

In-silico and In-vitro Evaluation of Anti-Cholelithiasis Activity for the Leaf extract of *Bryophyllum pinnatum* (Crassulaceae)

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doi.org/10.64643/IJIRTV13I5-209022-459

Abstract—Cholelithiasis remains a prevalent gastrointestinal disorder, with ursodeoxycholic acid (UDCA) serving as the standard pharmacological therapy for cholesterol gallstone dissolution. This study evaluated the anti-cholelithiasis potential of *Bryophyllum pinnatum* butanolic extract through integrated molecular docking and *in-vitro* gallstone dissolution assays. Molecular docking analysis revealed that phytoconstituents furostane and dammarane exhibited substantial binding affinities of -8.9 and -8.8 kcal/mol, respectively, approaching the standard UDCA value of -9.2 kcal/mol, suggesting favourable interactions with target receptors involved in cholesterol metabolism. *In-vitro* Cholesterol gallstone models were synthesized and treated with plant extract at 2mg and 4mg concentrations, alongside UDCA (2mg) and controls (water, model bile), over three weeks at 37°C. Results demonstrated concentration-dependent gallstone dissolution, with the 2mg extract reducing stone weight from 220mg to 20mg (90.9% dissolution), outperforming UDCA (210mg to 40mg; 81.0% dissolution) and 4mg extract (230mg to 30mg; 87.0% dissolution). Control groups showed minimal dissolution, confirming bile's inability to dissolve stones under cholelithiasis conditions. The 2mg extract exceeded UDCA efficacy by approximately 10%, while 4mg showed 6% greater activity, indicating superior litholytic potential at lower concentrations. These findings suggest that *B.pinnatum* butanolic extract possesses significant anti-cholelithiasis activity, potentially mediated by furostane and dammarane compounds, warranting further *in-vivo* validation and mechanistic exploration for herbal therapeutic development.

Index Terms—*Bryophyllum pinnatum*, Cholelithiasis, Gall Stones, Molecular docking, Dissolution

I. INTRODUCTION

Cholelithiasis: Cholelithiasis or gall stones disease, remains one of the most common and economically burdensome digestive disorders worldwide, with cholesterol gallstones representing its predominant form. ^[1] Gallstones are broadly classified as cholesterol stones, pigment stones, and mixed stones based on their biochemical composition. ^[2]

Limitations of Current Treatment: Current clinical management of cholelithiasis relies mainly on surgical cholecystectomy or pharmacological dissolution therapy with agents such as ursodeoxycholic acid. However, these approaches are limited by high recurrence rates, prolonged treatment duration, cost, and surgical risk, prompting growing scientific interest in plant-derived agents capable of inhibiting cholesterol nucleation and crystallization as safer, more economical alternatives.

***Bryophyllum pinnatum*:** Traditional Use and Relevance Medicinal plants have historically served as a rich source of bioactive molecules with hepatoprotective, choleretic, and anti-lithogenic potential. *Bryophyllum pinnatum* (Lam.) Oken (family Crassulaceae), commonly referred to as the “life plant” or “miracle leaf”, is a perennial succulent herb utilized widely in traditional medicine across tropical and sub-tropical regions. ^[3] It contains broad spectrum of secondary metabolites including terpenoids, flavonoids, alkaloids, saponins, and bufadienolides etc. Traditionally, the plant has been used across various systems of medicine for treating fever, smallpox, otitis, cough, asthma, headache,

convulsion, and general debility ^[4], and its extracts have additionally been investigated for their effect on calcium oxalate crystallization relevant to urolithiasis ^[5], indicating a broader anti-lithogenic potential relevant to stone-forming disorders in general.

Aim of the Present Study: The present study was therefore undertaken to carry out *in-vitro* assessment of the anti-cholelithiasis potential of *Bryophyllum pinnatum* leaf extract, with cholesterol stone dissolution activity, so as to provide a scientific basis for its traditional application and its viability as a candidate for future anti-cholelithiatic drug development.

II. MATERIALS AND METHODS

In-silico Molecular Docking Studies

Protein selection and preparation: FXR is a nuclear receptor is a nuclear receptor which is expressed in the gastrointestinal tract, bile acids are endogenous ligands of the FXR receptor which acts as bile acid sensor to control bile acid synthesis.^[6]

FXR is a type 2 receptor that closely relates to liver X receptor α and β (LXR α , NR1H3; LXR β , NR1H2), peroxisome proliferator-activated receptor α , γ and δ (PPAR α , NR1C1; PPAR γ , NR1C2; PPAR δ , NR1C3), pregnane X receptor (PXR, NR1I2) and constitutive androgen receptor (CAR, NR1I3). They form a heterodimeric complex with a common partner, RXR α .^[7]

The protein structure or receptor for this molecular docking using autodock vina was performed with the Farnesoid X receptor, it is the suitable candidate the anti-cholelithiasis activity because it regulates the bile homeostasis in the human body and helps in the metabolism of the cholesterol. The receptor structure is downloaded from the Protein Data Bank (<https://doi.org/10.2210/pdb6HL1/pdb>) in the legacy PDB format.

The protein structure is visualized in the autodock tools, then the water molecules are removed along with the heteroatoms, and protein chains in which the docking is not performed. Hydrogen atoms were added followed by the addition of Kollman charges. The ligand present in the binding site is removed in which the herbal active constituents are to be bound, save this as pdbqt format.^[8]

Now to determine the configurations for the grid box, the receptor is visualized in the discovery studios,

select the protein chain and select the attributes of the SBD site which gives the configurations of the XYZ, now enter these values in the grid box on the autodock tools, save this as a text file named "grid.txt".

Ligand selection and preparation: Dammarane (CID:9548714) and furostane (CID: 6857441) are the bioactive compounds present in the plant which produces anti-cholelithiasis activity which is copied from the PubChem in smiles. The standard drug for the comparison is UDCA (CID: 31401) which is also copied from the PubChem. These smiles are drawn into structures using chem3D.

The energy of the compounds are minimized and saved as PDB file, which is then converted into pdbqt file by using the autodock tools, before that Gasteiger charges are added for the ligand followed by checking the aromaticity criterion and marking the bond for the rotation to check for the best angle for the binding of ligand and protein. This file is saved as the pdbqt format.

Molecular docking: Protein and ligand structures are ready for the docking, now in the address bar of the file type "cmd" and enter. Open the command prompt in the system and type the following prompt,

"Vina.exe -config cconfig.txt.txt -log log.txt.txt"

After successfully running the docking, the files are saved as a single file for all possible interaction between the ligand and the protein, thus vina split is performed to separate the data of the protein and ligand interaction. It is carried out by the following prompt,

"vina_split.exe --input out.pdbqt"

In-vitro Cholesterol Gall Stone synthesis

Gall stones (or) cholelithiasis is formed by the capacity of bile to solubilize cholesterol is exceeded; thus, part of the cholesterol precipitates, presumably in the form of microcrystals. During the next step in gallstone formation microcrystals of cholesterol unite or grow to form macroscopic gallstones. These two steps probably proceed simultaneously. The ratio for cholesterol precipitation on the bile solution is 1:11, about 70-95% of the patients diagnosed with gall stones the ratio was above the 1:11 critical ratio, although this comparison is not completely reliable because normal bile had high ratios and cholelithiasis patient has normal bile ratio this is because the Issakon's ratio does not distinguish the functions of bile salt and lecithin.^[9]

Further the cholesterol gallstone formed by *in-vitro* method is categorized into,

- Liquid phase
- Cholesterol crystal phase
- Liquid crystal phase

The *in-vitro* synthesis of cholesterol gallstones composed of the following ingredients at different proportions,

- Bile salt (Sodium tauro cholate)
- Lecithin (soya bean)
- Cholesterol powder^[10]

The cholesterol is solubilized by lecithin and bile forming a micelle. According to Issakson, cholesterol is practically not soluble in the water, but it is soluble in human bile by forming a micelle made of bile salt and lecithin.^[11]

Here the bile salt acts as bile juice which dissolves the bile and lecithin is a phospholipid, bile salt helps in dissolving the cholesterol while lecithin helps by increasing the amount of cholesterol being digested. Lecithin plays an important role in fat digestion because the amount of lecithin is more than the normal amount it may cause supersaturation of the cholesterol.

In the absence of lecithin it takes about 97 molecules of bile salt to solubilize 3 molecules of cholesterol whereas in case of when the lecithin is increased the amount of cholesterol being solubilized is increased up to 10 molecules but, when the lecithin is increased to a ratio of 40 bile salts to 60 lecithin the amount of cholesterol being solubilized is nearly 0, the amount of cholesterol at low concentrations of lecithin tends to crystallize.^[9]

Cholesterol starts to build up more than what the lecithin and bile salt may solubilize, the cholesterol starts to form a lipid bilayer known as vesicles made up of cholesterol and phospholipids. This is the starting point to the gallstone formation. The vesicles are unstable and forms aggregates with the nearby molecule, once the vesicles overcome its own solubility it forms crystals and increases the concentration of the cholesterol which overtime forms gallstones.^[12]

The crystal in the bile is due to the poly unsaturated phospholipids which accelerate the cholesterol crystallization while the saturated phospholipids delay the process. The theory implies that the phospholipids

content can be increased from the diet which can prevent from the gallstones.^[13]

The hydrophobic hydrophilic balance of the bile salts classifies the cholesterol crystals into 7 different forms. The growth is decreased as the hydrophobicity of the bile salt decreases that is,

deoxycholate > chenodeoxycholate > cholate > taurocholate > ursodeoxycholic acid.^[14]

The hydrophobic bile salt with decreased micellar concentration induces rapid cholesterol rich vesicle formation that causes crystal precipitation, since UDCA is a hydrophilic salt, it prevents the cholesterol crystallization more effectively than the other bile salts.^[15]

The cholesterol gallstone dissolution of UDCA and CDCA are efficient similarly when taken orally and the microdose of the drug on chronic dose does not produce any serious side effects. The use of CDCA results in 22% dissolution while UDCA results in 25% dissolution and there is a 60% improvement in symptoms in the biliary patients.^[16]

The cholesterol dissolving capacity of the UDCA is simply not achieved by UDCA alone it is a complex process, Corrigan and his colleagues found that the tauro conjugate of UDCA along with the lecithin converts the solid crystalline phase into liquid crystalline phase which speeds up the cholesterol dissolution meanwhile the combination of UDCA with lecithin retards the solubility of cholesterol when lecithin is added.^[17]

Procedure

1. Preparation of model bile: In a 100ml beaker add the phosphate buffer pH 7.4 to dissolve the lecithin and bile salt in the required amount. Once the two compounds are completely soluble add the cholesterol and mix it thoroughly in the magnetic stirrer.
2. Supersaturation of the bile: Separate the bile into two equal portions and label them as normal and stone forming bile, to stone forming bile add double the amount of cholesterol than the normal bile. Incubate them in water bath at 37°C for 30-60 minutes.
3. Incubation: Store both the solutions in an incubator at 37°C for 15-20 days and observe the formation of the stones. The normal bile looks clear. Stone forming bile may have developed small aggregates, flakes and looks turbid^[18]

Table 1: Bile solution composition

Bile Solution	Cholesterol	Bile salt	Lecithin	Buffer	Appearance
Normal bile	50mg	150 mg	100 mg	10 ml	Clear
Stone forming bile	100 mg	150 mg	100 mg	10 ml	Turbid

In-vitro Evaluation of Cholesterol Gall Stone Dissolution Activity for *B. Pinnatum* extract

After 20 days of incubation period, uncalcified cholesterol sludge has been collected and centrifuged at 3500 RPM for 10 minutes. The supernatant liquid is removed, and the sediment is collected and dried in the hot air oven at 100° C until it dries. It is then collected

and stored in the desiccator for a period of 3 days. Finally, it is weighed and placed into the test and standard solution and incubated for 30 days. The weight of the stones is measured weekly once for 4 weeks to notice the dissolution of the cholesterol gallstones.

Table 2: Experimental Groups for *In-vitro* Cholesterol Gall Stone Dissolution Study

S. No	GROUP	INITIAL WEIGHT OF THE STONE	MEDIUM
1.	Control	170 mg	Water
2.	Disease control	190 mg	Bile juice
3.	Standard drug	210 mg	Ursodeoxycholic acid
4.	Test drug 1	220 mg	Plant extract (2mg/ml)
5.	Test drug 2	230 mg	Plant extract (4mg/ml)

III. RESULTS AND DISCUSSION

In-silico Molecular Docking studies

Furostane: A saponin group identified from the leaves of the plant *Bryophyllum pinnatum* which has the cholesterol lowering action has a 2D structure showing atomic connectivity and a 3D structure revealing its spatial arrangement and stereochemistry.

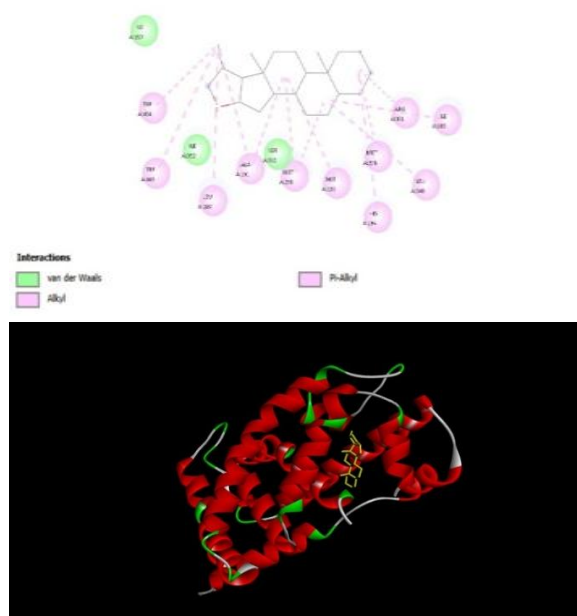


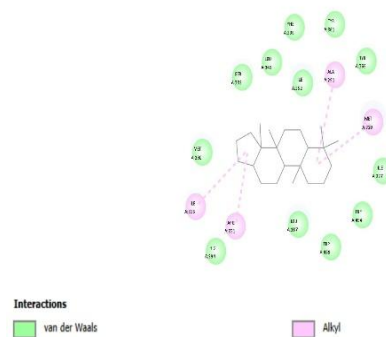
Figure 1: 2D binding analysis and 3D structure of the protein

Table 3: Binding affinity of furostane

mode | affinity | dist from best mode
| (kcal/mol) | rmsd l.b. | rmsd u.b.

mode	affinity	dist from best mode	
(kcal/mol)	rmsd l.b.	rmsd u.b.	
1	-8.9	0.000	0.000
2	-8.7	0.896	1.033
3	-8.1	1.793	2.590
4	-7.9	1.928	9.248
5	-6.9	2.058	9.459
6	-6.8	1.955	9.015
7	-6.7	2.248	8.807
8	-6.7	1.944	8.945
9	-6.0	2.051	9.276

Dammarane: 2D and 3D structures and arrangement of the saponin Dammarane



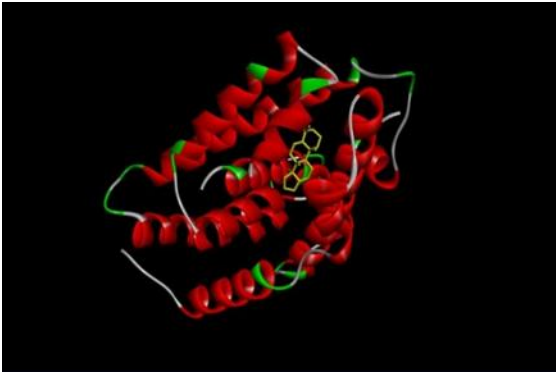


Figure 2: 2D binding analysis and 3D structure of the protein

Table 4: Binding affinity of dammarane
mode | affinity | dist from best mode
| (kcal/mol) | rmsd l.b.| rmsd u.b.

1	-8.8	0.000	0.000
2	-8.7	1.594	2.420
3	-8.4	1.821	8.460
4	-7.8	1.722	8.272
5	-7.7	1.559	2.921
6	-7.5	1.928	8.625
7	-7.2	2.056	3.607
8	-7.2	1.376	8.090
9	-6.6	1.350	3.294

Ursodeoxycholic acid: Standard 2D and 3D Structures of Urso deoxycholic acid Represent Its Molecular Connectivity and Spatial Arrangement.

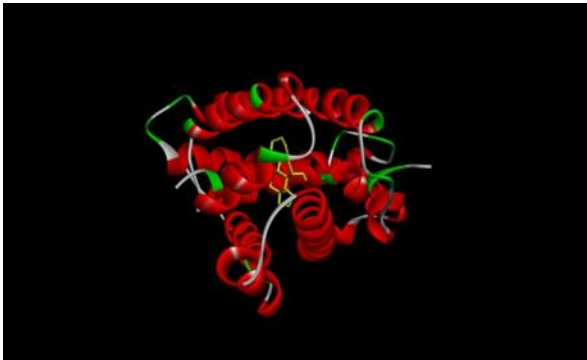
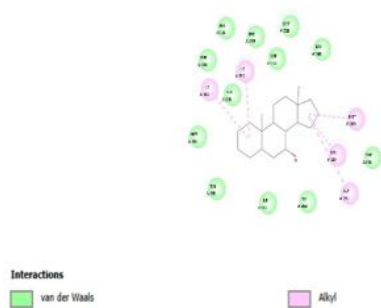


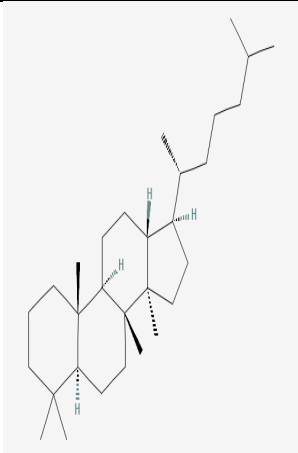
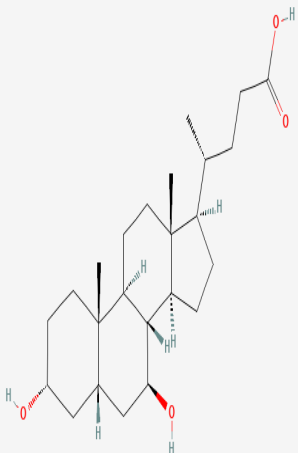
Figure 3: 2D binding analysis and 3D structure of the protein

Table 5: Binding affinity of ursodeoxy cholic acid
mode | affinity | dist from best mode
| (kcal/mol) | rmsd l.b.| rmsd u.b.

1	-9.2	0.000	0.000
2	-8.7	2.278	3.003
3	-8.5	2.088	7.343
4	-8.5	2.285	7.834
5	-8.2	2.658	3.796
6	-7.6	2.175	7.670
7	-7.6	2.729	7.946
8	-7.5	1.967	7.313
9	-7.5	2.903	4.718

Table 6: comparison of binding sites between the standard and plant extract

S.No	Ligand	Structure	Binding Affinity	Interacting residue
1.	Furostane		-8.9	Vander walls- • LEA:357 • ILEA:352 • SERA:332 Π alkyl interaction- • TRPA:454 • TRPA:469

				• LEUA:287 • ALAA:291
2.	Dammarane		-8.8	Vander walls- • PHEA:336 • TYRA:361 • LEUA:348 • SERA:332 • ILEA:352 Alkyl interactions- • ALAA:291 • META:328 • ILEA:335 • ARG A:331
3.	Ursodeoxycholic acid		-9.2	Vander walls- • PHE A:329 • PHE A:336 • MET A:328 • SER A:332 • LEU A:348 Alkyl interactions • ILE A:352 • MET A:290 • LEU A:287 • ALA A:291

The *in-silico* studies of the bioactive compound from plant *B.pinnatum* shows similar binding affinity when compared with the standard drug UDCA. The ligand binds to the A chain (JN 3501) with the grid box placed on the X, Y, Z (x = 11.313, y = -14.166, z = 12.478) for higher specificity. The compound with the most similar binding to that of the standard drug is furostane at -8.9 kcal/mol and RMSD 1 b value of 0.000 compared to standard with -9.2 kcal/mol and RMSD 1 b value of 0.000

These 3 drugs have common interaction residues which forms the core binding pocket for the ligand, the following amino acids interact with the ligand,

- SER A:332
- LEU A:348
- ILE A:357
- ILE A:352

- TRP A:454
- TRP A:469
- ALA A:291
- MET A:328
- MET A:290
- LEU A:287

Furostane has a unique binding affinity with MET A: 265 which accounts for its deeper penetration with π -alkyl interaction than the standard. On the other hand, dammarane matches the binding affinity of the UDCA.

In-vitro studies

The anti-cholelithiasis activity of the *Bryophyllum pinnatum* was analysed using *in-vitro* cholesterol gallstone analysis by synthesising the stone and the evaluation of the cholesterol gallstone dissolution at various concentration of 2mg and 4mg by comparing

with the standard UDCA, for 3 weeks at incubation of 37° C.



Figure 4: Cholesterol Gall stones



Figure 4: Flakes formation on Bile solution formed after incubation

Table 7: Cholesterol Gall stone weight reduction in different treatment groups

Cholesterol Gall Stone Model	Concentration	Weight (mg)	Wt. reduction after Week 1	Wt. reduction after Week 2	Wt. reduction after Week 3
Stone 1	Plant extract 2mg	220mg	60mg	50mg	20mg
Stone 2	Plant extract 4mg	230mg	70mg	60mg	30mg
Stone 3	Standard 2 mg (UDCA)	210mg	90mg	60mg	40mg
Stone 4	Water	170mg	90mg	70mg	60mg
Stone 5	Model Bile	190mg	160mg	110mg	70mg

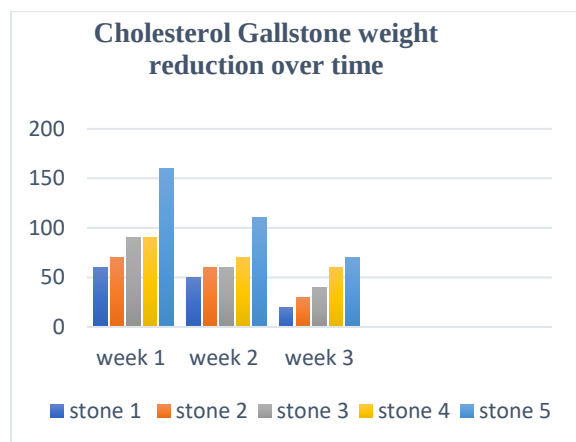


Figure 6: Histogram of the stone dissolution over time

The treatment with the plant extract showed a positive response against cholesterol gallstones. The butanolic extract was found to possess the anti-cholelithiasis activity. The cholesterol gallstone was synthesized by the *invitro* method, and its activity is measured by its dissolution of the cholesterol gallstone.

The above percentage of the extract shows that the plant extract with 2mg was found to be more effective than the standard drug and the 4mg extract produces action similar to that of the standard drug. The bile shows the least amount of dissolution which indicates the cholelithiasis condition that the bile cannot dissolve the cholesterol gallstone in the human body. The above *in-vitro* anti-cholelithiasis activity of the plant *Bryophyllum pinnatum* indicates that the plant extract is more efficient than the standard drug by more than 10% at 2mg concentration and by 6% more at 4mg concentration.

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