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Dual Targeting Of Cyclooxygenase And Lipoxygenase By Beta-Sitosterol: A Molecular Docking Study

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ABSTRACT

Inflammation is mediated by key enzymes, cyclooxygenase-2 (COX-2) and 5-lipoxygenase (LOX-5), which regulate prostaglandin and leukotriene biosynthesis. This study evaluated the anti-inflammatory potential and drug-likeness of β-sitosterol using in silico approaches. Drug-likeness was assessed via the Molinspiration tool based on Lipinski's Rule of Five, and molecular docking was performed against COX-2 and LOX-5. β-Sitosterol showed acceptable drug-likeness with a single lipophilicity violation. Docking revealed stable binding through hydrogen bonds and hydrophobic interactions within both enzymes' active sites. These results suggest β-sitosterol as a promising dual COX-2/LOX-5 inhibitor, warranting further experimental validation.

Keywords: Inflammation, cyclooxygenase, lipoxygenase, computational study, phytosterol

1. INTRODUCTION

Inflammation is a fundamental defence mechanism that enables the human body to respond to and counteract infections and tissue damage caused by infectious, physical, or chemical agents. It comprises a series of complex and tightly regulated sequential events aimed at eliminating the initial cause of cellular injury, clearing damaged tissues, and promoting repair and recovery. Under physiological conditions, inflammation plays a crucial role in maintaining tissue homeostasis and structural integrity in response to harmful stimuli. However, when the inflammatory response becomes dysregulated or persistent, inflammatory mediators may activate aberrant signal transduction pathways, leading to chronic pro-inflammatory states (Liu, 2017). Such sustained inflammation has been strongly associated with increased incidence and mortality in several chronic diseases, including diabetes, arthritis, cancer, obesity, cardiovascular disorders, multiple sclerosis, atherosclerosis, and ischemic conditions (Nunes CdR, 2020).

Inflammation is primarily mediated by bioactive lipid molecules derived from arachidonic acid (AA), a 20-carbon polyunsaturated fatty acid released from membrane phospholipids by phospholipase A_2. Upon release, AA is metabolized through two major enzymatic pathways: cyclooxygenase (COX) and lipoxygenase (LOX), leading to the biosynthesis of prostaglandins and leukotrienes, respectively (Wang B et al., 2021). Cyclooxygenases catalyze the conversion of AA into prostaglandin G_2 and subsequently prostaglandin H_2, a common precursor for various prostanoids including prostaglandins, prostacyclin, and thromboxane A_2, which play critical roles in fever, pain, inflammation, and platelet aggregation. While COX-1 is constitutively expressed and essential for physiological functions such as gastric mucosal protection, renal homeostasis, and platelet function, COX-2 is inducible and predominantly upregulated during

inflammatory states, making it a key therapeutic target for inflammation control (Qureshi O and Dua A, 2022).

Parallel to the COX pathway, 5-lipoxygenase (5-LOX) catalyzes the oxidation of arachidonic acid to 5-hydroperoxyeicosatetraenoic acid, which is further converted into leukotrienes, potent mediators of immune cell recruitment and chronic inflammatory responses. Leukotrienes, particularly LTB_4 and cysteinyl leukotrienes, are implicated in the pathogenesis of inflammatory disorders such as rheumatoid arthritis, asthma, and osteoporosis (Wisastra R, Dekker FJ, 2014). Consequently, excessive activation of either COX-2 or 5-LOX contributes to sustained inflammatory signaling and disease progression.

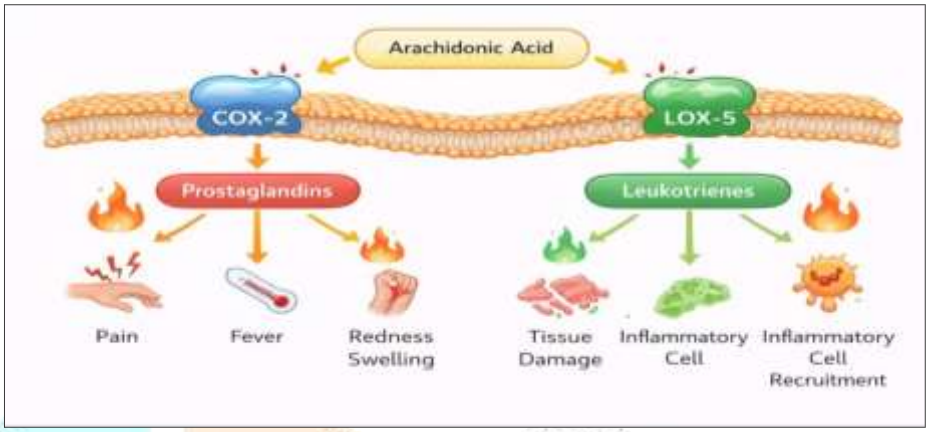


Fig 1 : Role of COX-2 and LOX-5 in inflammation

Although non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to alleviate pain and inflammation by inhibiting COX activity, long-term use of traditional NSAIDs is associated with significant gastrointestinal, renal, and cardiovascular adverse effects. This has led to increasing interest in natural products, particularly plant-derived compounds, have historically played a pivotal role in the discovery and development of modern therapeutic agents. Within this context, β-sitosterol, a naturally occurring phytosterol widely distributed in plant cell membranes and structurally analogous to cholesterol, has emerged as a promising candidate. β-Sitosterol has been reported to exhibit a broad spectrum of biological activities, including immunomodulatory, antioxidant, anticancer, and metabolic regulatory effects; however, its precise role in regulating inflammatory pathways remains insufficiently characterized (Shyamaladevi Babu & Selvaraj Jayaraman, 2020). Investigating the anti-inflammatory potential of β-sitosterol is therefore of particular significance, as it may offer a safer, naturally derived alternative to conventional NSAIDs by selectively modulating inflammatory mediators without compromising physiological homeostasis. Elucidating its interaction with key inflammatory enzymes and signaling pathways not only enhances the mechanistic understanding of its therapeutic action but also supports its potential development as a lead compound for the management of inflammation-associated disorders.

In this study, the anti-inflammatory potential of β-sitosterol is evaluated through molecular docking studies targeting key inflammatory mediators, particularly the cyclooxygenase pathway and lipoxygenase. In addition, the drug-likeness and pharmacokinetic properties of β-sitosterol are assessed using in-silico approaches to determine its suitability as a potential therapeutic candidate.

2. MATERIALS AND METHODS

2.1 Retrieval & Preparation of Protein Structure

Protein Selection and Preparation : Selection and preparation of the target protein constitute a critical prerequisite in structure-based computational biology approaches, particularly for molecular docking studies. This process involves identifying an appropriate protein structure and converting it into a suitable format to enable effective interaction analysis with the ligand of interest. In the present study, the crystal structure of human Cyclooxygenase-2 (COX-2) and lipoxygenase-5 (LOX-5) was retrieved from the Protein Data Bank (PDB ID: 5F19 and 3O8Y) (Md Idris, et al., 2022), determined at a resolution of 2.04 Å and 2.39 Å respectively, ensuring high structural accuracy for docking simulations. Prior to docking, the protein structure was prepared by removing crystallographic water molecules, bound ligands, co-crystallized complex molecules, and heteroatoms, in order to avoid steric interference and to expose the active binding site for ligand interaction.

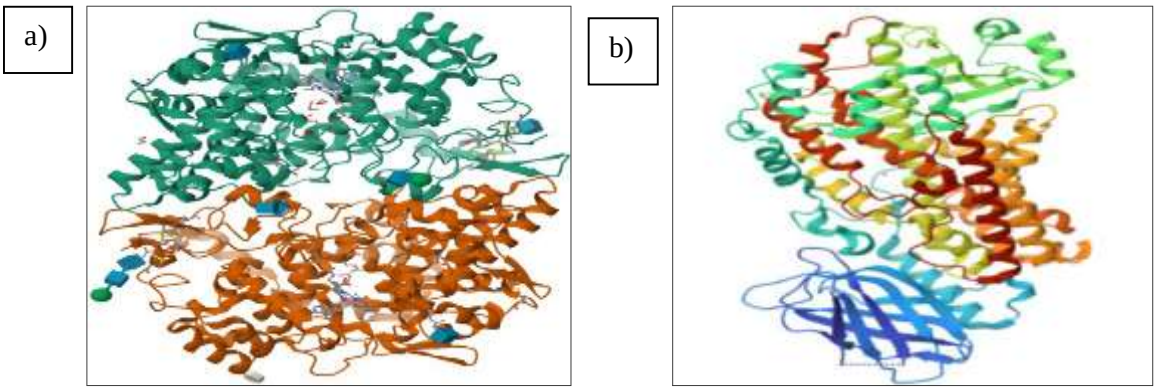


Fig 2 : a) 3D structure of COX-2 (PDB ID 5F19), b) 3D structure of LOX-5 (PDB ID 3O8Y) (source – RCSB Protein Data Bank)

Retrieval and Preparation of Ligand : The National Center for Biotechnology Information (NCBI) provides comprehensive online resources for accessing biological and chemical data. PubChem, a publicly available chemical database maintained by NCBI, offers detailed information on chemical structures, physicochemical properties, and biological assay data, and represents one of the largest repositories of freely accessible chemical information (Wang et al., 2009). Plant-derived steroids have been extensively investigated for their medicinal value, and several phytosterols are employed as therapeutic agents for the management and prevention of complex disease conditions. In the present study, the phytosterol β-sitosterol was selected to evaluate its potential anticancer activity. The three-dimensional structure of β-sitosterol was retrieved from the PubChem database. Ligand preparation, including structure optimization and conversion, was performed using Discovery Studio Visualizer, and the finalized ligand structure was saved in Protein Data Bank (PDB) format for subsequent molecular docking analysis.

Fig 3 : Structure of β-sitosterol (source – Pubchem)

1	Ligand	β- Sitosterol
2	Compound CID	222284
3	Molecular Formula	$C_{29}H_{50}O$
4	Molecular Weight	414.7g/mol
5	SMILES	C C [C @ H] (C C [C @ @ H] (C) [C@H]1CC[C@@H]2[C@@]1(CC[C@H]3[C@H]2CC=C 4[C@@]3(CC[C@@H](C4)O)C)C)C(C)C

Table 1 : Chemical description of β- Sitosterol

2.2 Ligand Validation & Pharmacokinetic Analysis

2.2.1 Determination of Bioactivity & Drug Score

The bioactivity score is used to evaluate the potential of the drug or the ligand to interact with different receptors, whether the compound is a G protein-coupled receptor (GPCR) ligand, ion channel modulator, enzyme, kinase or protease inhibitor or nuclear receptor ligand. This assessment of bioactivity score certifies the compound as a promising drug candidate and helps to assess whether the molecule is active, moderately active or inactive. The bioactivity of β-Sitosterol was determined using the online platform, Molinspiration Cheminformatics Software.

2.2.2 ADMET Profile of the Ligand

It is essential to assess the drug likeliness of a molecule before it is transformed into a drug. The ADMET profile of the molecule provides complete details on the absorption, distribution, metabolism, excretion and toxicity of the molecule. These properties provide insight on the various properties of the molecule including, physicochemical properties, lipophilicity, water solubility, GI absorption and ability to cross blood-brain barrier, the knowledge of which is pre requisite for computer aided drug designing. The SwissADME was used to determine the possible ADMET properties of the compound. Also, the toxicity, carcinogenicity and the drug score of the molecule was evaluated using Osiris property explorer.

2.3 MOLECULAR DOCKING

The binding affinity of β-Sitosterol to the targeted protein (COX-2) was analysed using docking studies, to generate the binding pose and affinity between the molecule (ligand) and the target (protein). Autodock Vina 4.2, Discovery Studio Visualizer (DSV), Pymol, Pubchem and RCSB PDB were used in the present study to investigate the binding affinities between the molecules.

The SDF format of the ligand (β-Sitosterol) and the PDB format of the target protein COX-2 (PDB ID : 5F19) and LOX-5 (PDB ID : 3O8Y) were obtained from Pubchem and RCSB PDB respectively. The ligand was converted to PDB format and the PDB of target proteins were prepared for docking by removing hetero atoms, native ligands and water molecules and also Kollman united atom charges, solvation parameters, and polar hydrogens were added.

AutoDock Vina requires pre-calculated grid maps for each atom type present in the ligand being docked, as these maps store the potential energy arising from interactions with the macromolecule. The grid must encompass the region of interest (active site) in the macromolecule. In this study, the binding site for grid was obtained from the X,Y,Z

parameters of the native ligands. Accordingly, the grid box was set for the x, y, and z axes, with the grid center coordinates at 28.56, 30.86, 64.27 and 4.09, 45.50, 3.10 respectively for COX-2 and LOX-5.

The docking simulations were performed using AutoDock Vina 4.2. the docked confirmations were visualized using PYMOL and Discovery Studio Visualiser tools. Also, using PyMol and Ligplus the nature of bonds formed and the bond distance were also identified.

3. RESULTS & DISCUSSION

3.1 Bioactivity and Drug Score of the Ligand

Computational advancements have greatly impacted the drug development process, with virtual screening methods being commonly employed to reduce both the cost and time involved. Molecular docking, a key technique, is used to identify new ligands for protein structures, making it an essential tool in structure-based drug design (Kitchen D.B. 2004).

The bioactivity score of the β-sitosterol was evaluated using Molinspiration Cheminformatics. Generally, a score greater than ‘0.00’ indicates that the molecule or ligand is significantly active, score between -0.5 to 0.00 infers that the molecule is moderately active and inactive if the score is less than -0.5. β-Sitosterol, was found to be significantly active as a GPCR ligand, Ion channel Modulator, Nuclear Receptor, Protease Inhibitor and Enzyme Inhibitor and moderately active as Kinase Inhibitor (Table 2).

Bioactivity	Score
GPCR Ligand	0.14
Ion Channel Modulator	0.04
Kinase Inhibitor	-0.51
Nuclear Receptor Ligand	0.73
Protease Inhibitor	0.07
Enzyme Inhibitor	0.51

Table 2 : Bioactivity score of β-Sitosterol

The drug-like properties of β-sitosterol were evaluated using the Molinspiration Cheminformatics platform based on Lipinski's Rule of Five, which is widely employed to predict the oral bioavailability of small-molecule drug candidates. The parameters assessed included molecular weight, hydrogen bond donors (HBD), hydrogen bond acceptors (HBA), lipophilicity (logP), and the number of rotatable bonds.

β-Sitosterol exhibited a molecular weight of 414.72 Da, which is within the acceptable threshold (<500 Da) defined by Lipinski's rule, suggesting favourable absorption and transport characteristics for oral administration (Lipinski et al., 2001). Molecular weight is a critical determinant of membrane permeability, and compounds with lower molecular mass generally demonstrate improved intestinal absorption.

Hydrogen bonding characteristics play a crucial role in receptor binding, solubility, and permeability. According to Lipinski's criteria, orally active compounds should possess fewer than five hydrogen bond donors and no more than ten hydrogen bond acceptors (Pollastri, 2010). In the present study, β-sitosterol was found to have one hydrogen bond donor and one hydrogen bond acceptor, indicating limited hydrogen bonding capacity. Reduced hydrogen bonding is often associated with enhanced passive diffusion across biological membranes, which may favour oral bioavailability (Veber et al., 2002). However, minimal polarity may also contribute to reduced aqueous solubility, a factor that must be considered during formulation development.

Lipophilicity, expressed as the logarithm of the partition coefficient (logP), is a key descriptor influencing drug absorption, distribution, and metabolic stability. β-Sitosterol exhibited a logP value of 8.62, which exceeds the recommended range of −2 to 5 for orally active drugs, thereby representing a single violation of Lipinski's rule. Elevated logP values indicate high hydrophobicity, which can enhance membrane permeability but may negatively impact solubility and bioavailability (Waring, 2009). Notably, several naturally derived bioactive compounds, including sterols and terpenoids, frequently violate logP criteria yet demonstrate significant pharmacological activity (Gleeson, 2008). Previous studies have reported that phytosterols such as β-sitosterol exert biological effects despite poor aqueous solubility, often requiring formulation strategies to improve their pharmacokinetic profiles (Shi et al., 2014).

Molecular flexibility was evaluated by assessing the number of rotatable bonds, which was found to be six for β-sitosterol. According to Veber's rule, compounds with fewer than ten rotatable bonds generally exhibit improved oral bioavailability due to reduced conformational entropy during membrane permeation (Veber et al., 2002). Lower flexibility is often associated with enhanced permeability and more favourable binding interactions with target proteins.

Despite exhibiting a high logP value, β-sitosterol satisfies the majority of Lipinski's criteria, with only a single rule violation. Lipinski's framework allows for one violation without disqualifying a compound as an orally active drug candidate (Lipinski et al., 2001). Importantly, β-sitosterol has been extensively reported to possess anti-inflammatory, anticancer, antioxidant, and immunomodulatory activities, supporting its pharmacological relevance (Bouic, 2001; Choi et al., 2003). The high lipophilicity of β-sitosterol may contribute to strong hydrophobic interactions within enzyme active sites, such as COX and LOX enzymes, which is particularly relevant for anti-inflammatory drug design.

Furthermore, several studies have emphasized that natural products often fall outside traditional drug-likeness rules, yet serve as valuable lead compounds due to their structural complexity and biological specificity (Newman & Cragg, 2020). In silico drug-likeness assessments should therefore be interpreted alongside biological activity and molecular docking results rather than as exclusionary criteria.

Overall, the Molinspiration-based analysis indicates that β-sitosterol possesses acceptable drug-like characteristics with respect to molecular weight, hydrogen bonding capacity, and molecular flexibility. Although the compound violates the logP criterion, this single violation does not preclude its development

as an oral therapeutic agent. Instead, it highlights the potential need for drug delivery optimization or formulation strategies to enhance solubility and bioavailability. Collectively, these findings support the candidacy of β-Sitosterol as a promising orally active bioactive compound warranting further pharmacokinetic and experimental validation.

Bioavailability Rules	**Score**	**Score for β-Sitosterol**
Molecular Weight	< 500	414.72
H Donor	≤ 5	1
Hydrogen Bond Acceptor	≤ 10 (5 X 2)	1
Log P	≤ 5	8.62
No. of Rotatable Atoms	< 10	6

Table 3 : Druglikeliness of β-Sitosterol

Also, the drug score of the selected molecule was found to be 0.134, using Osrir tool, it further revealed the negative tumorigenic, mutagenic and irritant effect of the molecule.

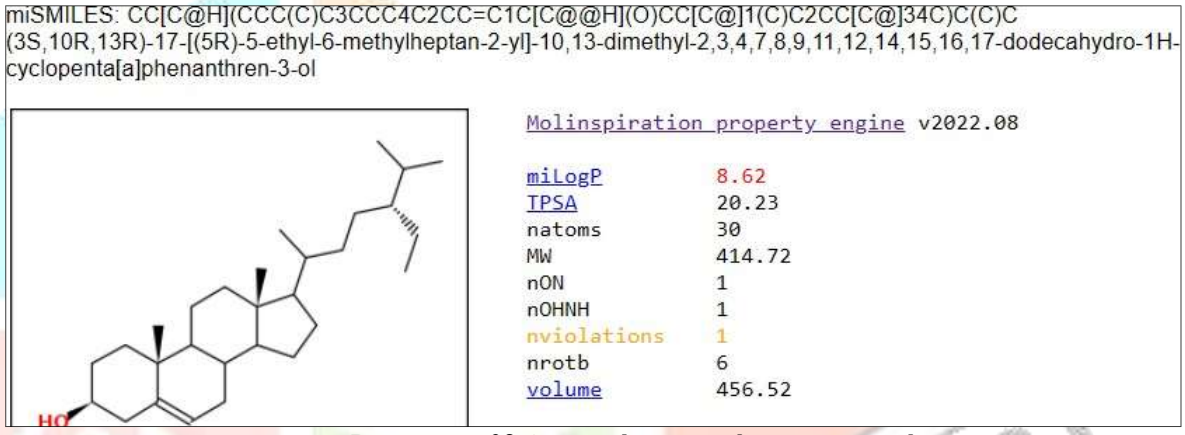


Fig 4 : Drug score of β-Sitosterol using Molinspiration Tool

3.2 ADMET Properties

The pharmacokinetic properties of the selected molecule, β-Sitosterol revealing its interaction in the human body was evaluated for its interaction with in the body by analysing its ADMET properties.

ADMET Properties	Values
BBB[a]	0.136
Caco[b]	-4.679
HIA[c]	0.002
MDCK[d]	1.1e-05
PPB[e]	93.38%
Toxicity[f]	Negative

Table 4 : ADMET properties of β-Sitosterol

a - Blood-Brain Barrier (BBB) penetration = [Brain]/[Blood].

b - Caco-2 cells are derived from human colon adenocarcinoma, possess multiple drug transport pathways through intestinal epithelium.

c - Human Intestinal Absorption (HIA) - is the sum of bioavailability and absorption evaluated from ratio of excretion or cumulative excretion in urine, bile and faeces.

d - MDCK cell system used as tool for rapid permeability screening.

e - % of drug binds to plasma protein.

f - In vitro Ames test by Metabolic & Non-metabolic activated TA100 & TA1535 strains collected from rat liver homogenate.

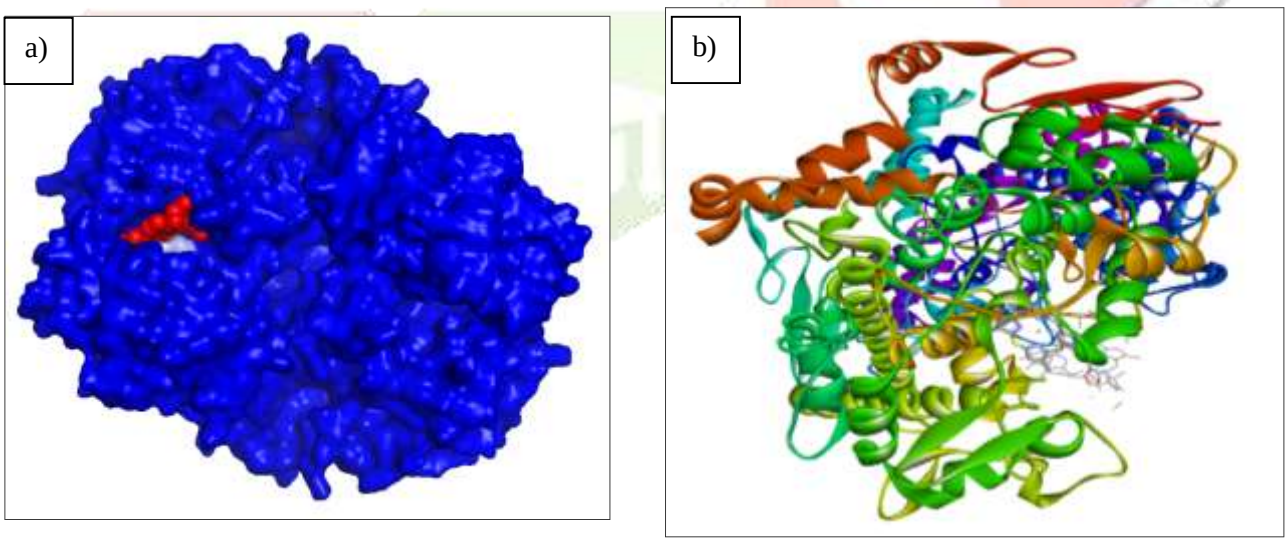
3.4 DOCKING STUDIES

3.4.1 Docking Interaction Analysis with COX-2

Molecular docking analysis revealed that β-sitosterol binds favourably within the active site of COX-2, forming both hydrogen bonding and hydrophobic interactions with key amino acid residues involved in enzymatic catalysis and substrate stabilization. Hydrogen bond interactions were observed with His241, Gln545, and His388, suggesting a stable anchoring of the ligand within the COX-2 binding pocket. These residues have been reported to play a significant role in ligand recognition and catalytic function, particularly in maintaining the geometry of the active site **(**Kurumbail et al., 1996; Rouzer & Marnett, 2009**).**

In addition to hydrogen bonding, β-sitosterol exhibited extensive hydrophobic and van der Waals interactions with residues such as Val282, Leu266, Gly262, Phe265, Tyr246, Ala290, Tyr264, Ala263, His260, and Thr210. The predominance of hydrophobic interactions is consistent with the highly lipophilic nature of β-sitosterol and the hydrophobic channel characteristic of the COX-2 active site. Previous crystallographic and docking studies have demonstrated that effective COX-2 inhibitors often rely on strong hydrophobic contacts within this channel to achieve enzyme inhibition **(**Picot et al., 1994; Warner et al., 1999**).**

Notably, residues such as Phe265, Tyr246, and Val282 are frequently implicated in stabilizing non-steroidal anti-inflammatory drugs (NSAIDs) and phytochemicals within the COX-2 binding cleft, suggesting that β-sitosterol may exert inhibitory activity via a mechanism similar to known COX-2 modulators **(Orlando et al., 2015**). The combined hydrogen bonding and hydrophobic interactions indicate a stable ligand–protein complex, supporting the anti-inflammatory potential of β-sitosterol through COX-2 inhibition.



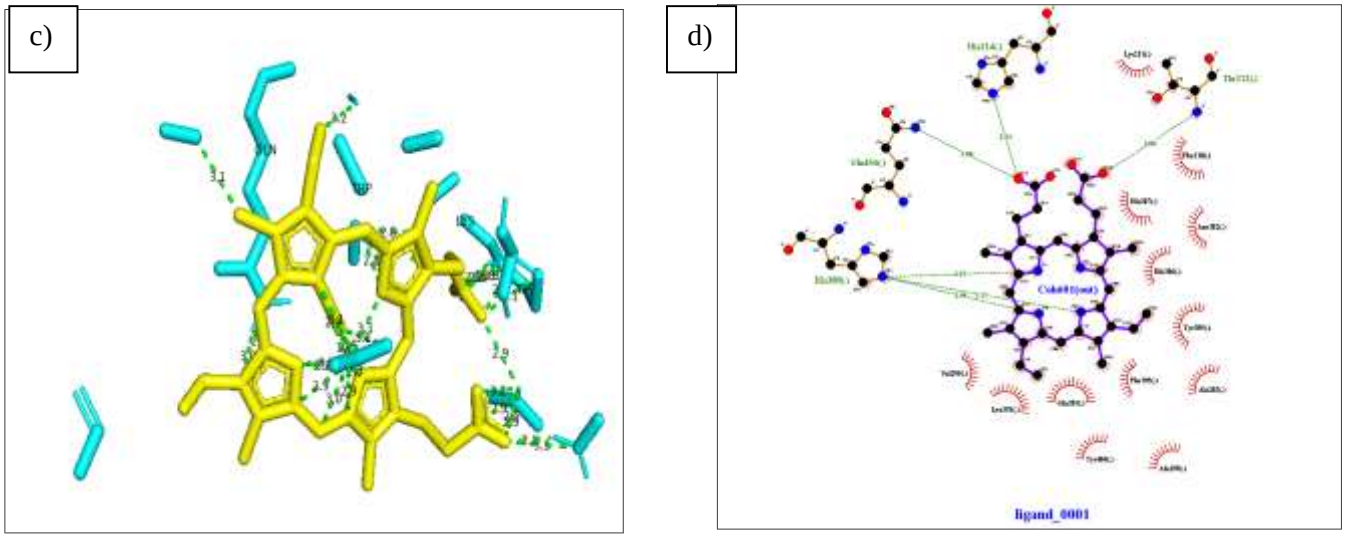


Fig 5: Molecular interactions of β-sitosterol with cyclooxygenase-2 (COX-2).
(a) Surface representation of the COX-2–β-sitosterol complex visualized using PyMOL;
(b) Three-dimensional binding orientation of β-sitosterol within the COX-2 active site as visualized using Discovery Studio Visualizer (DSV);
(c) Detailed interaction map showing amino acid residues of COX-2 involved in binding with β-sitosterol along with corresponding bond distances;
(d) Two-dimensional interaction diagram illustrating the nature of interactions and bond lengths generated using LigPlot⁺.

3.4.2 Docking Interaction Analysis with 5-LOX

Docking studies against 5-lipoxygenase (5-LOX) revealed that β-sitosterol interacts effectively within the enzyme's active site, forming a hydrogen bond with Arg457, a residue known to contribute to substrate positioning and enzymatic stability. Hydrogen bonding with Arg residues has been previously reported as a key determinant for effective 5-LOX inhibition by natural compounds (Gilbert et al., 2012; Gilbert & Newcomer, 2014).

In addition, β-sitosterol established multiple hydrophobic and van der Waals interactions with residues including Ala446, Ala453, Ala456, Ser447, Thr444, Asp442, Leu288, Glu287, Asp285, Val243, Arg370, Val361, and Leu244. The abundance of hydrophobic residues within the binding pocket highlights the importance of lipophilicity for effective 5-LOX inhibition. Studies have shown that the 5-LOX active site contains a largely hydrophobic cavity that accommodates fatty acid substrates and lipophilic inhibitors, making hydrophobic interactions critical for ligand stabilization (Haeggström & Funk, 2011).

Residues such as Leu288, Val243, and Leu244 have been frequently identified in docking studies involving natural anti-inflammatory compounds targeting 5-LOX, reinforcing the relevance of the observed interactions (Rådmark et al., 2015). The interaction profile suggests that β-sitosterol may effectively interfere with leukotriene biosynthesis by occupying the catalytic pocket and hindering substrate access.

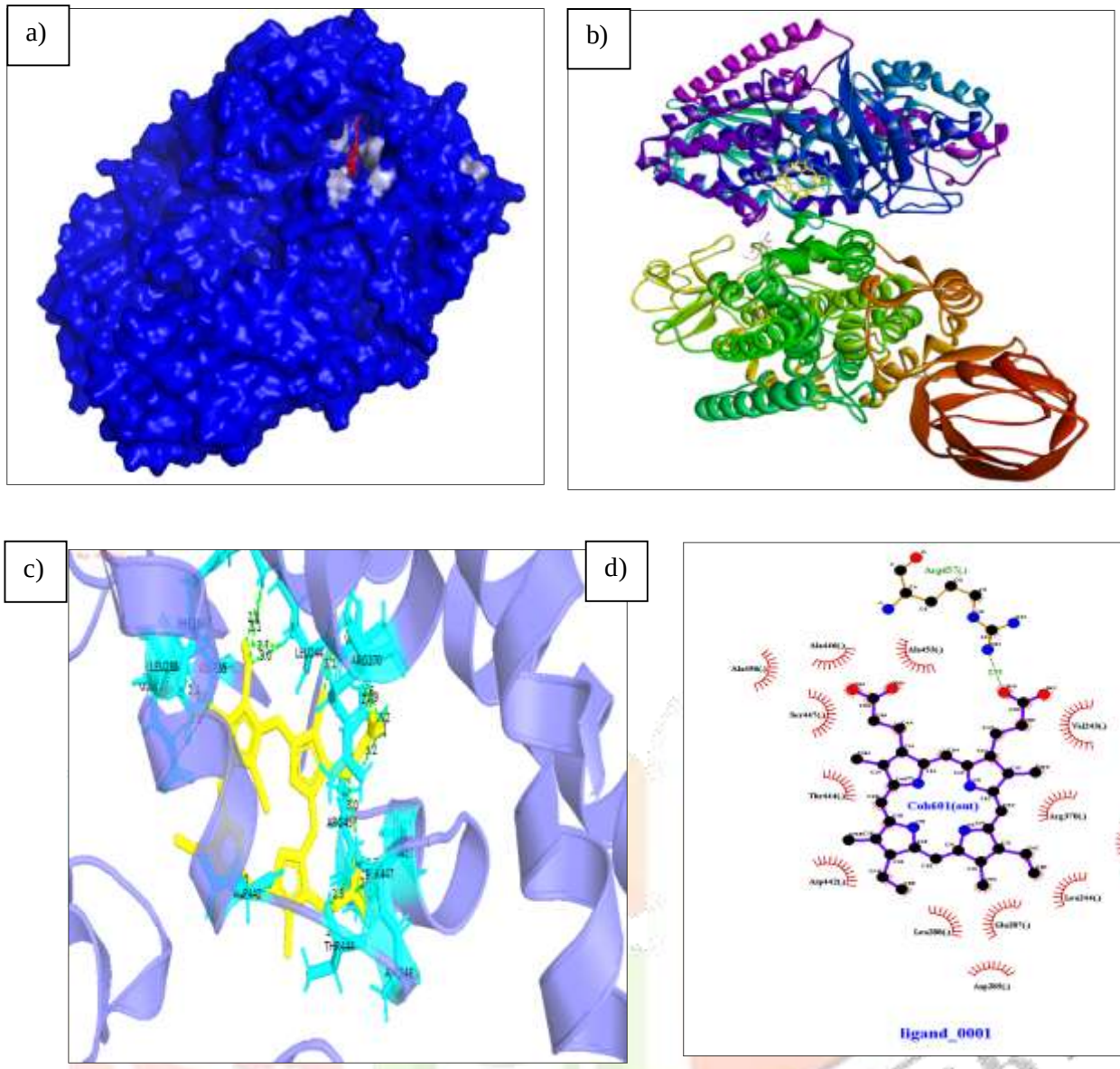


Fig 6 : Molecular interactions of β-sitosterol with lipooxygenase-5 (LOX-5).
(a) Surface representation of the LOX-5–β-sitosterol complex visualized using PyMOL;
(b) Three-dimensional binding orientation of β-sitosterol within the LOX-2 active site as visualized using Discovery Studio Visualizer (DSV);
(c) Detailed interaction map showing amino acid residues of LOX-2 involved in binding with β-sitosterol along with corresponding bond distances;
(d) Two-dimensional interaction diagram illustrating the nature of interactions and bond lengths generated using LigPlot⁺.

Protein	Type of Interaction	Amino acids involved
COX-2	Hydrogen Bond	His241, Gln545, His388
	Hydrophobic / van der Waals interactions	Val282, Leu266, Gly262, Phe265, Tyr246, Ala290, Tyr264, Ala263, His260, Thr210
LOX-5	Hydrogen Bond	Arg457
	Hydrophobic / van der Waals interactions	Ala446, Ala453, Ala456, Ser447, Thr444, Asp442, Leu288, Glu287, Asp285, Val243, Arg370, Val361, Leu244

Table 5 : Interaction of β-Sitosterol with COX-2 and LOX-5

The docking results demonstrate that β-sitosterol exhibits favourable binding interactions with both COX-

2 and LOX-2, supporting its potential as a dual-pathway anti-inflammatory agent. Dual inhibition of COX-2 and 5-LOX is considered a promising strategy to enhance anti-inflammatory efficacy while minimizing side effects associated with selective COX inhibition (Celotti & Laufer, 2001).

The predominance of hydrophobic interactions observed in both enzymes correlates well with the high lipophilicity of β-sitosterol, as indicated by its elevated logP value. While high lipophilicity may limit aqueous solubility, it enhances binding affinity within hydrophobic enzyme cavities, particularly for lipid-metabolizing enzymes such as COX-2 and 5-LOX (Waring, 2009). Moreover, the presence of stabilizing hydrogen bonds further strengthens the ligand–enzyme complexes, contributing to binding specificity and stability.

Overall, the molecular docking results provide mechanistic insight into the anti-inflammatory activity of β-sitosterol and support previously reported experimental evidence of its ability to modulate inflammatory mediators (Bouic, 2001; Choi et al., 2003). These findings suggest that β-sitosterol may serve as a promising lead compound for the development of novel anti-inflammatory agents targeting both prostaglandin and leukotriene pathways.

3.5 CONCLUSION

The present study employed in silico approaches to evaluate the anti-inflammatory potential and drug-likeness of the phytosterol β-sitosterol, with particular emphasis on its interaction with key inflammatory enzymes. Drug-likeness analysis revealed that β-sitosterol satisfies most of the criteria of Lipinski's Rule of Five, indicating acceptable oral bioavailability despite a single violation related to lipophilicity. Overall, the physicochemical profile supports its suitability as a potential therapeutic agent for inflammatory disorders.

Molecular docking studies demonstrated that β-sitosterol exhibits stable and favorable binding interactions with both cyclooxygenase-2 (COX-2) and 5-lipoxygenase (LOX-5), two pivotal enzymes involved in the biosynthesis of pro-inflammatory mediators such as prostaglandins and leukotrienes. The binding of β-sitosterol within the active sites of COX-2 and LOX-5 was stabilized through a combination of hydrogen bonding and extensive hydrophobic interactions with key amino acid residues, suggesting effective inhibitory potential against both enzymatic pathways. This dual interaction highlights the ability of β-sitosterol to modulate inflammation through simultaneous inhibition of COX-2 and LOX-5, a strategy known to enhance anti-inflammatory efficacy while potentially reducing adverse effects associated with selective COX inhibition.

In conclusion, the findings of this in silico investigation identify β-sitosterol as a promising natural lead compound with dual-target anti-inflammatory activity. The study supports its further exploration as an alternative or adjunct to conventional synthetic anti-inflammatory drugs. Nevertheless, comprehensive in vitro, in vivo, and pharmacokinetic evaluations are necessary to substantiate its efficacy, safety, and therapeutic applicability.

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