.3 – 2.6 Å. Overall, the RMSD analysis demonstrates that both Apigenin and Petasiphenol exhibit dynamic stability comparable to the standard inhibitor Linagliptin, supporting their potential as promising DPP-4 inhibitors.

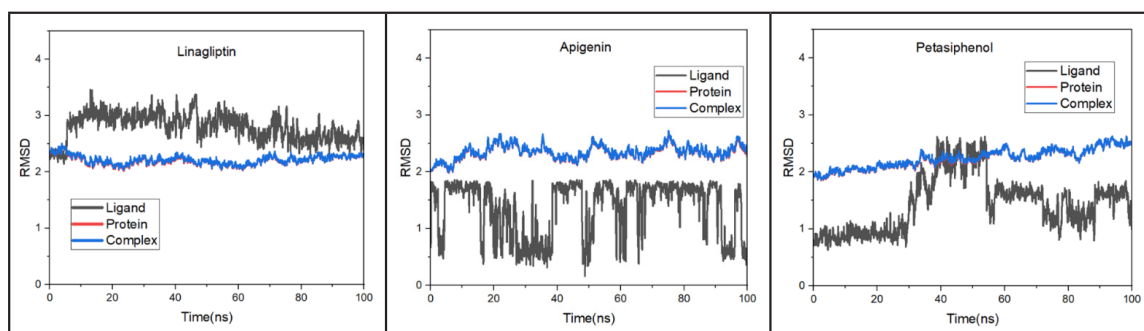


Figure 7. RMSD analysis of DPP-4 complexes with Apigenin, Linagliptin, and Petasiphenol over 100 ns MD simulation

RMSF (root mean square fluctuation) analysis

Root mean square fluctuation (RMSF) analysis was performed to examine the resi-

due-level flexibility of DPP-4 in complex with the standard inhibitor Linagliptin and the selected compounds Apigenin and Petasiphenol during the 100 ns molecular dynamics simulation (Fig-

ure 8). RMSF profiles provide insights into local conformational dynamics and identify flexible regions of the protein backbone that may be influenced by ligand binding. For the Linagliptin–DPP-4 complex, most residues exhibited low RMSF values, generally below ~ 1.0 Å, indicating a stable protein backbone throughout the simulation. Higher fluctuations were mainly observed at the N- and C-terminal regions and in solvent-exposed loop segments, which is typical for regions not directly involved in ligand binding. Importantly, residues within the catalytic and binding pocket regions showed minimal fluctuations, confirming effective stabilization of the active site by the standard inhibitor.

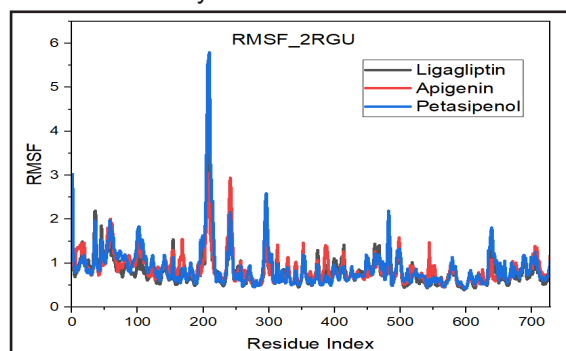


Figure 8. Root means square fluctuation (RMSF) profiles of DPP-4 residues in complex with Linagliptin (standard), Apigenin, and Petasiphenol over 100 ns molecular dynamics simulation, illustrating residue-level flexibility and stabilization of the binding pocket.

The Apigenin–DPP-4 complex displayed an RMSF pattern comparable to that of Linagliptin, with most residues maintaining low to moderate fluctuations and only minor increases in flexibility observed in selected loop regions. The binding site residues remained relatively rigid, indicating that Apigenin binding does not induce significant local destabilization. In contrast, the Petasiphenol–DPP-4 complex showed higher RMSF peaks at specific loop regions, reflecting increased local flexibility; however, these fluctuations were confined to non-critical regions, while residues near the binding pocket maintained low RMSF values.

Overall, the RMSF analysis demonstrates that both Apigenin and Petasiphenol preserve residue-level stability comparable to the standard inhibitor Linagliptin, supporting the formation of stable protein–ligand complexes and corroborating the RMSD results.

Radius of gyration (Rg) analysis

The radius of gyration (Rg) analysis was performed to evaluate the global structural compactness and stability of DPP-4 in complex with the standard inhibitor Linagliptin and the selected compounds Apigenin and Petasiphenol over the 100 ns molecular dynamics simulation (Figure 9). Rg reflects the overall distribution of the protein mass around its center of mass and serves as an indicator of large-scale conformational changes such as protein unfolding or compaction during the simulation.

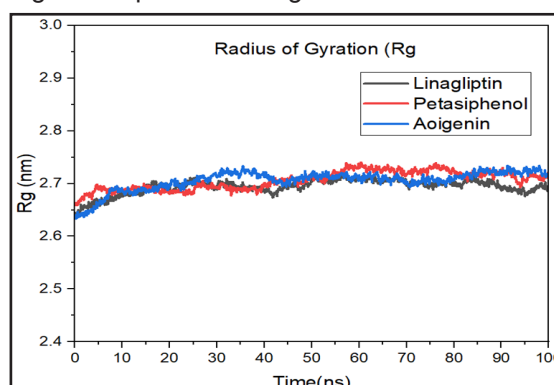


Figure 9. Radius of gyration (Rg) profiles of DPP-4 in complex with Linagliptin (standard), Apigenin, and Petasiphenol over a 100 ns molecular dynamics simulation.

As shown in the Rg profiles, all three complexes exhibited relatively stable Rg values throughout the simulation, with only minor fluctuations and no abrupt increases, indicating preservation of the global protein fold. The Linagliptin–DPP-4 complex maintained a consistent Rg profile, confirming its structural compactness and stability as the reference inhibitor. The Apigenin–DPP-4 and Petasiphenol–DPP-4 complexes showed Rg values comparable to the standard, with slightly higher but stable fluctuations that remained within a narrow range.

These observations suggest that both Apigenin and Petasiphenol do not induce large-scale structural expansion or destabilization of DPP-4. Overall, the Rg analysis supports the formation of stable protein–ligand complexes for Apigenin and Petasiphenol, consistent with the RMSD and RMSF results.

Solvent-accessible surface area (SASA) analysis

Solvent-accessible surface area (SASA) analysis was performed to evaluate changes in the overall solvent exposure of DPP-4 in complex with the standard inhibitor Linagliptin and the selected compounds Apigenin and Petasiphenol during the 100 ns molecular dynamics simulation (Figure 10). SASA provides insights into global conformational stability and potential unfolding events by monitoring the extent of protein surface exposed to the solvent.

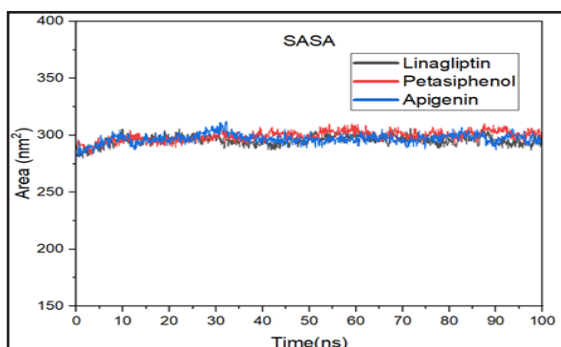


Figure 10. Solvent-accessible surface area (SASA) profiles of DPP-4 in complex with Linagliptin (standard), Apigenin, and Petasiphenol over a 100 ns molecular dynamics simulation

As shown in the SASA profiles, all three complexes exhibited stable SASA values throughout the simulation, with only moderate fluctuations and no continuous increasing trend. The Linagliptin–DPP-4 complex maintained a relatively constant SASA profile, indicating preserved structural compactness and stable solvent exposure. The Apigenin–DPP-4 and Petasiphenol–DPP-4 complexes showed SASA values comparable to the standard, with slightly higher but stable fluctuations that remained within a narrow range. These observations

suggest that neither Apigenin nor Petasiphenol induces significant structural expansion or unfolding of DPP-4. Overall, the SASA analysis supports the formation of stable protein–ligand complexes and is consistent with the RMSD, RMSF, and radius of gyration results.

In this study, molecular docking was employed to investigate the binding behavior of selected natural compounds from *Impatiens balsamina* and *Acmella oleracea* against the DPP-4 protein (PDB ID: 2RGU), using Linagliptin as the reference inhibitor. The docking results highlight the importance of ligand structural features, particularly hydroxyl groups, aromatic systems, and molecular compactness—in stabilizing protein–ligand interactions within the DPP-4 active site (25). Linagliptin, the standard inhibitor, exhibited the most extensive interaction network, forming multiple hydrogen bonds, π – π stacking interactions, and hydrophobic contacts with key residues such as Tyr158, Glu117, Trp193, and Pro159, which is consistent with its clinically established high affinity and inhibitory potency toward DPP-4 (26). The ability of Linagliptin to engage both polar and hydrophobic residues emphasizes the importance of multifunctional chemical scaffolds for effective DPP-4 inhibition.

Among the natural compounds evaluated, Petasiphenol demonstrated an interaction profile most closely resembling that of Linagliptin, forming several hydrogen bonds with residues including Arg669, Glu206, Ser630, Trp629, and Lys554, while additional aromatic interactions with Tyr547 and Phe357 further stabilized the ligand within the active pocket (27). These findings suggest that Petasiphenol possesses strong affinity for DPP-4 and may serve as a promising natural lead compound due to its capability to interact with both the catalytic site and neighboring subpockets. Flavonoids such as Apigenin also showed notable interactions, characterized by hydrogen bonding with Tyr631, Tyr669, and Tyr630 (28). Although the number of polar interactions was moderate compared to Petasiphenol, Apigenin's binding pattern re-

flects its planar aromatic scaffold, which allows effective accommodation within the DPP-4 binding pocket, in agreement with previous reports describing flavonoids as moderate natural DPP-4 inhibitors.

In contrast, the sesquiterpenoids Cedrenol and Salvia-1(14)-en-1-one displayed limited binding diversity, forming predominantly hydrophobic interactions with residues such as Ile102 and Phe240. The absence of hydrogen bond donors or acceptors in their structures restricts their ability to form polar interactions, resulting in weaker and less specific binding, highlighting the limitation of purely hydrophobic compounds in achieving strong or stable engagement with the DPP-4 catalytic cavity. Spathulenol, although structurally related to other sesquiterpenoids, showed a noticeably improved interaction profile due to the presence of a hydroxyl functional group (29). The formation of a hydrogen bond with Asn74, together with hydrophobic and π -alkyl interactions involving Phe95 and Ile102, suggests that even minimal incorporation of polar groups can significantly enhance binding affinity. Collectively, these results indicate that polar interactions—particularly hydrogen bonding—play a defining role in the stability and specificity of ligand binding to DPP-4 (30). Natural compounds possessing aromatic rings and hydroxyl groups, such as Petasiphenol, Apigenin, and Spathulenol, therefore exhibit stronger and more diverse interactions compared to purely hydrophobic molecules. Overall, the docking outcomes support the therapeutic potential of Apigenin and Petasiphenol as the most promising phytoconstituents of *Impatiens balsamina* and *Acmella oleracea*, respectively, due to their favorable binding affinity toward the DPP-4 protein (PDB ID: 2RGU) (25–30).

Molecular docking forecasts binding affinity, but molecular dynamics (MD) simulations offer critical insights into the long-term stability and durability of protein–ligand interactions in physiological circumstances. The stability of the apigenin–DPP-4 and petasiphenol–DPP-4 complexes was assessed in this study using 100 ns

MD simulations in comparison to the Linagliptin inhibitor (31). RMSD analysis demonstrated that apigenin maintained a stable trajectory (~2.2–2.5 Å), closely resembling Linagliptin (~2.1–2.3 Å), and indicating strong conformational stability within the binding pocket. Petasiphenol exhibited a longer equilibration phase but achieved a stable plateau after 40 ns, suggesting an induced-fit adaptation without disrupting the global structure. Consistently stable Radius of Gyration (Rg) values further confirmed that both natural ligands preserved the compact fold of DPP-4(32). RMSF profiles revealed reduced fluctuations in active-site residues for both apigenin and petasiphenol, comparable to Linagliptin, indicating the effective stabilization of the catalytic region. Higher flexibility was limited to solvent-exposed loop regions, which is typical and not functionally disruptive. Additionally, SASA analysis showed no significant increase over time, suggesting that ligand binding did not cause unfolding or structural expansion of the enzyme(33). Overall, these findings indicate that apigenin and petasiphenol form stable and persistent complexes with DPP-4, supporting their potential as promising natural scaffolds for the development of DPP-4 inhibitors.

Conclusion

This study demonstrates that natural compounds from *Impatiens balsamina* and *Acmella oleracea* exhibit varying affinities toward DPP-4, with Petasiphenol and Apigenin emerging as the most promising candidates. Docking analyses revealed that ligands containing hydroxyl groups and aromatic scaffolds establish stronger and more diverse interactions with key DPP-4 residues, while purely hydrophobic compounds show limited binding specificity. Importantly, molecular dynamics simulations validated the docking predictions, showing that Apigenin and Petasiphenol maintain stable protein–ligand complexes, preserved global compactness, and restrained residue-level fluctuations comparable to the standard inhibitor Linagliptin. These findings highlight the therapeutic potential of Apigenin and Petasiphenol

as natural lead compounds for DPP-4 inhibition and provide a robust structure–activity framework for further development of plant-derived antidiabetic agents.

Future recommendations

Future work should extend these findings through quantitative binding free-energy calculations (e.g., MM-PBSA/MM-GBSA) to rank ligand affinities and dissect energetic contributions. In-vitro DPP-4 inhibitory assays with IC₅₀ determination are essential to validate the predicted activities, followed by ADMET and toxicity profiling to assess pharmacokinetic suitability. Structure optimization of less active sesquiterpenoids by introducing hydrogen-bonding functionalities may enhance potency. Finally, *in-vivo* efficacy studies, along with QSAR and pharmacophore modeling, are recommended to guide the rational design of optimized natural or semi-synthetic DPP-4 inhibitors

Reference

1. Antar, S.A., Ashour, N.A., Sharaky, M., Khattab, M., Ashour, N.A., Zaid, R.T. *et al.* (2023) 'Diabetes mellitus: Classification, mediators, and complications; A gate to identify potential targets for the development of new effective treatments', *Bio-medicine & Pharmacotherapy*, 168(1), pp. 115734–115748.
2. Imran, M., Khan, H., Mushtaq, J. and Amjad, I. (1844) 'Diabetes mellitus type 2', 1844(1), pp. 1844–1851.
3. Ahmed, M., Zulfiqar, E., Shafiq, A., Shahzad, M., Hashmi, T.M., Ahmed, R., Rana, J.S., Sidney, S., Greene, S.J., Mentz, R.J. and Mar, M. (2025) 'Type 2 diabetes mellitus-related mortality in the United States, 1999 to 2023', 4(1), pp. 1–12.
4. Lu, X., Xie, Q., Pan, X., Zhang, R., Zhang, X., Peng, G. *et al.* (2024) 'Type 2 diabetes mellitus in adults: Pathogenesis, prevention and therapy', 12(1), pp. 1–25.
5. Makrilakis, K. (2019) 'The role of DPP-4 inhibitors in the treatment algorithm of type 2 diabetes mellitus: When to select, what to expect', *Drugs*, 79(2), pp. 123–134.
6. Ahrén, B. (2019) 'DPP-4 inhibition and the path to clinical proof', *Frontiers in Endocrinology*, 10(376), pp. 1–18.
7. El-Saadony, M.T., Saad, A.M., Mohamed, D.M., Korma, S.A., Alshahrani, M.Y., Ahmed, A.E. *et al.* (2025) 'Medicinal plants: Bioactive compounds, biological activities, combating multidrug-resistant microorganisms, and human health benefits – A comprehensive review', *Food Bioscience*, 65(1), pp. 1–37.
8. Qian, H., Wang, B., Ma, J., Li, C., Zhang, Q. and Zhao, Y. (2023) 'Impatiens balsamina: An updated review on the ethnobotanical uses, phytochemistry, and pharmacological activity', *Journal of Ethnopharmacology*, 303(1), pp. 115956–115972.
9. Paulraj, J., Govindarajan, R. and Palpu, P. (2013) 'Pharmacological properties: A review', *International Journal of Pharmaceutical Sciences Review and Research*, 2013(1), pp. 1–10.
10. Aktar, M.A., Bhuia, M.S., Molla, S., Chowdhury, R., Sarkar, C., Al Shahariar, M. *et al.* (2024) 'Pharmacological and phytochemical review of Acmella oleracea: A comprehensive analysis of its therapeutic potential', *Discover Applied Sciences*, 6(1), pp. 1–16.
11. Uthpala, T.G.G. and Navaratne, S.B. (2020) 'Acmella oleracea plant; Identification, applications and use as an emerging food source – Review', *Food Reviews International*, 36(7), pp. 1–16.
12. Atanasov, A.G., Waltenberger, B., Pferschy-Wenzig, E., Linder, T., Wawrosch, C., Uhrin, P. *et al.* (2015) 'Discovery and resupply of pharmacologically active plant-derived natural products: A review', *Biotechnology Advances*, 33(8), pp. 1582–1614.

13. Zhang, S., Liu, K., Liu, Y. and Hu, X. (2025) 'The role and application of bioinformatics techniques and tools in drug discovery', *Briefings in Bioinformatics*, 26(1), pp. 1–12.
14. Alkharboush, D.F., Kozielski, F., Wells, G. and Porta, E.O.J. (2025) 'Fragment-based drug discovery: A graphical review', *Current Research in Pharmacology and Drug Discovery*, 9(1), pp. 100233–100245.
15. Sliwoski, G., Kothiwale, S., Meiler, J. and Lowe, E.W. (2014) 'Computational methods in drug discovery', *Pharmacological Reviews*, 66(1), pp. 334–395.
16. Hollingsworth, S.A. and Dror, R.O. (2019) 'Molecular dynamics simulation for all', *Neuron*, 99(6), pp. 1129–1143.
17. Dao, T.M. and Rahman, T. (2025) 'Deep generative modeling of protein conformations: A comprehensive review', *Computational and Structural Biotechnology Journal*, 24(1), pp. 1–27.
18. Agoni, C., Fernández-Díaz, R., Timmons, P.B., Adelfio, A., Gómez, H. and Shields, D.C. (2025) 'Molecular modelling in bioactive peptide discovery and characterisation', *Briefings in Bioinformatics*, 26(2), pp. 1–45.
19. Leone, A. (2022) 'Antidiabetic phytochemicals from medicinal plants: Prospective candidates for new drug discovery and development', *Current Medicinal Chemistry*, 13(4), pp. 1–18.
20. Singh, N., Singh, S., Kumar, S. and Narasimhan, B. (2025) 'Molecular docking studies of trigonelline derivatives', *Bioorganic Chemistry*, 1331(1), pp. 141544–141556.
21. Gfeller, D., Wirth, M., Daina, A., Michielin, O. and Zoete, V. (2014) 'SwissTargetPrediction: A web server for target prediction of bioactive small molecules', *Nucleic Acids Research*, 42(W1), pp. 32–38.
22. Evaluations, P. (2023) 'Dipeptidyl peptidase 4 inhibitors in type 2 diabetes mellitus management: Pharmacophore virtual screening, molecular docking, pharmacokinetic evaluations, and conceptual DFT analysis', *Journal of Molecular Graphics and Modelling*, 124(1), pp. 1–20.
23. Hollingsworth, S.A. and Karplus, P.A. (2011) 'Standard structures in proteins', *Biomolecular Concepts*, 1(3–4), pp. 271–283.
24. Zhang, S.C., Yong, L., Cong, L., Karunakaran, R. and Ravichandran, V. (2022) 'Evaluation of coumarin based compounds targeting proteins involved in development of COVID-19', *Saudi Journal of Biological Sciences*, 29(5), pp. 103458–103470.
25. Istrate, D. and Crisan, L. (2022) 'Natural compounds as DPP-4 inhibitors: 3D-similarity search, ADME toxicity, and molecular docking approaches', *Symmetry*, 14(9), pp. 1–18.
26. Sortino, M.A., Sinagra, T. and Canonico, P.L. (2013) 'Linagliptin: A thorough characterization beyond its clinical efficacy', *Frontiers in Endocrinology*, 4(16), pp. 1–9.
27. Mani, S., Kaur, A., Jakhar, K., Kumari, G. and Sonar, S. (2020) 'Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19', *Journal of Infection and Public Health*, 2020(1), pp. 1–5.
28. Pan, J., Zhang, Q., Zhang, C., Yang, W., Liu, H. and Lv, Z. (2022) 'Inhibition of dipeptidyl peptidase-4 by flavonoids: Structure–activity relationship, kinetics and interaction mechanism', *Molecules*, 27(9), pp. 1–17.
29. Karatas, H., Townsend, E.C., Bernard, D., Dou, Y. and Wang, S. (2015) 'NIH public access', *Journal of Medicinal Chemistry*, 53(14), pp. 5179–5185.

30. Deogratias, G., Shadrack, D.M., Munisi, J.J.E., Kinunda, G.A., Jacob, F.R., Mtei, R.P. *et al.* (2022) 'Hydrophobic π - π stacking interactions and hydrogen bonds drive self-aggregation of luteolin in water', *Journal of Molecular Liquids*, 116(1), pp. 108243–108252.
31. Laverne, R.J., Shukla, S.K., Govender, P.P. and Mishra, A.K. (2026) 'DFT, molecular docking, molecular dynamics and ADMET integrated study reveal naturally occurring compound nardosinone as a potential drug candidate against ALS-related protease', *Next Research*, 1(1), pp. 101424–101438.
32. Id, S.B., Ghimire, M.P., Ram, T. and Id, L. (2023) 'Identification of potential human pancreatic α -amylase inhibitors from natural products by molecular docking, MM/GBSA calculations, MD simulations, and ADMET analysis', *PLOS ONE*, 18(4), pp. 1–13.
33. Shahraki, O., Zargari, F., Edraki, N., Firuzi, O. and Miri, R. (2017) 'Molecular dynamics simulation and molecular docking studies of 1,4-dihydropyridines as P-glycoprotein's allosteric inhibitors', *Journal of Biomolecular Structure and Dynamics*, 35(5), pp. 1102–1114.