International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Exploring the Potential of Moringa Oleifera Phytochemicals as Inhibitors of Dipeptidyl Peptidase-4 (DPP-4) in Diabetes Mellitus: A Molecular Docking Approach

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Abstract
Diabetes mellitus (DM) is a global health concern characterized by chronic hyperglycemia due to defects in insulin secretion, insulin action, or both. Type 2 diabetes (T2DM), the most prevalent form, is largely driven by obesity and a sedentary lifestyle, contributing to over 95% of diabetes cases worldwide. The current treatment regimens for T2DM, including Dipeptidyl Peptidase-4 (DPP-4) inhibitors, aim to enhance insulin secretion but are often associated with adverse side effects. This has intensified the search for natural, safer alternatives with comparable efficacy. Moringa oleifera, a highly valued plant with an extensive history in traditional medicine, has shown promising antidiabetic properties. This research explores the potential of Moringa oleifera-derived phytochemicals as natural DPP-4 inhibitors using in-silico molecular docking techniques. The study identifies and evaluates the binding affinities of these phytochemicals to the DPP-4 enzyme, aiming to predict their inhibitory potential and minimal side effects, thereby contributing to the development of novel, safer hypoglycemic drugs. The results indicate the compounds' potential as effective DPP-4 inhibitors with promising hypoglycemic properties. Specifically, piceatannol exhibited the highest binding affinity of -7.9 kcal/mol, whereas vanillic acid had the lowest at -5.7 kcal/mol. This study underscores the potential of *Moringa oleifera* as a natural, safer alternative in the development of novel anti-diabetic drugs, contributing to the growing body of research on plant-based therapeutics for diabetes management.

Keywords: Moringa oleifera, Phytochemicals, Diabetes Mellitus, Molecular Docking,

INTRODUCTION

Diabetes mellitus is a category of metabolic illnesses that cause hyperglycemia due to abnormalities in insulin secretion or activity[1]. DM is categorized into types 1 and type 2, with type 2 being the most frequent affecting over 95% of diabetic patients, predominantly caused by obesity and physical inactivity [2][3]. According to the World Health Organization (WHO), diabetes affects around 422 million people worldwide and causes 1.5 million deaths annually (source: https://www.who.int/, accessed October 26, 2023). Diabetes symptoms include frequent urination, thirst, and increased hunger.

If left untreated, diabetes can cause cardiovascular disease, stroke, chronic kidney disease, eye damage, nerve damage, and mental retardation [4]. Glucagon-like peptide-1 (GLP-1), a hormone produced by enteroendocrine L cells, pancreatic cells, and the body's nervous system, offers therapeutic benefits in the treatment of type 2 diabetes [5]. It regulates insulin release, glucose homeostasis, stomach emptying, and appetite regulation, facilitating weight loss. GLP-1 interacts with the GLP-1 receptor (GPCRs) on pancreatic β-cells, causing enhanced glucose-stimulated insulin production through protein kinase A(PKA) and exchange protein directly activated by cAMP (EPAC2) [6][7].

Incretin hormones, including GLP-1 and glucose-dependent insulinotropic polypeptide (GIP), promotes insulin production but are deactivated by dipeptidyl peptidase-4 (DPP-4), a type II transmembrane glycoprotein that involved in various physiological and pathological processes in the body [8][9][10]. Inhibiting DPP-4 enzyme has been a therapeutic approach for the management of type 2 diabetes. Current DPP-4 inhibitors, such as sitagliptin, saxagliptin and alogliptin exhibit potent hypoglycemic effects but are associated with certain side effects, like hypersensitivity reactions, rashes, and gastrointestinal issues[11]. In response to this concern, Food and Drug Administration (FDA) has encouraged the development of safer DPP-4 inhibitor, possibly derived from plant-based compounds (FDA Drug Safety Communication, 2015). A new drug development is a lengthy and costly process, with natural products historically serving as a source of novel drugs [12]. Modern drug discovery today incorporates bioinformatics and virtual screening, allowing for the quick evaluation of potential molecules. [13][14]. Molecular docking, a component of structure-based drug design (SBDD), is a significant computational technique for predicting the interaction of prospective drugs with target proteins[15][16][17][18].

Moringa oleifera Lam, popularly known as the "Miracle tree" has a variety of pharmacological effects, including antidiabetic properties [19][20].The aim of the research is to explore the antidiabetic potential of Moringa oleifera phytochemicals through in silico analysis, with a focus on their interaction with the DPP-4 enzyme. By using molecular docking, the study seeks to identify active compounds that can lead to the development of new, safer hypoglycemic drugs with less adverse effects.

METHODOLOGY

Phytochemicals in the Study

50 phytochemicals with reported medicinal properties were selected from different literatures. All these phytochemicals were subjected to various physicochemical and pharmacokinetic analyses.

Physiochemical and Pharmacokinetics Properties Analysis Using SwissADME and FAFDrug4 software

The individual ADME behaviors of the phytocompounds from the Moringa Oleifera plant were estimated using the SwissADME software (www.swissadme.ch) of the Swiss Institute of Bioinformatics (http://www.sib.swiss), which was accessible through a web server that shows the SwissADME Submission page in Google. The simplified molecular input line entry system (SMILES) defines the list, which has one input molecule per line with multiple inputs. The results are displayed for each molecule in tables and an Excel spreadsheet [21]. The shortlisted phytocompounds from SwissADME were also re-screened using FAFDrug4 software for the thorough Physiochemical and Pharmacokinetic Properties analysis.

Biological Activity predictions Using PASS Tool

The PASS (prediction of activity spectra for substances) tool allows for the investigation of potential bi-

ological characteristics of substances based on their chemical formula. It makes use 2D molecular fragments called multilevel neighbors of atoms (MNA) descriptors, which demonstrate how a chemical substance's molecular structure affects its biological activity. It calculates the prediction score for biological qualities using the ratio of 'probability to be active (Pa)' to 'probability to be inactive (Pi)'. A greater Pa indicates that a compound's biological feature is more likely to occur [22]

Biological Targets Prediction Using SwissTargetPrediction and Similar Ensemble Approach

SwissTargetPrediction website **(http://www.swisstargetprediction.ch)** was accessed. The molecule of interest was entered directly by pasting the molecule's SMILES string into the provided input field. After submitting the query by clicking the "Predict Targets" button, the tool examines the data and generates a list of expected protein targets. These targets are prioritized based on their probability scores, and each prediction includes detailed information such as the target name, uniprot ID, ChEMBL ID, Target class, associated organism, and probability score for the molecule

Changes in Gene Expression Profile Prediction Using DIGEP-Pred

DIGEP-Pred is a web-based tool that uses a compound's structural formula to predict in silico, how a drug and other chemical compounds would alter a gene expression profile. Prediction of Activity Spectra for Substances (PASS) program, accessible at http://www.way2drug.com/GE, was utilized to ascertain structure-activity relationships for the purpose of predicting drug-induced gene expression profiles. The training sets developed using data on drug-induced changes in gene expression profiles obtained from the Comparative Toxicogenomics Database (CTD), **https://ctdbase.org/**, serve as the foundation for the PASS Online software's prediction of drug-induced change in gene expression profiles of therapeutic candidates. When the compound's SMILE was pasted into the designated area and the software was run, the mRNA-based prediction result was shown, indicating both the up- and down-regulation of several genes do to their interaction with the potential drug candidate.

Ligand Preparation for Docking Analysis

OpenBabel version 2.4.1 was used to convert the 3D structures of the screened ligands (phytochemicals) from SDF format, which was obtained from the PubChem database, to PDB format. The ligands are subsequently converted to PDBQT format using the docking software **AutoDockTools version 1.5.7**. Lastly, the ligand structures were validated using PyMOL (version 3.0.3, Windows-x86_64), a molecular visualization software to ensure that there were no missing atoms, improper bonds, or other error.

Preparation of Protein for Docking Process

Protein 3D structure has been downloaded from the Protein Data Bank (PDB). The protein structure was then cleaned by eliminating any non-essential components, such as native ligands, water, and ions, which could interfere with docking. The missing atoms, polar hydrogen, and charges were added to achieve optimal binding affinity between protein and ligand. The protein was eventually saved in PDBQT format for docking. All protein processing was done using **AutoDockTools version 1.5.7**.

Protein-Ligand Docking using AutoDock Vina (version 1.5.7)

Following protein and ligand preparation, the grid box parameters were fully set up, covering the protein's active site, size, and xyz coordinates (blind docking). The AutoDock Vina was then run; it used a scoring system to assess possible ligand conformations within the binding region. The AutoDock eventually generated nine different ligand poses in the target cavities, with their affinity binding score evaluated in (Kcal/mol).

Analysis of Docked Models

The interaction between the ligands (i.e., phytochemicals) and the DPP-4 protein (4LKO) was examined using Discovery Studio **v24.1.0.23298** and PyMOL **v3.0.3**. Hydrogen and hydrophobic bonding interactions among others were all taken into consideration.

RESULTS AND INTERPRETATION

Table 1: Physiochemical Properties and Pharmacokinetic Parameters of Chemical Compounds (SwissADME and FAFDrug4)

Compound Properties	Ligands					
	Caffeic Acid	Gallic Acid	Vanillic Acid	Zeatin	Syringic acid	Piceatannol
Molecular Weight	180.16	170.12	168.15	219.24	198.17	244.24
Heavy atoms	13	12	12	16	14	18
Fraction Csp3	0	0	0.12	0.3	0.22	0
Rotatable bonds	2	1	2	4	3	2
H-bond acceptors	4	5	4	4	5	4
H-bond donors	3	4	2	3	2	4
MR	47.16	39.47	41.92	60.91	48.41	69.9
XLOGP3	1.15	0.7	1.43	0.67	1.04	2.86
WLOGP	1.09	0.5	1.1	0.51	1.11	2.46
ESOL Solubility (mg/ml)	2.32E+00	3.90E+00	1.60E+00	3.69E+00	2.84E+00	7.42E-02
ESOL Log S	-1.89	-1.64	-2.02	-1.77	-1.84	-3.52
GI absorption	High	High	High	High	High	High
BBB permeant	No	No	No	No	No	No
Pgp substrate	No	No	No	No	No	No
CYP2D6 Inhibitor	No	No	No	No	No	No
Lipinski violations	0	0	0	0	0	0
Ghose violations	0	2	0	0	0	0
Veber violations	0	0	0	0	0	0
Egan violations	0	0	0	0	0	0
Muegge violations	1	1	1	0	1	0
Bioavailability Score	0.56	0.56	0.85	0.55	0.56	0.55
Lead likeness violations	1	1	1	1	1	1
Synthetic Accessibility	1.81	1.22	1.42	2.54	1.7	2.09
Oral_Bioavailability_VEBER	Good	Good	Good	Good	Good	Good

Oral_Bioavailability_EGAN	Good	Good	Good	Good	Good	Good
Flexibility	0.2	0.13	0.22	0.27	0.3	0.13
Acceptability	Accepted	Accepted	Accepted	Accepted	Accepted	Accepted

Table 2: Biological Activities of Phytochemical Compounds

S. No.	Compounds	Biological Activity (Pa>Pi. Pa>0.7)		
		Pa	Pi	Activity
1	Caffeic Acid	0.977	0.001	Feruloyl esterase inhibitor
		0.945	0.003	Mucomembranous protector
		0.940	0.001	4-Hydroxybenzoate 3-monooxygenase inhibitor
		0.940	0.002	Benzoate 4-monooxygenase inhibitor
		0.882	0.002	Benzoylformate decarboxylase inhibitor
		0.881	0.002	Pyruvate decarboxylase inhibitor
		0.879	0.004	JAK2 expression inhibitor
		0.782	0.004	Antiseptic
		0.782	0.006	Vasoprotector
		0.711	0.014	Apoptosis agonist
2	Gallic Acid	0.955	0.002	Arylacetonitrilase inhibitor
		0.954	0.002	Chlordecone reductase inhibitor
		0.923	0.002	Fatty-acyl-CoA synthase inhibitor
		0.897	0.001	Aminobutyraldehyde dehydrogenase inhibitor
		0.885	0.003	Pterin deaminase inhibitor
		0.885	0.002	L-glutamate oxidase inhibitor
		0.770	0.027	Chymosin inhibitor
		0.782	0.016	CYP2J2 substrate
		0.770	0.027	Saccharopepsin inhibitor
3	Vanillic Acid	0.964	0.002	Chlordecone reductase inhibitor
		0.931	0.003	Feruloyl esterase inhibitor
		0.905	0.002	Preneoplastic conditions treatment
		0.898	0.003	Antiseptic
		0.885	0.015	Membrane integrity agonist
		0.871	0.004	JAK2 expression inhibitor
		0.834	0.003	Antimutagenic
		0.748	0.002	Urease inhibitor
		0.720	0.002	Antiinflammatory, intestinal
4	Syringic acid	0.949	0.002	Chlordecone reductase inhibitor
		0.907	0.004	Aldehyde oxidase inhibitor
		0.904	0.004	Sugar-phosphatase inhibitor
		0.874	0.002	Preneoplastic conditions treatment

		0.868	0.003	Spermidine dehydrogenase inhibitor
		0.868	0.004	Antiseptic
		0.821	0.004	Antimutagenic
		0.747	0.032	Saccharopepsin inhibitor
		0.728	0.044	Mucomembranous protector
		0.712	0.024	TP53 expression enhancer
5	Piceatannol	0.949	0.003	HIF1A expression inhibitor
		0.946	0.004	Membrane integrity agonist
		0.938	0.002	APOA1 expression enhancer
		0.936	0.003	Feruloyl esterase inhibitor
		0.921	0.003	JAK2 expression inhibitor
		0.892	0.005	Mucomembranous protector
		0.870	0.003	Antimutagenic
		0.824	0.007	Membrane permeability inhibitor
		0.801	0.008	Apoptosis agonist
		0.792	0.012	TP53 expression enhancer
6	Zeatin	0.910	0.003	DNA-(apurinic or apyrimidinic site) lyase inhibitor
		0.816	0.005	Nucleotide metabolism regulator
		0.761	0.019	Mannotetraose 2-alpha-N-acetylglucosaminyltransferase inhibitor
		0.734	0.020	Glucose oxidase inhibitor
		0.728	0.021	Sphinganine kinase inhibitor
		0.707	0.016	Immunosuppressant
		0.704	0.014	ADP-thymidine kinase inhibitor
		0.713	0.029	Methylenetetrahydrofolate reductase (NADPH) inhibitor

Table 3: Possible Adverse & Toxic Effects of phytochemical Compounds

S. No.	Compound Name	Possible Adverse & Toxic Effects (Pa>Pi. Pa>0.7)			S. No.	Compound Name	Possible Adverse & Toxic Effects (Pa>Pi. Pa>0.7)		
		Pa	Pi	Activity			Pa	Pi	Activity
1	Caffeic Acid	0.902	0.004	Urine discoloration	2	Gallic Acid	0.939	0.003	Hematemesis
		0.885	0.005	Hematemesis			0.934	0.003	Ulcer, aphthous
		0.853	0.018	Shivering			0.873	0.005	Urine discoloration
		0.794	0.008	Gastrointestinal hemorrhage			0.855	0.009	Muscle weakness
		0.78	0.00	Hypercholesterole			0.85	0.01	Shivering

		7	5	mic			9	6	
		0.787	0.010	Panic			0.822	0.004	Hypercholesterolemic
		0.789	0.027	Diarrhea			0.824	0.021	Diarrhea
		0.747	0.029	Sweating			0.792	0.010	Panic
		0.716	0.008	Sensitization			0.793	0.019	Reproductive dysfunction
		0.711	0.015	Hypomagnesemia			0.777	0.012	Hyperglycemic
		0.703	0.036	Hepatotoxic			0.766	0.008	Hyperuricemia
3	**Vanillic Acid**	0.910	0.003	Gastrointestinal hemorrhage	**4**	**Syringic acid**	0.907	0.003	Gastrointestinal hemorrhage
		0.899	0.004	Hematemesis			0.897	0.004	Hematemesis
		0.878	0.009	Acidosis, metabolic			0.885	0.008	Acidosis, metabolic
		0.860	0.003	Hypercholesterolemic			0.835	0.003	Hypercholesterolemic
		0.864	0.008	Postural (orthostatic) hypotension			0.835	0.024	Shivering
		0.824	0.008	Urine discoloration			0.797	0.014	Muscle weakness
		0.825	0.028	Shivering			0.799	0.019	Weakness
		0.789	0.007	Withdrawal			0.793	0.016	Postural (orthostatic) hypotension
		0.785	0.015	Muscle weakness			0.728	0.005	Spermicide
		0.772	0.029	Diarrhea			0.741	0.022	Nail discoloration
		0.755	0.013	Apnea			0.745	0.027	Reproductive dysfunction
5	**Piceatannol**	0.916	0.003	Urine discoloration	**6**	**Zeatin**	0.808	0.006	Thrombocytopoiesis inhibitor
		0.88	0.00	Hematemesis			0.81	0.02	Pure red cell

		6	4				6	1	aplasia
		0.885	0.009	Shivering					
		0.872	0.004	Panic					
		0.823	0.004	Hypercholesterolemic					
		0.826	0.012	Postural (orthostatic) hypotension					
		0.798	0.007	Gastrointestinal hemorrhage					
		0.771	0.003	Acidosis, lactic					
		0.746	0.017	Occult bleeding					
		0.742	0.015	Apnea					
		0.723	0.007	Irritation					

Table 4: Target Molecules for phytochemical Compounds

S. No.	Compound Name	Target	Common Name	Uniprot ID	ChEMBL ID	Target Class	Probability
1	**Caffeic Acid**	Carbonic anhydrase II	CA2	P00918	CHEMBL205	Lyase	0.739303938
		Arachidonate 5-lipoxygenase	ALOX5	P09917	CHEMBL215	Oxidoreductase	0.739303938
		Carbonic anhydrase VII	CA7	P43166	CHEMBL2326	Lyase	0.739303938
		Carbonic anhydrase I	CA1	P00915	CHEMBL261	Lyase	0.739303938
		Carbonic anhydrase VI	CA6	P23280	CHEMBL3025	Lyase	0.739303938
2	**Gallic Acid**	Carbonic anhydrase II	CA2	P00918	CHEMBL205	Lyase	0.999408275
		Carbonic anhydrase VII	CA7	P43166	CHEMBL2326	Lyase	0.999408275
		Carbonic anhydrase I	CA1	P00915	CHEMBL261	Lyase	0.999408275

		Carbonic anhydrase III	CA3	P07451	CHEMBL2885	Lyase	0.999408275
		Carbonic anhydrase VI	CA6	P23280	CHEMBL3025	Lyase	0.999408275
3	**Vanillic Acid**	Carbonic anhydrase II	CA2	P00918	CHEMBL205	Lyase	0.324627824
		Carbonic anhydrase VII	CA7	P43166	CHEMBL2326	Lyase	0.324627824
		Carbonic anhydrase I	CA1	P00915	CHEMBL261	Lyase	0.324627824
		Carbonic anhydrase XII	CA12	O43570	CHEMBL3242	Lyase	0.324627824
		Carbonic anhydrase XIV	CA14	Q9ULX7	CHEMBL3510	Lyase	0.324627824
4	**Syringic acid**	Carbonic anhydrase II	CA2	P00918	CHEMBL205	Lyase	1
		Carbonic anhydrase VII	CA7	P43166	CHEMBL2326	Lyase	1
		Carbonic anhydrase I	CA1	P00915	CHEMBL261	Lyase	1
		Carbonic anhydrase III	CA3	P07451	CHEMBL2885	Lyase	1
		Carbonic anhydrase VI	CA6	P23280	CHEMBL3025	Lyase	1
5	**Piceatannol**	Tyrosine-protein kinase LCK	LCK	P06239	CHEMBL258	Kinase	0.77053886
		Tyrosine-protein kinase SYK	SYK	P43405	CHEMBL2599	Kinase	0.77053886
		Cyclooxygenase-1	PTGS1	P23219	CHEMBL221	Oxidoreductase	0.561857771

Table 5: Cellular Gene Expressions Induce by the Phytochemical Compounds

S. No.	**Compound Name**	**(Pa>Pi. Pa>0.7)**			
		Pa	**Pi**	**Genes**	**Gene Expression**
1	**Caffeic Acid**	0.923	0.01	FTL	Up Regulation
		0.903	0.006	NQO1	Up Regulation

		0.897	0.014	PTGR1	Up Regulation
		0.884	0.016	OGG1	Up Regulation
		0.882	0.014	VASP	Up Regulation
		0.881	0.026	POR	Up Regulation
		0.798	0.033	CORO1C	Up Regulation
		0.791	0.038	GCLC	Up Regulation
		0.873	0.028	NOP56	Down Regulation
		0.831	0.021	CCDC93	Down Regulation
		0.831	0.021	STX1A	Down Regulation
		0.833	0.038	DEK	Down Regulation
		0.797	0.005	TBL1XR1	Down Regulation
		0.789	0.005	AK2	Down Regulation
		0.792	0.009	UBE2M	Down Regulation
2	**Gallic Acid**	0.839	0.033	SERPINB1	Up Regulation
		0.824	0.022	CCDC93	Up Regulation
		0.824	0.022	STX1A	Up Regulation
		0.824	0.027	ITGAV	Up Regulation
		0.807	0.037	GSS	Up Regulation
		0.794	0.028	TEFM	Up Regulation
		0.78	0.029	CAPRIN1	Up Regulation
		0.774	0.032	SLC30A1	Up Regulation
		0.893	0.015	PTGR1	Down Regulation
		0.879	0.021	AKR1B10	Down Regulation
		0.878	0.027	FTL	Down Regulation
		0.864	0.017	FECH	Down Regulation
		0.853	0.008	PLA2G6	Down Regulation
		0.86	0.022	OGG1	Down Regulation
		0.79	0.015	EP300	Down Regulation
3	**Vanillic Acid**	0.942	0.004	PTGR1	Up Regulation
		0.938	0.005	AKR1B10	Up Regulation
		0.934	0.008	FTL	Up Regulation
		0.929	0.004	FECH	Up Regulation
		0.88	0.009	PGD	Up Regulation
		0.875	0.016	VASP	Up Regulation
		0.839	0.009	GCLM	Up Regulation
		0.796	0.021	PLA2G6	Up Regulation
		0.897	0.014	SERPINB1	Down Regulation
		0.839	0.034	C6ORF48	Down Regulation
		0.83	0.05	H1FX	Down Regulation
		0.756	0.005	AKR1B1	Down Regulation
		0.756	0.033	TOB1	Down Regulation
		0.748	0.029	E2F5	Down Regulation

		0.741	0.028	KLF10	Down Regulation
4	**Syringic acid**	0.917	0.008	PTGR1	Up Regulation
		0.911	0.010	AKR1B10	Up Regulation
		0.902	0.006	FECH	Up Regulation
		0.907	0.015	FTL	Up Regulation
		0.863	0.019	VASP	Up Regulation
		0.834	0.017	PGD	Up Regulation
		0.788	0.024	GCLM	Up Regulation
		0.819	0.072	AURKA	Up Regulation
		0.866	0.022	SERPINB1	Down Regulation
		0.829	0.037	C6ORF48	Down Regulation
		0.740	0.006	AKR1B1	Down Regulation
		0.748	0.036	TOB1	Down Regulation
		0.724	0.031	KLF10	Down Regulation
		0.767	0.079	H1FX	Down Regulation
		0.719	0.049	CCDC93	Down Regulation
5	**Zeatin**	0.817	0.051	LST1	Up Regulation
		0.707	0.006	PIR	Up Regulation
		0.737	0.063	SQLE	Up Regulation
		0.757	0.037	SLC15A1	Down Regulation
6	**Piceatannol**	0.935	0.004	TCF12	Up Regulation
		0.932	0.002	EP300	Up Regulation
		0.925	0.006	OGG1	Up Regulation
		0.918	0.003	PLA2G6	Up Regulation
		0.896	0.005	GDPD5	Up Regulation
		0.887	0.004	FGFR1	Up Regulation
		0.889	0.006	ERBB2	Up Regulation
		0.728	0.093	MLPH	Up Regulation
		0.933	0.005	MANSC1	Down Regulation
		0.924	0.005	WWP2	Down Regulation
		0.929	0.011	C9ORF40	Down Regulation
		0.920	0.003	RNFT2	Down Regulation
		0.895	0.005	SAT	Down Regulation
		0.894	0.004	GPLD1	Down Regulation
		0.892	0.004	ITGB8	Down Regulation

Table 6: Molecular Docking Analysis with Different Visualization Software (Discovery Studio and PyMOL)

Docking Software	Visualization Software	Protein	Ligand	Binding Affinity (Kcal/mol)	Amino Acid Residue with H-Bond Interaction	Amino Acid Residue with Hydrophobic and other Interaction (A & B are Protein Chains)
Auto Dock Vina	Discovery Studio	4LKO 4LKO	Caffeic Acid	-6.7	PRO510A, ASN562A, THR565A	LYS512A, PHE559A
			Gallic Acid	-6.4	GLN123A, GLU205A, ASN710A, ASP739A	ARN125A, ASP709A
			Vanillic Acid	-5.7	PRO475A	LYS512A, PHE559A
			Zeatin	-6.3	ASN74A, GLU91A, ASN92A, ASP96A	ILE76A, LEU90A, ILE102A
			Syringic acid	-6.1	PRO475A, ARG560A, THR565A	MET509A, PRO510A, LYS512A, ILE529A, VAL558A, PHE559A
			Piceatannol	-7.9	PRO475A, PRO510A, GLN527A	LYS512A, ILE529A, PHE559A, ARG560A
			Alogliptin	-6.6	ASP501A	LEU477A, LEU504A, PHE559A
	PyMOL		Caffeic Acid	-6.7	ASN562A, PRO510A	LYS512A, PHE559A, PRO475A
			Gallic Acid	-6.4	ARG125A, LYS122A, ASP739A, ASN710A, GLU205A	TRP205A, TRP124A

			Vanillic Acid	-5.7	PRO475A, ARG560A	PHE559A, LYS512A
			Zeatin	-6.3	GLU91A, ASP96A, ASN92A	LEU90A, ILE76A, ILE102A, ASN74A
			Syringic acid	-6.1	ARG560A, THR565A, PRO475A	PHE559A, ILE529A, LYS512A, VAL558A, PRO510A
			Piceatannol	-7.9	GLN527A, PRO510A, PRO475A	LYS512A, ILE529A, PHE559A, ARG560A, ASP556A

SwissADME and FAFDrug4 were used to assess the physicochemical characteristics and pharmacokinetic parameters of six phytochemicals: caffeine, gallic acid, vanillic acid, zeatin, syringic acid, and piceatannol. Zeatin has the greatest molecular weight among all, ranging from 168.15 to 244.24 g/mol. All the compounds, except piceatannol, which was the least soluble and most lipophilic, showed a moderate degree of flexibility and favorable lipophilicity. There is a lower chance of drug-drug interactions because none of the substances were predicted to inhibit CYP2D6 or cross the blood-brain barrier. Lipinski's rule of five was followed by all substances, showing their potential for oral administration due to their good gastrointestinal absorption and bioavailability.

The chemicals exhibited diverse inhibitory actions on enzymes, namely in pathways related to metabolism and cancer. Vanillic acid and syringic acid showed antibacterial qualities, whereas caffeic acid and piceatannol showed significant anti-cancer potential. But there were also possible side effects, including weakening in the muscles, hypercholesterolemia, and gastrointestinal problems. Zeatin, in particular, had a noticeable impact on platelet production.

Similar binding patterns were found in the protein-ligand interactions examined with PyMOL and Discovery Studio; piceatannol showed the highest binding affinity (-7.9 kcal/mol) with 4LKO protein.

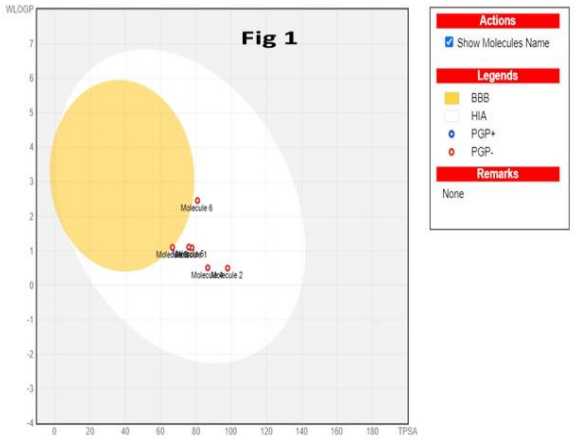
DISCUSSION

This study evaluated 50 prospective phytocompounds from various sections of the *Moringa Oleifera* plant, which include leaves, flowers, stems, roots, and pods, that appear to have medicinal applications and have been extracted from various literatures [23][24]. They were assessed for their physiochemical and pharmacokinetic properties. Therefore, using Lipinski's "rule of five," several phytochemicals with less reasonable physicochemical features were removed. This screening process identified 6 promising drug candidates (Table 1), all having molecular weights less than 500 Da, which is a prime property of drug-likeness.

Zeatin has the highest fraction of sp^3 hybridized carbons (0.3) and the most rotatable bonds (4), indicating more molecular flexibility and potential for binding interactions. The compounds generally have a low fraction of sp^3 carbons, which aligns with findings showing that increasing sp^3 hybridization can increase drug-likeness by decreasing planarity [25]. The majority of the compounds contain a

similar number of hydrogen bond acceptors and donors, which aids in their high gastrointestinal absorption and favorable traits for oral bioavailability. Piceatannol, which has the most hydrogen donors (4), may have better interaction with biological targets, though its highest logP (2.86) suggests increased hydrophobicity, potentially affecting solubility and permeability [26], [27]. They do not penetrate the barrier between blood and brain (BBB), which is beneficial for non-CNS-targeted therapies because it minimizes the possibility of CNS-related side effects [28]. In addition, none of them inhibit CYP2D6 activity; according to Li et al. (2019) [29], inhibiting CYP2D6 can alter the metabolism of other drugs, leading to drug-drug interactions and, which may eventually result in toxicity and other side effects in the body. All 6 candidates showed no violations of Lipinski's, Veber's, or Egan's Rule, though minor deviations were noted in Muegge's criteria.

Fig 1 depicts a BOILED-Egg model that can be utilized to evaluate passive gastrointestinal absorption (HIA) and blood-brain barrier penetration (BBB). Five molecules caffeic acid, gallic acid, zeatin, syringic acid, and piceatannol, are completely within the white region of the BOILED-Egg, suggesting high gastrointestinal tract absorption. However, vanillic acid lightly touched the yellow egg yolk of the BOILED-Egg, indicating a very minimal likelihood of Blood-Brain Barrier penetration (BBB). All compounds are colored red, implying non-substrate status for P-glycoprotein. This means that, as predicted, these compounds will be readily absorbed into the [30]. While Fig. 2 depicts the bioavailability radar profile of the six compounds. The red lines indicate how well each molecule meets the optimal bioavailability requirements for each of those qualities[31].



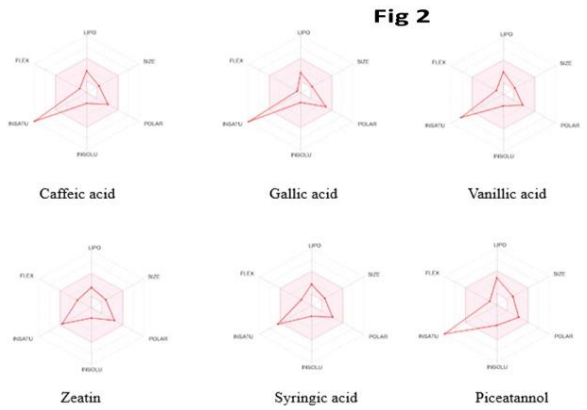


Fig 1: Schematic representation of perceptive evaluation of intestinal absorption (HIA) and Brain permeation (BBB) with molecules in the WLOGP-versus-TPSA using BOILED-Egg.

Fig 2: Bioavailability radar system for Drug Likeness of Molecules (lipophilicity: XLOGP3 between -0.7 and +5.0, size: MW between 150 and 500 g/mol, polarity: TPSA between 20 and 130 A2, solubility: log S not greater than 6, saturation: fraction of carbons in the sp3 hybridization not less than 0.25, and flexibility: no more than 9 rotatable bonds).

PASS (Prediction of Activity Spectra for Substances) software analysis (Table 2) revealed that all 6 phytocompounds exhibit strong inhibitory activities against various enzymes, including feruloyl esterase, 4-hydroxybenzoate 3-monooxygenase, pyruvate decarboxylase, arylacetonitrilase esterase, chlordecone reductase, glucose oxidase, fatty acyl-CoA synthase, and several dehydrogenases at probability of activity Pa>0.7. This is a promising hint that some of these drug candidates may limit the biological activity of the DPP-4 enzyme, a key target in diabetes management. Certain features, such as antioxidant activity, metabolic control, glucose oxidase inhibition, and anti-inflammatory properties that

have a major role in diabetes mellitus management were all considered[32]. Additionally, compounds like piceatannol and syringic acid have shown antimutagenic properties and the ability to increase TP53 expression, indicating possible anticancer activities [33]. The role of zeatin as a DNA-(apurinic or apyrimidinic site) lyase inhibitor further highlights its potential in DNA repair and cancer therapy [34]. Table 3 outlines the predicted adverse effects, with zeatin exhibiting the fewest side effects among the candidates at Pa>0.7 probability of activity.

Swiss Target Prediction (Table 4) identified several protein targets for the compounds. Caffeic and gallic acids showed a high affinity for carbonic anhydrases (CA2, CA7, CA1, CA3, CA6), with gallic acid demonstrating a particularly high interaction (Pa = 0.999). Syringic acid has a perfect probability score (1.0) for these enzymes, suggesting a great potential to influence carbonic anhydrase-related pathways. Piceatannol targets tyrosine-protein kinases LCK and SYK (Pa = 0.77) and cyclooxygenase-1 (PTGS1, Pa = 0.56), suggesting possible multi-target effects. In contrast, vanillic acid showed a lower probability (0.32) of interacting with the same carbonic anhydrases, though it may still interact [35].

DIGEP-Pred analysis (Table 5) demonstrated significant changes in gene expression caused by the phytocompounds, especially those associated with diabetes mellitus. Caffeic acid increased the expression of genes associated with cellular defense and oxidative stress responses, implying that it may have a role in decreasing diabetes implications.STX1A and DEK, on the other hand, were downregulated, potentially influencing metabolic or signaling pathways involved in disease progression. Gallic acid regulates gene upregulation (SERPINB1) and downregulation (PTGR1), indicating anti-inflammatory and metabolic regulatory functions. Vanillic acid increases the expression of genes relevant to glucose metabolism, such as PTGR1 and VASP. This could be linked to its role in metabolic and cellular stress pathways [36]Syringic acid increased AKR1B10 and FECH expression, which is associated with its regulatory effects on the metabolism of lipids and oxidative stress control, both of which are essential in enhancing insulin sensitivity [36]. Zeatin predominantly upregulated LST1, PIR, and SQLE, genes related to immunological responses, transcriptional regulation, and cholesterol synthesis, while downregulating SLC15A1; indicating impacts on nutrition absorption pathways important for diabetes management. Piceatannol greatly upregulated OGG1 and EP300, genes involved in DNA repair and epigenetic regulation, respectively, while downregulating SAT and GPLD1, which play roles in lipid and glycemic control processes, presenting novel therapeutic possibilities [37][38].

Table 6 reveals the result of phytochemicals, along with Alogliptin, were docked with the DPP-4 protein (PDB ID: 4LKO) using an AutoDockVina. Table 6 displays the binding affinity of each ligand, as well as the amino acid residues that established hydrogen and hydrophobic interactions with them. The visualization was done using Discovery Studio and PyMOL. All the phytocompounds showed a binding affinity for the 4LKO protein (Figure 3-14). Alogliptin, a pre-existing synthetic DPP-4 enzyme inhibitor, was employed as a positive control [39]. The binding affinities range from -5.7 kcal/mol (vanillic acid) to -7.9 kcal/mol (piceatannol), demonstrating different degrees of interaction intensity with the protein. Alogliptin, the positive control, has a binding affinity of -6.6 kcal/mol which is close to certain phytochemicals particularly caffeic acid and gallic acid (Table 4.6). Protein-ligand interactions rely heavily on hydrogen bonds to stabilize the complex through specific, non-covalent interactions. They improve binding affinity and specificity by enabling the ligand's precise positioning within the protein's binding site [40].

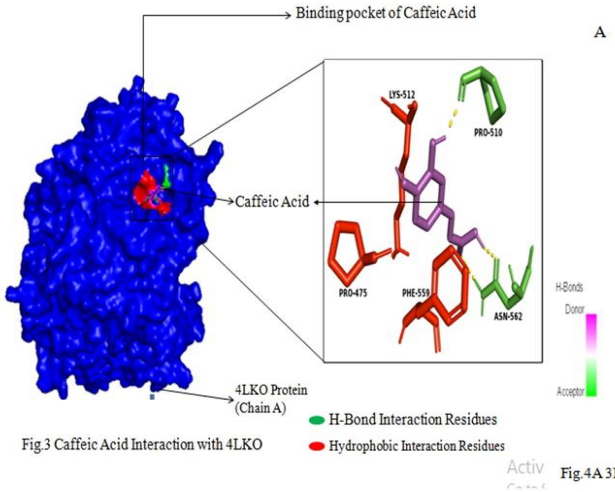


Fig.3 Caffeic Acid Interaction with 4LKO

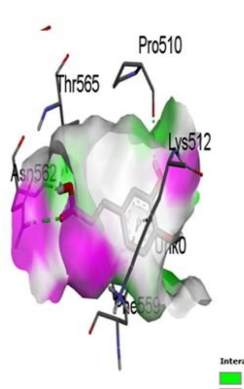


Fig.4A 3D of Caffeic Acid Interaction with 4LKO

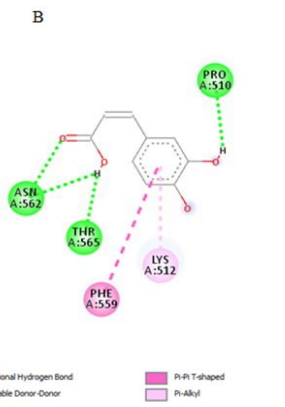


Fig.4B 2D of Caffeic Acid Interaction with 4LKO

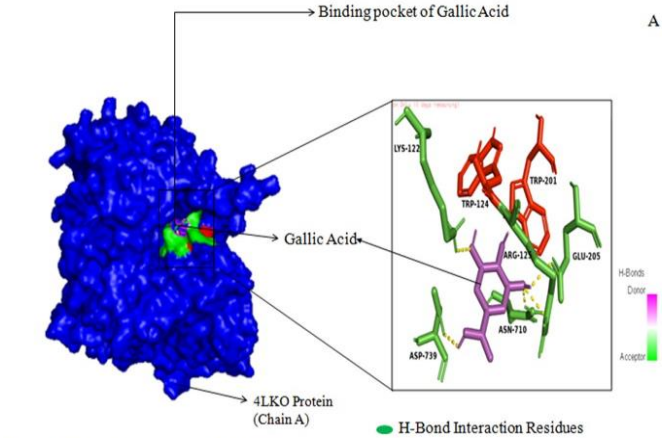


Fig.5 Gallic Acid Interaction with 4LKO

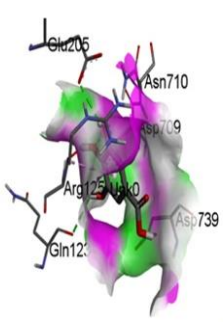


Fig.6A 3D of Gallic Acid Interaction with 4LKO

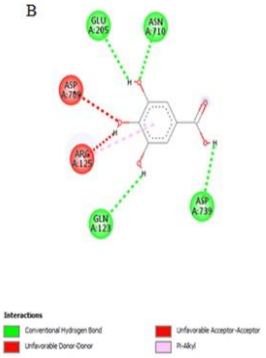


Fig.6B 2D of Gallic Acid Interaction with 4LKO

Residues such as PRO510A, ASN562A, GLU91A, and ASP739A appear to be participated in most of hydrogen bond-mediated ligands interaction. Hydrophobic and other interactions mainly include residues such as LYS512A, PHE559A, and ILE76A, highlighting their importance in ligand binding stability. None of the ligands showed identical interaction residues as alogliptin (Fig 15A & 15B), but all of the amino acids involved in the interaction are within the catalytic domain of the DPP-4 protein, which may be among residues that have inhibitory activity in the enzyme 4LKO protein [41]. Caffeic Acid and Gallic Acid have competing binding affinities (-6.7 and -6.4 kcal/mol, respectively) and form interactions that are stable similar to alogliptin, whereas Piceatannol has a higher binding affinity (-7.9 kcal/mol) than all the phytochemicals and alogliptin, implying that it may provide enhanced DPP-4 inhibition. This analysis points out that piceatannol and other phytochemicals could be effective DPP-4 inhibitors.

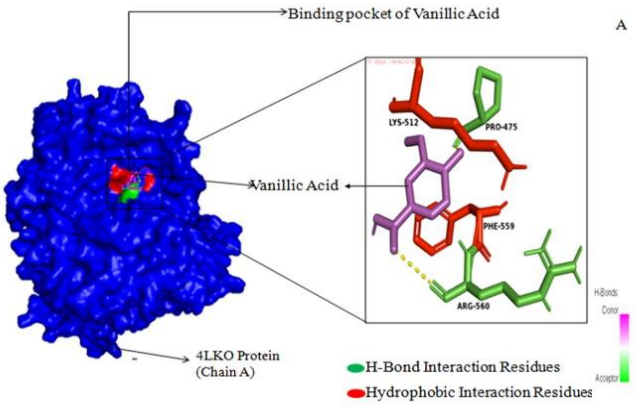


Fig.7 Vanillic Acid Interaction with 4LKO

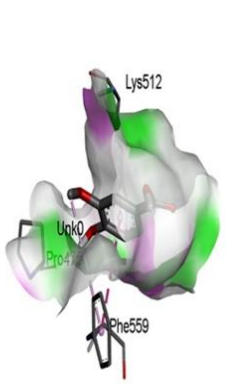


Fig.8A 3D of Vanillic Acid Interaction with 4LKO

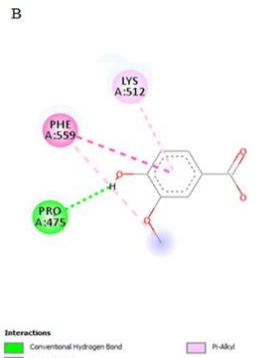


Fig.8B 2D of Vanillic Acid Interaction with 4LKO

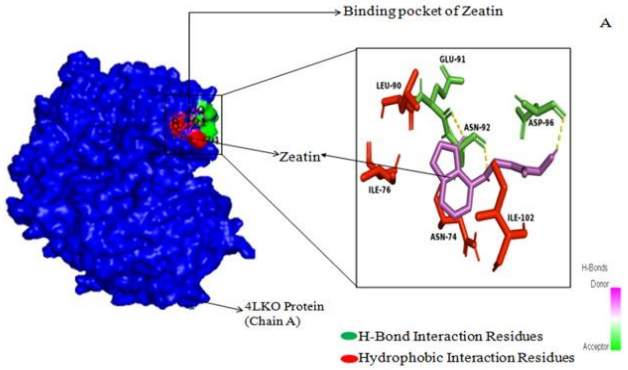


Fig.9 Zeatin Interaction with 4LKO

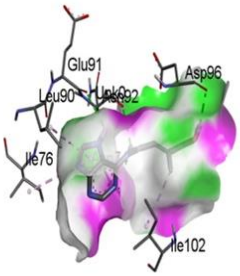


Fig.10A 3D of Zeatin Interaction with 4LKO

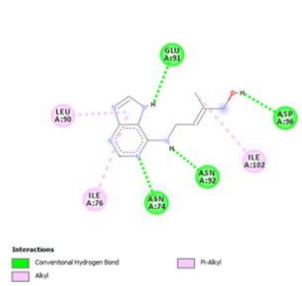


Fig.10B 2D of Zeatin Interaction with 4LKO

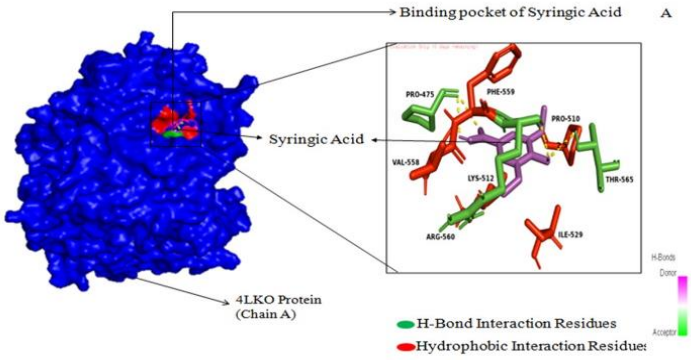


Fig.11 Syringic Acid Interaction with 4LKO

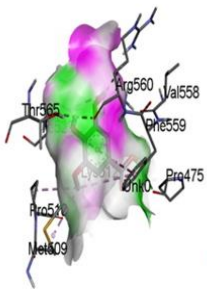


Fig.12A 3D of Syringic Acid Interaction with 4LKO

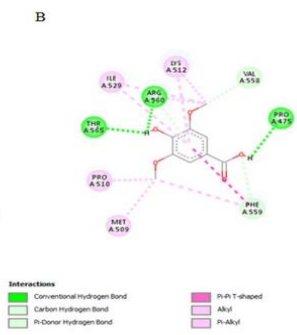


Fig.12B 2D of Syringic Acid Interaction with 4LKO

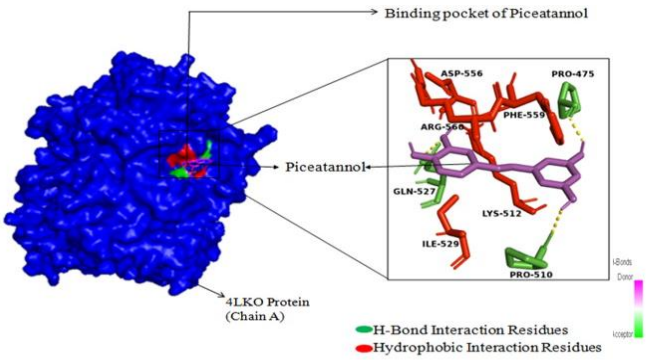


Fig.13 Piceatannol Interaction with 4LKO

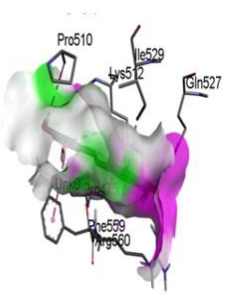


Fig.14A 3D of Piceatannol Interaction with 4LKO

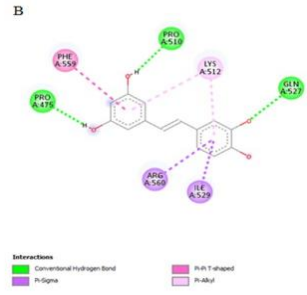


Fig.14B 2D of Syringic Acid Interaction with 4LKO

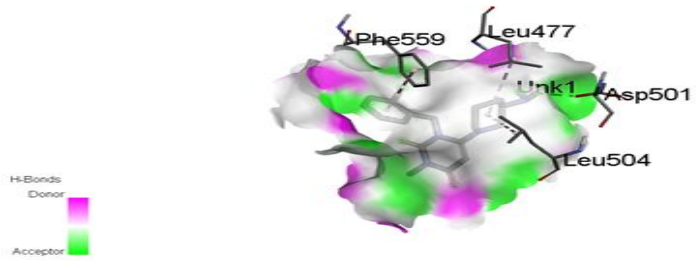


Fig.15A 3D of Alogliptin Interaction with 4LKO

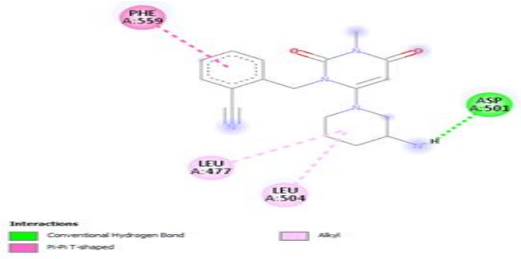


Fig.15B 2D of Alogliptin Interaction with 4LKO

Conclusion

According to this study, piceatannol from Moringa oleifera outperforms synthetic alogliptin in terms of binding affinity and anticipated efficacy, making it a promising inhibitor of the DPP-4 enzyme. These substances have advantageous drug-like characteristics, such as high solubility and minimal side effect risk. The study backs up the potential of Moringa oleifera in treating diabetes and recommends more research to validate these results and improve the solubility and bioavailability of the components for improved therapeutic application.

Conflict of Interest

There was no conflict of interest in this research study.

Acknowledgements

We would like to thank the management of Mewar University, Chittorgarh, Rajasthan, India, for their invaluable assistance with this research project.

Funding

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

Ethical Approval

Ethical approval is not applicable in this research study.

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