nutrición clínica y Dietética Hospitalaria

Artículo Original

Nutr Clín Diet Hosp. 2026; 46(1):178-185
DOI: 10.12873/461baulia

In silico investigation of DLBS3233 bioactives targeting inflammatory, metabolic, and cholesterol transport pathways in gallstone (cholelithiasis) pathogenesis

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Recibido: 29/diciembre/2025. Aceptado: 31/enero/2026.

ABSTRACT

Background: Gallstone disease (cholelithiasis) remains a prevalent hepatobiliary disorder with rising incidence globally and limited pharmacologic options[1,2]. Ursodeoxycholic acid (UDCA), the mainstay therapy, is restricted to small non-calcified stones, requires prolonged administration, and has a high recurrence rate[3]. DLBS3233 an Indonesian bioactive fraction derived from Lagerstroemia speciosa and Cinnamomum burmannii is known for its metabolic benefits through PPARγ modulation, suggesting potential therapeutic relevance in cholesterol-related diseases such as gallstone formation[4]. This study aimed to explore, using an in silico approach, the potential molecular interactions of DLBS3233 bioactive compounds with key molecular targets involved in cholelithiasis pathophysiology[5,6].

Methods: An exploratory computational study was conducted using molecular docking (CB-Dock2) to evaluate the interactions of two DLBS3233 bioactives, Cinnamtannin B1 (C1) and Ellagic acid (C2), with ten targets related to cholesterol transport, metabolism, nuclear receptor regulation, inflammation, and vascularization. Bioactivity prediction (PASS), toxicity (ProTox-II), and drug-likeness analyses were performed as preliminary screening tools. No molecular dynamics simulations or experimental validations were conducted[16].

Conclusion: This exploratory in silico study suggests that DLBS3233 bioactives derived from *Lagerstroemia speciosa* and *Cinnamomum burmannii* may interact with multiple molecular targets involved in cholesterol transport, lipid metabolism, inflammatory signaling, and hepatobiliary vascular regulation. The findings generate hypotheses and encourage additional validation via experiments and molecular dynamics simulations.

KEYWORDS

Molecular docking, cholesterol metabolism, PPARγ, hepatobiliary inflammation, plant bioactives, computational pharmacology.

INTRODUCTION

Gallstones (cholelithiasis) are a common hepatobiliary disorder worldwide. In the United States, the prevalence of cholelithiasis is estimated at 10–15% of the adult population, with a higher incidence in women, obese individuals, and those with diabetes mellitus[1]. Globally, 6% of the population have gallstones, with higher rates in females and in South America[2]. The incidence of gallstones may be increasing. In developing countries like Indonesia, the incidence of gallstones continues to increase due to lifestyle changes, high-fat diets, and urbanization. Research at Dr. Cipto Mangunkusumo National Hospital (RSUPN) noted that cholelithiasis is one of the most common causes of right upper abdominal pain and elective cholecystectomy[3]. Serious complications such as acute cholecystitis, pancreatitis, and cholangitis can occur if not treated promptly and appropriately.

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The main pharmacological therapy for cholelithiasis is the use of ursodeoxycholic acid (UDCA), which works to dissolve cholesterol stones. However, the efficacy of this therapy is limited to small, non-calcified stones, and requires a long treatment period of 6–24 months[3]. Side effects such as diarrhea, nausea, and mild liver dysfunction have also been reported. Furthermore, after discontinuation of therapy, gallstone recurrence can occur in >50% of patients within 5 years[4]. While invasive approaches such as cholecystectomy are effective, they carry risks of infection, bleeding, and post-cholecystectomy syndrome.

The National Agency of Drug and Food Control, Republic of Indonesia, has approved the new insulin-sensitizing drug DLBS3233, which is presently being sold for the treatment of type 2 diabetes. DLBS3233 is a combination- bioactive-fraction generated from Lagerstroemia speciosa and Cinnamomum burmannii, two Indonesian herbs[5,6]. PI-3-kinase (phosphatidylinositol-3-kinase), GLUT-4 (glucose transporter-4), Akt, PPAR-gamma, and PPAR-delta were all expressed at the mRNA level in 3T3 Swiss Albino preadipocyte cells as a result of DLBS3233, according to preclinical investigations. At the mRNA level, it also decreased the expression of the resistin gene and raised the expression of GLUT-4 and adiponectin[7]. When DLBS3233 was administered to insulin-resistant Wistar rats, the levels of blood glucose, insulin, as well as triglycerides, total cholesterol, high-density lipoprotein, and low-density lipoprotein, were all found to be under control. Studies on teratogenicity, subchronic poisoning, and toxic effect have validated the reliability of DLBS3233[8].

This study was conducted to evaluate the clinical benefits of DLBS3233 as an add-on therapy in cholelithiasis patients. There are study from Rajendran, S., & Vellapandian[9] showed that physiological proteins involved in gallstone formation are Liver X Receptor (LXR), Farnesoid X Receptor (FXR), PPARRγ, and Niemann-Pick C1-Like 1 (NPC1L1). The pathogenesis of cholelithiasis, or gallstone formation, is the result of a complex interaction between genetic, environmental, and metabolic factors[10]. This process involves changes in bile composition, impaired gallbladder motility, stone nucleation and growth, and the influence of chronic inflammation on hepatobiliary tissue. An imbalance between cholesterol, bile salts, and bilirubin causes bile to become supersaturated, triggering the formation of cholesterol crystals that then develop into gallstones. In addition to these mechanistic factors, molecular pathways and cellular signaling also play a crucial role in the development of cholelithiasis. From other study suggests that ABCG8 D19H (G/C) and ABCG5 Q604E (C/C) genotypes may play a significant role in gallstone diseases[11]. Genetic variants in *CYP7A1* which are associated with increased levels of LDL cholesterol, are associated with an increased risk of GSD[12].

One modern approach to understanding this mechanism is through the ligand-protein interaction approach, in which bioactive compounds or ligands interact with protein receptors involved in various physiological processes. Several receptors known to be associated with the pathogenesis of cholelithiasis include: 1) Vascular Endothelial Growth Factor Receptor 2 (VEGFR-2), which plays a role in vascularization and perfusion of hepatobiliary tissue. Activation or inhibition of this receptor can affect blood flow and tissue repair in conditions of gallbladder inflammation, 2) Tumor Necrosis Factor Alpha (TNF-α) and 3) Interleukin-6 (IL-6) are receptors that play a role in the inflammatory response. Increased expression of these two receptors is often associated with chronic inflammation of the gallbladder wall (cholecystitis), which can alter bile composition and increase the risk of stone formation[9].

Peroxisome Proliferator-Activated Receptor Gamma (PPARγ) plays a role in regulating lipid and cholesterol metabolism. Imbalanced PPARγ activity can lead to cholesterol accumulation in the bile, increasing the risk of cholelithiasis. Hydroxymethylglutaryl-CoA Reductase (HMG-CoA Reductase) is a key enzyme in cholesterol biosynthesis. Excessive activity of this enzyme can increase cholesterol levels in the bile, contributing to gallstone formation[9].

METHODS

Materials & Methods

This research was designed as a purely exploratory in silico study**.** All analyses were computational and aimed to predict potential molecular interactions between DLBS3233 bioactives and proteins involved in gallstone pathogenesis. The selected targets represent heterogeneous molecular classes, including enzymes, membrane transporters, nuclear receptors, soluble cytokines, and receptor tyrosine kinases. Therefore, docking results were interpreted descriptively and not intended for direct quantitative comparison across different protein classes. Using a ligand protein approach, receptors such as ATP-binding cassette G5/G8 (ABCG5/8), Kolesterol 7α-hidroksilase (CYP7A1), Farnesoid X Receptor (FXR), Liver X Receptor (LXRa), 3-hidroksi-3-metilglutaril-koenzim A (HMGCoA), peroxisome proliferatoractivated receptor gamma (PPAR γ), Interleukin6 (IL-6), Tumour necrosis factor alpha (TNFα), and vascular endothelial growth factor receptor 2 (VEGFR2) were docked with several compounds from combination- bioactive-fraction generated from Lagerstroemia speciosa and Cinnamomum burmannii: Proanthocyanidin A trimer (Cinnamtannin B1) and Ellagic acid (Benzoaric acid). CB-Dock2 was selected for its automated cavity-detection-guided blind docking capability, suitable for exploratory multi-target screening. However, no re-docking, RMSD calculation, or structural validation using molecular dynamics simulations was conducted.

2.2 Evaluation of in silico study

Prediction of Bioactivity, Toxicity, and Drug-Likeness

The bioactive potential of compounds derived from *Lagerstroemia speciosa* and *Cinnamomum burmannii* was

predicted using the WAY2DRUG PASS online platform (https://www.way2drug.com/PassOnline/predict.php, accessed on October 31, 2025)[16]. This tool was employed to evaluate the compounds' ability to specifically target cholelithiasis. A Structure–Activity Relationship (SAR) analysis was conducted to compare the tested compounds with established bioactive molecules. The prediction output, represented by the Pa (Probability of Activity) value, indicates the likelihood that a compound exhibits a specific biological activity. Higher Pa scores correspond to more reliable predictions. Thus, this study focused on compounds exhibiting Pa values above 0.4. In addition, toxicity and drug-likeness evaluations were performed to determine the pharmacokinetic profiles and possible adverse effects of the compounds. Drug-likeness was assessed based on Lipinski's Rule of Five (Ro5). Toxicity and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) analyses were carried out using the Protox II database (https://tox-new.charite.de/protox_II/index.php?site=compound_input, accessed on October 31, 2025) and the ADMETLab 2.0 platform (https://admetmesh.scbdd.com/service/evaluation/index, accessed on October 31, 2025). The SMILES (Simplified Molecular Input Line Entry System) notations for each compound were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov, accessed on October 31, 2025) and used as input for these analyses.

Simulated molecular docking

The molecular docking simulation was conducted using CB-Dock2**,** an enhanced version of the CB-Dock server that employs cavity-detection-guided blind docking to predict protein–ligand interactions[13,14]. This method integrates cavity identification, molecular docking, and homologous template fitting. CB-Dock2 automates the docking process by detecting potential binding pockets, calculating their coordinates and dimensions, adjusting the docking box according to the ligand's size, and executing the docking simulations. The CurPocket algorithm applies curvature-based cavity detection to predict the binding sites of target proteins, while CB-Dock2 determines the corresponding ligand binding positions. For detailed methodological principles, the referenced publication can be consulted. Proteins with the highest centrality within their respective signaling pathways were selected for molecular docking analysis. The receptor proteins used in this study included ABCG5/8 (PDB ID: 8CUB)**,** NPC1L1 (PDB ID: 3QNT)**,** CYP7A1 (PDB ID: 3SN5), FXR (PDB ID: 3FLI)**,** LXRα (PDB ID: 1UHL)**,** HMG-CoA reductase (PDB ID: 1R31)**,** PPARγ (PDB ID: 2ATH)**,** IL-6 (PDB ID: 1P9M)**,** TNFα (PDB ID: 2AZ5)**,** and VEGFR-2 (PDB ID: 3V2A)[13,14]. Prior to docking, the CB-Dock2 server automatically removed water molecules and other heteroatoms from the protein structures. All proteins were treated as receptors, while the compounds acted as ligands. The protein structures in .pdb format were retrieved from the RCSB Protein Data Bank (https://www.rcsb.org; accessed on October 31, 2025), and the ligand structures in .sdf format were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov, accessed on October 31, 2025). By integrating cavity detection, docking, and template fitting, CB-Dock2 enhances docking precision and facilitates the prediction of ligand binding sites and affinities—an essential step in drug discovery. The resulting binding affinity (ΔG) values were used to assess ligand efficacy and compare the compounds with standard control drugs.

RESULTS

3.1 These results are presented in table 1 of the study. Additionally, in silico or computational studies were conducted to explore structure-activity relationship (SAR) predictions, Pa values, computational toxicity analysis, and drug-like properties.

3.2 Biological Activity Analysis and Toxicity Prediction of Compounds C1 and C2

Based on the predicted biological activity (Pa level), the two compounds exhibit distinct pharmacological profiles in several enzymatic targets. Compound C1 has a high probability of activity (Pa) as a kinase inhibitor (0.987) and CYP2A11 substrate (0.864), indicating a strong potential for inhibiting kinase enzymes involved in cellular signal phosphorylation and interacting with the cytochrome P450 enzyme system. Furthermore, C1 also has potential as a peroxidase inhibitor (Pa = 0.810) and UGT16A substrate (Pa = 0.757), indicating its ability to modulate oxidative activity and glucuronidation conjugation in the liver. Meanwhile, compound C2 also showed high potential biological activity, with a value of Pa = 0.861 as a peroxidase inhibitor, Pa = 0.792 as a CYP2A11 substrate, Pa = 0.849 as a kinase inhibitor, and Pa = 0.732 as a UGT16A substrate. These values indicate that C2 has a more balanced potential activity

Table 1.

No	Observed compounds	Molecular formula	Molecular weight (g/mol)	Substance ID (PubChem)
C_1	Proanthocyanidin A trimer (Cinnamtannin B1)	C45H36O18	864.76 g/mol	475277
C_2	Ellagic acid (Benzoaric acid)	C14H6O8	302.19 g/mol	5281855

on the four biological targets compared to C1, although slightly lower in kinase inhibition activity.

Toxicity predictions were based on the estimated LD_{50} (Lethal Dose 50) and toxicity classification. Compound C1 has an LD_{50} of 2500 mg/kg, classifying it as a toxicity class 5, while C2 has an LD_{50} of 2991 mg/kg, class 4. Based on the OECD classification, an LD_{50} value above 2000 mg/kg indicates low to very low toxicity, indicating that both compounds are relatively safe at potential therapeutic doses. However, the slightly higher toxicity value for C2 indicates a higher level of safety compared to C1.

Drug-likeness testing was conducted using three main criteria: Lipinski's Rule of Five, the Pfizer filter, and the GSK filter. Based on the analysis results, compound C1 did not meet the Lipinski criteria (Rejected) or the GSK filter (Rejected), but was accepted by the Pfizer criteria. This indicates that although C1 has high biological activity, its oral bioavailability and pharmacokinetic stability may still require structural optimization to meet ideal drug characteristics. Conversely, C2 met all drug-likeness evaluation criteria—accepted by the Lipinski, Pfizer, and GSK filters—indicating that C2 has a better pharmacokinetic profile and more closely aligns with the parameters of an ideal drug compound, including molecular size, lipophilicity, and biological membrane permeability.

Thus, the data from this analysis are presented in Table 2 of the study. This study found that both compounds from Lagerstroemia speciosa and Cinnamomum burmanii exhibited Probability Activity Scores (Pa) greater than 0.40 as peroxidase inhibitors, CYP2A11 Substrate, Kinase inhibitor and UGT16A Substrate as shown in Table 2. Furthermore, the cytotoxicity prediction values placed these compounds in toxicity classes 4 and 5 (greater than 3), indicating their potential to be developed as functional food candidates.

The study examines seven compounds: two experimental compounds labeled C1 and C2, four established pharmaceuti-

Table 2.

	Pa level*				Analysis of toxicity computation**		Drug Likeness***		
	Peroxidase Inhibitor	CYP2A11 Substrate	Kinase inhibitor	UGT16A Substrate	LD_{50} prediction (mg/kg)	Class of toxicity	Lipinski	Pfizer	GSK
C_1	0,810	0,864	0,987	0,757	2500mg/kg	5	Rejected	Accepted	Rejected
C_2	0,861	0,792	0,849	0,732	2991mg/kg	4	Accepted	Accepted	Accepted

* Way2Drug; ** Protox; *** ADMET.

Table 3.

Compound and controls as ligands	Cholestrol Transport- related receptor protein		Metabollic-related receptor protein					Inflammation-related receptor protein		Vascularisation-receptor protein	Overall
	ABCG5/8 (8CUB)	NPC1L1 (3QNT)	CYP7A1 (3SN5)	FXR (3FLI)	LXRα (1UHL)	HMGCoA (1R31)	PPARγ (2ATH)	IL-6 (1-P9M)	TNFα (2AZ5)	VEGFR-2 (3V2A)	
C_1	-9,8	-8,5	-12,7	-8,7	-9,9	-9,3	-12	-10,9	-10,3	-9,7	–101.8
C_2	-9,1	-7,2	-8,8	-8,7	-9,4	-8,6	-8,5	-7,7	-7,9	-6,8	–82.7
UDCA	-9,4	-9,1	-6,6	-8,9	-8,3	-7,3	-9,4	-9,2	-9,5	-7,7	–85.4
Pioglitazone	-8,9	-6,9	-8,4	-8,6	-9,4	-8,2	-7,8	-7,4	-7,8	-7,0	–81.4
Metformin	-5,2	-5,5	-5,2	-5,3	-4,9	-5,1	-5	-5,5	-5,3	-4,8	-51,8
Simvastatin	-9,4	-6,6	-9,1	-7,2	-8	-7,6	-9,3	-7,9	-8,2	-6,8	-80,1
Gemfibrozil	-7,6	-7,2	-7,6	-6,9	-7,6	-6,5	-7,4	-6,2	-7	-6,1	-70,1

cal drugs (UDCA, Pioglitazone, Metformin, Simvastatin and Gemfibrozil). These compounds are evaluated against ten protein targets that play important roles in various metabolic and inflammatory pathways to gallstone. The targets include cholesterol metabolism proteins (ABCG5/8, NPC1L1, and CYP7A1), nuclear receptors involved in lipid regulation (FXR, LXRα, and PPARγ), metabolic enzymes (HMG CoA), and inflammatory markers (IL-6, TNFα, and VEGFR-2). Each binding affinity value represents the calculated energy of interaction between a specific compound and its target protein.

Results of Ligand Interaction Analysis with Receptors

Results of Ligand Interaction Analysis with Receptors Related to Cholesterol Transport

As shown in Table 1, **C1 (Cinnamtannin B1)** demonstrated strong binding affinities toward **ABCG5/8 (–9.8 kcal/mol)** and **NPC1L1 (–8.5 kcal/mol)**, comparable to the reference drug **UDCA (–9.4 and –9.1 kcal/mol)**. These findings suggest that C1 may influence cholesterol transport across the hepatobiliary system in a manner similar to UDCA, which is known to reduce biliary cholesterol supersaturation.

Both **ABCG5/8** and **NPC1L1** play key roles in maintaining cholesterol balance—ABCG5/8 facilitates cholesterol efflux into bile, whereas NPC1L1 mediates intestinal and hepatic cholesterol uptake. Inhibition or modulation of these receptors can reduce cholesterol accumulation, thereby decreasing the likelihood of gallstone formation.

Conversely, **C2 (Ellagic acid)** exhibited slightly higher binding energy values (–9.1 and –7.2 kcal/mol), suggesting weaker interactions but still indicative of potential activity in cholesterol transport regulation. Together, these results imply that DLBS3233 compounds may collectively contribute to the stabilization of cholesterol homeostasis within the hepatobiliary environment.

Results of Ligand Interaction Analysis with Metabolic Receptors

C1 displayed the strongest binding affinity toward **CYP7A1 (–12.7 kcal/mol)** and **HMG-CoA reductase (–9.3 kcal/mol)** among all tested ligands. These two enzymes serve as central regulators of cholesterol metabolism. **CYP7A1** catalyzes the conversion of cholesterol to bile acids, while **HMG-CoA reductase** governs cholesterol biosynthesis. Strong inhibition of these enzymes indicates that C1 may act through a dual mechanism—enhancing bile acid synthesis while concurrently reducing hepatic cholesterol production.

In comparison, **C2** also interacted favorably with CYP7A1 (–8.8 kcal/mol) and HMG-CoA reductase (–8.6 kcal/mol), suggesting a supportive metabolic role. However, given its superior pharmacokinetic and toxicity profile, C2 may serve as a safer adjunct candidate for long-term metabolic modulation. This complementary relationship between the two bioactives highlights the potential of DLBS3233 as a multi-component system targeting multiple aspects of lipid regulation.

Results of Ligand Interaction Analysis with Receptors Associated with Inflammatory Processes

Chronic inflammation is recognized as a significant contributor to gallstone pathogenesis. Docking simulations revealed that **C1** had exceptionally strong interactions with **IL-6 (–10.9 kcal/mol)** and **TNF-α (–10.3 kcal/mol)**, the reference compound UDCA (–9.2 and –9.5 kcal/mol). These results suggest that C1 may effectively inhibit key inflammatory mediators responsible for hepatobiliary inflammation and gallbladder wall damage.

In contrast, **C2** showed moderate binding affinities (–7.7 and –7.9 kcal/mol) but maintained relevant activity levels, implying that it could complement C1's stronger anti-inflammatory effects. Collectively, these findings support the potential role of DLBS3233 bioactives as natural anti-inflammatory agents that may mitigate chronic inflammation and bile compositional changes leading to lithogenesis. However, these observations are based solely on in silico predictions and require experimental validation.

Results of Ligand Interaction Analysis with Nuclear Receptors

The nuclear receptors **FXR**, **LXRα**, and **PPARγ** are pivotal in maintaining lipid and bile acid homeostasis. C1 demonstrated high binding affinities toward **FXR (–8.7 kcal/mol)**, **LXRα (–9.9 kcal/mol)**, and particularly **PPARγ (–12.0 kcal/mol)**. PPARγ activation is known to improve insulin sensitivity and modulate lipid metabolism, suggesting that C1 may exhibit insulin-sensitizing and lipid-lowering properties similar to those of pioglitazone. This finding aligns with previous in vivo reports indicating that DLBS3233 enhances PPARγ expression and glucose utilization.

Meanwhile, **C2** also bound effectively to FXR (–8.7 kcal/mol), LXRα (–9.4 kcal/mol), and PPARγ (–8.5 kcal/mol), albeit with lower affinity. These consistent interactions across nuclear receptor targets indicate that both bioactives may synergistically modulate lipid metabolism through coordinated receptor signaling, ultimately preventing cholesterol supersaturation in bile.

Results of Ligand Interaction Analysis with Receptors Associated with Vascularization

The **VEGFR-2** receptor plays an essential role in angiogenesis and tissue repair processes during hepatobiliary inflammation. C1 exhibited strong binding affinity toward VEGFR-2 (–9.7 kcal/mol), outperforming both **C2 (–6.8 kcal/mol)** and **UDCA (–7.7 kcal/mol)**. Such interaction may enhance mi-

crovascular perfusion and promote tissue regeneration in inflamed gallbladder and hepatic tissues. Improved vascularization could facilitate healing and reduce oxidative damage in chronic cholecystitis, suggesting an additional protective mechanism of C1 in gallstone-related pathology.

DISCUSSION

Gallstone formation associated with supersaturated cholesterol, bilirubin, or not enough bile salts, causing these substances to crystallize and harden into stones. The gallbladder may not empty completely or often enough, which concentrates bile and promotes stone formation. In this case metabolism of cholesterol play important role as main problem of gallstone. If the liver excretes more cholesterol than the bile can dissolve, the excess cholesterol can form crystals that eventually harden into stones. The other if the gallbladder doesn't empty completely or frequently enough because of excess cholesterol, bile can become too concentrated, contributing to stone formation. When bile is supersaturated, small crystals precipitate and can get trapped in the gallbladder's mucus, leading to the formation of gallbladder sludge, which eventually coalesces into stones.

Many therapeutic modalities address excess cholesterol, which can impact gallstone formation. UDCA, as the primary medication for gallstone treatment, does not provide a preventive effect, but its therapeutic effect is limited to small, non-calcified stones and requires a long treatment period of 6–24 months. Furthermore, after discontinuation of therapy, gallstone recurrence can occur in >50% of patients within 5 years. Cholesterol therapies such as statins, fibrates, and gemfibrozil have been shown to be effective in gallstone formation, while other existing therapeutic modalities, such as DLBS3233, have been primarily studied as a treatment for type 2 diabetes mellitus.

Mohani et al, explained that DLBS3233 has the potential to work through the PPAR gamma pathway[15]. PPAR-gamma regulates cholesterol metabolism by influencing cholesterol uptake and efflux, primarily through its interactions with other nuclear receptors like LXR-alpha. PPAR-gamma increases the removal of cholesterol from cells. It achieves this by inducing the expression of LXR-alpha, which in turn activates genes like ABCA1 and ABCG1 that facilitate cholesterol transport out of the cell. PPAR-gamma also influences the uptake of cholesterol from the blood. It has been shown to regulate the expression of scavenger receptors such as CD36 and SRA-I, which are involved in the uptake of oxidized low-density lipoprotein (oxLDL). As result, activating PPAR-gamma has been explored as a therapeutic strategy for managing metabolic disorders, as it can favorably impact cholesterol and can reduce excess cholesterol as gallstone formation[15].

When all docking scores were combined, C1 (–101.8 kcal/mol) displayed the highest cumulative binding affinity among all compounds tested, outperforming standard therapeutic agents such as UDCA (–85.4 kcal/mol), pioglitazone (–81.4 kcal/mol), simvastatin (–80.1 kcal/mol), and metformin (–51.8 kcal/mol). These data emphasize the multi-target binding potential of C1 across metabolic, inflammatory, and vascular pathways involved in gallstone formation. Although C2 (–82.7 kcal/mol) exhibited lower overall binding strength, its excellent drug-likeness and low toxicity (LD_{50} = 2991 mg/kg, class 4) make it a promising complementary candidate for oral or long-term use. Taken together, these results highlight the synergistic nature of DLBS3233 bioactives: C1 provides potent pharmacological effects through strong multi-target interactions, while C2 ensures safety, stability, and favorable pharmacokinetic behavior.

Through our study, DLBS3233 has been shown to have a broader potential effect, particularly on cholesterol metabolism as a preventive pathway and as an add-on therapeutic agent with UDCA to prevent and reduce excess cholesterol, thus improving gallstone therapy.

To our knowledge, this is the first study to examine DLBS3233 in silico in cholesterol metabolism and link it to gallstone therapy. The results of this study can be further developed into clinical trials so that the effects of DLBS3233 in gallstone therapy can be more clearly demonstrated.

This silico investigation reveals that the two key bioactives of DLBS3233 which contains Cinnamtannin B1 (C1) and Ellagic acid (C2) engage multiple molecular pathways involved in gallstone formation. Gallstone pathogenesis is driven by cholesterol supersaturation, impaired bile acid regulation, and chronic hepatobiliary inflammation, processes regulated by metabolic enzymes, nuclear receptors, and cytokine networks[1,10]. C1 exhibited the strongest overall binding affinity among all ligands (–101.8 kcal/mol), UDCA (–85.4 kcal/mol), particularly through robust interactions with CYP7A1 and HMG-CoA reductase, suggesting suppression of cholesterol synthesis and enhanced bile acid conversion—two essential mechanisms to reduce crystallization risk[12]. C1 also bound strongly to IL-6 and TNF-α, indicating a potential role in mitigating chronic cholecystitis, a key driver of lithogenesis[4]. Its high affinity toward PPARγ aligns with earlier findings demonstrating DLBS3233-mediated upregulation of PPARγ and improvements in lipid and glucose homeostasis[7,8]. Although C2 showed lower binding strength, its optimal drug-likeness profile and low toxicity (LD_{50} 2991 mg/kg) support its suitability for long-term modulation of cholesterol and inflammatory pathways.

Compared with current therapies, which rely heavily on UDCA and suffer from long treatment durations and high recurrence rates[3,4], DLBS3233 offers a broader mechanistic reach. C1 showed numerically lower predicted binding energy values across several targets compared with selected reference compounds[7,8]. Notably, C1's strong affinity for VEGFR-2 points to a vascular-protective mechanism not previously considered in gallstone therapy.

These findings highlight the potential of DLBS3233 as an adjunct therapy to UDCA enhancing bile acid homeostasis and reducing hepatic cholesterol load while also serving as a preventive agent in individuals with metabolic syndrome or obesity, both major gallstone risk factors[4]. The multi-target engagement across cholesterol transport (ABCG5/8, NPC1L1), metabolism (CYP7A1, HMG-CoA reductase), nuclear receptors (FXR, LXRα, PPARγ), inflammatory signaling (IL-6, TNF-α), and vascular remodeling (VEGFR-2) supports its broad therapeutic relevance. These mechanisms also align with genetic evidence showing the involvement of ABCG5/8, NPC1L1, and CYP7A1 variants in gallstone susceptibility[11,12].

Nevertheless, computational modeling has inherent limitations. However, these results represent docking score predictions only and should not be interpreted as evidence of pharmacological superiority. Only two compounds were assessed, despite DLBS3233 containing multiple bioactives with potential synergistic effects. The absence of molecular dynamics simulation may also limit prediction accuracy. Thus, in vitro validation, cellular assays, animal models, and clinical trials are required to confirm the metabolic, anti-inflammatory, and vascular effects of C1 and C2 and to determine their translational potential in gallstone management.

CONCLUSION

Overall, this exploratory in silico study suggests that DLBS3233 bioactives derived from *Lagerstroemia speciosa* and *Cinnamomum burmannii* may interact with multiple molecular targets involved in cholesterol transport, lipid metabolism, inflammatory signaling, and hepatobiliary vascular regulation. These predicted interactions provide a computational framework supporting further investigation of DLBS3233 in gallstone-related pathways. Experimental validation through molecular dynamics simulations, in vitro assays, in vivo models, and clinical studies is required to determine its translational relevance.

LIMITATION OF THIS STUDY

This study has several important limitations. First, all analyses were conducted exclusively using computational approaches, without molecular dynamics simulations, experimental validation, or statistical comparison between ligands. Second, the protein targets analyzed represent heterogeneous molecular classes, limiting direct comparison of docking scores. Third, molecular docking provides static interaction predictions and does not account for protein flexibility, solvent effects, metabolism, or in vivo pharmacokinetics. Fourth, bioactivity, toxicity, and drug-likeness predictions are probabilistic tools and do not confirm therapeutic applicability. Therefore, the findings should be considered hypothesis-generating and require further validation.

ACKNOWLEDGEMENT

We would like to thank PT Dexa Medica for collaborating with us to conduct this research.

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