

In-silico screening of the plant-based compounds for diabetes from *Cinnamomum verum*

Ajay Kumar

CSJM University

Shweta Singh Verma

CSJM University

Shivam Kumar

S J Institute of Pharmacy

Jagat Pal Yadav

Sam Higginbottom University of Agriculture, Technology and Sciences

Ankit Kumar Singh

Sam Higginbottom University of Agriculture, Technology and Sciences

Mashooq Ahmad Bhat

King Saud University

Habibullah Khalilullah

Qassim University

Amita Verma

`amitaverma.dr@gmail.com`

Sam Higginbottom University of Agriculture, Technology and Sciences

Research Article

Keywords: In-silico, Protein-kinase, molecular docking, ADMET prediction, and diabetes mellitus

Posted Date: June 16th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-5057197/v1>

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

[Read Full License](#)

Additional Declarations: No competing interests reported.

In-silico* screening of the plant-based compounds for diabetes from *Cinnamomum verum

Ajay Kumar^{1*}, Shweta Singh Verma¹, Shivam Kumar², Jagat Pal Yadav³, Ankit Kumar Singh^{3,4}, Mashooq Ahmad Bhat⁵, Habibullah Khalilullah⁶, Amita Verma^{*3}

¹ School of Pharmaceutical Sciences, CSJM University, Kanpur, 208024, India.

² S J Institute of Pharmacy, Ramaipur, Kanpur Nagar, 209214, India.

³ Bioorganic and Medicinal Chemistry Research Laboratory, Department of Pharmaceutical Sciences, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, 211007 India

⁴ Department of Pharmaceutical Sciences, School of Health Sciences and Technology, University of Petroleum and Energy Studies (UPES), Dehradun, 248007, India

⁵ Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia.

⁶ Department of Pharmaceutical Chemistry and Pharmacognosy, College of Pharmacy, Qassim University, Buraydah, 51911, Saudi Arabia

Corresponding author

Prof. Dr. Amita Verma*

Bioorganic and Medicinal Chemistry Research Laboratory, Department of Pharmaceutical Sciences, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, 211007, India.

Email: amitaverma.dr@gmail.com, amita.verma@shiats.edu.in

ORCID: <https://orcid.org/0000-0001-6653-7335>

Dr. Ajay Kumar*

School of Pharmaceutical Sciences, CSJM University, Kanpur, 208024, India.

Email: ajaykumar@csjmu.ac.in

Abstract

Background: Diabetes mellitus remains a critical global health challenge, characterized by dysregulated insulin secretion and metabolic mismanagement. In 2019 alone, the disease was responsible for 1.5 million deaths, significantly impacting the population under 70 years of age and contributing to a high incidence of cardiovascular and renal complications. Adenosine monophosphate-activated protein kinase (AMPK) serves as a pivotal therapeutic target due to its role in enhancing glycolysis while inhibiting gluconeogenesis and glycogenolysis.

Methods: In this study, nine bioactive phytoconstituents from *Cinnamomum verum* were subjected to molecular docking against AMPK using AutoDock 1.5.6. Pharmacokinetic suitability was evaluated via ADMET profiling using the pkCSM server. To transition from static models to dynamic validation, comprehensive all-atom molecular dynamics (MD) simulations were conducted using GROMACS 2025. Triplicate 200 ns production runs (totaling 600 ns per complex) were performed for the top-scoring compounds—Oleic acid, Gallic acid, and Protocatechuic acid—under a 310 K temperature gradient and Nose-Hoover pressure coupling (1 bar). Structural stability was quantified through backbone RMSD, residual flexibility via RMSF, and polar interaction persistence using the *gmx hbond* utility, with data visualization executed in QtGrace.

Results: Molecular docking identified Oleic acid as the strongest binder with a binding energy of -6.6 kcal/mol, while Gallic and Protocatechuic acids exhibited slightly higher energies (-4.7 kcal/mol) but superior hydrogen bond density. ADMET predictions revealed that Gallic and Protocatechuic acids possess favorable pharmacokinetic profiles, whereas Oleic acid's high molecular weight and aliphatic nature suggest limited oral bioavailability. MD simulations corroborated these findings, showing that while the AMPK–Oleic acid complex was stable (average RMSD 0.38 nm), it relied on transient hydrogen bonds (1–2 bonds). In contrast, Gallic and Protocatechuic acids formed highly rigid complexes (RMSD < 0.25 nm) stabilized by a persistent network of 3–6 hydrogen bonds with key residues such as ASN A:195 and ASN B:238. RMSF analysis confirmed that all three ligands effectively stabilized the catalytic core, with localized fluctuations remaining below 0.15 nm.

Conclusion: This integrated *in-silico* study demonstrates that while Oleic acid is a potent binder, Gallic acid and Protocatechuic acid emerge as the most promising therapeutic leads due to their superior dynamic stability, robust hydrogen bond persistence, and favorable ADMET properties. These phenolic compounds effectively "lock" the AMPK active site into a stable conformation, suggesting high inhibitory potential. Our findings identify these *Cinnamomum verum* derivatives as viable scaffolds for the development of novel, orally bioavailable AMPK activators for the management of hyperglycemia in Type 2 diabetes.

Keywords: *In-silico*, Protein-kinase, molecular docking, ADMET prediction, and diabetes mellitus.

1. Introduction: Insulin is the hormone that keeps blood glucose levels within normal ranges by stimulating processes that use glucose, like glycolysis and glycogenesis, and blocking those that produce glucose, including gluconeogenesis and glycogenolysis (Petersen and Shulman, 2002; Shepherd and Kahn, 1999). The pancreatic β -cell produces and secretes it to keep blood glucose levels within a specific range. Hypoglycemia results from excessive insulin secretion, whereas diabetes mellitus is linked to insufficient insulin secretion (Fonseca, 2003). In type 1 diabetes, there is a complete lack of insulin due to the loss of pancreatic β -cells, whereas in type 2 diabetes, peripheral resistance to insulin action and insufficient secretory response by pancreatic β -cells coexist (Janghorbani et al., 2007; Ozougwu et al., 2013). Treatment options for diabetes mellitus include insulin-sensitizing medications that target the nuclear receptor peroxisome proliferator-activated receptor (PPAR) γ (Janghorbani et al., 2007). Diabetes mellitus is caused by a combination of insulin resistance and β -cell failure (Ozougwu et al., 2013; Zaccardi et al., 2016). The majority of medications used to treat diabetes today try to increase the body's production of insulin, which is essential for controlling metabolism (Petersen and Shulman, 2002). For example, thiazolidinediones are insulin-sensitizing medications that aid in restoring insulin's sensitivity to glucose, whereas insulin secretagogues are insulin-secreting medications that function by activating pancreatic β -cells (Gulcin et al., 2019). Serious side effects including hypoglycemia, weight gain, anaemia, and congestive heart failure are associated with several of these oral antidiabetic medications (Kazeem and Davies, 2016). The hunt for safer and more effective antidiabetic medications is a critical step in managing diabetes and its consequences, given the high cost of insulin therapy and the negative effects of oral antidiabetic medications (Rozenfeld et al., 2008). Natural items, particularly food plants, have received a lot of interest lately as potential sources of safer and more effective antidiabetic medications (Kazeem and Davies, 2016). It has been demonstrated that certain plants used in traditional medicine include substances that can increase insulin sensitivity or insulin-signaling pathways, such as PPAR α and PPAR γ activity (Abdel-Moneim and Fayez, 2015; Dewanjee et al., 2020; Ungurianu et al., 2019). The enzyme 5' AMP-activated protein kinase, also known as AMPK or 5' adenosine monophosphate-activated protein kinase, is involved in maintaining the homeostasis of cellular energy (Raj et al., 2016). It primarily functions by promoting the uptake and oxidation of glucose and fatty acids during periods of low cellular energy. Our goal in this work is to create a novel phytoconstituent derived from *Cinnamomum verum* (Abdel-Moneim and Fayez, 2015; El-Desoky et al., 2012). It

demonstrated that *verum* had previously been utilized in South Asian folk medicine (Kamran et al., 2019; Mahmood et al., 2021; Puangpronpitag and Sittiwet, 2009; Rattanachaikunsopon and Phumkhachorn, 2010; Shori and Baba, 2011). On the basis of this, we developed the theory that the anti-diabetic action of the plants is due to phytoconstituents found on them (Jung et al., 2006; Rafiullah et al., 2006; Singab et al., 2014). We screened out several phytoconstituents found on them from various literature survey and choose the particular polyphenolic, flavonoids and acids (Gulcin et al., 2019; Neupane et al., 2022b; Pathak and Sharma, 2021). The purpose of selecting particularly those phytoconstituents is due to their affinity of forming hydrogen bonds with the amino acid's residue. The hydrogen plays the crucial role in the binding of the ligand in the protein binding pocket.

The ability of AMP-activated protein kinase (AMPK) to bind to AMP allows it to sense the state of cellular energy and regulate enzymatic activity in order to maintain the equilibrium between ATP production and consumption in eukaryotic cells (Fryer et al., 2002; Gruzman et al., 2009). Additionally, it plays crucial roles in the establishment and maintenance of cell polarity as well as the control of cell growth and proliferation (Hardie, 2011). Because of these crucial roles, AMPK is now a popular drug target for the treatment of cancer, type 2 diabetes, and obesity. The crystal structures of a phosphorylated kinase domain from *Saccharomyces cerevisiae* (Snf1-pKD) and an unphosphorylated fragment of the AMPK alpha-subunit (KD-AID) (Gruzman et al., 2009) from *Schizosaccharomyces pombe* that contains both the catalytic kinase domain and an autoinhibitory domain (**Fig. 1**) (AID) (Marx et al., 2010; Muller et al., 2005).

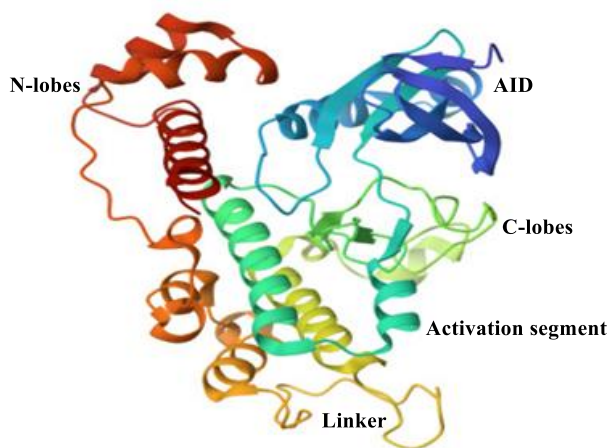


Figure 1. Schematic and surface representations of *S. pombe* KD-AID. The *N*- and *C*-lobes of the kinase domain are colored in red and green, respectively, the activation segment is in magenta, the linker is in yellow, and the AID is in blue.

2. Materials and Methods:

2.1 Preparation of Ligand: The ligands were selected by screening various previously published literature on Google Scholar (Abdalla and Abdelgadir, 2016; Adarsh et al., 2020; Al-Mijalli et al., 2023; Batiha et al., 2020; Bernard et al., 2014; Hameed et al., 2016; Singh et al., 2021) and assigned the correct 2D orientation in ChemDraw Ultra 6.0 (ChemOffice package), and each compound's structure was examined for connection errors in bond order. The chemical structures of all the compounds are listed in **Fig. 2**. After drawing the structure, we performed the energy minimization of the ligand using Chem 3D Ultra 6.0. Thus, prepared ligand structure are converted to .pdbqt form by using Autodock 1.5.6.

2.2 Preparation of protein: The crystal structure of pombe AMP-activated protein kinase (AMPK) protein (PDB ID: 3H4J) has been downloaded from protein data bank (PDB) (Marx et al., 2010) website and were prepared by using Autodock tools-1.5.6. To prepare protein, we have added Kollman charges to each atom, non-polar hydrogens were merged, atom types were determined. The more importantly, all the hetero-atoms and water was removed and some of the missing amino acid residue were fixed and grid box is generated around the active site of the amino acids with dimension of (60*60*60) in all the axis.

2.3 Molecular Docking: The receptor and ligand interaction through virtual molecular docking have been performed through AutoDock Tools-1.5.6. All the molecules have been docked to active site 1 of AMP-activated protein kinase (AMPK) receptor. Ligand-receptor interaction was viewed through Discovery Studio 2020 Client (BIOVIA) and re-docking of all the compounds was done to assure reproducibility (Neupane et al., 2021, 2022a).

2.4 ADMET Properties: The drug-likeness properties of the selected ligands from *Cinnamomum verum* were analyzed using the Swiss ADME server (Ootuechere et al., 2022). The physicochemical properties included Lipinski's rule of 5, molecular weight, log P_{o/w}, molar refractivity, TPSA, number of H-bond acceptor, donor, and rotatable bonds. Nine compounds selected from *Cinnamomum verum*, namely eugenol, pyrogallol, cinnamic acid, ferulic acid, caffeic acid, gallic acid, protocatechuic acid, oleic acid, and *p*-Hydroxybenzoic acid were selected based on previously reported phytochemical analysis and chemical identification of the plant. The pharmacokinetic parameters absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiles of the compounds were determined using pkCSM ADMET descriptors algorithm protocol.

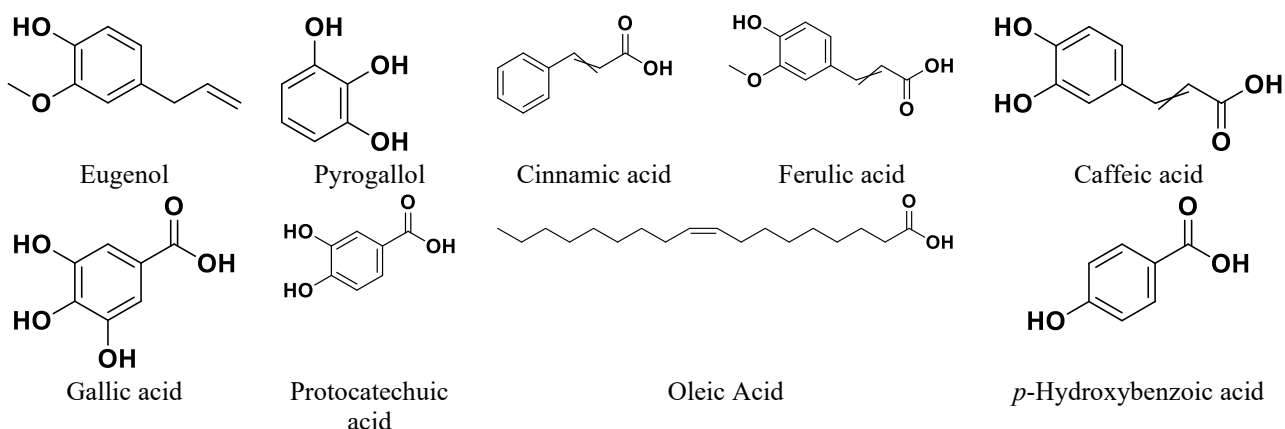


Figure 2: Phytoconstituents from *Cinnamomum verum* with chemical structures

2.5 Molecular Dynamics Simulations: To evaluate the structural stability and interaction dynamics of the AMPK complexes with Oleic acid (A), Gallic acid (B), and Protocatechuic acid (C), all-atom molecular dynamics (MD) simulations were performed. These three compounds were selected for MD analysis based on their superior hydrogen-bonding profiles and lowest binding energies obtained from docking studies.

All simulations were executed using the GROMACS 2025 package, employing the CHARMM36 all-atom force field for the protein and CGenFF (CHARMM General Force Field) for ligand parameterization. Each protein-ligand complex was solvated in a cubic box using the TIP3P water model, maintaining a minimum distance of 1.0 nm between the protein surface and the box edges. The systems were neutralized and adjusted to a physiological ionic strength of 0.15 M by adding Na^+ and Cl^- ions.

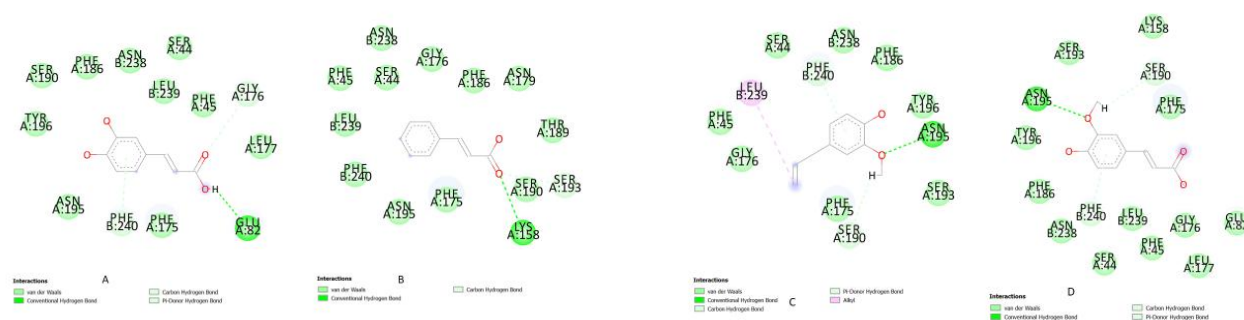
Energy minimization was carried out using the steepest descent algorithm until the maximum force converged below 1000 kJ/mol/nm. To ensure a stable starting configuration, equilibration was performed using a gradual temperature and pressure gradient. First, a 100 ps NVT ensemble was conducted to reach a target temperature of 310 K using the V-rescale thermostat. This was followed by a 100 ps NPT equilibration at 1 bar using the Nose-Hoover pressure coupling scheme. During these phases, position restraints were applied to all heavy atoms of the protein and ligand.

Production MD simulations were then conducted for 200 ns in triplicate (three independent runs per compound) to ensure statistical reproducibility, with initial velocities randomized for each replicate. A 2-fs integration timestep was utilized, and all covalent bonds involving hydrogen atoms were constrained using the LINCS algorithm. Long-range electrostatic interactions were

treated with the Particle Mesh Ewald (PME) method, while a 1.2 nm cutoff was applied for van der Waals and short-range Coulombic forces. Trajectories were analyzed using GROMACS built-in utilities, and all data visualization, including RMSD, RMSF, and H-bond plots, was performed using QtGrace to ensure high-resolution graphical representation.

3. Results and Discussion

3.1 Molecular Docking: The molecular docking was performed using the Autodock Tools 1.5.6 and result had validated by re-docking in the same receptor at similar condition. The molecular docking study showed that oleic acid has lowest binding energy (-6.6 Kcal/mol) with the AMP-activated protein kinase (AMPK) protein. However, oleic acid has only two hydrogen bonds that are GLU B: 42 and SER B: 44. Further, oleic acid has high molecular weight therefore, cell permeability and oral bioavailability have issues. Protocatechuic acid and gallic acid showed the strongest interaction in-terms of hydrogen bonding. These two compounds form three hydrogen bonds each with the amino acid residues. Protocatechuic acid formed hydrogen bonding with these three amino acids ASN A: 195, PHE B: 240, and ASN B: 238 and the gallic acid have ASN A: 179, SER A: 193, and ASN B: 238 amino acid interaction. The data showed that oleic acid has lowest binding energy, but higher molecular weight opt out as suitable drug choices. Therefore, protocatechuic acid (-4.7 Kcal/mol) and gallic acid (-4.7 Kcal/mol) based on binding pose with the AMPK protein makes it ideal choice to make as a drug for diabetes mellitus.



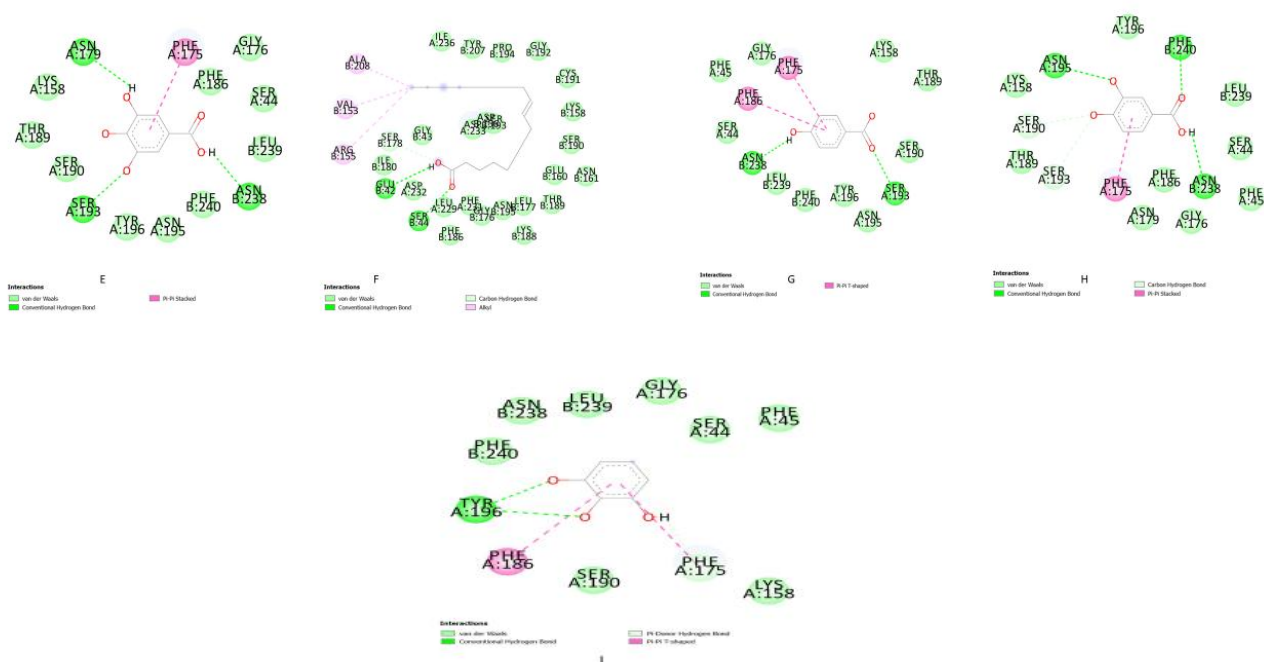


Figure 3. Schematic representation of the **A)** Caffeic acid with AMPK protein **B)** Cinnamic acid with AMPK protein **C)** Eugenol with AMPK protein **D)** ferulic acid with AMPK protein **E)** gallic acid with AMPK protein **F)** Oleic acid with AMPK protein **G)** *p*-hydroxybenzoic acid with AMPK protein **H)** Protocatechuic acid with AMPK protein **I)** Pyrogallol with AMPK protein

3.2 Ligand binding affinity with protein: Molecular docking study shows that oleic acid is the most potent inhibitor, however, it forms a smaller number of hydrogen bonds which is crucial because stability of other bonds is weak and can't rely completely. However, hydrogen bonds are stable in nature. Therefore, we selected the two compounds that have a greater number of hydrogen bonds i.e. protocatechuic acid and gallic acid. In our results, it showed that some of the amino acid residues from AMPK protein are common for various ligands. Therefore, we can predict that those amino acid are crucial for the binding of the ligand to our protein. Those amino acids are TYR A: 196, ASN B: 238, SER A: 193, ASN A: 195, SER B: 44, and SER A: 193. However, there are other amino acid residues that also play a crucial role in the binding of our molecules but based on common interaction on protein-ligand complexes. Further, **Table 1** illustrates the detailed insight of docking score, number of hydrogen bonds, and amino acids that form hydrogen bonds with respective ligands.

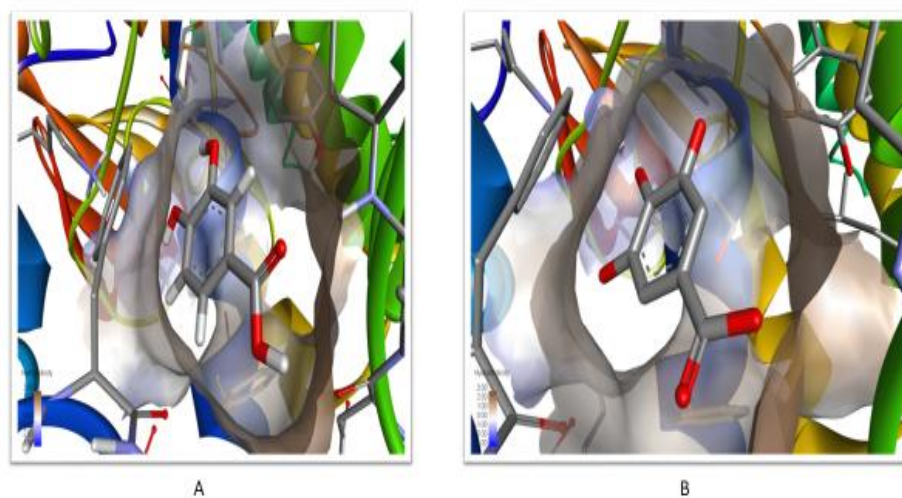


Figure 4: Three-dimensional interaction of the **A)** protocatechuic acid **B)** and gallic acid in the active site binding pose of the AMPK protein

Table 1: The docking score report of selected phytoconstituents from the *Cinnamomum verum* with h-bonding and interacting amino acid residues of crystal structure of pombe AMP-activated protein kinase (AMPK) protein (PDB ID: 3H4J).

S.N.	Name of compounds	Docking Score (- Kcal/mol)	Number of H-bonds	H-bond forming amino acid residues
1	Caffeic acid	4.7	1	GLU A: 82
2	Cinnamic acid	4.7	1	LYS A: 158
3	Eugenol	5.1	1	ASN A: 195
4	Ferulic acid	5.2	1	ASN A:195
5	Gallic acid	4.7	3	ASN A:179, SER A:193, and ASN B:238
6	Oleic Acid	6.6	2	GLU B: 42 and SER B:44
7	<i>p</i> -Hydroxybenzoic acid	4.6	2	ASN B:238 and SER A:193
8	Protocatechuic acid	4.7	3	ASN A:195, PHE B:240, and ASN B:238
9	Pyrogallol	4.2	2	TYR A:196 and TYR A:196

3.3 ADMET Predictions: Parameters such as water solubility, Caco-2 permeability, intestinal absorption, skin permeability, glycoprotein substrate, and glycoprotein inhibitor were used to predict the absorption pharmacokinetics. A log Papp coefficient >10⁻⁶ is an indication of high

Caco-2 permeability. Our observations revealed that gallic acid had the lowest Caco-2 permeability. Human intestinal absorption is an important ADMET prediction parameter that models the transport of xenobiotics to their ultimate targets. Intestinal absorption of less than 30% is considered poor (Sjögren et al., 2013). All selected ligands showed optimum absorption from the intestinal walls except gallic acid which showed about half intestinal absorption than other selected ligands. Moreso, ligands with less than 2.5 log P are considered to be readily permeable from the skin; hence, eugenol showed the highest skin permeability. P-glycoprotein, a major efflux pump in humans, is highly associated with the ADMET properties of drugs and drug candidates. Our results suggested that none of our compounds are substrates of p-glycoprotein, and they have no effect on p-glycoprotein type I and II inhibitors. Diabetes mellitus is a chronic metabolic disorder characterized by elevated levels of glucose (or blood sugar) in the blood (Abdel-Moneim and Fayez, 2015; Bnouham et al., 2006; Chaudhury et al., 2017). This condition occurs when the body cannot produce enough insulin or does not use the insulin properly. Insulin hormone produced by the pancreas plays the major role in the controlling of diabetes (Fonseca, 2003; Hawley et al., 2002; Janghorbani et al., 2007). In our study, we screened out the nine phytochemicals from the plant *Cinnamomum verum* and studied using the *In-silico* techniques. The molecular study showed that oleic acid has the lowest binding energy among all selected compounds while the bonding interactions showed that compound has very low binding affinity with the protein. Therefore, I went and performed the ADMET predictions and saw the physiochemical properties of the drug molecule. The ADMET parameters are important for the synthesis and behaviors of the compounds in the biological system. From the results, it showed that oleic acid has high molecular weight to permit the biological membrane system (Otuechere et al., 2023). Furthermore, partition coefficient of the drug is also quite low to formulate the drug in the dosages form. Based on overall molecular docking profile and ADMET properties, we selected the protocatechuic acid and gallic acid potential inhibitor to AMPK protein. This pathway helps to clear out blood glucose level by glycolysis and glycogenesis and further also prevent gluconeogenesis and glycogenolysis. The selection of these compounds is based on their docking scores, binding affinity of the compound with the protein and ADMET profile. **Fig.3** shows their binding interactions with different amino acids of the protein and **Fig.4** shows the three-dimensional pose of both ligands in the protein. Furthermore, **Table 2** shows ADMET properties. Based on our data, these compounds show significant activity against AMPK protein.

3.4 Molecular Dynamics Simulations:

3.4.1. Fundamental Concepts of RMSD and RMSF Analysis

In the field of computational structural biology, the Root Mean Square Deviation (RMSD) serves as the primary quantitative metric for evaluating the global structural stability of a protein-ligand complex throughout a simulation trajectory. By calculating the time-dependent average displacement of the protein's backbone atoms (typically C-alpha) relative to the initial minimized starting structure, RMSD provides a clear indication of whether the system has reached a state of thermodynamic equilibrium. A low and plateauing RMSD value signifies that the complex has converged to a stable conformation without significant structural deformation. Complementary to this, the Root Mean Square Fluctuation (RMSF) is utilized to assess the local flexibility of individual amino acid residues. By mapping the fluctuations of each residue over the simulation time, RMSF identifies rigid and flexible regions within the protein. Lower RMSF values in the active site typically indicate that the binding of a ligand has effectively "locked" the catalytic residues into a stable orientation, thereby restricting the conformational entropy of the binding pocket.

3.4.2. Global Stability and Convergence (RMSD)

The results of the 200 ns triplicate MD simulations demonstrated that all three AMPK-ligand complexes attained structural convergence within the first 40–50 ns, confirming the reliability of the trajectories. For the AMPK–Oleic acid (Compound A) complex, the RMSD profile exhibited a gradual ascent during the initial 25 ns equilibration phase before stabilizing at an average value of 0.38 nm. Although the system remained stable, Oleic acid displayed slightly higher fluctuations relative to the phenolic compounds. This behavior is likely due to the long, flexible aliphatic hydrocarbon chain of fatty acid, which facilitates continuous conformational sampling and subtle shifting within the hydrophobic regions of the pocket. In contrast, the complexes involving Gallic acid (Compound B) and Protocatechuic acid (Compound C) exhibited notably lower and more rigid RMSD profiles, plateauing at 0.22 nm and 0.25 nm, respectively. The rapid equilibration and negligible deviations observed for these phenolic acids suggest the formation of a highly rigid and structurally stable complex, which is a key characteristic of high-affinity inhibitory molecules.

3.4.3. Local Flexibility and Binding Pocket Integrity (RMSF)

Analysis of the per-residue RMSF provided a comprehensive map of protein flexibility, revealing that the structural integrity of the AMPK catalytic core remained intact across all three simulated

systems. The β -sheets and α -helices maintained low fluctuation values, generally remaining below 0.15 nm, which signifies a highly stable protein fold. Crucially, the residues previously identified as critical during the docking studies, specifically ASN A:195, SER A:193, and ASN B:238—showed a marked reduction in flexibility compared to the unbound apo-protein state. This localized stabilization indicates that the ligands effectively anchor these residues, potentially interfering with the dynamic conformational shifts required for the natural activation of the AMPK enzyme. While the binding pocket remained rigid, higher fluctuations (RMSF > 1.5 nm) were restricted to the distal N- and C-terminal loop regions. These fluctuations are consistent with standard protein behavior in an aqueous environment and do not compromise the integrity of the ligand-binding site.

3.4.4. Hydrogen Bond Persistence and Evolution

The transition from static molecular docking to dynamic simulation elucidated the underlying strength and persistence of the polar interactions between the ligands and the receptor. Gallic acid and Protocatechuic acid distinguished themselves by maintaining a dense and robust network of 3 to 6 hydrogen bonds consistently throughout the 200 ns duration. This remarkable persistence is facilitated by the presence of multiple hydroxyl (-OH) groups on their benzene rings, which allow for a "hydrogen bond hopping" mechanism; in this process, bonds can reform rapidly with adjacent residues even if a single contact is momentarily disrupted by thermal motion. Conversely, while Oleic acid achieved a favorable docking score, the MD trajectory revealed that its hydrogen bonds were significantly more transient, fluctuating between 1 and 2 bonds. The binding of Oleic acid is primarily driven by non-specific hydrophobic forces, which, while energetically favorable, lack the directional specificity and stability provided by the intricate hydrogen-bond networks observed in the phenolic acids. Consequently, the phenolic compounds B and C appear to offer more reliable and stable inhibitory potential.

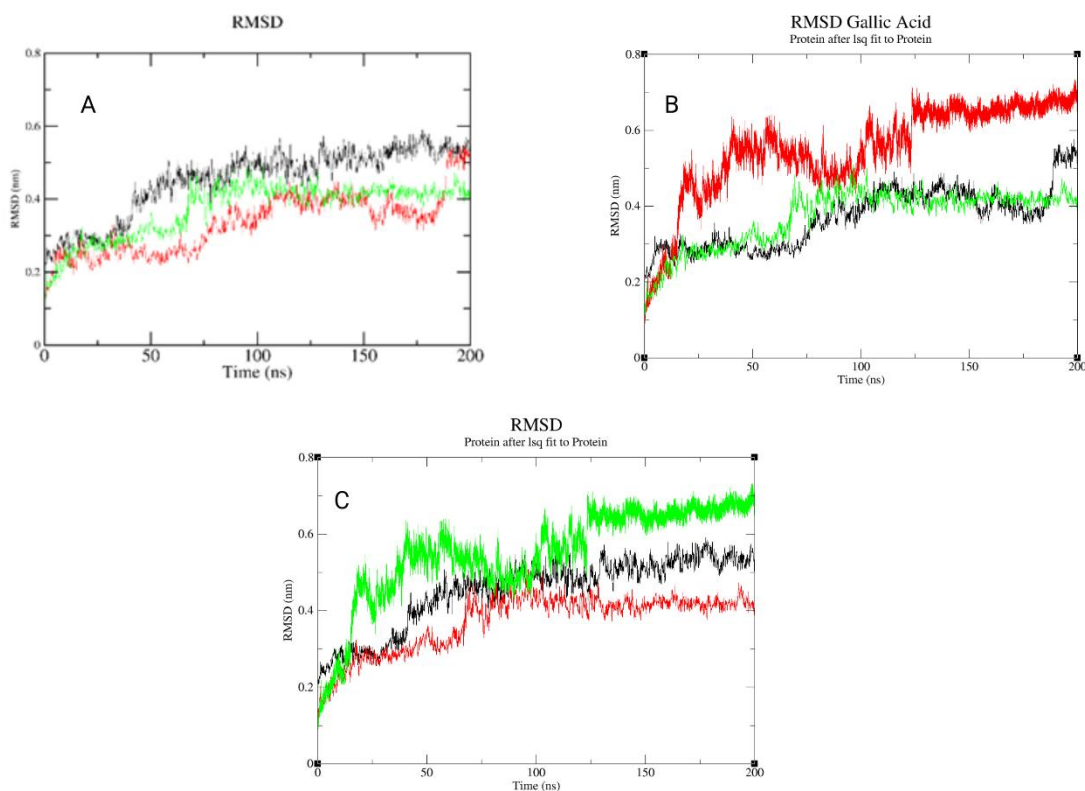


Figure 5. Root Mean Square Deviation (RMSD) of AMPK-Ligand Complexes.

In **Fig.5** The plots illustrate the structural stability of the AMPK protein backbone during 200 ns molecular dynamics simulations in triplicate. (A) Oleic acid (red/green/blue trajectories) showing equilibration after 30 ns with an average RMSD of 0.38 nm; (B) Gallic acid showing high structural rigidity with a lower plateau at 0.22 nm; (C) Protocatechuic acid displaying steady convergence at 0.25 nm. The overlapping nature of the triplicate runs confirms the thermodynamic stability and reproducibility of the binding modes for all three phytoconstituents.

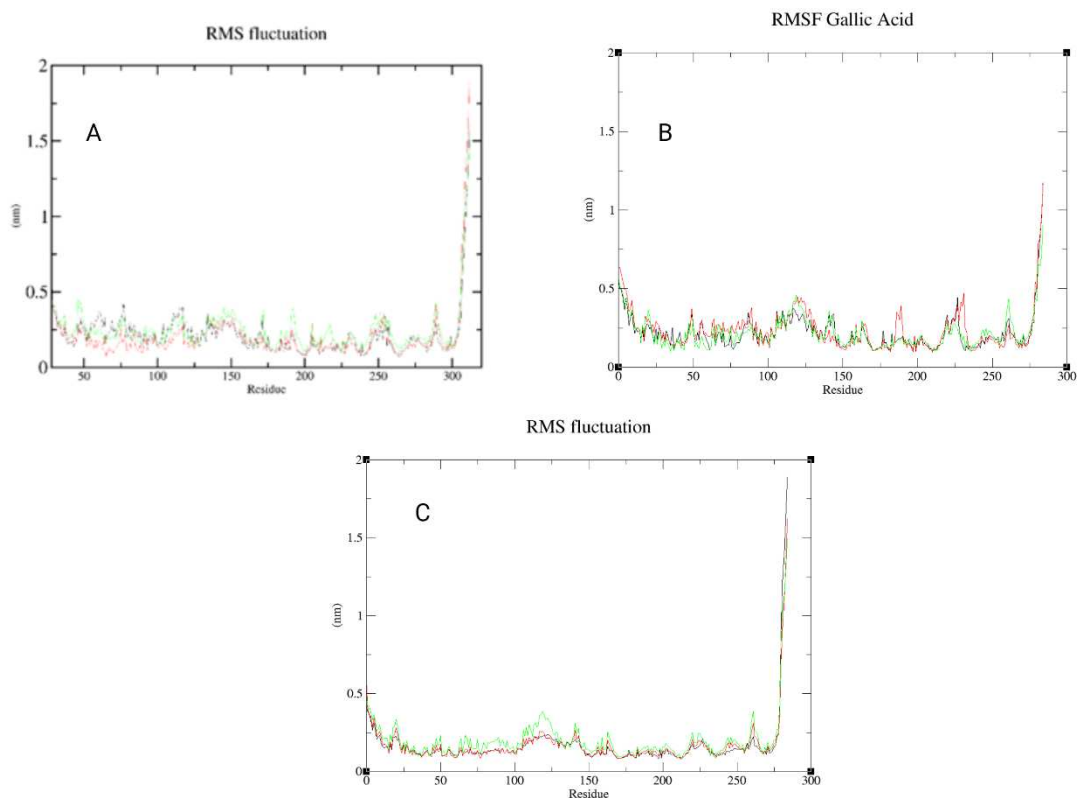


Figure 6. Root Mean Square Fluctuation (RMSF) of AMPK Residues.

In **Fig.6** Per-residue fluctuation analysis comparing the local flexibility of the AMPK protein when bound to (A) Oleic acid, (B) Gallic acid, and (C) Protocatechuic acid. The catalytic core and binding pocket residues (ASN A:195, SER A:193, and ASN B:238) exhibit minimal fluctuations (<0.15 nm), indicating significant stabilization upon ligand binding. High-magnitude fluctuations are localized to the distal loop regions, consistent with standard protein dynamics in a solvated system.

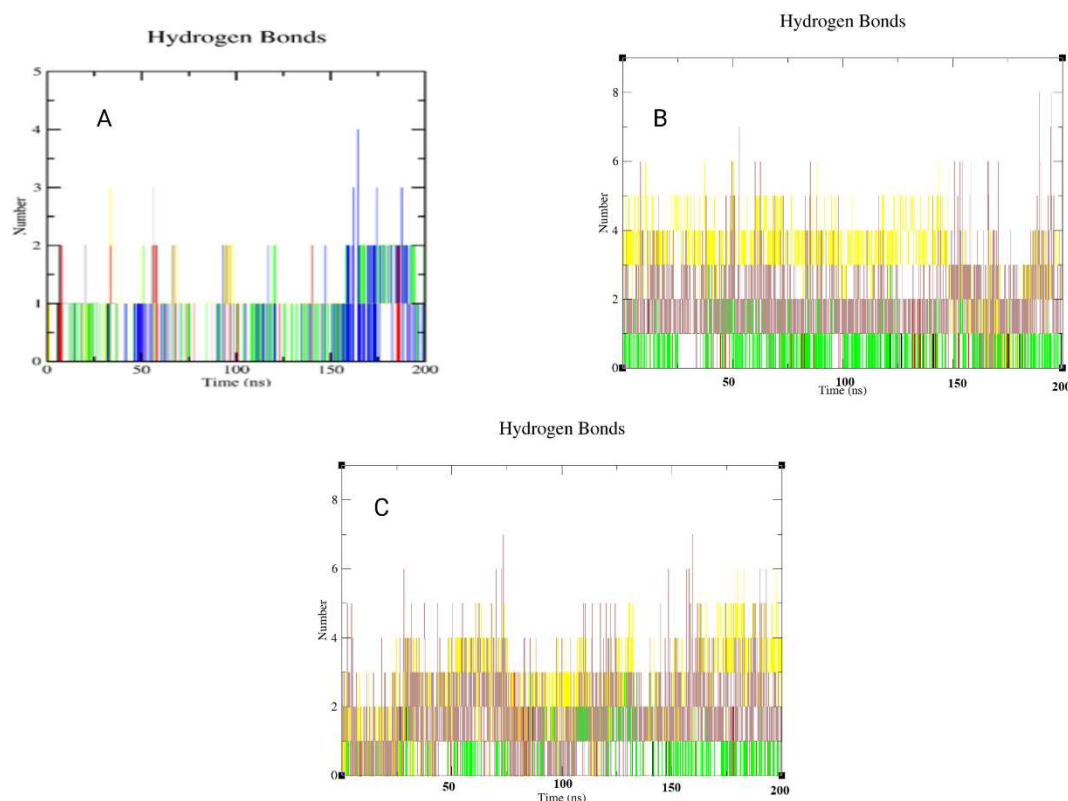


Figure 7. Time-Dependent Hydrogen Bond Evolution.

Analysis of the intermolecular hydrogen bonds formed over the 200 ns simulation trajectory. In **Fig.7** (A) **Oleic acid** maintains intermittent polar contacts (average 1–2 bonds) primarily through its carboxylate headgroup; (B) Gallic acid and (C) Protocatechuic acid demonstrate a dense and persistent network of 3–6 hydrogen bonds. The high residence time of these polar interactions, particularly for the phenolic acids, underscores their superior anchoring capability within the AMPK active site.

Table 2: Predicted ADMET parameters of selected nine ligands of *Cinnamomum verum*.

Compounds name	Eugenol	Pyrogallol	Cinnamic acid	Ferulic acid	Caffeic acid	Gallic acid	Protocatechuic acid	Oleic acid	<i>p</i> -Hydroxybenzoic acid
Water solubility (log mol/L)	-2.25	-1.408	-2.608	-2.817	-2.33	-2.56	-2.069	-5.924	-1.877
Caco2 permeability (log Papp in 10 ⁻⁶ cm/s)	1.559	1.122	1.717	0.176	0.634	-0.081	0.49	1.563	1.151
Intestinal absorption	92.041	83.549	94.833	93.685	69.407	43.374	71.174	91.823	83.961

(human) (%) Absorbed)									
Skin	-2.207	-2.751	-2.695	-2.72	-2.722	-2.735	-2.727	-2.725	-2.723
Permeability (log Kp)									
p-glycoprotein substrate	No	No	No	No	No	No	No	No	No
p-glycoprotein I inhibitor	No	No	No	No	No	No	No	No	No
p-glycoprotein II inhibitor	No	No	No	No	No	No	No	No	No

4. Conclusion: The present study utilized a comprehensive *in-silico* framework—integrating molecular docking, ADMET profiling, and high-performance molecular dynamics (MD) simulations—to evaluate the antidiabetic potential of phytoconstituents from *Cinnamomum verum* against the AMPK target. Initial virtual screening and docking identified Oleic acid as the lead compound with the highest binding affinity (−6.6 kcal/mol). However, a deeper analysis of its physicochemical properties revealed that its relatively high molecular weight and flexible aliphatic nature, coupled with limited hydrogen bond stabilization, pose significant challenges for its development as a high-efficiency oral drug candidate.

To address these limitations, a comparative 200 ns triplicate MD simulation was conducted to evaluate the dynamic stability of Oleic acid alongside two promising phenolic compounds: Gallic acid and Protocatechuic acid. The trajectory analysis revealed that while Oleic acid maintains a stable plateau (RMSD ~0.38 nm), it relies heavily on non-specific hydrophobic interactions. In contrast, Gallic acid and Protocatechuic acid exhibited superior structural rigidity (RMSD < 0.25 nm) and maintained a dense, persistent network of 3–6 hydrogen bonds throughout the 200 ns duration. This "multi-point" anchoring system, particularly with residues ASN A:195 and ASN B:238, underscores the higher residence time and target specificity of these phenolic acids within the AMPK active site.

In conclusion, although Oleic acid serves as a strong binder in static models, Gallic acid and Protocatechuic acid emerge as the most viable lead candidates from *Cinnamomum verum*. Their balanced profile of favorable ADMET properties, lower molecular weight (complying with Lipinski’s Rule of Five), and exceptional dynamic stability makes them ideal scaffolds for the development of novel, orally bioavailable AMPK activators. These findings provide a robust computational foundation for future *in-vitro* and *in-vivo* pharmacological validations to confirm their therapeutic efficacy in the management of Type 2 diabetes.

Conflict of Interest: Authors declare no conflict of Interest regarding this article.

Funding: N/A

Ethics approval and consent to participate: NA

Consent for publication: Not applicable

Availability of data and materials: All data repository available within the manuscript.

Acknowledgements: The authors are thankful to Sam Higginbottom University of Agriculture, Technology and Sciences, for providing the necessary facilities to execute this manuscript.

Authors' contributions: Conceptualization: Amita Verma, Ajay Kumar; Data collection/Writing the manuscript/Sketching of figures and data interpretation: Shweta Singh Verma, Shivam Kumar, Jagat Pal Yadav, Ankit Kumar Singh; Writing, review and final editing of the manuscript: Mashooq Ahmad Bhat, Habibullah Khalilullah, Amita Verma, Ajay Kumar.

References

- Abdalla, R.M., Abdelgadir, A.E., 2016. Antibacterial activity and phytochemical constituents of *Cinnamomum verum* and *Matricaria chamomilla* from Sudan. *Bio Bulletin* 2, 01–0.
- Abdel-Moneim, A., Fayez, H., 2015. A review on medication of diabetes mellitus and antidiabetic medicinal plants. *Int J Bioassays* 4, 4002–4012.
- Adarsh, A., Chettiyar, B., Kanthesh, B., Raghu, N., 2020. Phytochemical screening and antimicrobial activity of “*Cinnamon zeylanicum*.” *Int. J. Pharm. Res. Innov* 13, 22–33.
- Al-Mijalli, S.H., Mrabti, H.N., El Hachlafi, N., El Kamili, T., Elbouzidi, A., Abdallah, E.M., Flouchi, R., Assaggaf, H., Qasem, A., Zengin, G., 2023. Integrated analysis of antimicrobial, antioxidant, and phytochemical properties of *Cinnamomum verum*: A comprehensive In vitro and In silico study. *Biochemical Systematics and Ecology* 110, 104700.
- Batiha, G.E.-S., Beshbishy, A.M., Guswanto, A., Nugraha, A., Munkhjargal, T., M. Abdel-Daim, M., Mosqueda, J., Igarashi, I., 2020. Phytochemical characterization and chemotherapeutic potential of *Cinnamomum verum* extracts on the multiplication of protozoan parasites in vitro and in vivo. *Molecules* 25, 996.
- Bernard, D., Asare, I.K., Ofori, D.O., Daniel, G.A., Elom, S.A., Sandra, A., 2014. The effect of different drying methods on the phytochemicals and radical scavenging activity of Ceylon cinnamon (*Cinnamomum zeylanicum*) plant parts. *European Journal of Medicinal Plants* 4, 1324.
- Bnouham, M., Ziyyat, A., Mekhfi, H., Tahri, A., Legssyer, A., 2006. Medicinal plants with potential antidiabetic activity-A review of ten years of herbal medicine research (1990-2000). *Dubai Diabetes and Endocrinology Journal* 14, 1–25.
- Chaudhury, A., Duvoor, C., Reddy Dendi, V.S., Kraleti, S., Chada, A., Ravilla, R., Marco, A., Shekhawat, N.S., Montales, M.T., Kuriakose, K., 2017. Clinical review of antidiabetic drugs: implications for type 2 diabetes mellitus management. *Frontiers in endocrinology* 8, 6.
- Dewanjee, S., Chakraborty, P., Mukherjee, B., De Feo, V., 2020. Plant-based antidiabetic nanoformulations: the emerging paradigm for effective therapy. *International journal of molecular sciences* 21, 2217.

- El-Desoky, G.E., Aboul-Soud, M.A., Al-Numair, K.S., 2012. Antidiabetic and hypolipidemic effects of Ceylon cinnamon (*Cinnamomum verum*) in alloxan-diabetic rats. *Journal of Medicinal plants research* 6, 1685–1691.
- Fonseca, V.A., 2003. Management of diabetes mellitus and insulin resistance in patients with cardiovascular disease. *The American journal of cardiology* 92, 50–60.
- Fryer, L.G., Parbu-Patel, A., Carling, D., 2002. The anti-diabetic drugs rosiglitazone and metformin stimulate AMP-activated protein kinase through distinct signaling pathways. *Journal of Biological Chemistry* 277, 25226–25232.
- Gruzman, A., Babai, G., Sasson, S., 2009. Adenosine monophosphate-activated protein kinase (AMPK) as a new target for antidiabetic drugs: a review on metabolic, pharmacological and chemical considerations. *The review of diabetic studies: RDS* 6, 13.
- Gulcin, I., Kaya, R., Goren, A.C., Akincioglu, H., Topal, M., Bingol, Z., Cetin Çakmak, K., Ozturk Sarikaya, S.B., Durmaz, L., Alwasel, S., 2019. Anticholinergic, antidiabetic and antioxidant activities of cinnamon (*cinnamomum verum*) bark extracts: polyphenol contents analysis by LC-MS/MS. *International Journal of Food Properties* 22, 1511–1526. <https://doi.org/10.1080/10942912.2019.1656232>
- Hameed, I.H., Altameme, H.J., Mohammed, G.J., 2016. Evaluation of antifungal and antibacterial activity and analysis of bioactive phytochemical compounds of *Cinnamomum zeylanicum* (Cinnamon bark) using gas chromatography-mass spectrometry. *Oriental Journal of Chemistry* 32, 1769.
- Hardie, D.G., 2011. Sensing of energy and nutrients by AMP-activated protein kinase. *The American journal of clinical nutrition* 93, 891S-896S.
- Hawley, S.A., Gadalla, A.E., Olsen, G.S., Hardie, D.G., 2002. The antidiabetic drug metformin activates the AMP-activated protein kinase cascade via an adenine nucleotide-independent mechanism. *Diabetes* 51, 2420–2425.
- Janghorbani, M., Van Dam, R.M., Willett, W.C., Hu, F.B., 2007. Systematic review of type 1 and type 2 diabetes mellitus and risk of fracture. *American journal of epidemiology* 166, 495–505.
- Jung, M., Park, M., Lee, H.C., Kang, Y.-H., Kang, E.S., Kim, S.K., 2006. Antidiabetic agents from medicinal plants. *Current medicinal chemistry* 13, 1203–1218.
- Kamran, U., Bhatti, H.N., Iqbal, M., Jamil, S., Zahid, M., 2019. Biogenic synthesis, characterization and investigation of photocatalytic and antimicrobial activity of manganese nanoparticles synthesized from *Cinnamomum verum* bark extract. *Journal of Molecular Structure* 1179, 532–539.
- Kazeem, M.I., Davies, T.C., 2016. Anti-diabetic functional foods as sources of insulin secreting, insulin sensitizing and insulin mimetic agents. *Journal of functional foods* 20, 122–138.
- Mahmood, H., Ali, Q., Hafeez, M.M., Malik, A., 2021. Antioxidant activity of *Syzygium aromaticum* and *Cinnamomum verum* seed extracts. *Biological and Clinical Sciences Research Journal* 2021.
- Marx, A., Nugoor, C., Panneerselvam, S., Mandelkow, E., 2010. Structure and function of polarity-inducing kinase family MARK/Par-1 within the branch of AMPK/Snf1-related kinases. *FASEB j.* 24, 1637–1648. <https://doi.org/10.1096/fj.09-148064>

- Muller, L., Gorter, K.J., Hak, E., Goudzwaard, W.L., Schellevis, F.G., Hoepelman, A.I.M., Rutten, G., 2005. Increased risk of common infections in patients with type 1 and type 2 diabetes mellitus. *Clinical infectious diseases* 41, 281–288.
- Neupane, N.P., Karn, A.K., Mukeri, I.H., Pathak, P., Kumar, P., Singh, S., Qureshi, I.A., Jha, T., Verma, A., 2021. Molecular dynamics analysis of phytochemicals from *Ageratina adenophora* against COVID-19 main protease (Mpro) and human angiotensin-converting enzyme 2 (ACE2). *Biocatalysis and Agricultural Biotechnology* 32, 101924.
- Neupane, N.P., Kushwaha, A.K., Karn, A.K., Khalilullah, H., Khan, M.M.U., Kaushik, A., Verma, A., 2022a. Anti-bacterial efficacy of bio-fabricated silver nanoparticles of aerial part of *Moringa oleifera* lam: Rapid green synthesis, In-Vitro and *In-silico* screening. *Biocatalysis and Agricultural Biotechnology* 39, 102229.
- Neupane, N.P., Yadav, E., Verma, A., 2022b. Cultural, Practical, and Socio-Economic Importance of Edible Medicinal Plants Native to Central India, in: Masoodi, M.H., Rehman, M.U. (Eds.), *Edible Plants in Health and Diseases*. Springer Nature Singapore, Singapore, pp. 181–207. https://doi.org/10.1007/978-981-16-4880-9_8
- Otuechere, C.A., Adewuyi, A., Salau, T.B., Neupane, N.P., Adebayo, O.L., Egunjobi, M., Verma, A., 2022. Polyathia longifolia: Redox potential of a cellulose nanocrystal derivative and ADMET predictions of selected compounds. *Biocatalysis and Agricultural Biotechnology* 40, 102295.
- Otuechere, C.A., Neupane, N.P., Adewuyi, A., Pathak, P., Novak, J., Grishina, M., Khalilullah, H., Jaremko, M., Verma, A., 2023. Green Synthesis of Genistein-Fortified Zinc Ferrite Nanoparticles as a Potent Hepatic Cancer Inhibitor: Validation through Experimental and Computational Studies. *Chemistry & Biodiversity* 20, e202300719. <https://doi.org/10.1002/cbdv.202300719>
- Ozougwu, J.C., Obimba, K.C., Belonwu, C.D., Unakalamba, C.B., 2013. The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus. *J Physiol Pathophysiol* 4, 46–57.
- Pathak, R., Sharma, H., 2021. A review on medicinal uses of *Cinnamomum verum* (Cinnamon). *Journal of Drug Delivery and Therapeutics* 11, 161–166.
- Petersen, K.F., Shulman, G.I., 2002. Pathogenesis of skeletal muscle insulin resistance in type 2 diabetes mellitus. *The American journal of cardiology* 90, 11–18.
- Puangpronpitag, D., Sittiwet, C., 2009. Antimicrobial properties of *Cinnamomum verum* aqueous extract. *Asian Journal of Biological Sciences* 2, 49–53.
- Rafiullah, M.R.M., Siddiqui, A.W., Mir, S.R., Ali, M., Pillai, K.K., Singh, S., 2006. Antidiabetic Activity of Some Indian Medicinal Plants. *Pharmaceutical Biology* 44, 95–99. <https://doi.org/10.1080/13880200600591972>
- Raj, R.R.R., Sahay, S.S.S., Tripathi, J.T.J., 2016. Medications of diabetes mellitus and antidiabetic medicinal plants: a review. *International Journal of Indigenous Herbs and Drugs* 19–28.
- Rattanachaikunsopon, P., Phumkhachorn, P., 2010. Potential of cinnamon (*Cinnamomum verum*) oil to control *Streptococcus iniae* infection in tilapia (*Oreochromis niloticus*). *Fish Sci* 76, 287–293. <https://doi.org/10.1007/s12562-010-0218-6>

- Rozenfeld, Y., Hunt, J.S., Plauschinat, C., Wong, K.S., 2008. Oral antidiabetic medication adherence and glycemic control in managed care. *The American journal of managed care* 14, 71–75.
- Shepherd, P.R., Kahn, B.B., 1999. Glucose Transporters and Insulin Action — Implications for Insulin Resistance and Diabetes Mellitus. *N Engl J Med* 341, 248–257. <https://doi.org/10.1056/NEJM199907223410406>
- Shori, A.B., Baba, A.S., 2011. Cinnamomum verum improved the functional properties of bioyogurts made from camel and cow milks. *Journal of the Saudi Society of Agricultural Sciences* 10, 101–107.
- Singab, A.N., Youssef, F.S., Ashour, M.L., 2014. Medicinal plants with potential antidiabetic activity and their assessment. *Med Aromat Plants* 3, 2167–0412.
- Singh, N., Rao, A.S., Nandal, A., Kumar, S., Yadav, S.S., Ganaie, S.A., Narasimhan, B., 2021. Phytochemical and pharmacological review of Cinnamomum verum J. Presl-a versatile spice used in food and nutrition. *Food Chemistry* 338, 127773.
- Ungurianu, A., Șeremet, O., Gagniuc, E., Olaru, O.T., Guțu, C., Grădinaru, D., Ionescu-Tîrgoviște, C., Margină, D., Dănciulescu-Miulescu, R., 2019. Preclinical and clinical results regarding the effects of a plant-based antidiabetic formulation versus well established antidiabetic molecules. *Pharmacological Research* 150, 104522.
- Zaccardi, F., Webb, D.R., Yates, T., Davies, M.J., 2016. Pathophysiology of type 1 and type 2 diabetes mellitus: a 90-year perspective. *Postgraduate medical journal* 92, 63–69.