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Short Communication

# INVESTIGATION ON THE INTERACTION OF β-SITOSTEROL AND LUTEOLIN-7-GLUCOSIDE BINDING TO BOVINE SERUM ALBUMIN

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**ABSTRACT**

**Objective:** The study ondrug–protein interactions is an important field of interest because of the prospective of unraveling of drug action mechanisms and the possibility of designing novel medicines. Bovine serum albumin (BSA) has been studied extensively because of its structural homology with human serum albumin (HSA). The objective of the work was to study the interaction between β-sitosterol and Luteolin–7-glucoside with bovine serum albumin (BSA) investigated by molecular docking.

**Methods:** Docking studies were carried out using a crystal structure of bovine serum albumin complexed with naproxen (pdb code-4OR0). Auto dock 4.2 was used to perform molecular docking. Ligands were found flexible during the docking process, and protein was kept rigid.

**Results:** Molecular docking studies revealed that the β-sitosterol can bind in the large hydrophobic cavity of BSA, mainly by the hydrophobic interaction but also by hydrogen bond interactions between the hydroxyl (OH) group of β-sitosterol to SER 488 with hydrogen bond distance of 2.1Å. Luteolin-7-glucoside molecule interact by hydrophobic interaction with LYS 431, ARG 427, ALA 193 amino acids of Bovine Serum Albumin. The amino acids ARG 458, ARG 435, ARG 185 are involved in forming a hydrogen bond with hydroxyl oxygens, carbonyl carbon of Luteolin-7-glucoside with hydrogen bond distance of 2.4, 2.3 and 1.9 Å, respectively.

**Conclusion:** Study indicated that hydrophobic and hydrogen bonding interactions were mostly responsible for albumin interaction. Further research of the pharmaceutical potential of plant molecules will be valuable for monitoring their biological functions.

**Keywords:** β-sitosterol, Luteolin–7-glucoside, Bovine serum albumin, Molecular docking


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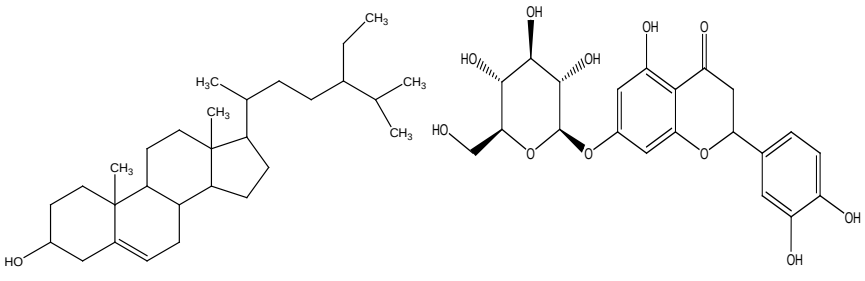
β-Sitosterol is a phytosterol or plant sterol compound, found in a variety of fruits, vegetables, and seeds, that has a variety of pharmacological properties [1]. The molecular mass is 414.7 Da and its molecular formula is $C_{29}H_{50}O$ (fig. 1A) and similar to the cholesterol structure. It blocks cholesterol absorption resulting in lower serum cholesterol levels [2], and also prevents the oxidation of LDL cholesterol, thereby reducing the risk of atherosclerosis [3, 4].

Luteolin 7-glucoside is a glycosyloxy flavone that is luteolin substituted by a beta-D-glucopyranosyl moiety at position 7 via a glycosidic linkage. It has a role as an antioxidant and a plant metabolite [5]. The molecular mass is 450.39 Da and its molecular formula is $C_{21}H_{22}O_{11}$ (fig. 1B)

Knowledge of interaction mechanisms between drugs and plasma proteins is of crucial importance for us to understand the pharmacodynamic and pharmacokinetic properties of a drug. Drug binding influences the distribution, excretion, metabolism, and interaction with the target tissues. Considering the fact that β-sitosterol and Luteolin-7-glucoside products play a major role in many biological processes [1], it is important to understand the interactions of these compounds with a major carrier protein such as human serum albumin (HSA). Due to the structural similarity of bovine serum albumin (BSA) to HSA, it is often used as a probe to investigate the binding properties of drugs with BSA. BSA is composed of three linearly arranged and structurally homologous sub-domains. The binding sites of BSA for endogenous and exogenous ligands may be in these domains and the principal regions of drugs binding sites of albumin are often located in hydrophobic cavities in sub-domains IIA and IIIA. [6, 7]. Few attempts have been done related to the study of the interaction of synthetic and natural molecules by protein denaturation [8-11]. Even the attempts have been made to dock flavonoids on HAS and phytosterols on BSA to seek information related to binding interactions and disaggregate effect [12, 13]. The present work deals with molecular docking studies of isolated and identified molecules viz β-sitosterol from the leaves of *Feronia Limonia* and Luteolin-7-glucoside from leaves of *Bougainvillea spectabilis*.




**A)** **B)**

**Fig. 1: Structure of A) β-Sitosterol and B) luteolin-7-glucoside**

Docking studies were carried out using Crystal Structure of Bovine Serum Albumin complexed with naproxen (pdb code-4OR0). It was resolved by X-ray diffraction technique with a resolution of 2.58 Å. We retrieved it from RCSB Protein Data Bank. A typical PDB protein complex structure, as downloaded from the Research Collaboratory for Structural Bioinformatics (RCSB) website [14], has no hydrogen and may have residues in unusual charge states. Therefore, comprehensive protein preparation to ensure chemical correctness and optimization of protein structure was done in order to achieve best results.4OR0. pdb file was loaded in Autodock. The bovine serum albumin structure consists of two protein chains (Dimer), some water molecules and a ligand naproxen. A single protein chain was extracted (Chain A) from the crystallized dimer bovine serum albumin. The attached ligands from downloaded structures were removed to avoid unwanted interaction during molecular docking. All water molecules were removed. Also, the addition of polar hydrogens and energy minimization was performed using Autodock tools (version 1.5.6). Total 0.278 Kollman charges were added to the protein. The optimized chain 'A' of the BSA structure is shown in fig. 2A) and the structure of cocrystalized ligand naproxen is shown in fig. 2B).

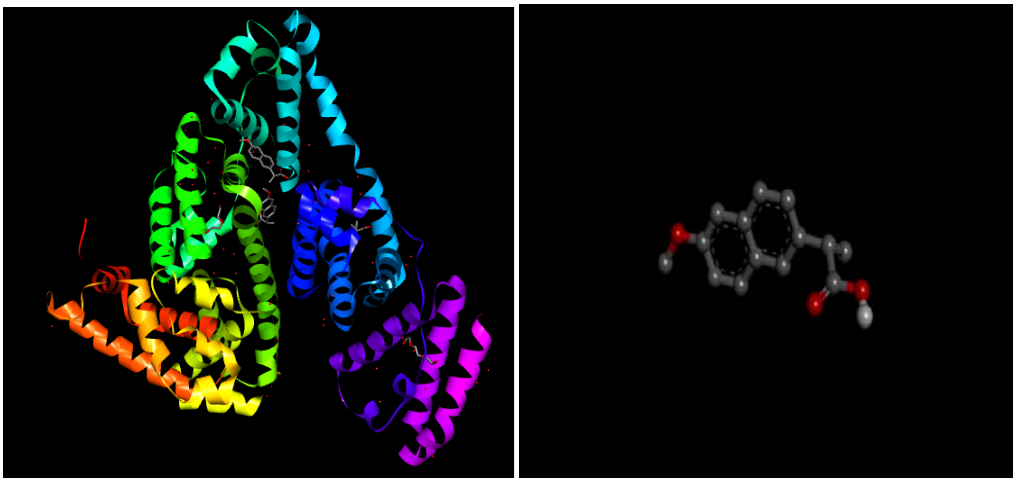


**Fig. 2: A) Chain A of BSA fig. B) Structure of co-crystalized naproxen**

Ligands were downloaded from the pubchem database. 3D structures of β-sitosterol (Pubchem ID: 222284) and Luteolin-7-glucoside (Pubchem ID: 5280637) were retrieved in SDF format from Pubchem. Using Open babel GUI software (version 2.4.1) SDF formats were converted into pdbqt format. Further, the ligands were prepared by detecting the torsion root, setting up the number of torsions and choosing torsions using Autodock Tools (version 1.5.6).

Following parameters were set for both ligands. Number of active torsions and Number of rotatable bonds were set to 7 for β-Sitosterol; while set to 11 for Luteolin-7-glucoside**.** Grid parameters were set as, spacing of 0.375 Å, box size of 60x60x60 A °and coordinates as x = 10.809, y = 14.539, z = 117.794. Auto dock 4.2 was used to perform molecular docking. Ligands were found flexible during the docking process, and protein was kept rigid. Numbers of Geometric Algorithm were set as 10 and population size was 100.

The Binding energy (kcal/mol) of molecules was found to be-7.23 for *β*-sitosterol and-5.38 for Luteolin-7-glucoside. Amino acids in the active protein site interacting with ligands were identified using Pymol 2.3. Biovia Discovery Studio 2021 was used to visualize and study the ligand-receptor interactions. *β*-sitosterol molecule interact by hydrophobic interaction with LEU 452, LEU 429, VAL 432, PHE 402, CYS 437, CYS 391, LEU 386 amino acids of Bovine Serum Albumin. The amino acid THR 448, ILE 387, CYS 436, ASN 390, GLY 433, PHE 394, ARG 409, LEU 406, TYR 410, LYS 413, PHE 487 of Bovine Serum Albumin interact with β-sitosterol by means of vandewalls interaction. The SER 488 formed hydrogen bond with hydroxyl OH of *β*-sitosterol with hydrogen bond distance of 2.1Å.

Luteolin-7-glucoside molecule interact by hydrophobic interaction with LYS 431, ARG 427, ALA 193 amino acids of Bovine Serum Albumin. The amino acid ARG 196, SER 192, HIS 145, ILE 522, GLU 424, of Bovine Serum Albumin interact with Luteolin-7-glucoside by means of Vandewalle interaction. The amino acids ARG 458, ARG 435, ARG 185 are involved in forming hydrogen bond with hydroxyl Oxygen, Carbonyl carbon of Luteolin-7-glucoside with hydrogen bond distance of 2.4, 2.3 and 1.9 Å, respectively.

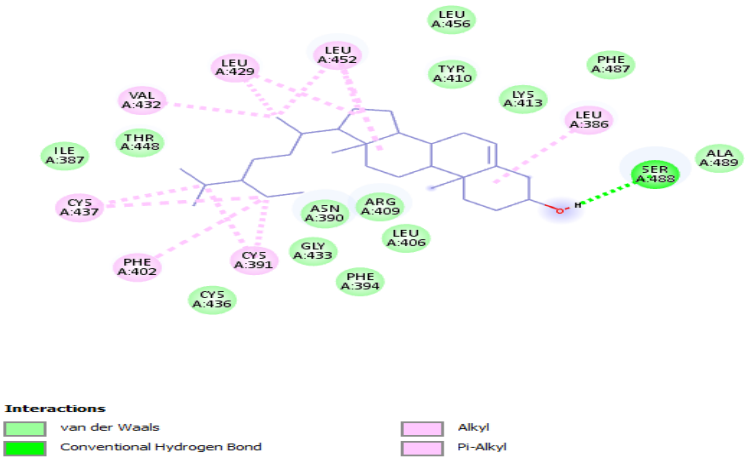



**Fig. 3: Interaction of *β*-sitosterol with binding pocket of bovine serum albumin**

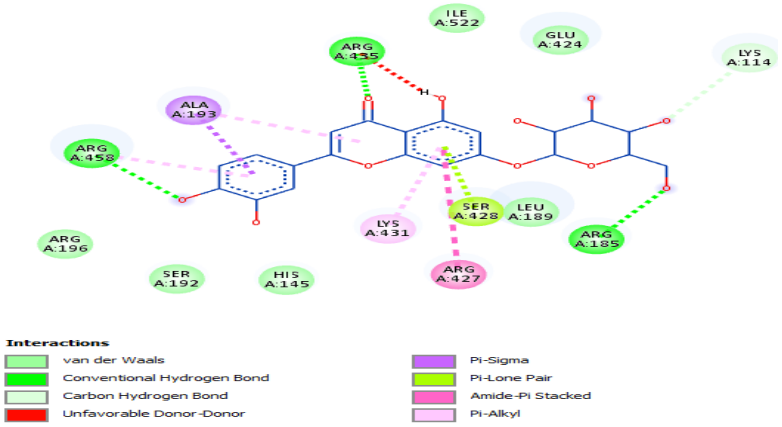



**Fig. 4: Interaction of luteolin 7-glucoside with binding pocket of bovine serum albumin**

The work [12] done shown that the following relationship may exist between the structure and binding interaction of the flavonoids with HAS with structure-binding relationships explored. The study [13] reports phytosterols to bind with some lysyl and arginine glycation sites of BSA, via Hydrogen-bonding with their OH groups and pi-pi interactions of their steroid core. The work done by us further explores and suggest hydrophobic and vandewalls interactions involved in the binding of *β*-sitosterol and luteolin-7-glucoside to bovine serum albumin.

Naturally occurring *β*-sitosterol was purified from leaves of *Feronia Limonia* and Luteolin-7-glucoside from leaves of *Bougainvillea glabra* and has therapeutic potential to cure many diseases. Here we have tested these compounds with BSA since it plays a major role in transporting the drug molecules to the target places. Analysis of parameters indicated that hydrophobic and hydrogen bonding interactions were mostly responsible for albumin association. It is our hope that the results presented here provide new grounds for further investigations of the pharmaceutical potential of these plant molecules and will be useful for monitoring its biological functions *in vivo*.

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**AUTHORS CONTRIBUTIONS**

SVG designed the work. SVG contributed for the analysis and data collection parts of the work. SVG and SSP contributed to the interpretation of the results.

**CONFLICT OF INTERESTS**

Declared none

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