# A Multi-Target Approach for Identifying Natural Inhibitors of Metabolic Syndrome Proteins (AMPK, PPAR-Γ, IRS-1) Using Molecular Docking and in-Silico Screening

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## ABSTRACT

Metabolic syndrome is a complex cardiometabolic disorder driven by coordinated dysregulation of energy balance, insulin signaling, and lipid metabolism. Central regulatory proteins—including AMP-activated protein kinase (AMPK), peroxisome proliferator-activated receptor-γ (PPAR-γ), and insulin receptor substrate-1 (IRS-1) - represent interconnected molecular nodes within this network, yet current therapeutic strategies largely rely on single-target modulation. Natural products offer structurally diverse scaffolds capable of engaging multiple targets, providing a rational basis for multi-target drug discovery. In this study, a systematic in silico multi-target screening strategy was employed to evaluate phytochemicals derived from Hyptis verticillata against AMPK, PPAR-γ, and IRS-1. Molecular docking was performed using AutoDock Vina against crystallographic structures of AMPK and PPAR-γ, while a homology-modeled structure of IRS-1 was utilized. Binding affinities and protein–ligand interaction profiles were analyzed, followed by in silico assessment of drug-likeness and pharmacokinetic properties using SwissADME. Docking analyses revealed binding energies ranging from −3.8 to −8.6 kcal/mol across the targets. Dehydropodophyllotoxin, oleanolic acid, cadina-4,10(15)-dien-3-one, aromadendr-1(10)-en-9-one, and squalene consistently exhibited favorable binding across multiple proteins. Interaction mapping indicated that ligand stabilization was dominated by hydrophobic and π-alkyl interactions within functionally relevant binding regions. Pharmacokinetic profiling suggested acceptable oral drug-likeness for several top-ranking compounds, particularly oleanolic acid. Collectively, these findings identify H. verticillata phytochemicals as promising multi-target molecular scaffolds relevant to metabolic regulation. While the results reflect predicted molecular recognition rather than functional modulation, this work establishes a robust computational framework for prioritizing natural compounds for experimental validation and supports the utility of multi-target in silico approaches in metabolic syndrome drug discovery.

**Keywords:** Metabolic syndrome; Multi-target drug discovery; Molecular docking; AMPK; PPAR-γ; IRS-1; Phytochemicals; ADMET profiling

## INTRODUCTION

Metabolic syndrome (MetS) is a complex, multifactorial disorder characterized by a cluster of metabolic abnormalities, including central obesity, insulin resistance, dyslipidemia, and hypertension, which collectively increase the risk of cardiovascular diseases and type 2 diabetes mellitus (Alberti et al., 2009). The prevalence of MetS has risen dramatically worldwide, primarily due to sedentary lifestyles, high-calorie diets, and genetic predisposition, making it a major public health concern (Grundy, 2016). The pathophysiology of MetS

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involves the interplay of several signaling pathways and molecular targets, with key proteins such as AMP-activated protein kinase (AMPK), peroxisome proliferator-activated receptor gamma (PPAR-γ), and insulin receptor substrate 1 (IRS-1) playing critical regulatory roles in energy homeostasis, glucose metabolism, and lipid storage (Hardie et al., 2012; Saltiel & Kahn, 2001).

AMPK serves as a central energy sensor that maintains cellular energy balance by activating catabolic pathways and inhibiting anabolic processes under low-energy conditions. Activation of AMPK enhances glucose uptake, fatty acid oxidation, and mitochondrial biogenesis, making it a key therapeutic target for insulin resistance and obesity-related complications (Hardie et al., 2012). PPAR-γ, a nuclear receptor, regulates adipocyte differentiation, lipid metabolism, and insulin sensitivity, and its modulation has been shown to improve metabolic parameters in obesity and type 2 diabetes (Tontonoz & Spiegelman, 2008). IRS-1, an essential mediator of insulin receptor signaling, propagates downstream phosphorylation events that regulate glucose uptake, glycogen synthesis, and lipid metabolism. Dysregulation of IRS-1 contributes significantly to insulin resistance and hyperglycemia (Saltiel & Kahn, 2001).

Despite the availability of pharmacological agents such as metformin and thiazolidinediones, which target AMPK and PPAR-γ respectively, current therapies often face limitations including side effects, single-target specificity, and variable patient responses (Ashraf et al., 2019). Consequently, there is a growing interest in identifying natural compounds with multi-target potential, which could simultaneously modulate key proteins involved in MetS, offering safer and more effective therapeutic strategies (Li et al., 2020). Natural products, particularly phytochemicals such as flavonoids, alkaloids, and polyphenols, exhibit structural diversity and biological activity that allow interactions with multiple molecular targets, making them attractive candidates for multi-target drug discovery.

Molecular docking and in-silico screening have become indispensable tools in modern drug discovery, allowing rapid assessment of potential interactions between small molecules and protein targets. Docking predicts binding affinities and identifies key residues involved in ligand stabilization, providing insight into the molecular mechanism of inhibition or activation (Sliwoski et al., 2014). Multi-target in-silico screening further facilitates the identification of compounds capable of modulating several proteins simultaneously, which is particularly relevant for complex diseases like MetS that involve interconnected signaling pathways (Hopkins, 2008).

In this study, we aimed to employ a multi-target computational approach to identify natural inhibitors of AMPK, PPAR-γ, and IRS-1. By screening a library of phytochemicals for binding affinity, drug-likeness, and ADMET properties, we sought to prioritize compounds with the potential to modulate multiple key proteins involved in metabolic syndrome. This approach offers a rational strategy to discover novel natural compounds with therapeutic potential, while reducing the reliance on single-target pharmacological interventions.

# METHODOLOGY

### Software and Databases

This study employed a combination of computational tools and databases to perform ligand and protein preparation, molecular docking, and pharmacokinetic analysis. The crystal structures of AMPK (PDB ID: 4CFE) and PPAR-γ (PDB ID: 3DZY) were retrieved from the Protein Data Bank (PDB) (Berman et al., 2000). For IRS-1, no experimentally resolved structure was available; therefore, a homology model was generated using the Swiss-Model server, based on the amino acid sequence retrieved from UniProt (P35568). Natural compounds were sourced from PubChem and ChEMBL, and their 2D structures were converted to 3D using Open Babel. Molecular docking was conducted using PyRx (AutoDock Vina) (Dallakyan & Olson, 2015), and protein-ligand interactions were visualized using Discovery Studio Visualizer. Drug-likeness and ADMET properties were evaluated using SwissADME (Daina et al., 2017).

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**Ligand Preparation**

A library of natural compounds with reported anti-diabetic, anti-obesity, and insulin-sensitizing effects was compiled. The 2D structures of these compounds were downloaded in SDF format and converted to 3D geometries using Open Babel. Energy minimization of each ligand was performed using the MMFF94 force field to ensure optimal geometry and proper charge distribution for docking. Ligands were then saved in PDBQT format, the standard input format for AutoDock Vina, after the addition of polar hydrogens and assignment of Gasteiger charges.

Table 1: Library of phytocompounds derived from *hyptis verticillata*

| S/N | Compound Name |
|---|---|
| **1** | Aromadendr-1(10)-en-9-one |
| **2** | Cadina-4,10(15)-dien-3-one |
| **3** | Dehydropodophyllotoxin |
| **4** | Oleanolic Acid |
| **5** | Thymol |
| **6** | 3a.4,5,6,7,7a-hexahydro-4,7-methanoindene |
| **7** | 4,7- methanon-1H-indene |
| **8** | R-R,R-E- trans-Phytol |
| **9** | Squalene |
| **10** | 9,12,15-octadecatrien-1-ol |
| **11** | 1-octadecyne |
| **12** | 1-fluorodecane |

**Protein Preparation**

Protein preparation involved removing all water molecules, ions, and co-crystallized ligands to prevent interference with docking. Hydrogen atoms were added to maintain correct protonation states at physiological pH. Structural minimization was performed using Chimera to relieve steric clashes and ensure stability. For AMPK and PPAR-γ, the active sites were defined based on co-crystallized ligands in the PDB structures and reported catalytic residues. For the homology-modeled IRS-1, the phosphotyrosine binding (PTB) domain was designated as the docking site, based on literature reports of functional interaction regions (Saltiel & Kahn, 2001).

Table 2: Protein Targets and Their PDB Information

| Protein Target | PDB ID |
|---|---|
| **AMPK** (AMP-activated protein kinase) | **4CFE** |
| **PPAR-γ** (Peroxisome proliferator-activated receptor gamma) | **2P4Y** |
| **IRS-1** (Insulin receptor substrate-1) | **5U1M** |

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### Molecular Docking Protocol

Molecular docking was performed using PyRx (AutoDock Vina). For each target protein, a grid box was defined around the active site to ensure exploration of the catalytic and ligand-binding regions. The docking protocol used an exhaustiveness parameter of 8, allowing adequate sampling of conformational space. The binding affinity for each ligand was recorded in kcal/mol, with more negative values indicating stronger predicted interactions. The top-ranking ligands for each protein were further analyzed using Discovery Studio Visualizer to assess hydrogen bonds, hydrophobic interactions, π-π stacking, and other stabilizing interactions within the active site.

### Multi-Target Screening and ADMET Profiling

Ligands with high binding affinities to more than one target were prioritized for multi-target potential, as simultaneous modulation of AMPK, PPAR-γ, and IRS-1 is desirable for addressing the complex pathophysiology of metabolic syndrome. Top compounds were subjected to ADMET profiling using SwissADME to evaluate absorption, distribution, metabolism, excretion, and toxicity properties. This ensured that selected compounds were not only effective in binding but also had favorable drug-like properties for potential development.

### Data Analysis

Binding affinity data and protein-ligand interaction analyses were tabulated and compared across all targets. Compounds were ranked based on binding energy and multi-target potential. Interaction maps highlighting hydrogen bonding and hydrophobic contacts were generated for the top candidates to illustrate the molecular basis of their predicted inhibitory effects.

## RESULTS AND DISCUSSION

### Molecular docking results

The results of molecular docking against the selected receptor are shown below as represented by the docking scores. The docking scores of the compounds range from -3.9 to – 8.6.

Table 2: Docking score of phytochemicals from *Hyptis Verticillata* with receptor

| **LIGAND** | **2P4Y** | **4CEF** | **5U1M** |
|---|---|---|---|
| 1-fluorodecane | -4.6 | -4.5 | -4.5 |
| 1-octadecyne | -5.1 | -3.9 | -3.9 |
| 3a,4,5,6,7,7a-hexahydro-4,7-methanoindene | -6 | -6 | -6 |
| 4,7,methanon-1H-indene | -6.1 | -6.1 | -6.1 |
| 9,12,15-octadecatrien-1-ol, | -6.4 | -5 | -5 |
| Aromadendr-1(10)-en-9-one | -7.7 | -7.5 | -7.5 |
| **Cadina-4,10(15)-dien-3-one** | **-7.4** | **-8.2** | **-8.2** |
| **Dehydropodophyllotoxin** | **-8.6** | **-8.2** | **-8.2** |
| **Oleanolic_Acid** | **-8.2** | **-8.1** | **-8.1** |

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| | | | |
|---|---|---|---|
| R-R-R-E-trans-Phytol | -6.3 | -5.3 | -5.3 |
| Squalene | -7.9 | -6.5 | -6.5 |
| Thymol | -5.9 | -6.1 | -6.1 |

**2D structure of compounds with high binding affinity**

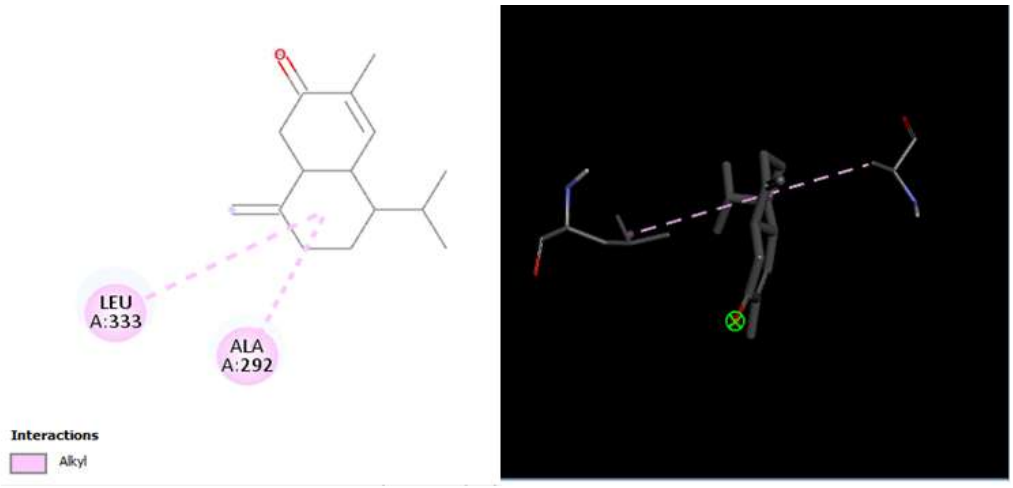


Figure 1: 2D and 3D interactions of Cadina-4,10(15)-dien-3-one with 2PY4 protein

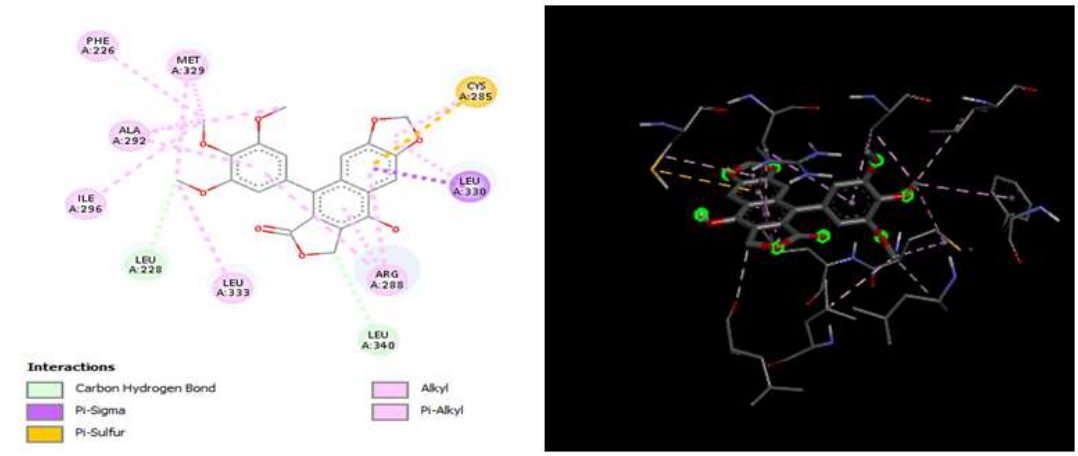


Figure 2: 2D and 3D interactions of Dehydropodophyllotoxin with 2PY4 protein

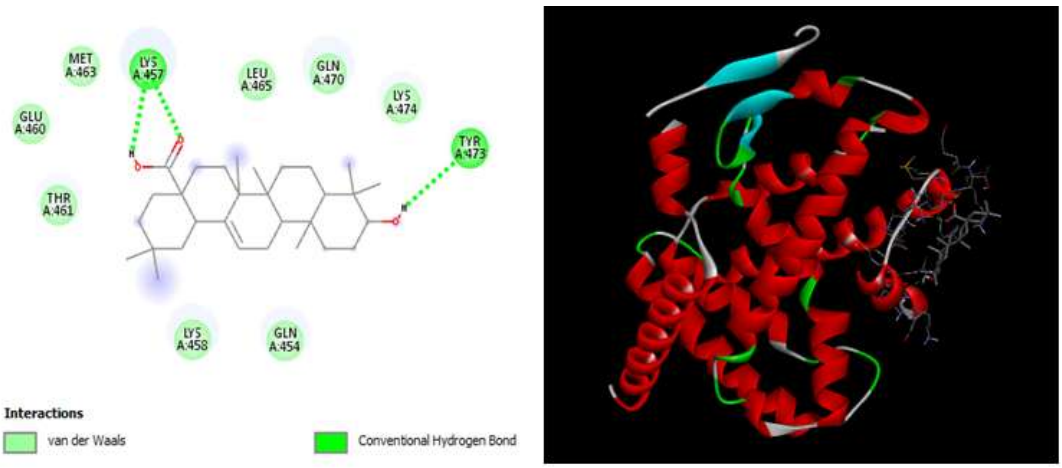


Figure 3: 2D and 3D interactions of Oleanolic_Acid with 2PY4 protein

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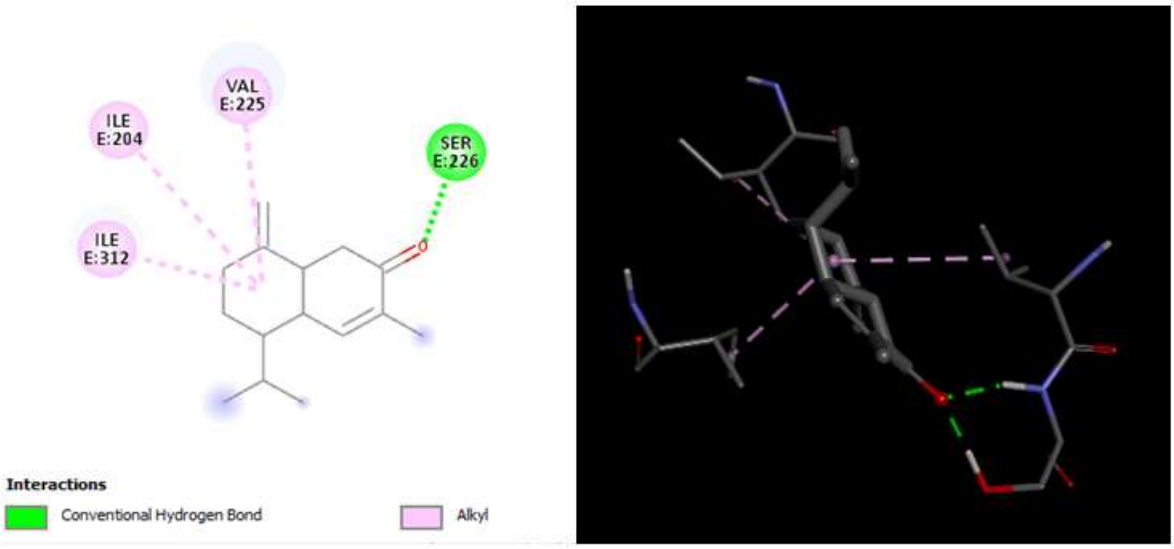


Figure 4: 2D and 3D interactions of Cadina-4,10(15)-dien-3-one with 4CEF protein

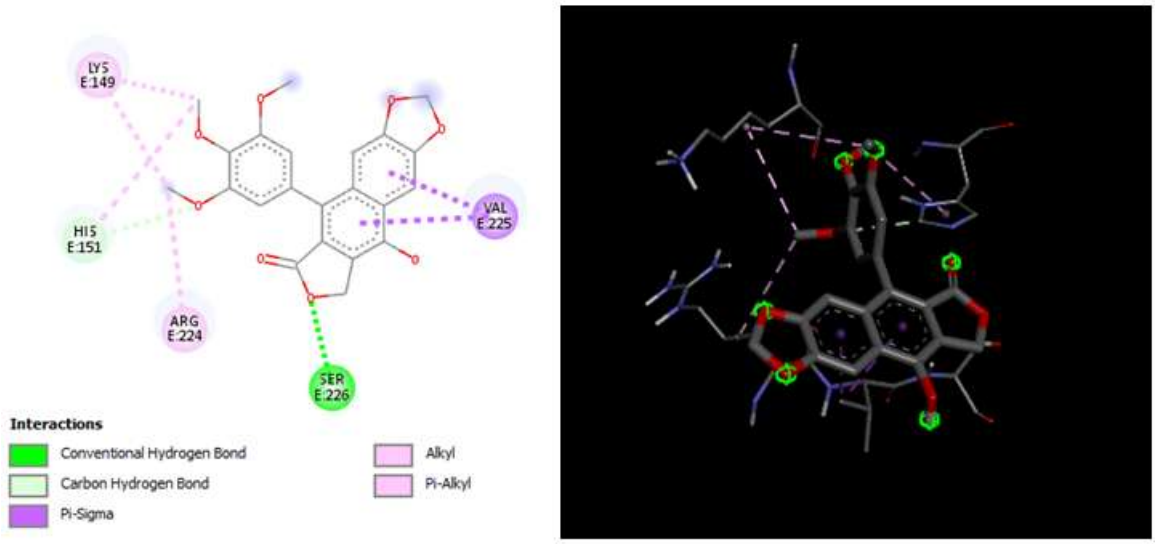


Figure 5: 2D and 3D interactions of Dehydropodophyllotoxin with 4CEF protein

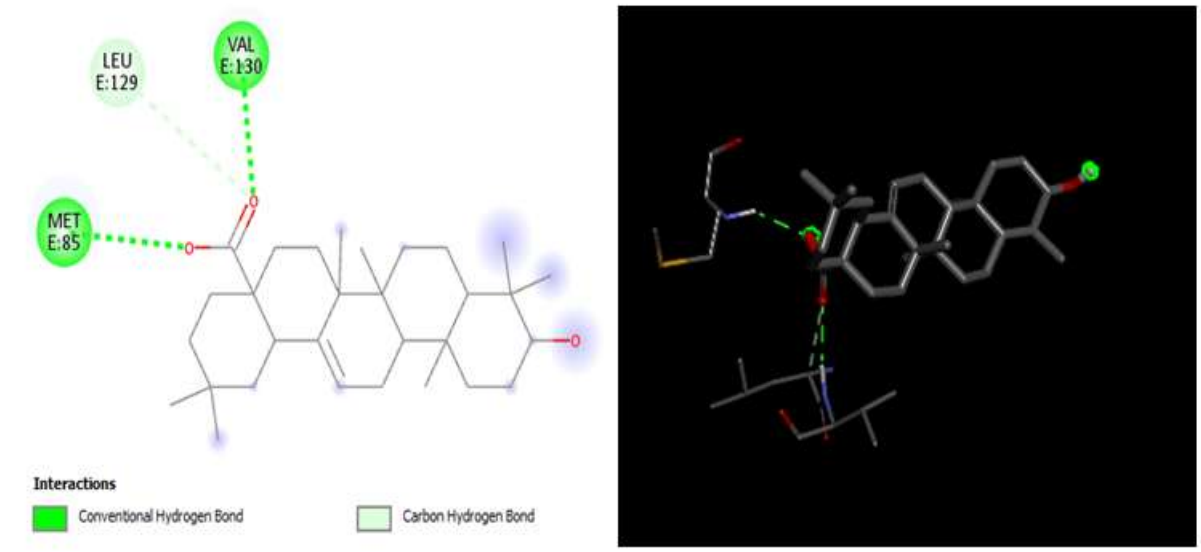


Figure 6: 2D and 3D interactions of Oleanolic Acid with 4CEF protein

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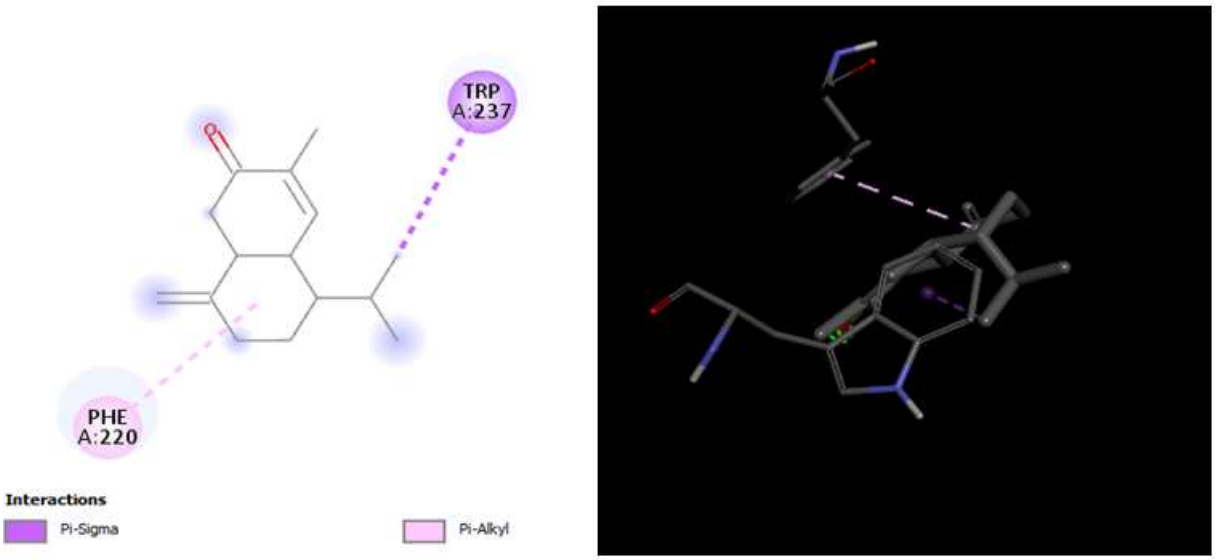


Figure 7: 2D and 3D interactions of Cadina-4,10(15)-dien-3-one with 4CEF protein

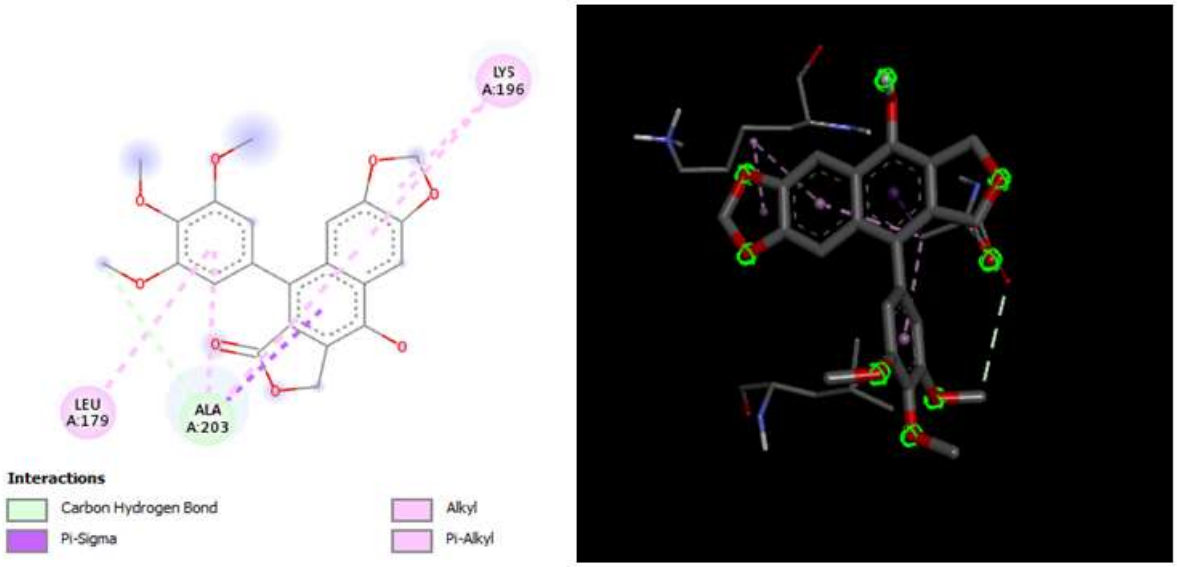


Figure 8: 2D and 3D interactions of Dehydropodophyllotoxin with 4CEF protein

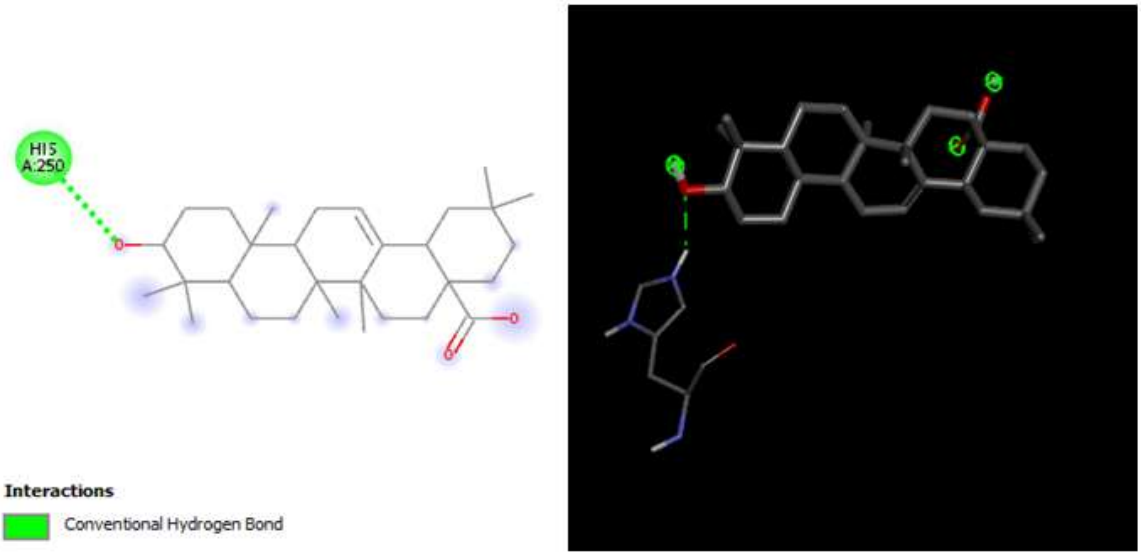


Figure 9: 2D and 3D interactions of Oleanolic_Acid with 4CEF protein

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**ADMET results of high binding compounds**

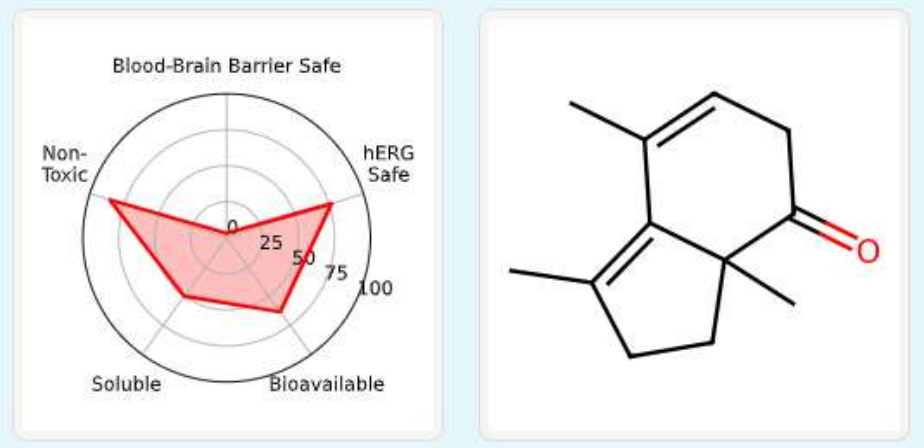


Figure 10: Admet result of Cadina-4,10(15)-dien-3-one

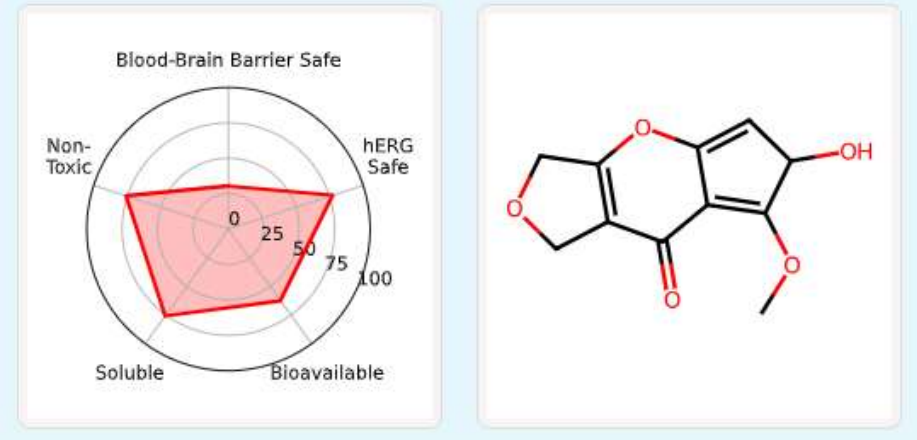


Figure 11: Admet result of Dehydropodophyllotoxin

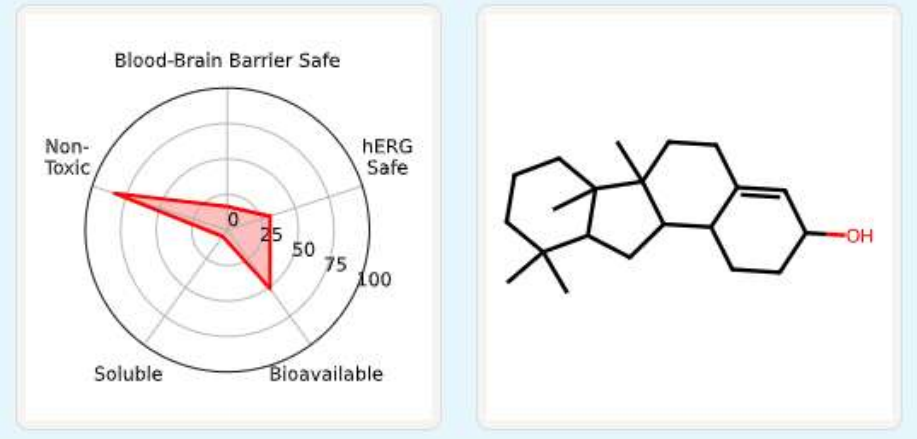


Figure 12: Admet result of Oleanolic_Acid

## DISCUSSION

The present study investigated the binding interactions of selected phytochemicals from *Hyptis verticillata* with three key proteins involved in metabolic regulation—AMPK, PPAR-γ, and IRS-1—using a multi-target molecular docking strategy. These proteins were selected due to their central roles in energy homeostasis, insulin sensitivity, and lipid metabolism, which collectively underpin the pathophysiology of metabolic syndrome. By integrating docking scores, interaction profiling, and in silico pharmacokinetic evaluation, this

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work aimed to prioritize phytochemical candidates with potential relevance to metabolic modulation at the molecular level.

**Molecular Docking Outcomes and Binding Trends**

The docking results demonstrated that the phytochemicals exhibited binding energies ranging from −3.8 to −8.6 kcal/mol across the three protein targets, reflecting variable degrees of predicted binding affinity. Notably, compounds such as dehydropodophyllotoxin, oleanolic acid, cadina-4,10(15)-dien-3-one, aromadendr-1(10)-en-9-one, and squalene consistently ranked among the top-scoring ligands. The recurrence of these compounds across multiple targets suggests a degree of structural compatibility with conserved or functionally relevant regions of the proteins examined.

Oleanolic acid and dehydropodophyllotoxin demonstrated particularly strong binding affinities, with docking scores exceeding −8.0 kcal/mol for AMPK, PPAR-γ, and IRS-1. These values are comparable to those reported for natural product scaffolds in exploratory docking studies targeting metabolic proteins and indicate favorable ligand–receptor complementarity. The observed binding patterns support the notion that triterpenoids and lignan-like structures possess physicochemical features conducive to interacting with metabolically relevant proteins, likely due to their hydrophobic cores and capacity for stabilizing non-covalent interactions.

**Interaction Profiles and Structural Stabilization**

Detailed interaction analysis revealed that ligand stabilization within the binding sites was predominantly mediated through hydrophobic contacts, π–alkyl interactions, and, to a lesser extent, hydrogen bonding. These interaction types are consistent with the known ligand-binding environments of AMPK and PPAR-γ, where hydrophobic pockets play a critical role in ligand accommodation and receptor conformational stabilization. For PPAR-γ in particular, the observed interaction profiles align with its established preference for lipophilic ligands capable of occupying the large Y-shaped ligand-binding domain.

In the case of IRS-1, docking interactions were localized primarily within regions associated with the phosphotyrosine-binding domain. While IRS-1 is not a classical enzymatic target for small-molecule inhibition, the observed interactions suggest surface complementarity that may influence protein–protein interactions or conformational accessibility. These findings should be interpreted as indicative of potential molecular engagement rather than direct functional inhibition, highlighting the exploratory nature of docking in the context of adaptor proteins.

**Multi-Target Binding and Network Pharmacology Implications**

The identification of compounds with consistent binding across AMPK, PPAR-γ, and IRS-1 supports a multi-target interaction profile, which is increasingly recognized as advantageous for complex metabolic disorders. Metabolic syndrome arises from interconnected dysregulation across multiple signaling pathways; therefore, compounds capable of engaging several nodes within this network may offer broader modulatory potential than single-target agents.

Rather than implying simultaneous or equivalent biological effects across all targets, the multi-target docking results suggest that certain *H. verticillata* phytochemicals possess structural flexibility compatible with diverse protein environments. This characteristic is particularly relevant for natural products, which often exert pleiotropic effects through moderate interactions with multiple proteins rather than high-affinity engagement with a single target.

**Pharmacokinetic Considerations and Drug-Likeness**

In silico ADMET profiling revealed that several top-ranking compounds exhibit physicochemical properties consistent with oral bioavailability and acceptable safety profiles. Oleanolic acid, in particular, demonstrated favorable drug-likeness parameters, aligning with previous reports of its metabolic and pharmacological relevance. However, some compounds displayed limitations related to lipophilicity and molecular size, which

may influence absorption and distribution. These findings underscore the importance of integrating pharmacokinetic screening with docking analysis to refine compound prioritization. While ADMET predictions do not replace experimental validation, they provide an additional layer of selection that enhances the translational relevance of computational screening.

**Biological Relevance and Interpretation of Findings**

Collectively, the results indicate that selected *Hyptis verticillata* phytochemicals exhibit structurally plausible binding interactions with proteins central to metabolic regulation. The docking outcomes suggest that these compounds may serve as molecular scaffolds capable of engaging AMPK- and PPAR-γ-associated binding regions, with potential indirect relevance to IRS-1–mediated signaling. Importantly, the observed binding interactions should be interpreted as indicative of molecular recognition rather than definitive functional modulation.

The findings provide a computational basis for further investigation into the metabolic relevance of *H. verticillata*, particularly in the context of multi-target natural product research. Future studies incorporating molecular dynamics simulations, biochemical assays, and cellular models will be essential to elucidate the functional consequences of these interactions and to determine whether binding translates into measurable metabolic effects.

## CONCLUSION

In summary, this study presents a systematic in silico evaluation of *Hyptis verticillata* phytochemicals against AMPK, PPAR-γ, and IRS-1, identifying several compounds with favorable binding profiles and drug-likeness characteristics. By combining molecular docking with interaction analysis and pharmacokinetic screening, the work contributes to the growing body of evidence supporting the use of computational approaches to prioritize natural products for metabolic research. The results establish a rational foundation for experimental validation and further exploration of *H. verticillata* as a source of metabolically relevant bioactive compounds.

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