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Computational Screening of *Vitex negundo* Compounds for Potential Arthritis Therapies in India

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Abstract

Objective: *Arthritis is a pervasive medical condition that manifests as inflammation and discomfort within the joints. As of September 2021, arthritis has affected an estimated 180 million people in India, making it a substantial public health concern with a considerable impact on individuals' quality of life and healthcare systems. Therefore, using molecular docking, drug-likeness prediction, and ADME analysis, an effort was made to identify natural compounds from Vitex negundo, which have many medicinal properties in Indian Ayurveda, to avoid this condition.* ***Methodology:*** *A computational approach was employed to evaluate the inhibitory potential of several phytochemicals on the target proteins, 1G0Y (IL-1), 1Alu (IL-6), and 5vqp (TFG-B), which were sourced from the PDB database. Docking was carried out systematically using IMPAAT, PubChem, PDB, Open Bable, BIOVIA Discovery Studio Visualizer, PDB sum generate, PyRx, and ADMETlab 2.0 after the removal of the ligands with weak binding and compounds that can disrupt docking.* ***Results:*** *The results of the docking analysis showed that the selected ligands possessed improved binding affinities for each of the three target proteins. The statistical distribution of protein combinations with backbone dihedral angles is shown by a Ramachandran plot. Three compounds from Vitex negundo were identified through molecular docking studies as having a potential binding affinity to provide anti-inflammatory effects. All three compounds were found to be safe and to have drug-like qualities according to the ADMET profile and drug-likeness prediction.* ***Conclusion:*** *This computer study examined how Vitex negundo compounds can be used to treat arthritis in India. Our comprehensive molecular docking, drug similarity prediction, and ADME analyses showed that these drugs bound strongly to arthritic target proteins. Lutein and beta-sitosterin showed promising anti-inflammatory properties, bringing hope for arthritis therapies and enhanced quality of life while relieving India's healthcare system.*

Keywords: Arthritis, Vitex negundo, 1G0Y(IL-1), 1Alu (IL-6), and 5vqp (TFG-B), luteolin, beta-sitosterin, elixene, molecular docking, ADMET

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INTRODUCTION

Arthritis, an intricate constellation of inflammatory joint disorders, is a significant facet of autoimmune and rheumatic conditions. The prevalence of arthritis varies globally, with estimations indicating millions are affected by its debilitating effects [1]. The interplay among genetics, environmental triggers, and immune responses contributes to its intricate pathogenesis. Incidence rates continue to increase, underscoring the urgent need for comprehensive research and management strategies [2].

Within the realm of inflammatory disease, arthritis leaves an indelible mark. Its diverse forms include rheumatoid arthritis and ankylosing spondylitis, engendered pain, stiffness, and functional limitations. These circumstances not only diminish the quality of life for those affected but also present significant economic challenges for healthcare systems [3]. As a significant contributor to disability, arthritis has prompted ongoing efforts to unravel its molecular underpinnings, identify novel therapeutic targets, and enhance patient outcomes. A deeper understanding of the role of arthritis in inflammatory cascades holds promise for advancing medical interventions and ultimately alleviating its societal impact.

Arthritis, which encompasses various inflammatory joint disorders, arises from complex pathological processes involving immune dysregulation, genetic predisposition, and environmental triggers [4]. Cytokines, such as tumor necrosis factor (TNF), interleukin-1 (IL-1), and interleukin-6 (IL-6) are crucially involved in the development of arthritis and are key targets for treatment strategies.

Pathologically, arthritis involves immune cells infiltrating the synovial tissues, triggering a cascade of pro-inflammatory cytokines. IL-1, IL-6, and TNF are the chief contributors to this inflammatory milieu. IL-1 promotes joint inflammation and cartilage [5]. IL-6 stimulates immune response and synovial inflammation, thereby amplifying joint damage. TNF drives synovitis, osteoclast activity, and tissue destruction [6].

Targeted therapies have revolutionized arthritis treatment by neutralizing these cytokines. Biological drugs, including anti-TNF agents, IL-1 receptor antagonists, and IL-6 receptor blockers, impede cytokine actions, reduce inflammation, and prevent joint destruction [7]. This precise approach has significantly improved disease management, alleviated symptoms, preserved joint function, and enhanced patient quality of life. The intricate involvement of IL-1, IL-6, and TNF underscores their indispensable roles as therapeutic targets for combating arthritis [8].

In the intricate landscape of arthritis, interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) are key players that orchestrate a cascade of molecular events that contribute to inflammation, tissue damage, and disease progression [9].

IL-1, a potent pro-inflammatory cytokine, binds to its receptors in synovial cells. This interaction triggers the release of inflammatory mediators, such as prostaglandins and matrix metalloproteinases, which promote cartilage degradation and bone resorption [10–14]. Additionally, IL-1 fuels synovial cell proliferation and angiogenesis and further perpetuates joint inflammation.

IL-6 is a multifunctional cytokine that influences immune cell activation and differentiation. It stimulates B cells to produce antibodies, enhances T cell activity, and fuels the production of acute-phase reactants such as C-reactive protein. This amplifies the systemic inflammation, exacerbates synovial hyperplasia, and contributes to joint destruction [15].

Transforming growth factor-beta (TGF-β) plays a dual role in arthritis, initially contributing to anti-inflammatory responses and tissue repair but potentially promoting tissue fibrosis and joint deterioration in advanced stages of the disease [16]. Its intricate effects underscore its importance as a therapeutic target for achieving a balanced immune and tissue remodeling response in arthritis management.

Targeting these cytokines has revolutionized the treatment of arthritis. Biological agents, including anti-TGF-β therapies, IL-1 receptor antagonists, and IL-6 receptor blockers disrupt the inflammatory cascade. Anti-TNF drugs neutralize TGF-β, reduce joint inflammation, and prevent tissue damage. IL-1 inhibitors suppress cartilage degradation and bone erosion, whereas IL-6 receptor blockers dampen immune responses and inflammation [17].

By interrupting these cytokine-mediated pathways, treatment not only alleviates pain and swelling but also modifies disease progression. The preservation of joint function and prevention of irreversible damage is achieved, offering a transformative impact on the lives of individuals with arthritis [18].

Vitex negundo, commonly known as "Five-Leaved Chaste Tree" or "Nirgundi," has garnered significant attention owing to its anti-inflammatory properties. This medicinal plant, which is native to Asia and widely used in traditional medicine, holds promise because of its diverse phytochemical composition. Vitex negundo extracts have been explored for their ability to mitigate inflammatory responses by targeting various molecular pathways [19]. Compounds such as flavonoids, alkaloids, and terpenoids found in Vitex negundo possess potential anti-inflammatory effects, making them a subject of scientific inquiry for their role in managing inflammatory conditions. As researchers have delved deeper into its mechanisms of action and interactions with immune processes, the potential of Vitex negundo as a natural anti-inflammatory agent continues to spark interest and investigation [20].

The main objective of this study was to assess the anti-inflammatory and inhibitory effects of the 30 selected phytocompounds from V. negundo on three target proteins, 1G0Y(IL-1), 1Alu (IL-6), and 5vqp (TFG-B). In addition, to study the pharmacological and therapeutic efficacy, the results obtained from these studies will be compared to those of the commonly used drugs, celecoxib and ketorolac, which are approved by the FDA.

METHODOLOGY

Protein Preparation

The crystal structures of three target proteins, 1G0Y (IL-1), 1Alu (IL-6), and 5vqp (TFG-B), were acquired from the Protein Data Bank (PDB) maintained by the Research Collaboratory for Structural Bioinformatics (RCSB) (*https://www.rcsb.org/*). The abbreviation "1G0Y" refers to Interleukin-1 (IL-1). The protein under investigation was composed of a single chain, denoted as chain A, comprising 312 amino acids. The device exhibited a crystal resolution of 3.00 A. Before the optimization of hydrogen bonds and removal of atomic conflicts, protein crystal structures were prepared for docking. Alu (interleukin-6, IL-6). The protein possesses a singular polypeptide Chain A denoted as Chain A comprises 186 amino acid residues. The crystal resolution was determined to be 1.90 angstroms. Before the optimization of hydrogen bonds and removal of atomic conflicts, protein crystal structures were prepared for docking. The provided text "5vqp (TFG-B)" lacks any valuable information or context. Therefore, the protein in question cannot be composed of a single chain, denoted as chain A, consisting of 363 amino acids. The crystal exhibits a resolution of 2.90 A. Before the optimization of hydrogen bonds and the removal of atomic conflicts, protein crystal structures were prepared for docking. Protein preparation was conducted using the established procedure in Discovery Studio Visualizer 21.1. Water molecules and heteroatoms present in the proteins were eliminated, and polar hydrogen atoms were subsequently introduced. Additionally, the active sites of the proteins were predicted. The purified protein was subsequently stored in the pdb file format, which was employed to derive the 2-dimensional structure and Ramachandran plot using the PDBsum creation tool (*https://bio.tools/pdbsum_generate*). Additionally, a hydrophobicity plot was generated using the BIOVIA Discovery Studio software.

Ramachandran Plot

The Ramachandran plot illustrates the torsional angles psi and phi linked to the amino acids present in a peptide. The Ramachandran plot was analyzed using the web-based Ramachandran plot server provided by PDBsum (*https://bio.tools/pdbsum_generate*). The PDB files of three target proteins, 1G0Y(IL-1), 1Alu (IL-6), and 5vqp (TFG-B), were uploaded, and analysis of the Ramachandran plot was run with outliers labeled by residue type, residue number, and chain and displaying all the labels.

Retrieval of Ligands

Thirty phytocompounds from V. negundo were derived from Indian Medicinal Plants, Phytochemistry, and Therapeutics 2.0 (IMPPAT 2.0) (*https://cb.imsc.res.in/imppat/*). For further analysis. The canonical SMILES, PubChem CID, and two-dimensional (2D) models of these compounds along with the two standard drugs were retrieved in SDF format using the PubChem database (*https://pubchem.ncbi.nlm.nih.gov/*), and all the structures in the SDF files were then converted to PDB file format using Open Babel software. Ligand preparation was carried out by optimizing the ligand, energy minimization, and conversion of ligands to the 3D PDB format using the PyRx tool.

Molecular Docking

Molecular docking techniques enable the examination of how small molecules interact within the binding sites of target proteins, facilitating a deeper understanding of fundamental biochemical processes through simulations at the atomic level. PyRx software, a virtual screening tool, was used for molecular docking studies. This software facilitates the docking of small-molecule libraries to identify potential lead compounds and their activities. AutoDock Vina was used to execute the molecular docking process after retrieving the proteins and ligands. It is a fully accessible virtual screening tool that can evaluate libraries of phytocompounds against a particular therapeutic target and is primarily used for computer-aided drug design (CADD) techniques. The selected 30 phytocompounds and two standard drugs were loaded as ligands, and the three target proteins were loaded as macromolecules, namely 1G0Y(IL-1), 1Alu (IL-6), and 5vqp (TFG-B). The loaded ligand was energy minimized and converted to pdbqt form, and the grid was generated for the targeted protein, which followed the grid for the center, as shown in Table 1. All ligands were docked with the target protein discretely and evaluated based on binding affinity, which is the strength of protein-ligand binding. That is, the lowest binding indicates the best docking conformation (kcal/mol). After completion of docking, we obtained a table consisting of the binding affinity of each ligand. The top three ligands were selected for further study based on their highest binding affinities.

Visualization

The top three ligands with the lowest binding affinities for each protein were selected, and the best model for each ligand was saved in the PDB file format from PyRx. These were visualized using BIOVIA Discovery Studio software, the three-dimensional (3D) structure and non-bond interactions were observed, and the 3D model was extracted in png file format.

Physiochemical Studies (ADMET Analysis)

In the fields of pharmacokinetics and pharmacology, the acronym ADME, which stands for absorption, distribution, metabolism, and excretion, is used to describe the processes by which a drug is eliminated from an organism. The efficacy and pharmacological effects of a compound as a pharmaceutical agent are influenced by four key criteria, as they collectively influence drug concentrations and the dynamics of drug distribution in various tissues. Toxicity, commonly referred to as ADMET toxicity, is occasionally considered. For this study, three compounds with the greatest binding affinity were selected for drug similarity testing and Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) analysis. Drug-likeness and ADMET analyses were conducted using SWISS-ADME (*http://www.swissadme.ch/*) and ADMETLAB (*https://admetmesh.scbdd.com/service/evaluation/index*). The Lipinski rule of three was used as a criterion for conducting absorption, distribution, metabolism, and excretion (ADME) studies. Lipinski's rule of three is a predictive model used to determine the likelihood of a drug candidate's success based on its adherence to two or more specific conditions.

Table 1. Ligand loaded with energy minimization**.**

PDB Protein ID	X	Y	Z
1g0y (IL-1)	24.39	8.60	140.39
1Alu (IL-6)	2.66	-19.93	8.83
5vqp (TFG-B)	86.74	43.99	36.19

The criteria for compound selection were as follows: the molecular mass should be less than 500 Da, the logarithm of the partition coefficient (logP) should be less than 4.15, the number of hydrogen bond donors should be less than 5, the number of hydrogen bond acceptors should be less than 10, and molar refractivity should fall within the range of 40 to 130.

RESULT

Selection of Phytocompounds

A total of 30 phytocompounds of V. negundo were chosen from IMPAAT, of which only three were selected based on docking results and the two-dimensional (2D) chemical structure shown in Figure 1. To evaluate the inhibitory activity of these compounds against target proteins, two standard drugs, celecoxib, and ketorolac, were retrieved (Figure 1).

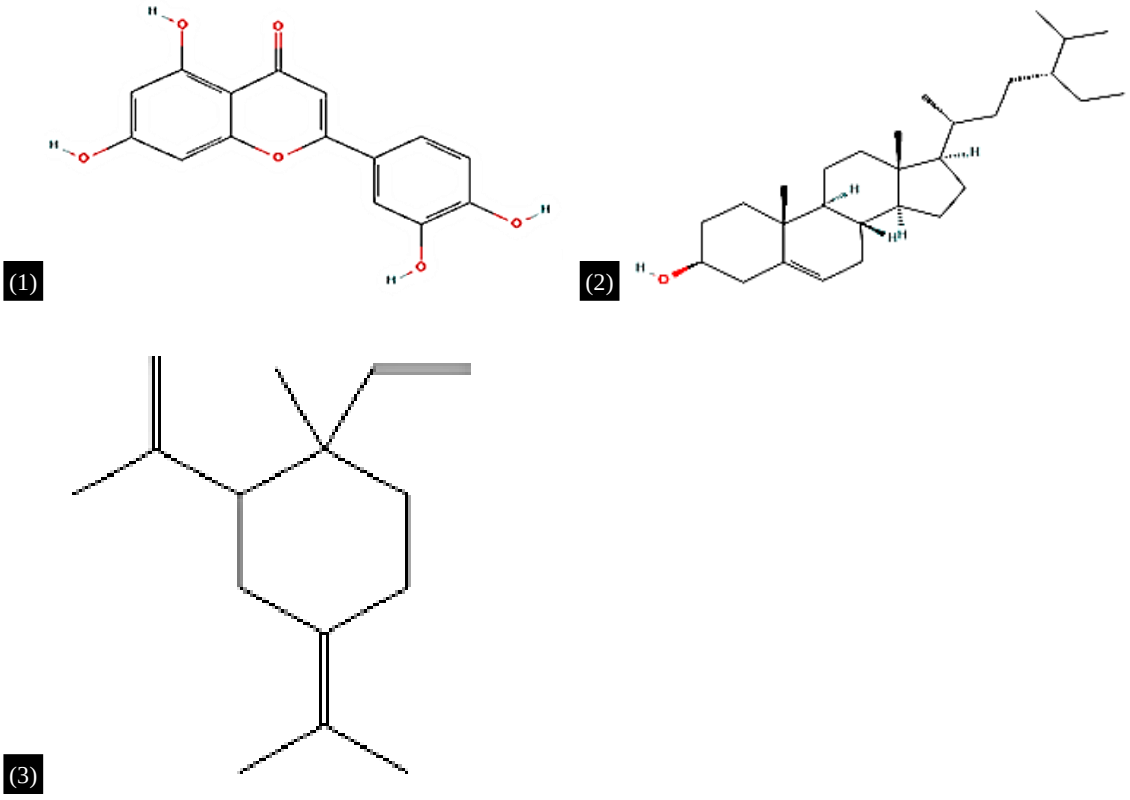


Figure 1. Chemical structures of the top three phytocompounds and standard drugs. (1) Luteolin, (2) Beta-Sitosterin, (3) Elixene (a) celecoxib and (b) ketorolac.

Protein Retrieval and Purification

Three-dimensional (3D) crystal structures of three target proteins retrieved from PDB. Subsequently, the proteins IL-1 (4GAF), IL-6 (409H), and TNFA (7KPA) were purified using BIOVIA Discovery software and subjected to structural analysis, as shown in Figures 2, 3(a,b), and 4, respectively. In the structural analysis, the Ramachandran plot, Secondary Structure, and hydropathy plot were analyzed.

The Ramachandran Plots

IL-1

As shown in Figure 2, the 3D structure had 77.5% of the residues in the region that were most preferred, 17.7% in the extra allowed region, 3.4% in the generously allowed region, and 1.4% in the rejected region.

Il-6

As shown in Figure 3 (a,b), the 3D structure had 95.2% of the residues in the region that were most preferred, 4.8% in the extra allowed region, 0% in the generously allowed region, and 0% in the rejected region.

TFG-B

As shown in Figure 4, the 3D structure had 87.8% of the residues in the region that were most preferred, 10.4% in the extra allowed region, 1.4% in the generously allowed region, and 0.4% in the rejected region.

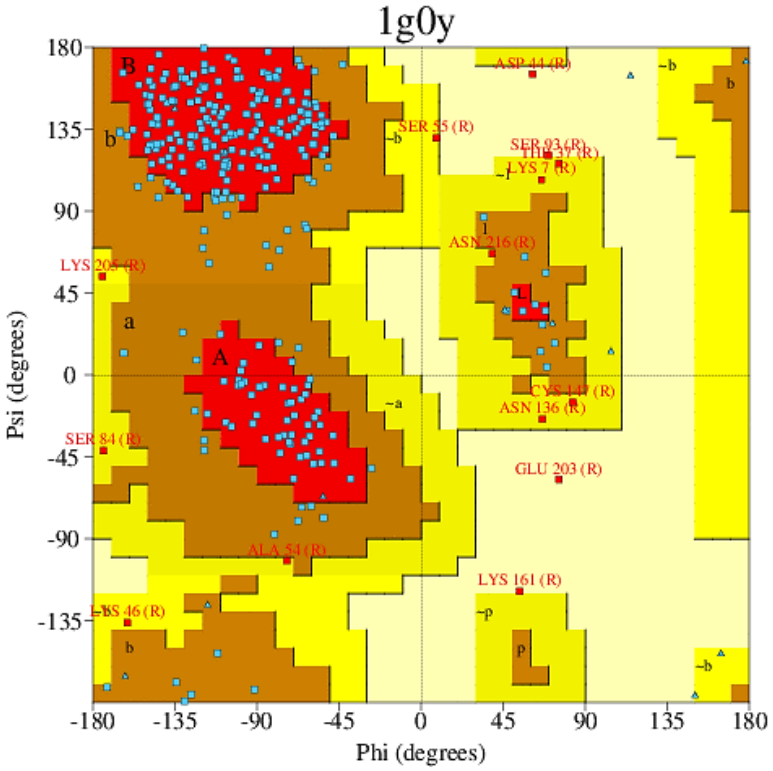
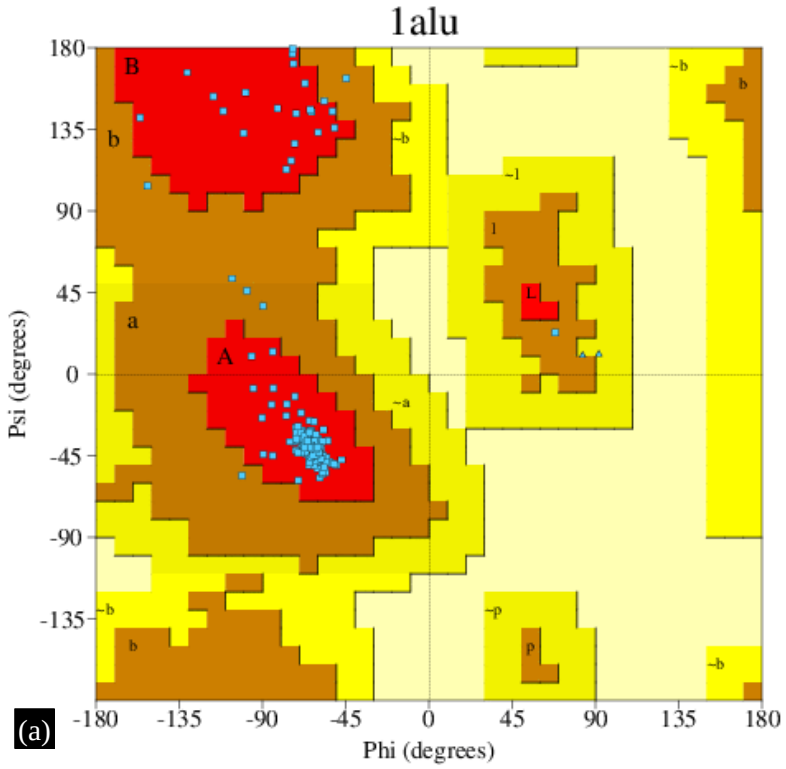


Figure 2. Ramachandran Plot for 1g0y (IL-1).



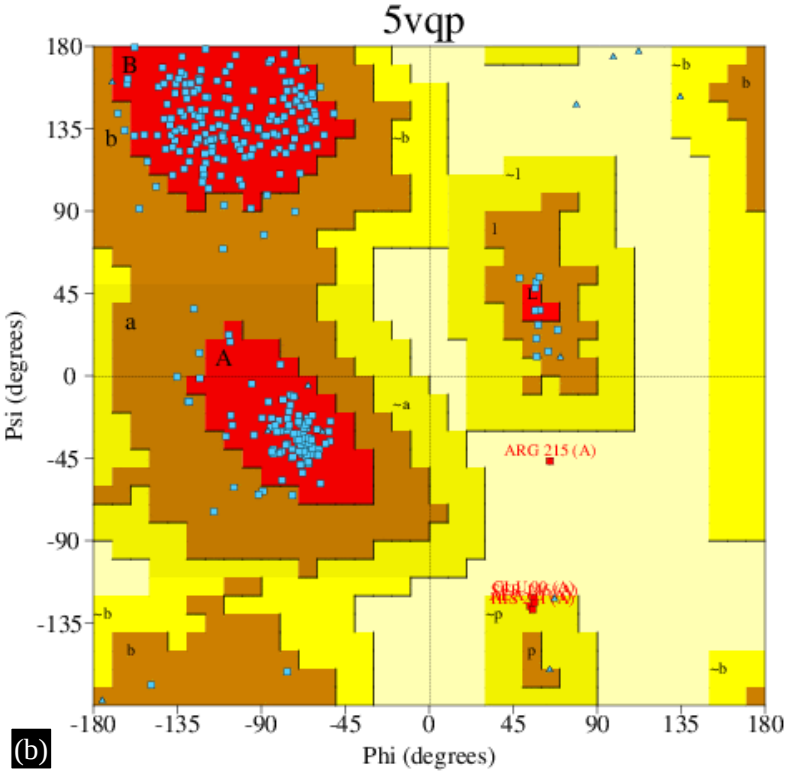


Figure 3. (a) Ramachandran Plot for 5vqp (TFG-B), (b) Ramachandran Plot for 1alu (IL-6).

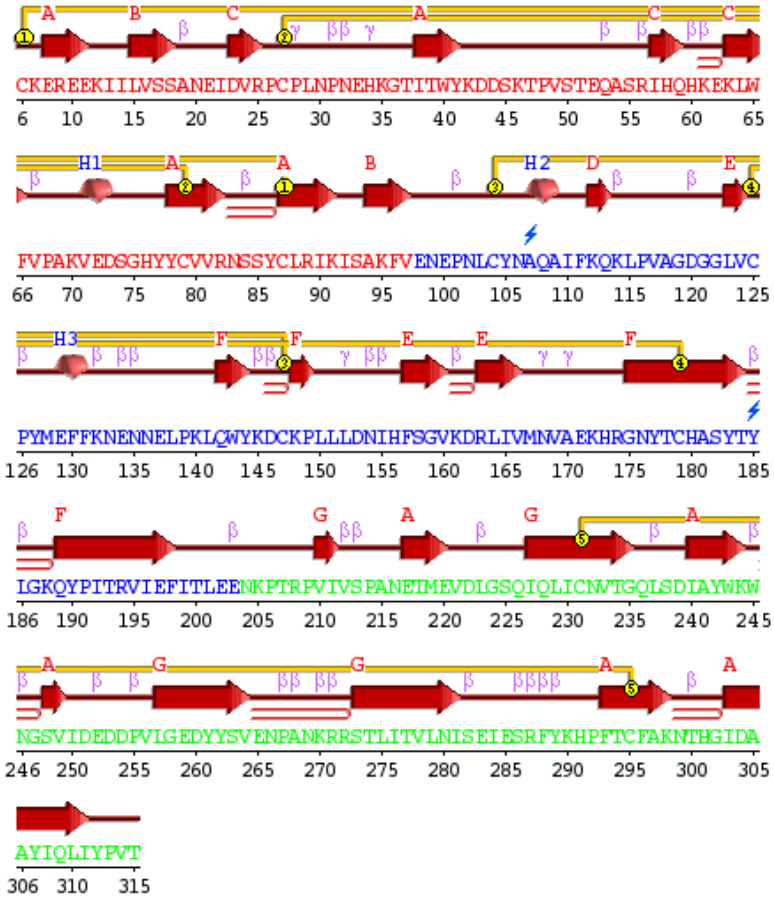


Figure 4. Predicted secondary structure 1g0y (IL-1).

Secondary Structure

IL-1

As shown in Figure 4, the secondary structure predicted the presence of seven sheets, eight beta hairpins, one beta bulge, 26 strands, three helices, 42 beta turns, and seven gamma turns.

IL-6

As shown in Figure 5, its secondary structure predicts the presence of six helices, seven helix-helix interactions, and three beta turns.

TFG-B

As shown in Figure 6, its secondary structure predicts the presence of four sheets, three beta hairpins, six beta bulges, 14 strands, 29 beta turns, seven helices, two helix-helices, and two gamma turns.

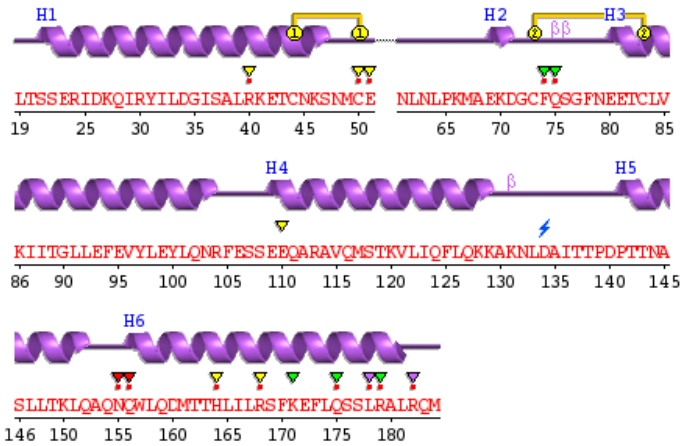


Figure 5. Predicted secondary structure of 1alu (IL-6).

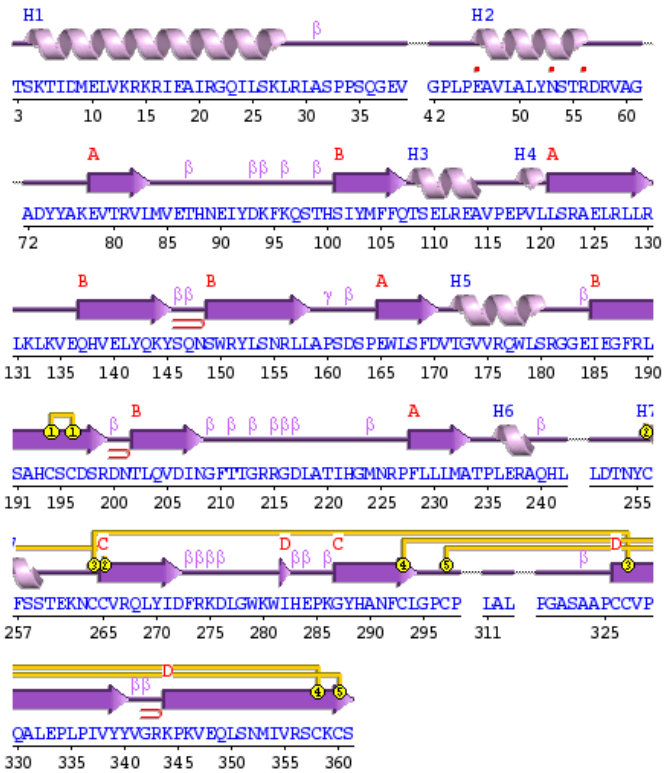


Figure 6. Predicted secondary structure 5vqp (TFG-B).

Hydropathy Plot

Amino acid sequences with hydrophobic and hydrophilic tendencies are shown in a hydropathy plot, which is used for quantitative analysis. The hydropathy plots of all three target proteins, IL-1, IL-6, and TFG-B, were analyzed using the BIOVIA Discovery Studio Visualizer, as shown in Figures 7, 8, and 9, respectively.

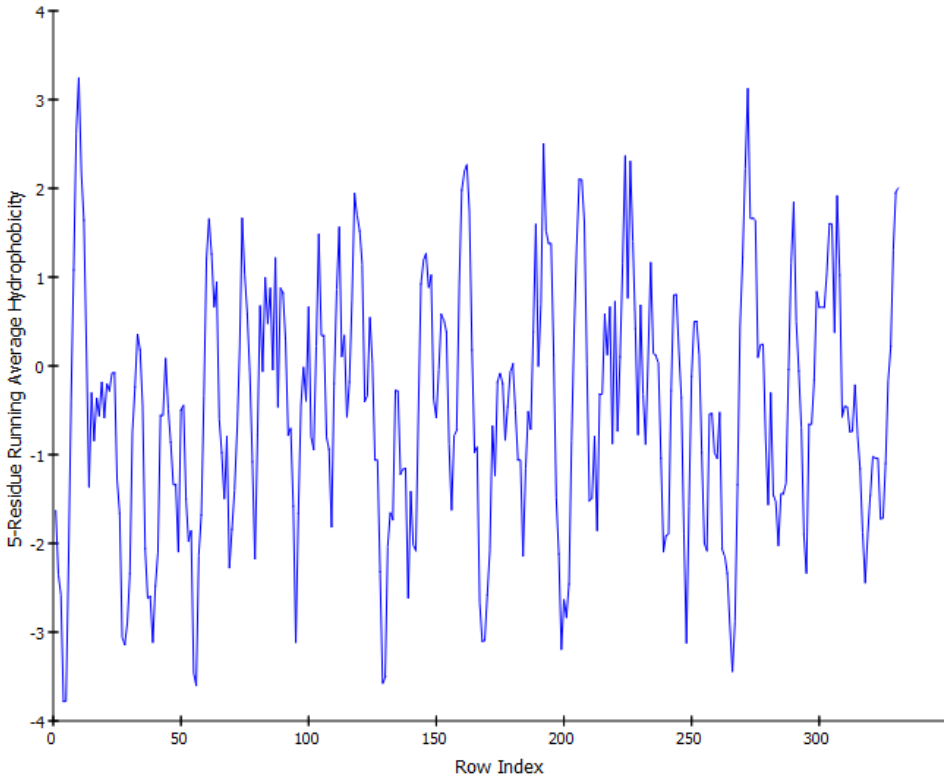


Figure 7. Hydropathy plot analysis of 1g0y (IL-1): protein.

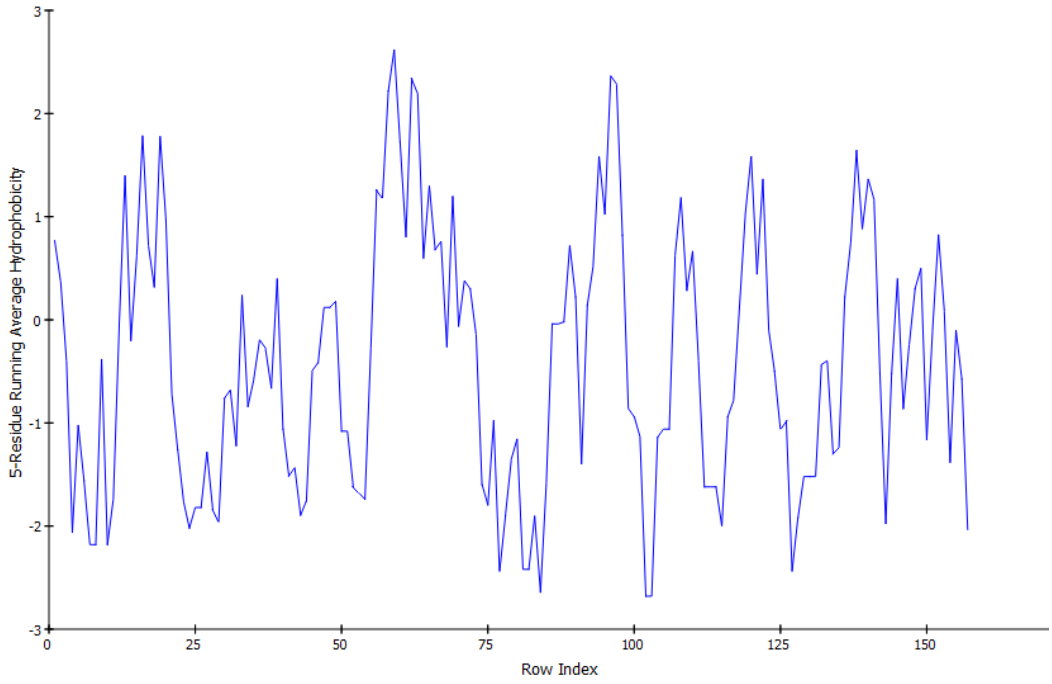


Figure 8. Hydropathy plot analysis of 1ALU (IL-6): protein.

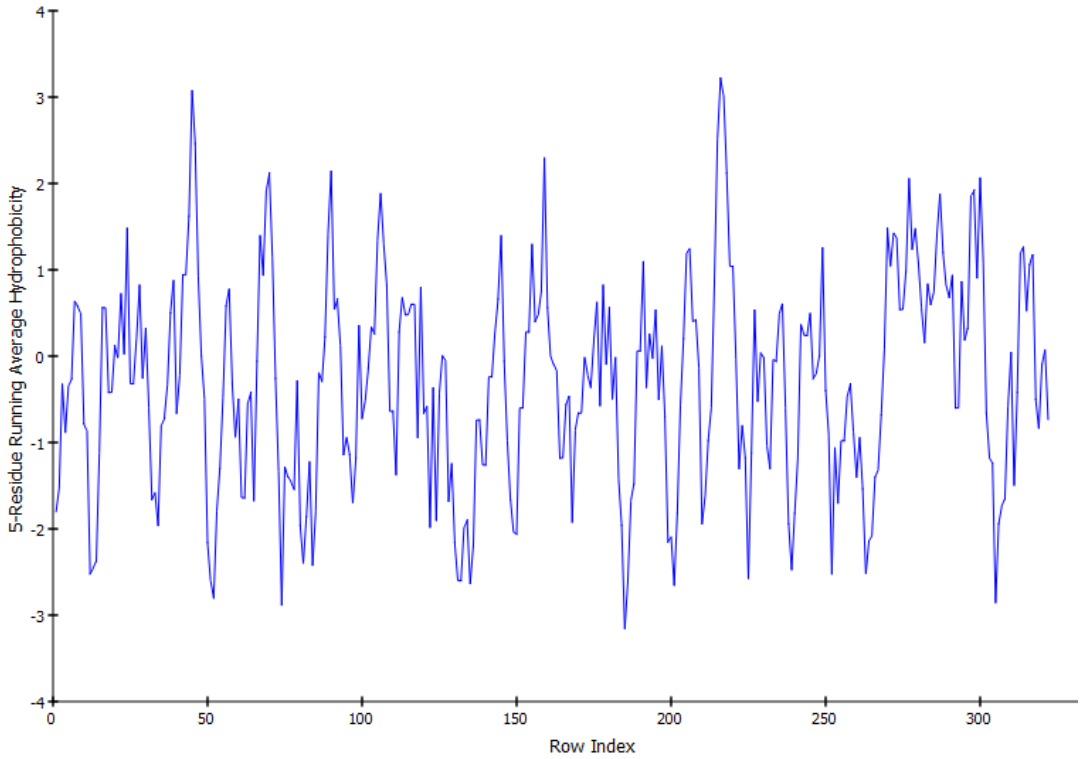


Figure 9. Hydropathy plot analysis of 5VQP (TFG-B): protein.

Molecular Docking

Twenty-one ligands were docked using PyRx software against IL-1, IL-6, and TFG-B for this docking study. The conformation with the lowest binding affinity and zero root mean square deviation (RMSD) was selected as the compound's optimal docking orientation upon the docking. After docking was completed, RMSD and binding affinity were recorded. Among the twenty-one phytocompounds examined, only those displaying lower binding affinities (less than 7) across all three target proteins were chosen. The selected phytocompounds, along with standard drugs, were docked to each protein, and their corresponding binding affinities are detailed in Table 2.

Visualization

Among the three phytocompounds, the top three compounds were selected for each protein and visualized using BIOVIA Discovery Studio Visualizer. 3D models of interactions and information about the category and type of interaction, along with the bond for the corresponding amino acid residues in the ligand, were also obtained.

Molecular Docking Interaction with 1g0y

The 3d interactions of 1g0y with the top three ligands, luteolin, beta-sitosterin, and elixene, were visualized. Luteolin forms a conventional hydrogen bond with amino acids tyr127, gln15, asn216, and ile211 with bond distances of 1.53649, 1.52317, 1.538, and 1.548, respectively, and a limited number of van der Waals contacts are likewise analogously established by the remaining residues, as shown in Figure 10. Therefore, the remaining ligands did not form hydrogen bonds with the amino acids. However, Luteolin exhibited better conventional hydrogen bonding with amino acids.

Molecular Docking Interaction with 1Alu

The 3d interaction of 1Alu with the top three ligands, luteolin, beta-sitosterin, and elixene, was visualized. Luteolin forms a conventional hydrogen bond with amino acids GLN175 and ARG30 with bond distances of 1.55963 and 1.55477, and a limited number of van der Waals contacts are likewise analogously established by the remaining residues, as shown in Figure 11. Beta-sitosterin is bound to

proteins by a conventional hydrogen bond with LYS150. The bond distance is 1.54064, as shown in Figure 11.

Table 2. The binding affinity of the top 3 phytocompounds and standard drug towards all 3-target proteins.

Phytocompounds	**IL-1**	**IL-6**	**TFG-B**
Luteolin	-9.0	-7.1	-6.9
Beta.-sitosterin	-9.4	-7.3	-6.2
Elixene	-9.1	-7.8	-9.1
Binding affinity for FDA-approved drugs			
Celecoxib	-9.6	-5.9	-6.8
Ketorolac	-8.0	-6.7	-6.5

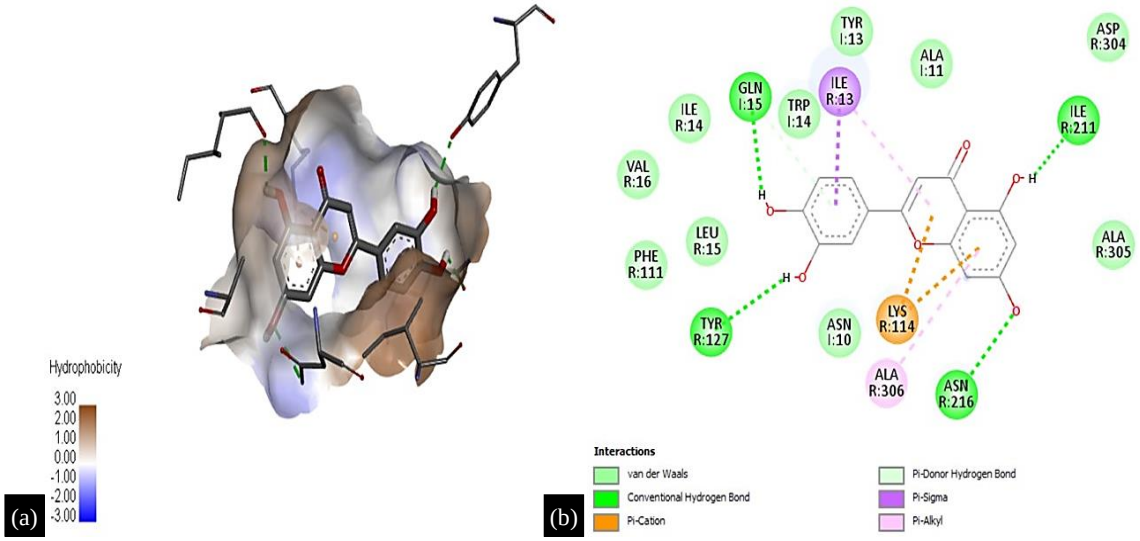
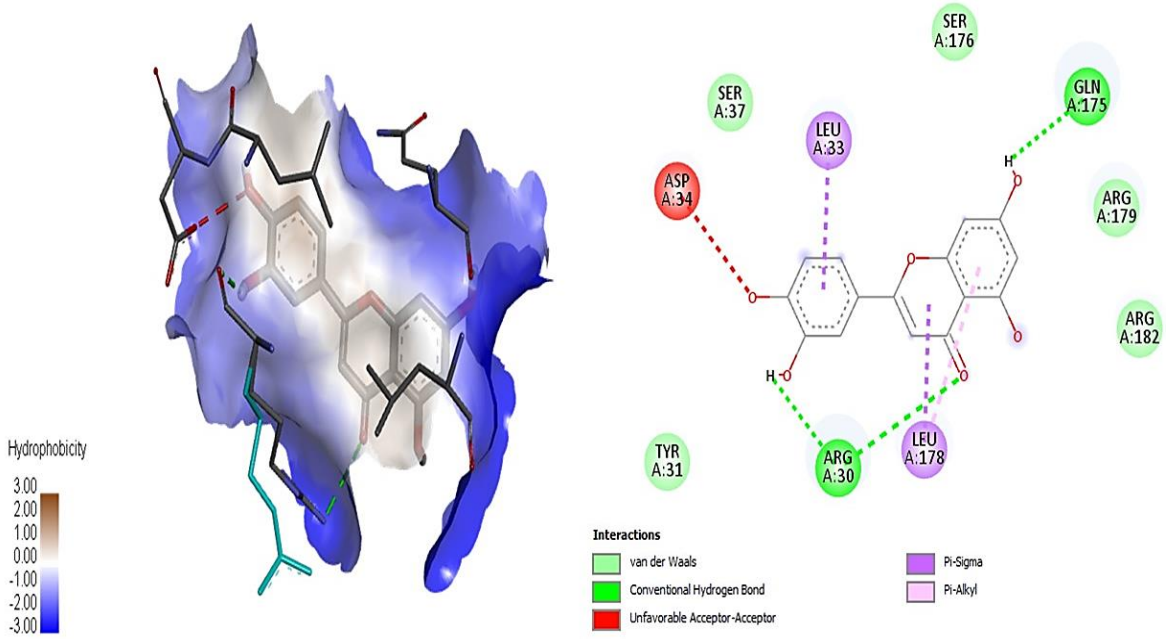
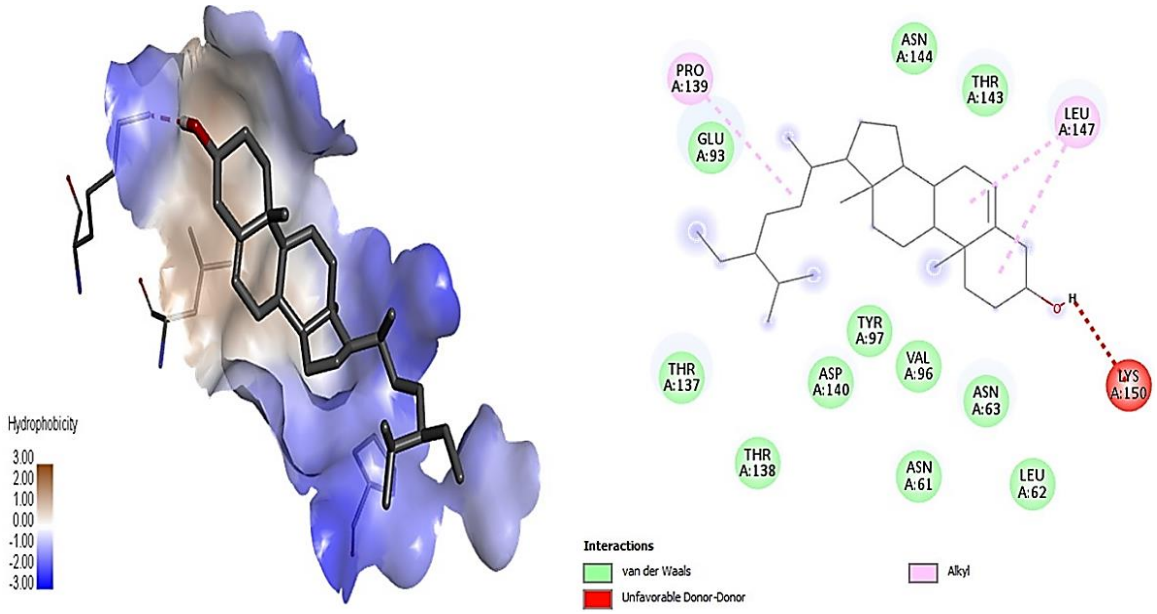


Figure 10. Protein-ligand 3D and 2D interactions of protein 1g0y with luteolin and two standard drugs. (a) 1 g0y with luteolin and (b) luteolin.



(a) 1ALU of Luteolin



(b) 1Alu with beta-sitosterol

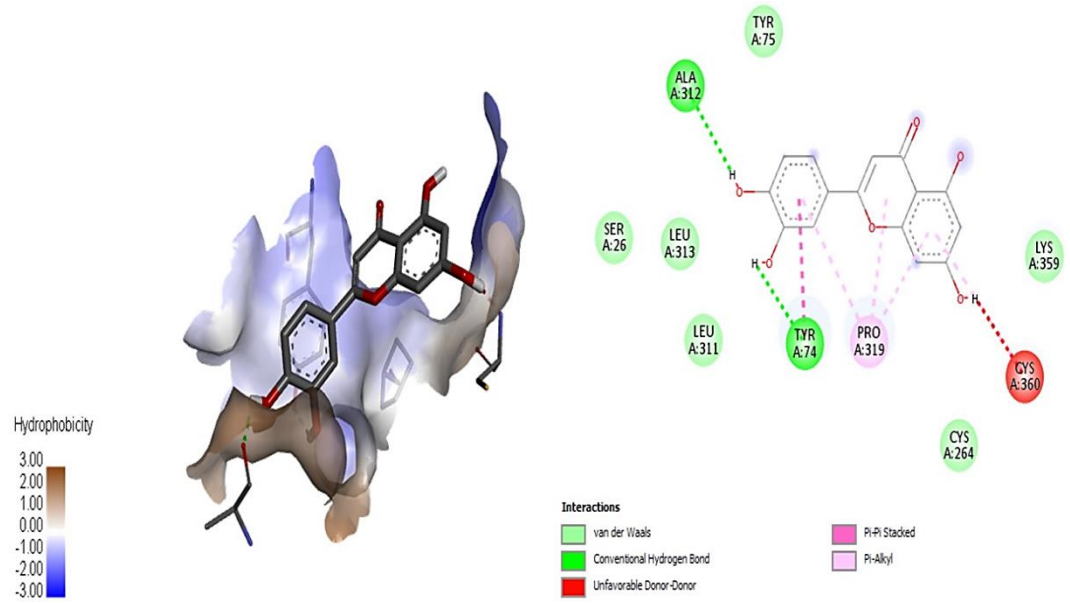
Figure 11. Protein-ligand 3D interaction of protein 1Alu: (a) luteolin and (b) beta-sitosterin.

Molecular docking interaction with 5vqp

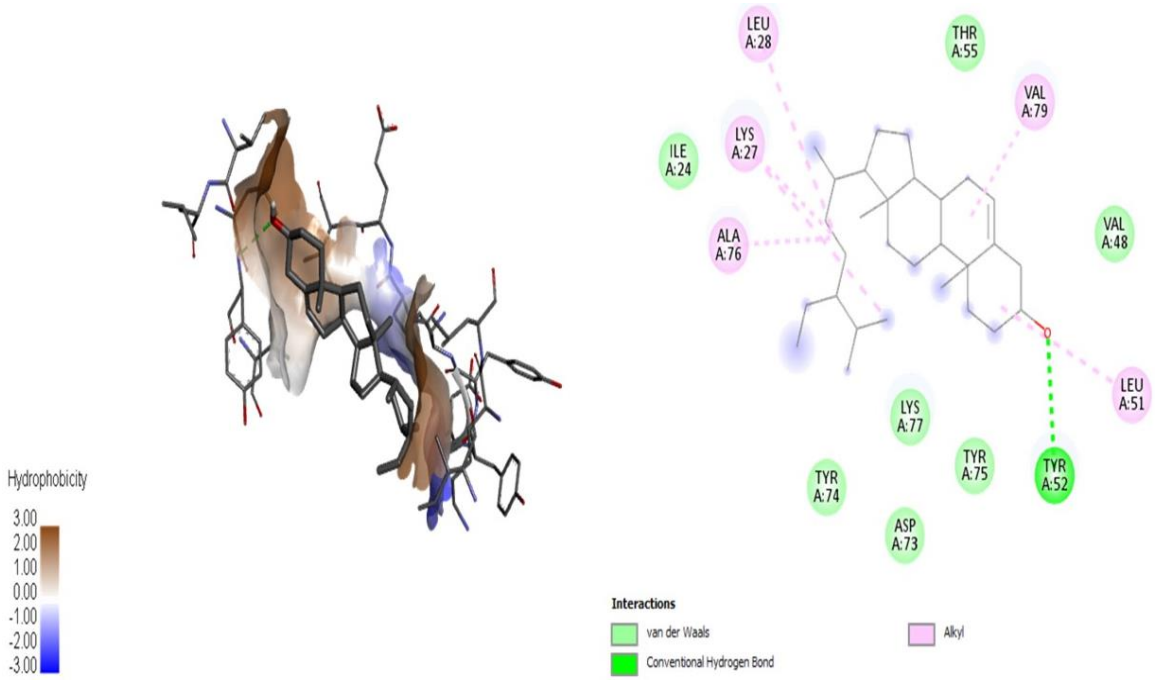
The 3d interaction of 1Alu with the top three ligands, luteolin, beta-sitosterin, and elixene, was visualized. Luteolin forms a conventional hydrogen bond with amino acids ALA312 and TYR74 with bond distances of 1.52863 and 1.52806, and a limited number of van der Waals contacts are likewise analogously established by the remaining residues, as seen in Figure 12. Beta-sitosterin is bound to proteins by a conventional hydrogen bond with TYR52. As shown in Figure 12, the bond distance was 1.5292.

ADMET Analysis

Eight phytocompounds were selected along with their PubChem ID. Physiochemical characteristics, medicinal chemistry, absorption, distribution, metabolism, excretion, and toxicity were examined using ADMETlab 2.0, as shown in Tables 3, 4, 5, 6, 7, and 8.



(a) 5vqp with Luteolin.



(b) 5vqp with beta.-sitosterin

Figure 12. Protein-ligand 3D and 2D interaction of protein 5vqp: (a) luteolin, (b) beta.-sitosterin, and two standard drugs.

Table 3. Physiochemical properties of the top three ligands.

Phytocompounds	MW	Vol	Dense	nHA	nHD	TPSA	nRot	nRing	Max Ring	nHet	fChar	nRig	Flex	LogS
Luteolin	286.05	273.977	1.044	6	4	111.13	1	3	10	6	0	18	0.056	-3.629
Beta.-sitosterin	204.19	251.53	0.812	0	0	0	4	1	6	0	0	8	0.5	-5.671
Elixene	204.19	251.53	0.812	0	0	0	2	1	6	0	0	9	0.222	-4.893

Table 4. Medicinal chemistry of top 3 ligands.

Phytocompounds	QED	Synth	Fsp3	PAINS	Lipinski
Luteolin	0.511	2.42	0	1	Accepted
Beta.-sitosterin	0.563	3.431	0.6	0	Accepted
Elixene	0.562	4.313	0.6	0	Accepted

Table 5. Absorption of the top three ligands.

Phytocompounds	Pgp-inh	Pgp-sub	HIA	F (20%)	F (30%)	Caco-2	MDCK
Luteolin	0.004	0.274	0.047	0.998	1	-5.028	1.00E-05
Beta.-sitosterin	0.765	0	0.003	0.915	0.957	-4.473	1.35E-05
Elixene	0.896	0	0.003	0.796	0.014	-4.542	1.88E-05

MDCK, Madin-Darby Canine Kidney Cells, PgP-inh: P-glycoprotein inhibitor; PgP-sub: PgP Substrates, HIA: Human Intestinal absorption; F (20%), Human Oral Bioavailability, 20%.

Table 6. Distribution of the top three ligands.

Phytocompounds	BBB	PPB	VDss	Fu
Luteolin	0.009	95.44%	0.533	5.98%
Beta.-sitosterin	0.544	97.65%	4.646	3.40%
Elixene	0.157	95.88%	4.07	4.34%

BBB, blood-brain barrier; PBB, plasma protein binding; VDss, volume distribution; FU, unbound fraction in plasma.

Table 7. Metabolism and excretion of the top three ligands.

Phytocompounds	CYP1A2-inh	CYP1A2-sub	CYP2C19-inh	CYP2C19-sub	CYP2C9-inh	CYP2C9-sub	CYP2D6-inh	CYP2D6-sub	CYP3A4-inh	CYP3A4-sub	CL
Luteolin	0.981	0.154	0.124	0.046	0.576	0.842	0.568	0.559	0.549	0.092	8.146
Beta.-sitosterin	0.865	0.333	0.395	0.621	0.253	0.852	0.051	0.664	0.226	0.229	13.439
Elixene	0.391	0.494	0.327	0.92	0.231	0.582	0.165	0.66	0.598	0.312	10.922

Table 8. Toxicity of the top three ligands.

Phytocompounds	hERG	H-HT	DILI	Ames	Carcinogenicity	Respiratory	IGC50	LC50
Luteolin	0.064	0.084	0.905	0.536	0.095	0.22	4.432	5.222
Beta.-sitosterin	0.022	0.875	0.05	0.005	0.64	0.043	3.335	4.955
Elixene	0.02	0.07	0.025	0.007	0.134	0.09	3.722	5.138

HER: Human Ether-a-go-go related gene, H-HT: Human Hepatotoxicity, DILI: Drug-induced liver injury, IGC50:50% growth inhibition, LC50%: 50% death

DISCUSSION

Arthritis is a widespread and debilitating medical condition that primarily affects joints and causes inflammation, pain, and restricted movement. Arthritis manifests in diverse forms, each possessing distinct features and root causes [21]. The condition tends to deteriorate with age, substantially affecting the well-being of the affected individuals. Among the prevalent types is osteoarthritis, arising from the gradual degradation of joint cartilage, resulting in discomfort, stiffness, and diminished joint mobility [22]. Rheumatoid arthritis is another common form distinguished by an autoimmune reaction that targets the synovium and lining around the joints. This leads to inflammation, discomfort, and risk of joint deformities. Other types of arthritis, such as psoriatic arthritis, ankylosing spondylitis, and gout, exhibit unique symptoms and underlying causes [23]. The risk factors associated with the onset of arthritis include genetic predisposition, advanced age, excessive weight, joint trauma, and specific infections. Although there is no treatment for arthritis, there is a range of treatments aimed at alleviating symptoms and enhancing joint functionality. These treatments include medications to alleviate pain, physical therapy, lifestyle adjustments, and, in more severe instances, surgical procedures such as joint replacement. Arthritis can have a profound impact on an individual's ability to perform daily activities, thus affecting both physical and mental well-being. As a chronic condition, it requires ongoing management and care to minimize its effects and maintain a good quality of life [24].

Interleukin-1 (IL-1), Interleukin-6 (IL-6), and transforming growth factor-beta (TGF-β) are critical cytokines in arthritis and play pivotal roles in its development and progression. IL-1 and IL-6 are pro-inflammatory cytokines that contribute to the inflammatory response within the joints in various forms of arthritis, including rheumatoid arthritis and osteoarthritis. They promote the recruitment of immune cells and stimulate the release of other inflammatory molecules, leading to joint tissue damage and pain [25, 26]. Targeting these cytokines with medication can help mitigate inflammation and alleviate symptoms of arthritis. On the other hand, TGF-β has a dual role. In the early stages of arthritis, it may exert anti-inflammatory effects by suppressing immune response and promoting tissue repair. However, in later stages, TGF-β can contribute to tissue fibrosis, joint destruction, and worsening of arthritis [27]. Balancing the effects of TGF-β is crucial for preventing excessive tissue remodeling. Understanding the intricate roles of IL-1, IL-6, and TGF-β in arthritis is essential for the development of targeted therapies that modulate the activity of these cytokines. By controlling the levels and actions of these cytokines, researchers aim to better manage inflammation, slow joint damage, and improve the quality of life of individuals with arthritis [28].

As observed in the result Luteolin and Elixene are two phytocompounds that have the best binding affinity with all three target proteins. The phytocompounds were extracted from *Vitex negundo*, which has been proven to have anti-inflammatory activity in various studies. Luteolin and Elixene, two

phytocompounds, are thought to have anti-inflammatory properties, as luteolin is an anti-inflammatory compound that may play a role in arthritis prevention [29].

Luteolin has garnered attention owing to its potential therapeutic benefits in arthritis. Owing to its anti-inflammatory, antioxidant, and immunomodulatory properties, luteolin holds promise as a remedy for debilitating conditions. Its capacity to suppress the production of inflammatory molecules, such as cytokines and chemokines, offers the potential to mitigate inflammation, pain, and joint damage characteristic of arthritis [30]. Additionally, luteolin's role as an antioxidant counteracts oxidative stress, which contributes to joint tissue deterioration. By influencing immune responses, luteolin may aid in maintaining balanced immune reactions that prevent the excessive inflammation seen in arthritis. Moreover, the ability of luteolin to inhibit enzymes implicated in joint tissue breakdown could help safeguard the integrity of these structures [31]. Although preliminary research in animal models and laboratory settings suggests positive outcomes, clinical trials involving human subjects are necessary to establish the efficacy and safety of luteolin supplementation for managing arthritis. Consultation with a healthcare professional is recommended before considering luteolin as a potential treatment option, particularly if there are existing medical conditions or concurrent medication use [32].

The phytocompounds studied were compared with celecoxib and ketorolac, which are FDA-approved, to determine whether they provided better results. Celecoxib and ketorolac are nonsteroidal anti-inflammatory drugs (NSAIDs) with distinct roles in the management of arthritis. Celecoxib belongs to a class of selective COX-2 inhibitors that primarily target the COX-2 enzyme responsible for inflammation and pain. It offers effective pain relief and reduces inflammation in arthritis while potentially minimizing the gastrointestinal side effects commonly associated with traditional NSAIDs. Celecoxib is particularly useful in cases where risk factors for gastrointestinal complications are a concern [33]. On the other hand, ketorolac is a potent NSAID with both analgesic and anti-inflammatory properties. It is often used for short-term pain management in moderate-to-severe arthritis cases and provides rapid relief. Ketorolac can be administered orally or via injection, making it suitable for cases in which rapid pain control is necessary. Both celecoxib and ketorolac play vital roles in alleviating the pain and inflammation associated with various forms of arthritis. However, their use should be approached with caution because of potential side effects, particularly gastrointestinal and cardiovascular risks. Individuals must consult their healthcare providers before starting these medications to ensure appropriate use and to manage potential interactions with other medications or existing health conditions [34].

According to these findings, the top three phytochemicals for each protein had lower binding affinities than the drugs, which means that they had a more effective inhibitory effect on the target proteins than the chosen drug. Therefore, the phytocompounds luteolin, beta-sitosterin, and elixene in *Vitex negundo*. can be further studied in vitro and in vivo to identify novel anti-inflammatory, antioxidant, and immunomodulatory properties [35]. Although the remaining phytocompounds had lower binding affinities than the drugs, they did not show good results in the ADMET analysis, whereas other phytocompounds such as Luteolin, Beta.-sitosterin, and Elixene showed good results in the ADMET analysis. When the visualization findings were compared, it was clear that some protein amino acids interacted with all the ligands and either one or both standard drugs. Therefore, if both in vitro and *in vivo* studies yield positive results, we can infer that the phytocompounds luteolin, beta-sitosterin, and elixene may be ideal for use as multi-target medications [36].

CONCLUSION

The results of this study show that the ligand luteolin (-9) has the best binding affinity with IL-1, followed by beta-sitosterin (-9.4) and TNFG (-9.1). The ligand luteolin (-7.1) had the best binding affinity with IL-6, followed by beta-sitosterin (-7.3) and elixene (-7.8). Luteolin (-6.9) had the best binding affinity with TNFG, followed by beta-sitosterin (-6.2) and elixene (-9.1). The best binding affinity for the proteins was for beta-sitosterin (-9.4), as shown in Table 2.

According to these findings, luteolin, beta-sitosterin, and elixene are common ligands that effectively bind to all three proteins. Furthermore, two standard drugs, Celecoxib and Ketorolac, were used to compare the binding affinities of the ligands, as shown in Table 2. The binding affinities of these two drugs were higher than those of their ligands, indicating that the selected periwinkle phytocompounds had more potent inhibitory effects on IL-1, IL-6, and TFG-B. In addition, the three common ligands listed in Table 2 were subjected to ADMET analysis, in which all ligands were examined for their physiochemical properties. These results indicate that these ligands have potential for future use in the treatment of breast cancer. Further investigations are warranted to explore the efficacy of these ligands through in vitro and in vivo analyses, to develop innovative arthritis treatments.

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Abbreviations

2D—two-dimensional

ADMET—absorption, distribution, metabolism, excretion, toxicity

CID—compound identification

COX-2—cyclooxygenase-2

FDA—Food and Drug Administration

IL-1—Interleukin-1

IL-6—Interleukin-6

NSAIDs—nonsteroidal anti-inflammatory drugs

PDB—Protein Data Bank

RMSD—root mean square deviation

SDF—Structure-Data File

SMILES—Simplified Molecular Input Line Entry System

TFG-B—transforming growth factor-beta

REFERENCES

1. Alamanos Y, Drosos AA. Epidemiology of adult rheumatoid arthritis. Autoimmunity Rev. 2005;4(3):130–6. DOI: 10.1016/j.autrev.2004.09.002. PubMed: 15823498.
2. Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis. Lancet. 2016;388:2023–38. DOI: 10.1016/S0140-6736(16)30173-8. PubMed: 27156434.
3. Firestein GS. Evolving concepts of rheumatoid arthritis. Nature. 2003;423:356–61. DOI: 10.1038/nature01661. PubMed: 12748655.
4. Cross M, Smith E, Hoy D, Carmona L, Wolfe F, Vos T, Williams B, Gabriel S, Lassere M, Johns N, Buchbinder R, Woolf A, March L. The global burden of rheumatoid arthritis: Estimates from the Global Burden of Disease 2010 study. Ann Rheum Dis. 2014;73:1316–22. DOI: 10.1136/annrheumdis-2013-204627. PubMed: 24550173.
5. McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N Engl J Med. 2011;365:2205–19. DOI: 10.1056/NEJMra1004965. PubMed: 22150039.
6. McInnes IB, Schett G. Cytokines in the pathogenesis of rheumatoid arthritis. Nat Rev Immunol. 2007;7:429–42. DOI: 10.1038/nri2094. PubMed: 17525752.
7. van Vollenhoven RF. Treatment of rheumatoid arthritis: State of the art 2009. Nat Rev Rheumatol. 2009;5:531–41. DOI: 10.1038/nrrheum.2009.182. PubMed: 19798027.

8. Choy EH, Panayi GS. Cytokine pathways and joint inflammation in rheumatoid arthritis. N Engl J Med. 2001;344:907–16. DOI: 10.1056/NEJM200103223441207. PubMed: 11259725.
9. Kalliolias GD, Ivashkiv LB. Overview of the biology of type I interferons. Arthritis Res Ther. 2010;12(Suppl 1). DOI: 10.1186/ar2881. PubMed: 20392288.
10. Scott DL, Kingsley GH. Tumor necrosis factor inhibitors for rheumatoid arthritis. N Engl J Med. 2006;355:704–12. DOI: 10.1056/NEJMct055183. PubMed: 16914706.
11. Feldmann M, Maini RN. Anti-TNF alpha therapy of rheumatoid arthritis: What have we learned? Annu Rev Immunol. 2001;19:163–96. DOI: 10.1146/annurev.immunol.19.1.163. PubMed: 11244034.
12. Brennan FM, McInnes IB. Evidence that cytokines play a role in rheumatoid arthritis. J Clin Invest. 2008;118(11):3537–45. DOI: 10.1172/JCI36389. PubMed: 18982160.
13. Silman AJ, Pearson JE. Epidemiology and genetics of rheumatoid arthritis. Arthritis Res. 2002;4(Suppl 3):S265–S272. DOI: 10.1186/ar578. PubMed: 12110146.
14. Singh JA, Saag KG, Bridges SL Jr, Akl EA, Bannuru RR, Sullivan MC, Vaysbrot EE, McNaughton C, Osani M, Shmerling RH, Curtis JR, Furst DE, Parks D, Kavanaugh A, O'Dell J, King C, Leong A, Matteson EL, Schousboe JT, Drevlow B, Ginsberg S, Grober J, St Clair EW, Tindall E, Miller AS, McAlindon T. 2015 American College of Rheumatology guideline for the treatment of rheumatoid arthritis. Arthritis Rheumatol. 2016;68:1–26. DOI: 10.1002/art.39480. PubMed: 26545940.
15. Singh D, Aggarwal A. Role of tumor necrosis factor inhibitors in rheumatoid arthritis. Clin Med Insights Arthritis Musculoskelet Disord. 2019;12:1179544119835883. DOI: 10.1177/1179544119835883.
16. McInnes IB, Schett G. The Pathogenesis of Rheumatoid Arthritis. N Engl J Med. 2011;365:2205–19. DOI: 10.1056/NEJMra1004965.
17. Scally SW, Law SC, Ting YT, Heemst JV, Sokolove J, Deutsch AJ, Bridie Clemens E, Moustakas AK, Papadopoulos GK, van der Woude D, Smolik I, Hitchon CA, Robinson DB, Ferucci ED, Bernstein CN, Meng X, Anaparti V, Huizinga T, Kedzierska K, Reid HH, Raychaudhuri S, Toes RE, Rossjohn J, El-Gabalawy H, Thomas R. Molecular basis for increased susceptibility of Indigenous North Americans to seropositive rheumatoid arthritis. Ann Rheum Dis. 2017;76:1915–23. DOI: 10.1136/annrheumdis-2017-211300. PubMed: 28801345.
18. Henderson J, O'Reilly S. Inflammasome lights up in systemic sclerosis. Arthritis Res Ther. 2017;19:205. DOI: 10.1186/s13075-017-1420-z. PubMed: 28923085.
19. Mao H, Chen M, Su Y, Wu N, Yuan M, Yuan S, Brestic M, Zivcak M, Zhang H, Chen Y. Comparison on Photosynthesis and Antioxidant Defense Systems in Wheat with Different Ploidy Levels and Octoploid Triticale. Int J Mol Sci. 2018;19. DOI: 10.3390/ijms19103006. PubMed: 30279334.
20. Scanzello CR, Goldring SR. The role of synovitis in osteoarthritis pathogenesis. Bone. 2012;51:249–57. DOI: 10.1016/j.bone.2012.02.012. PubMed: 22387238.
21. van Laar JM, van der Woude D. Role of TGF-β signaling in control of T-cell–mediated inflammation in rheumatoid arthritis. F1000Res. 2017;6:625. DOI: 10.12688/f1000research.10540.1.
22. Conaghan PG, Cook AD, Hamilton JA, Tak PP, Bridges SL Jr. Pathogenesis of osteoarthritis. Nat Rev Rheumatol. 2018;14:627–41. DOI: 10.1038/s41584-018-0073-0.
23. Arend WP, Firestein GS. Pre-rheumatoid arthritis: Predisposition and transition to clinical synovitis. Nat Rev Rheumatol. 2012;8(10):573–86. DOI: 10.1038/nrrheum.2012.134. PubMed: 22907289.
24. Goldring MB, Goldring SR. Osteoarthritis. J Cell Physiol. 2007;213:626–34. DOI: 10.1002/jcp.21258. PubMed: 17786965.
25. Inglis JJ, McNamee KE. Cytokines in rheumatoid arthritis. Inflamm Regen. 2009;29:250–6. DOI: 10.2492/inflammregen.29.250.
26. Li J, Hsu HC, Mountz JD. Managing macrophages in rheumatoid arthritis by reform or removal. Curr Rheumatol Rep. 2012;14:445–54. DOI: 10.1007/s11926-012-0272-4. PubMed: 22855296.

27. Kinne RW, Stuhlmüller B, Burmester GR. Cells of the synovium in rheumatoid arthritis. Macrophages. Arthritis Res Ther. 2007;9:224. DOI: 10.1186/ar2333. PubMed: 18177511.
28. Hamm-Alvarez SF, Janga SR, Edman MC, Madrigal S, Shah M, Frousiakis SE, Renduchintala K, Zhu J, Bricel S, Silka K, Bach D, Heur M, Christianakis S, Arkfeld DG, Irvine J, Mack WJ, Stohl W. Tear cathepsin S as a candidate biomarker for Sjögren's syndrome. Arthritis Rheumatol. 2014;66:1872–81. DOI: 10.1002/art.38633. PubMed: 24644101.
29. Miceli-Richard C, Gottenberg JE, Mariette X. CTLA-4 polymorphism and primary Sjögren's syndrome: Authors' response. Arthritis Res Ther. 2007;9. DOI: 10.1186/ar2199.
30. Trentham DE, Townes AS, Kang AH. Autoimmunity to type II collagen an experimental model of arthritis. J Exp Med. 1977;146:857–68. DOI: 10.1084/jem.146.3.857. PubMed: 894190.
31. Klareskog L, Lundberg K, Malmström V. Autoimmunity in rheumatoid arthritis: Citrulline immunity and beyond. Adv Immunol. 2013;118:129–58. DOI: 10.1016/B978-0-12-407708-9.00003-0. PubMed: 23683943.
32. Zhang Z, Xing X, Hensley G, Chang LW, Liao W, Abu-Amer Y, Sandell LJ. Resistin induces expression of proinflammatory cytokines and chemokines in human articular chondrocytes via transcription and messenger RNA stabilization. Arthritis Rheum. 2010;62:1993–2003. DOI: 10.1002/art.27473.
33. Ermoza K, Glatigny S, Jah N, Camilo V, Mambu Mambueni H, Araujo LM, Chiocchia G, Breban M. Tolerogenic XCR1+ dendritic cell population is dysregulated in HLA-B27 transgenic rat model of spondyloarthritis. Arthritis Res Ther. 2019;21:46. DOI: 10.1186/s13075-019-1827-9. PubMed: 30717755.
34. Zhang JM, An J. Cytokines, inflammation, and pain. Int Anesthesiol Clin. 2007;45:27–37. DOI: 10.1097/AIA.0b013e318034194e. PubMed: 17426506.
35. Schett G, Neurath MF. Resolution of chronic inflammatory disease: Universal and tissue-specific concepts. Nat Commun. 2018;9:3261. DOI: 10.1038/s41467-018-05800-6. PubMed: 30111884.
36. Burmester GR, Pope JE. Novel treatment strategies in rheumatoid arthritis. Lancet. 2017;389(10086):2338–48. DOI: 10.1016/S0140-6736(17)31491-5. PubMed: 28612748.

