In-silico profiling of phenolic acids for gallstone prevention: a basis for in-vitro studies in high physical activity and rapid weight loss

Perfiles in silico de ácidos fenólicos para la prevención de cálculos biliares: una base para estudios in vitro en actividad física intensa y pérdida de peso rápida

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Abstract

Background: Cholelithiasis is increasingly seen in individuals with rapid weight loss or high physical stress, such as athletes. Existing therapies like ursodeoxycholic acid have limited efficacy, prompting interest in safer natural options.
Aim: To assess the anti-cholelithiatic potential of selected phenolic acids via molecular docking and pharmacokinetic profiling.
Methodology: Molecular docking, ADMET analysis, and molecular dynamics simulations were performed for sinapic, p-coumaric, caffeic, and ferulic acids, focusing on targets involved in cholesterol and bile acid regulation (LXR, FXR, PPAR-γ, NPC1L1). Tools used included AutoDock 4.2, SwissADME, and GROMACS 2019.4.
Results: Sinapic acid showed the strongest binding with LXR (-6.65 kcal/mol), suggesting enhanced cholesterol efflux, while p-coumaric acid showed significant interaction with FXR (-4.86 kcal/mol), implicating a role in bile acid regulation. Both compounds exhibited stable binding and favourable pharmacokinetics with low toxicity.
Conclusion: Sinapic and p-coumaric acids appear promising for gallstone prevention, particularly in active individuals or those with rapid weight loss, warranting further in-vitro, in-vivo, and clinical studies.

Keywords

ADMET; bile acid metabolism; gallstone disease; molecular docking; phenolic acids; physical activity; rapid weight loss; sports medicine; sinapic acid.

Resumen

Antecedentes: La colelitiasis se observa cada vez con más frecuencia en personas con pérdida de peso rápida o alto estrés físico, como los deportistas. Las terapias existentes, como el ácido ursodesoxicólico, tienen una eficacia limitada, lo que ha suscitado interés en opciones naturales más seguras.
Objetivo: Evaluar el potencial anticolelitiasis de ácidos fenólicos seleccionados mediante acoplamiento molecular y perfil farmacocinético.
Metodología: Se realizaron acoplamiento molecular, análisis ADMET y simulaciones de dinámica molecular para los ácidos sinápico, p-cumárico, cafeico y ferúlico, centrándose en dianas implicadas en la regulación del colesterol y los ácidos biliares (LXR, FXR, PPAR-γ, NPC1L1). Las herramientas utilizadas fueron AutoDock 4.2, SwissADME y GROMACS 2019.4.
Resultados: El ácido sinápico mostró la unión más fuerte con el LXR (-6,65 kcal/mol), lo que sugiere un aumento en la eliminación del colesterol, mientras que el ácido p-cumárico mostró una interacción significativa con el FXR (-4,86 kcal/mol), lo que implica un papel en la regulación de los ácidos biliares. Ambos compuestos mostraron una unión estable y una farmacocinética favorable con baja toxicidad.
Conclusión: Los ácidos sinápico y p-cumárico parecen prometedores para la prevención de cálculos biliares, especialmente en personas activas o con pérdida de peso rápida, lo que justifica la realización de más estudios in vitro, in vivo y clínicos.

Palabras clave

ADMET; ácidos fenólicos; acoplamiento molecular; ácido sinápico; ácidos fenólicos; actividad física; enfermedad de cálculos biliares; medicina deportiva; metabolismo de los ácidos biliares; pérdida de peso rápida.

Introduction

Gallstone disease (GSD) is among the most common gastrointestinal disorders worldwide and a leading cause of hospital admissions, contributing significantly to both healthcare burden and economic costs (Lammert et al., 2016; Stinton & Shaffer, 2010). While classically associated with abdominal pain and biliary colic, gallstones are now increasingly linked to more severe complications, including a heightened risk of gallbladder cancer, cardiovascular events, and all-cause mortality (Shabanzadeh et al., 2017; Zheng et al., 2018). The pathogenesis of gallstones involves cholesterol supersaturation, biliary stasis, and mucin hypersecretion, which together promote the nucleation and retention of cholesterol crystals (Di Ciaula et al., 2018; Hofmann, 1988). These processes are modulated by a range of genetic, hormonal, metabolic, and lifestyle factors.

High-intensity exercise may be associated with a high frequency of gastrointestinal symptoms: a prevalence (proportion affected) reported in the literature of over 80%, particularly for forms of high-intensity exercise like running. This is initially because of the diversion of blood from the gut to skeletal muscle and thermoregulatory systems and secondarily due to sympathetic nervous and hormonal activation with changes in intestinal motility, transit, and nutrient absorptive capacity (Bertuccioli et al., 2024). Swimming, rowing, cycling, triathlons, and several long-distance running disciplines are a few examples of activities that may necessitate training for four to six hours every day, six days a week (Clark A. et al., 2016; MacKinnon, L. T. 2000). This cascade of effects eventually initiates a local inflammatory event with derangement of the macrosymbiotic microbiota as well as long-term systemic inflammation (Bertuccioli, A. et al., 2024).

In these cases, obesity and rapid weight loss represent a paradoxical dual risk for gallstone formation. In obesity, insulin resistance and overactivity of hepatic HMG-CoA reductase contribute to increased biliary cholesterol output. Conversely, rapid weight loss, especially post-bariatric surgery or through extreme calorie restriction, causes excessive mobilisation of cholesterol from adipose stores and hypomotility of the gallbladder, resulting in bile stasis and supersaturation (Ilton, 2024; Bicilioglu et al., 2017). Patients undergoing bariatric surgery who lose over 1.5 kg/week have an elevated risk of developing gallstones, with some studies reporting an eightfold increase in incidence (Johansson et al., 2014; Ribeiro Jr., 2024). Alarmingly, even adolescents engaging in intense weight loss protocols or elite sports training have shown increased susceptibility to gallstones due to metabolic shifts involving bile composition and gallbladder function (Bicilioglu et al., 2017). Additionally, impaired gallbladder motility contributes to bile stasis, promoting the development of biliary sludge, a precursor to gallstone formation (Gebhard RL et al., 1996; Shiffman ML et al., 1992).

These insights are particularly relevant in the context of sports and physical activity. Athletes and fitness enthusiasts often engage in intense training regimens and adopt strict dietary strategies to manage weight and performance. These interventions, while beneficial for physical conditioning, may inadvertently place stress on hepatobiliary physiology and increase the risk of cholelithiasis. Despite this growing concern, research exploring non-invasive gallstone prevention specifically for physically active individuals remains limited.

Currently, laparoscopic cholecystectomy is the standard treatment, but it is unsuitable as a preventive measure, particularly in asymptomatic individuals. Pharmacological options, such as ursodeoxycholic acid (UDCA), have limited efficacy, poor solubility in bile, and are associated with gastrointestinal side effects (Ursodeoxycholic Acid, 2019). Recurrence rates after UDCA therapy are substantial, ranging from 30% to 50% within five years and up to 70% over twelve years (Guarino et al., 2013). Although generally well tolerated, UDCA may still cause abdominal discomfort, allergic reactions, and upper respiratory symptoms (1mg, 2025). These limitations underscore the urgent need for safer, more effective, and preferably preventive strategies, particularly for populations engaged in high-performance physical activity.

Plant-derived compounds, especially phenolic acids, have emerged as promising candidates in the search for such alternatives. These naturally occurring compounds are abundant in fruits, vegetables, and whole grains and exhibit antioxidant, anti-inflammatory, and lipid-modulating properties (Kumar et al., 2019; Chambers et al., 2019). Vanillic acid, for example, has been shown to support hepatic cholesterol metabolism and improve bile flow. Among these, sinapic acid (SA), a cinnamic acid derivative,

has demonstrated antioxidant, anti-inflammatory, antifibrotic, and anticancer properties, partly mediated through nitric oxide signalling pathways (Ansari et al., 2021; Chen et al., 2017). SA and its derivatives (e.g., 4-vinylsyringol) also exhibit neuroprotective, antimutagenic, and antiglycemic activities.

However, the specific anti-cholelithiatic potential of phenolic acids has not been adequately characterized in the context of weight loss and high physical stress. In this regard, computational methods such as molecular docking, ADMET (absorption, distribution, metabolism, excretion, and toxicity) prediction, and molecular dynamics simulations offer valuable tools for preclinical screening. These in silico approaches are time-efficient and cost-effective and can help prioritize bioactive molecules for experimental validation (Kantari et al., 2024).

To address this research gap, the present study investigates the in silico anti-cholelithiatic potential of four phenolic acids - sinapic acid, ferulic acid, caffeic acid, and p-coumaric acid, using a vesicle model of cholelithiasis. The study aims to evaluate their potential in preventing gallstone formation, particularly under physiological conditions resembling intensive exercise or rapid weight loss, where bile stasis and cholesterol supersaturation are prevalent. The ongoing in vivo study justifies our findings and strengthens our hypothesis.

Method

Molecular Docking Analysis

This study evaluates the binding affinity of selected phenolic acids for key proteins involved in gallstone formation, namely Liver X Receptor (LXR), Farnesoid X Receptor (FXR), Peroxisome Proliferator-Activated Receptor Gamma (PPAR-γ), and Niemann-Pick C1-Like 1 (NPC1L1) through molecular docking. Docking simulations were performed using AutoDock (version 4.2.6, Scripps Research Institute). The overall docking workflow is illustrated in Figure 1.

Reconstitution and Solubilization of Target Proteins

The 3D crystal structures of the target proteins were obtained from the Protein Data Bank (PDB) (https://www.rcsb.org; accessed March 12, 2025). PDB IDs used were: (i) LXR: 6S4T, (ii) FXR: 10SH, (iii) PPAR-γ: 2VST, and (iv) NPC1L1: 3QNT.

Protein preparation was carried out using AutoDock Tools (ADT version 1.5.6), including water molecule removal, hydrogen atom addition, charge assignment (Gasteiger), and grid box adjustment to fully encompass the active site region.

Preparation of Ligands

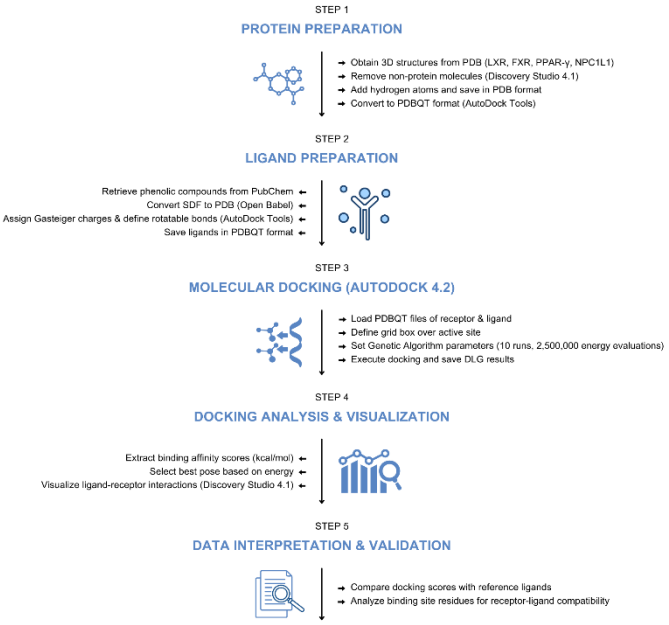
The 3D structures of the phenolic acids, including sinapic acid, caffeic acid, ferulic acid, and p-coumaric acid, were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/; accessed March 13, 2025). Open Babel (version 3.1.1) and AutoDock Tools were used to convert the ligands into the PDBQT format.

Docking Protocol

Docking was carried out with the Lamarckian Genetic Algorithm (LGA) using the following parameters: number of runs: 50, population size: 150, energy evaluations: 2,500,000, and grid spacing: 0.375 Å, as recommended for medium-affinity ligand binding studies [Morris GM et al., 2009]. Post-docking interaction analysis (hydrogen bonds, van der Waals, hydrophobic contacts) was performed using Discovery Studio Visualizer (version 4.1, BIOVIA).

Docking Validation: The protocol was validated for molecular docking by redocking known ligands into their respective binding sites to compare the predicted binding conformations with experimentally determined crystal structures. The accuracy of the docking process was validated by low RMSD values between the docked and experimental poses. RMSD values <2.0 Å confirmed acceptable precision of the docking setup.

Figure 1. Molecular Docking Workflow



Notes: This figure presents the workflow of the molecular docking studies performed to determine the binding affinities of phenolic acids to target receptors, including protein preparation, ligand preparation, and docking simulations.

ADMET Analysis and Pharmacokinetics

Computational tools were used to determine the drug-likeness and putative oral bioavailability of the phenolic acids based on their pharmacokinetic profiles and toxicity.

Pharmacokinetic Screening

The pharmacokinetic properties of the compounds were predicted using SwissADME (http://www.swissadme.ch; March 15, 2025), a freely available online tool for ADME analyses. It has been applied in the prediction of important pharmacokinetic parameters, such as GI absorption, BBB penetration, CYP450 inhibition, logP, and TPSA. To visualize GI absorption and BBB permeability and compare the individual absorption properties of each compound, a boiled egg model was used to evaluate and gain insights into the suitability of the compound for oral administration.

Toxicity Assessment

Toxicity profiles were evaluated using Cheminfo Property Explorer (https://www.cheminfo.org; accessed March 15, 2025), an open-access cheminformatics platform. To ensure safety, toxicity endpoints, such as mutagenicity, tumorigenicity, irritancy, and reproductive toxicity, were studied. However, the tool has limitations, as its predictions rely on an underlying algorithm and dataset and should be viewed with caution; experimental validation is needed. Moreover, it may not address all toxicological endpoints, which will require the use of additional tools to build a complete profile.

Molecular Dynamics Simulation

Simulation Environment

Molecular dynamics (MD) simulations were performed in GROMACS version 2019.4, using Newtonian mechanics to study time-dependent atomic interactions and protein-ligand complex stability.

Force Fields

The GROMOS force field was selected for simulations based on its proven compatibility with protein-ligand studies involving phenolic compounds. Ligand topologies were generated using the Automated Topology Builder (ATB, https://atb.uq.edu.au; accessed March 16, 2025).

System Preparation

The selected protein-ligand complex was energy-minimized using 1500 steps of the steepest descent algorithm. Solvation was done in a cubic box with a 0.5 nm margin using the SPC water model. The system was neutralized with 0.15 M NaCl to mimic physiological conditions.

Equilibration and Production Run

Equilibration was performed under the NPT ensemble (constant pressure and temperature) for 1 ns. A 100 ns production run was followed to capture dynamic behavior, which is considered standard for evaluating ligand-binding stability in comparable systems.

Trajectory Analysis

Post-MD analysis was conducted using built-in GROMACS utilities to compute: (i). Root Mean Square Deviation (RMSD), which represents general structural stability; (ii). Root Mean Square Fluctuation (RMSF), which determines the flexible regions of the molecule; and (iii). The radius of gyration (Rg) indicates compactness of the protein structure (iv). Solvent Accessible Surface Area (SASA): Analyzes the exposure of the protein to the solvent and (v). Hydrogen bonding (H-bond) interactions for binding stability and interactions. Trajectory analysis was performed as previously described.

These were benchmarked against reference values from literature and previous simulations to ensure consistency.

Statistical and Ethical Considerations

As an in silico study, no animal or human data were used, eliminating the need for Institutional Review Board approval. All analyses adhered to the FAIR principles (Findable, Accessible, Interoperable, and Reusable). While no inferential statistics were applied (as this was not an experimental or clinical study), RMSD and interaction values were compared across all ligands and proteins to ensure interpretative consistency. Where possible, averages and standard deviations were computed for trajectory metrics.

Results

Molecular Docking Analysis

The structural importance of phenolic acids was systematically studied for their binding affinities to physiological proteins involved in gallstone formation, that is, Liver X Receptor (LXR), Farnesoid X Receptor (FXR), PPARRγ, and Niemann-Pick C1-Like 1 (NPC1L1). The binding energies (kcal/mol) of the compounds examined are listed in Table 1.

Table 1. Binding Energies (kcal/mol) of Phenolic Acids with Key Receptors

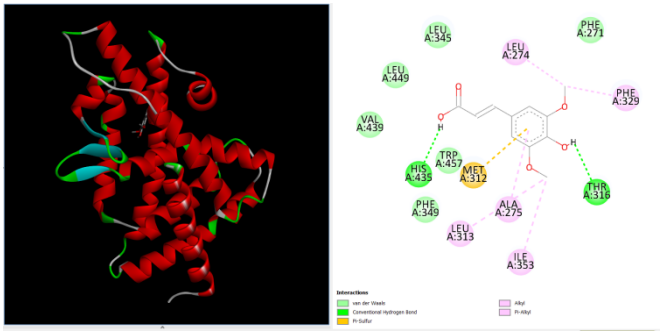
Drug	LXR	FXR	PPAR-γ	NPC1L1
Caffeic acid	-4.56	-4.57	-3.94	-4.30
P-coumaric acid	-5.18	-4.86	-4.72	-4.56
Ferulic acid	-5.07	-4.01	-3.94	-3.60
Sinapic acid	-6.65	-4.45	-4.08	-3.84
Gallic acid	-4.18	-3.39	-3.58	-2.89
Vanillic acid	-3.84	-3.67	-3.79	-3.99
Protocatechuic acid	-4.38	-3.69	-3.87	-3.71
Syringic acid	-3.79	-4.04	-3.43	-3.53
Gentisic acid	-4.71	-3.71	-4.48	-3.81
Rosemarinic acid	-3.42	-2.41	-3.18	-3.48
Ursodeoxycholic acid	-4.95	-4.98	-6.01	-4.47

Notes: This table shows the binding affinities of various phenolic acids (e.g., sinapic acid and caffeic acid) with important receptors (LXR, FXR, PPAR-γ, and NPC1L1) for gallstone formation. Sinapic acid exhibited maximum binding affinity with LXR (-6.65 kcal/mol), indicating its probable role in cholesterol metabolism and bile acid synthesis.

Among the phenolic acids, sinapic acid exhibited the highest binding affinity toward LXR (-6.65 kcal/mol), surpassing p-coumaric acid (-6.11 kcal/mol) and ferulic acid (-6.00 kcal/mol). With FXR, p-coumaric acid showed the best interaction (-4.86 kcal/mol), slightly ahead of sinapic acid (-4.82 kcal/mol). For PPAR-γ, UDCA displayed the highest affinity (-6.01 kcal/mol), followed by p-coumaric acid (-4.72 kcal/mol). In the case of NPC1L1, p-coumaric acid (-4.56 kcal/mol) and sinapic acid (-4.32 kcal/mol) again emerged as top-performing ligands.

The 2D and 3D binding interactions are visualized in Figure 2. These show hydrogen bonding and hydrophobic interactions stabilizing the ligand–receptor complexes, particularly in the case of sinapic acid with LXR.

Figure 2. Structural insights into protein-ligand interactions: docking study of LXR with sinapic acid.



Notes: This figure illustrates the interactions between sinapic acid and LXR-β, including the hydrogen bonds and the contribution of hydrophobic interactions to strong binding.

Key Findings

1. LXR Binding: The docking of sinapic acid resulted in the best binding energy (-6.65 kcal/mol) compared to the reference drug ursodeoxycholic acid (UDCA) (-4.95 kcal/mol) among the ligands against LXR. The higher affinity of sinapic acid, on the other hand, can be attributed to the formation of stable H-bond interactions between sinapic acid and the key amino acid residues residing within the active site of the receptor. Moreover, hydrophobic interactions and π–π stacking can be further compensated for by the incorporation of conjugated double bonds and hydroxyl groups of sinapic acid, which can increase the stability of a single compound within the binding pocket. This probably led to strong hydrogen-bonding interactions, resulting in stronger bonding interactions relative to UDCA, which has a much more limited hydrogen-bonding profile. Considering the function of LXR in regulating cholesterol output and bile acid synthesis, these results indicate that sinapic acid can be effectively responsible for the modulation of cholesterol homeostasis, contributing to gallstone prevention. Here, we describe the structural details of the protein-ligand interactions (Fig. 2).
2. FXR Binding: The affinity of p-coumaric acid (-4.86 kcal/mol) for binding to FXR was the highest, followed by sinapic acid (-4.45 kcal/mol). The results showed that UDCA had the most negative binding free energy (-4.98 kcal/mol) toward FXR. Sinapic acid has a relatively weak binding affinity for FXR, which may be secondary to bile acid metabolism and is mediated primarily by LXR activation.
3. PPAR-γ Binding: p-Coumaric acid (-4.72 kcal/mol) and gentisic acid (-4.48 kcal/mol). The highest binding energy (-6.01 kcal/mol) was shown by UDCA for the PPAR-γ target. As PPAR-γ is a lipid metabolism modulator, these findings suggest that phenolic acids have the potential for further benefits in gallstone disease through the modulation of the lipid profile.
4. NPC1L1 Binding: p-coumaric acid (-4.56 kcal/mol) bound best to NPC1L1, a target of cholesterol absorption. Sinapic acid (NG, 3037902) displayed a moderate binding affinity (−3.84

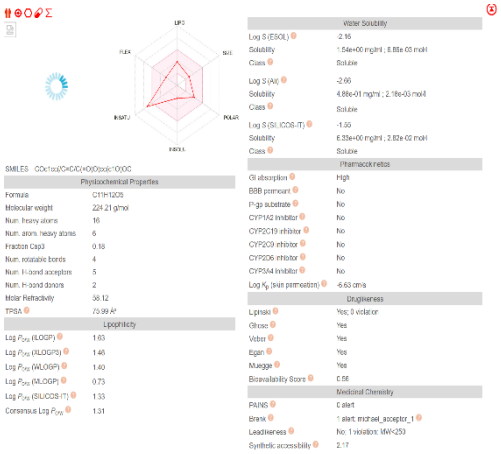
kcal/mol) with NPC1L1; consequently, it would likely exhibit a limited effect on the inhibition of intestinal lipid absorption.

Pharmacokinetics and ADMET Properties

In addition, pharmacokinetic and toxicity analyses were performed to evaluate the drug-likeness of sinapic acid. It has non-mutagenic and non-tumorigenic drug profiles, making it a possible drug candidate. Sinapic acid is water-soluble (logP 1.58) and has a relatively low molecular weight (224.21 g mol^{1}), which may facilitate its penetration through cell membranes. Its high gastrointestinal (GI) absorption shows its potential as an oral drug, and its inability to pass the blood-brain barrier (BBB) decreases the chances of central nervous system-related side effects. Moreover, it lacks CYP450 inhibition, which minimizes possible drug-drug interactions.

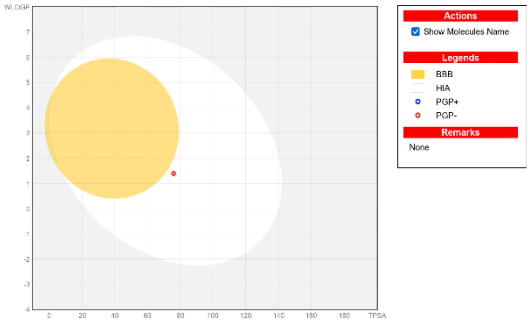
These are delineated in Figure 3, which follows Figure 4 to present the Boiled Egg model, corroborating sinapic acid as a putative therapeutic candidate.

Figure 3. Visualization of Key Pharmacokinetic Parameters in Drug Absorption and Distribution.



Notes: The figure presents an overview of the important pharmacokinetic parameters (i.e., GI absorption and BBB penetration) of sinapic acid represented by drug candidates with oral availability and lower CNS-induced side effects (summary).

Figure 4. Boiled Egg Model: Cell Membrane Structure and Function Representation.



Notes: The boiled egg model suggests drug GI (gastrointestinal) absorption with low BBB (blood-brain barrier) penetration of sinapic acid.

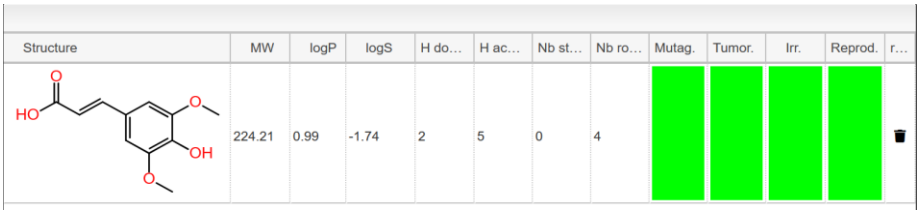
Clinical Relevance: This means that LXR interacts strongly with sinapic acid with a binding affinity of -6.65 kcal/mol, and this strong interaction may mediate effectiveness in promoting cholesterol metabolism. These affinities are comparable to or greater than those of many established cholesterol-modulating agents, establishing sinapic acid as a novel lead for drug development. Its computational in silico binding is strong, but its bioavailability, metabolic stability, and in vivo efficacy need to be proven for clinical effectiveness; however, all require experimental validation.

Toxicity Analysis

Sinapic acid was evaluated using the Property Explorer tool and showed adequate physicochemical and toxicity properties. The molecular weight of this compound was 224.21 g/mol, which is sufficient for drug-like compounds. A log P value of 0.99 indicates moderate lipophilicity (as would be desirable for a compound with good permeability through biological membranes and reasonable solubility). A logS value of -1.74 was calculated, which indicates moderate solubility in water, favoring oral bioavailability. In addition, sinapic acid meets Lipinski's rule of five with two hydrogen bond donors and five hydrogen bond acceptors. The molecule has four rotatable bonds and is relatively flexible, which can increase its binding to biological targets.

Toxicology assessments also indicated no potential for (green) mutagenicity, tumorigenicity, irritancy, or reproductive toxicity. These findings indicate that sinapic acid has a favorable toxicity profile (as shown in Figure 5), supporting further pharmacological evaluation of the compound concerning its potential anti-cholelithiatic picomotor action.

Figure 5. Toxicity Prediction and Analysis Using Property Explorer: Assessing Drug Safety.



Notes: This figure shows that sinapic acid is non-mutagenic, non-tumorigenic, and possesses a therapeutic window suitable for experimental pharmacological potentials, such as analgesic and antioxidant efficacies.

Molecular Dynamics Simulation

The stability and dynamics of the protein-ligand complexes were evaluated using 100 ns all-atom molecular dynamics (MD) simulations. Two protein-ligand complexes, 6S4T-LIG1 (Sinap) and 6S4T-LIG1 (Standard), were examined in terms of their structural features, including root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), and solvent-accessible surface area (SASA). The mean values of these parameters are listed in Table 2.

Table 2. Structural and dynamic comparison of 6S4T-LIG1 with Sinap and Standard Ligands.

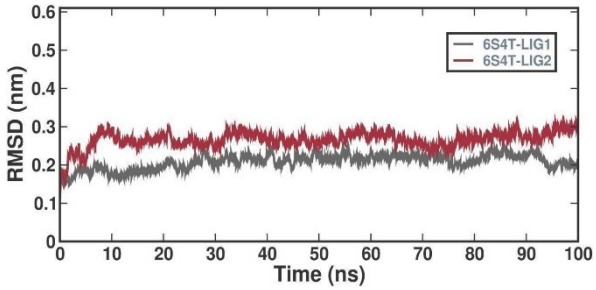
No	Protein	Average RMSD (nm)	RMSF (nm)	Radius of gyration (nm)	Average SASA (nm^2)
1	6S4T-L1G1 (Sinap)	0.19 +/- 0.01	0.14 +/- 0.05	1.87 +/- 0.02	138.46 +/- 2.20
2	6S4T-L1G1 (Standard)	0.24 +/- 0.01	0.14 +/- 0.05	1.85 +/- 0.02	136.46 +/- 2.15

Notes: In this table, the structural and dynamic attributes (RMSD, RMSF, Rg, and SASA) of the 6S4T-LIG1 complex with sinapic acid and a representative ligand are compared. These results indicate that sinapic acid binds more stably and preserves a better structure than the standard ligands, as shown by the lower RMSD.

Root Mean Square Deviation (RMSD)

We concluded that RMSD is a part of determining the stability of the protein-ligand complex based on the RMSD plot that we generated and can be computed by calculating the degree of displacement of the atoms in the protein-ligand complex, depending on time, as a function of the calculation. The lower the RMSD, the more stable the conformation. Both complexes showed stable RMSD values over the 100 ns, which were 0.19 ± 0.01 nm for the 6S4T-LIG1 (sinap) complex, lower than the 6S4T-LIG1 (standard) complex average RMSD of 0.24 ± 0.01 nm. This indicates that the sinap-bound complex is more stable and undergoes fewer conformational changes. The RMSDs showed that both complexes reached equilibrium states relatively early in the simulations and displayed little structural divergence. Figure 6 shows the root-mean-square deviation of the backbone atoms during the simulation.

Figure 6. Root mean square deviation of backbone atoms

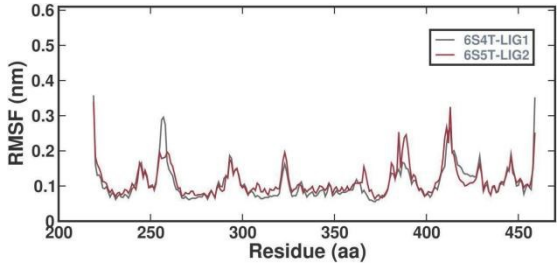


Notes: RMSD analysis of protein-ligand complexes over a 100 ns simulation reveals the stability of the sinapic acid-bound complex over the standard ligand.

Root Mean Square Fluctuation (RMSF)

This means that the Sinap-bound complex is more stable and has fewer conformational changes. The RMSDs exhibit that both complexes reached equilibrium states relatively early on in the simulations and displayed little structural divergence. Figure 6 shows the root mean square deviation of backbone atoms during the simulation. Both complexes showed localized fluctuations, mainly in the loop and stable core structural regions. Ligand binding did not cause notable destabilization of the corresponding protein, confirming the stability of the complexes. It depicts the root-mean-square fluctuation of the C-alpha atoms in these complexes, as shown in Figure 7.

Figure 7. Root mean square fluctuation of c-alpha atoms of Complexes

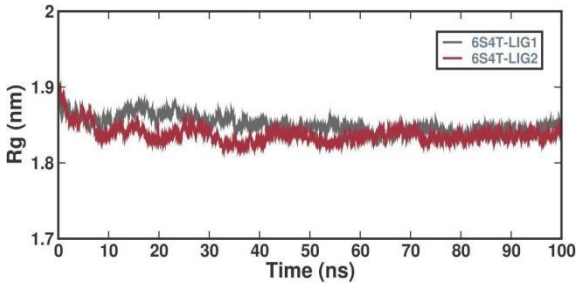


Notes: Root mean square fluctuation (RMSF) of C-alpha atoms reveals localized flexibility of the loop regions and stability of the core regions of the secondary structure, which indicates the stability of the sinapic acid-bound complex.

Radius of Gyration (Rg)

The radius of gyration (Rg) indicates the global compactness of the protein-ligand complex, where lower values indicate tighter structural packing. Throughout the simulation, the trends in the Rg profiles for both complexes were comparable with minor deviations. For the standard ligand complex, the Rg determined is 1.85 ± 0.02 nm, while the 6S4T-LIG1 (Sinap) complex's corresponding Rg was found within 1.87 ± 0.02 nm, implying a slightly elongated conformation. These findings indicated that the characterized complexes exhibited stable organization, and no major expansion or contraction was observed during the simulation. The nearly constant Rg values further support the idea that the protein did not unfold or undergo significant conformational changes during ligand binding and remained in a native state. Figure 8 shows the mean radius of gyration (Rg) of the backbone atoms as a function of time during the simulation (Rg = 0.836 nm).

Figure 8. RG of backbone atoms

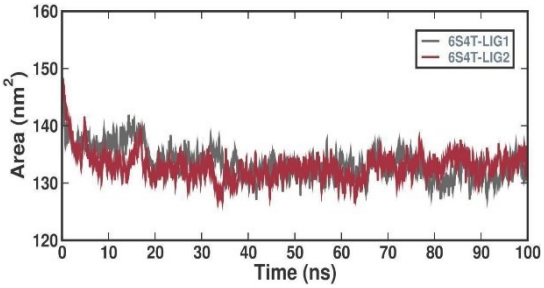


Notes: Plotted radii of gyration (Rg) show the compactness of protein-ligand complexes. There is only a relatively extended but stable conformation of sinapic acid.

Solvent-Accessible Surface Area (SASA)

SASA represents the solvent-accessible surface area of the protein and correlates with the compactness of the structural conformation and conformational changes upon ligand binding. SASA values were mostly unchanged, and the predicted complex bound to Sinap has a higher SASA than that from the standard ligand (138.46 ± 2.20 nm^2 vs. 136.46 ± 2.15 nm^2). This subtle adjustment indicated that ligand binding did not substantially alter the surface accessibility of the protein. These stable values of SASA suggest that, while there may be some local movement, the overall structure of both proteins remains preserved, and the proteins do not exhibit significant conformational changes during the simulation. Figure 9 shows the time evolution of SASA for both complexes.

Figure 9. SASA of backbone atoms

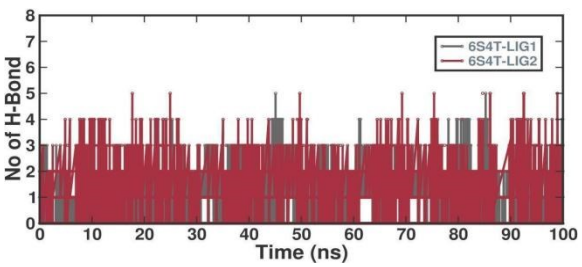


Notes: The SASA values show that the complexes are stable, as there is little change in solvent access upon ligand binding.

Hydrogen Bonding Analysis

Hydrogen bonding plays a pivotal role in stabilizing protein-ligand interactions, which ultimately helps the integrity of the complex and binding affinity. A key output of MD was to verify that the hydrogen bonds formed during molecular docking persisted in the simulation. The hydrogen bonds formed between proteins and ligands in the dynamic analysis were consistent with the stability of the complexes. Reproducing the interactions that were present during docking under physiologically relevant conditions further supported the reliability of the docking predictions. The hydrogen bond patterns observed during the simulations for both complexes are shown in Fig. 10.

Figure 10. H-Bond



Notes: The interaction diagrams of hydrogen bonds show interactions between proteins and ligands, which persisted and confirmed the interactions that were observed during docking and contributed to the stability of the complexes.

Discussion

In this study, eight phenolic acids – sinapic, p-coumaric, ferulic, caffeic, gallic, vanillic, protocatechuic, and syringic acids – were docked against major targets in cholesterol metabolism and bile acid transport: liver X receptor (LXR), peroxisome proliferator-activated receptor gamma (PPAR-γ), farnesoid X receptor (FXR), and Niemann-Pick C1-like 1 (NPC1L1). The compound with the highest binding affinity for LXR (-6.65 kcal/mol) was sinapic acid, which continuously formed hydrogen bonds and displayed hydrophobic interactions during molecular dynamics simulations. Furthermore, p-coumaric acid showed good docking to FXR (-6.18 kcal/mol), which is necessary for the feedback mechanisms that limit the synthesis of bile acids. Ferulic acid's moderate binding affinities across a range of targets and its remarkable RMSF and Rg stability in MD simulations suggested a degree of conformational flexibility. Caffeic acid, on the other hand, demonstrated lower binding affinities (-5.88 kcal/mol to LXR) and higher atomic fluctuations in simulations, indicating less target engagement, despite its reputation as an antioxidant. Gallic and vanillic acids showed binding energies between -5.7 and -5.9 kcal/mol, but they lacked sustained stability measures under MD simulation, indicating less favourable dynamics. Protocatechuic acid primarily interacts with NPC1L1, despite having a lower affinity, suggesting its significance in intestinal cholesterol absorption. In comparison to ursodeoxycholic acid (UDCA), sinapic and p-coumaric acids showed superior ADMET profiles and competitive or better docking scores. The idea that certain phenolic acids might cooperate to prevent gallstones through complementary or synergistic mechanisms like transporter inhibition, bile acid regulation, and cholesterol efflux is supported by this more in-depth study.

Among other relevant bile-regulatory targets, the secondary candidates p-coumaric acid, caffeic acid, and ferulic acid also demonstrated promising docking affinities with PPAR-γ, FXR, and NPC1L1. P-coumaric acid's interaction with FXR points to a role in bile acid feedback inhibition, while its binding to NPC1L1 suggests a possible reduction in intestinal cholesterol uptake. All of these pathways suggest a multifaceted approach to bile cholesterol management. Molecular dynamics analysis confirmed the long-lasting hydrogen bonding and structural stability of these ligand–target complexes. These interactions are in line with findings by Pawar SH et al. (2023), who reported polyphenol multi-receptor interactions using similar computational techniques. Even though these interactions are structurally favourable, biological tests are required to validate functional outcomes, such as physiological effects, gene activation, and modifications in protein expression.

Furthermore, a dual mechanism of increased hepatic excretion and decreased intestinal absorption was achieved by interacting with NPC1L1, a crucial gut cholesterol transporter, which enabled reduced cholesterol reabsorption. The stability of the RMSD (from 1.4 to 2.3 Å) and continuous hydrogen bonding with minimal atomic fluctuation (RMSF) in MD simulation validate the structural dependability of the docked complex under physiological conditions. Rg values indicated a well-folded protein-ligand structure, while SASA data of the complexes demonstrated higher dynamic stability and stable exposure of the ligands to the solvent environment, particularly for the sinapic acid-LXR complex. The phenolic acids need to have good ADMET and drug-like properties [Pawar SH et al., 2023]. Due to sinapic acid's high gastrointestinal absorption, lack of blood-brain barrier crossing, and lack of interaction with cytochrome P450, the likelihood of metabolic drug-drug interactions was reduced. Thankfully, it also satisfied Lipinski's Rule of Five, indicating that it has the potential to be both bioavailable and effective when taken orally. Since none of these compounds were predicted to be mutagenic, tumorigenic, irritants, or reproductive toxicants, it was suggested that they might be appropriate for use as preventive supplementation if necessary (e.g., by populations with extreme metabolic strain, such as those undergoing a bariatric procedure or elite athletes).

Gallstones can also develop as a result of increased hepatic cholesterol secretion and gallbladder hypomotility brought on by rapid weight loss [Di Ciaula A et al., 2016]. Post-bariatric surgery, crash dieting, and high levels of physical activity in athletes all encourage lipolysis [Horowitz JF 2000; Zhu JY 2024;

Thompson D et al., 2012]. Additionally, biliary secretion increases the risk of hepatic cholesterol overload and cholesterol saturation. In this case, sinapic acid's activation of LXR is particularly important. Through the induction of reverse cholesterol transport and the expression of ABCA1 and ABCG5/ABCG8, LXR facilitates biliary cholesterol efflux (Yu, L. et al., 2002). This mechanism may help to reduce the cholesterol saturation of bile and prevent the formation of gallstones during a time of rapid metabolic change. At the molecular level, it was shown that sinapic acid and the LXR ligand-binding domain had strong hydrogen bonds and hydrophobic contacts, indicating efficient conformational stability and the potential for receptor activation. FXR and p-coumaric acid work together to control bile acid production through feedback inhibition by inhibiting CYP7A1 and FGF19 signaling.

Given that rapid weight loss raises the risk of gallstones, these findings are pertinent to the bariatric and sports medicine populations. Due to changes in lipid metabolism and excessive adipose tissue turnover, weight-cycling athletes, such as boxers, wrestlers, and military personnel, are especially vulnerable to elevated biliary cholesterol saturation. Sinapic acid, found in foods like whole grains and seeds (Nicifo-rovic, N. 2014), can be utilized as a dietary supplement or functional food additive in these groups. Any substance used by athletes must also follow safety regulations, avoid impairing performance, and not interfere with other supplements or ergogenic drugs. Because of its predicted safety profile and compatibility with food sources, sinapic acid is a good choice for these treatments.

The most common medication used to dissolve gallstones is ursodeoxycholic acid (UDCA). However, in addition to limited bile solubility and ineffectiveness in preventing recurrence, it causes gastrointestinal adverse effects, including nausea, diarrhoea, and abdominal discomfort. Furthermore, the UDCA + PUFA combination therapy performs better than UDCA alone in terms of response and cholesterol gallstone dissolving rates [Lee SY et al., 2024]. While docking scores and pharmacological profiles suggest promise, direct comparative efficacy with UDCA requires clinical and animal studies.

The high rate of recurrence is the upper limit of UDCA therapy for gallstone dissolution. Recurrences are common after successful treatment, especially in people with many stones; studies have shown that recurrence rates range from 30% to 50% after five years and from 50% to 70% after twelve years [Portincasa P et al., 2012; Lazaridis KN et al., 2001; and Villanova N et al., 1989]. For the past few decades, UDCA has been a controversial treatment option for patients with gallbladder stones due to these factors. However, the efficacy of this bile acid as a medicinal agent has been reevaluated on numerous occasions, both in terms of its ability to dissolve and its anti-inflammatory qualities.

This increases their target range, and for phenolic acids such as sinapic acid, they can also be affected by intestinal absorption (NPC1L1), cholesterol efflux (LXR), or bile acid feedback loops (FXR). For any month-long preventive action, this makes them a potentially safer and nutraceutical-compatible option. Because physically active people, particularly those who weight-cycle (e.g., wrestlers, bodybuilders, and military personnel), are more likely to develop gallstones, adding phenolic acids to functional foods or as dietary supplements may be a promising preventive measure [Chambers KF et al., 2019]. Sinapic acid, which is dietary-compatible and can be made into a beverage, pill, or fortified food targeted at high-risk individuals, is found in whole grains, seeds, and vegetables. Medication-targeted therapies that are non-invasive, performance-compatible, have minimal side effects, and may work in concert to improve hepatic function and antioxidant defence should also be prioritized in sports medicine.

This chemical interaction suggests that sinapic acid may favourably modulate LXR signaling, an important regulator of biliary secretion and cholesterol homeostasis. LXR activation promotes cholesterol outflow from hepatocytes by upregulating ABCG5/ABCG8 transporters, which may reduce biliary cholesterol saturation, a critical step in the formation of gallstones.

Although the in silico technique clarifies likely binding affinities, molecular stability, and pharmacokinetics (the study of metabolism, mechanism of action, and toxicity), a significant drawback of the study is the experimental validation. Computational docking can't tell us how well something will work in the real world on its own, especially when it comes to the complex physiological conditions that high-performance athletes have to deal with. Both in vitro and in vivo, we need to keep a close eye on how metabolism, the gut microbiota, and the breakdown of chemicals change when they are under physical stress.

This is very important in sports medicine, as people often lose or gain weight quickly, work out hard, and vary their diets. Wrestlers, bodybuilders, and military personnel are more likely to have gallstones

because they have too much cholesterol in their livers and too much lipolysis. One option to minimise this risk is to use sinapic acid to activate LXR and let cholesterol exit the body. However, this will only work if the bioavailability problems can be fixed. Nanoemulsions and encapsulation are two examples of lipid-based delivery systems that can help the body absorb things better.

Our ongoing in vitro experiments will confirm the pathways that control bile acids and move cholesterol out of the liver. This will make it possible for athletes to acquire specific supplements. But before the medicine can be used in real life, we need to know how safe and tolerable it is for people, especially since competitive sports have rigorous laws about additives and how they affect the body.

The next step, based on these encouraging in silico results and current lab research, is to build a robust preclinical foundation that supports phenolic acid-based treatments, functional meals, or supplements for people who are active. Additionally, their preventive effects will need to be tested in subpopulations in athletes and those undergoing rapid changes in body composition, and the therapeutic decisions made accordingly.

Conclusions

This study provides in silico evidence highlighting sinapic acid as a novel natural gallstone-retardant agent, especially in stress conditions such as crash diets and rigorous exercise regimes. The discovery of high LXR binding affinity, a good ADMET profile, and stable molecular properties indicates that this compound could be developed as a novel mechanism-based therapeutic lead. Furthermore, the influence of p-coumaric acid on FXR illustrates the mollifying effect of multi-targeted prevention of FXR through phenolic acids. These insights will provide a framework for future development in sports medicine and metabolic health.

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