

In silico Rationalization for Leads from *Oldenlandia umbellata* L. to Inhibit Multiple Molecular Targets Regulating Osteoporosis

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Abstract

Background: Osteoporosis is a chronic metabolic bone disease caused due to the dysregulation in the functioning of osteoblast and osteoclast cells leading to increased bone resorption predominantly in postmenopausal women and the elderly, thereby affecting a significant global population.

Purpose: The present study aims to computationally screen the phytochemical constituents of *Oldenlandia umbellata* Linn against the unique molecular therapeutic targets of osteoporosis and validate the results using Molecular Dynamics (MD) Simulation.

Materials and Methods: The molecular docking analysis of the selected phytoconstituents against the molecular targets were performed using AutoDock 4.2.6 – AutoDock Tools 1.5.6. In addition, the drug likeliness, ADME, bioactivity, and toxicity were predicted using Molinspiration ADMELAB2.0, ProTox-II and Orisis DataWarrior online tools. Molecular Dynamic Simulation studies were performed using WebGRO macromolecular simulation server.

Results: Molecular docking results and data analysis revealed that deacetylasperuloside possesses good drug-likeness, ADME properties, and bioactive scores and did not indicate any toxicity compared to other phytochemicals exhibiting binding energies below -8.00 Kcal/mol against the targets. Together, the study emphasized that deacetylasperuloside could bind with the molecular targets of osteoporosis, and the lead is a potential therapeutic candidate for osteoporosis treatment. Further, molecular dynamic simulation analysis for 100 ns revealed that the ligand–protein complexes, including glycogen synthase kinase 3β (GSK3 β)-deacetylasperuloside and cathepsin K (CTSK)-deacetylasperuloside complexes, were stable and highly compact assessed from their trajectories. Hence, it can be emphasized that deacetylasperuloside could be a potential therapeutic molecule that could inhibit the targets, including GSK3 β and CTSK.

Conclusion: *Oldenlandia umbellata* L. is a potential candidate for identifying therapeutic leads for osteoporosis treatment. Further, *in vitro* and *in vivo* studies are needed as an output of this research to evaluate its therapeutic efficacy.

Keywords

Deacetylasperuloside, docking, *in silico*, molecular dynamics simulation, osteoporosis, *Oldenlandia umbellata*

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Introduction

Osteoporosis is a chronic metabolic bone disease caused due to an imbalance in bone formation and resorption affecting millions globally. In 1993, the World Health Organization (WHO) elucidated osteoporosis as a “progressive systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture” (Noh et al., 2020). Osteoporosis is highly predominant among the elderly, especially postmenopausal women, resulting in an increased risk of fracture and mortality (Sun et al., 2020). Generally, bone homeostasis is governed

by orchestrated functioning of osteoblast and osteoclast cells regulating bone formation and resorption to aid the continuous remodeling of the bone to maintain its architecture (Sun et al., 2020). Increased osteoclast or decreased osteoblast activity leads to increased bone resorption, causing osteoporosis.

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Mesenchymal stem cells (MSC) are the precursor of osteoblasts, which differentiates from the latter depositing the bone-forming extracellular matrix proteins such as collagen Type I, subsequently mineralizing the bone (Noh et al., 2020).

The major signaling molecules that play a crucial role in the differentiation of osteoblasts include β -catenin, glycogen synthase kinase 3 β (GSK3 β) (Saad, 2020), runt-related transcription factors (Runx2), and activator protein 1 (AP-1) (Chau et al., 2009). On the other hand, osteoblasts produce receptor activator of nuclear factor-kappa-B ligand (RANKL), osteoprotegerin (OPG), a decoy receptor for RANKL, and macrophage colony-stimulating factor (M-CSF), which stimulates osteoclast differentiation, thereby regulating osteoclastogenesis. Osteoclast cells are differentiated from the progenitor, that is, hematopoietic stem cells (HSC), upon M-CSF and RANKL stimulation. Various signaling molecules, including cathepsin K (CTSK), AP-1, tartrate-resistant acid phosphatase (TRAP), nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), and TNF receptor-associated factor 6 (TRAF-6) and α v β ₃ integrin contribute to osteoclastogenesis and osteoclast survival, thereby increases the bone resorption (Györi & Mócsai, 2020). Hence, in general, among various targets, the signaling molecules play crucial roles in aggravating the pathogenesis of the disease.

Currently, estrogen replacement therapy is the most effective method for treating osteoporosis in postmenopausal women, whereas it also has an adverse effect leading to uterine and breast cancer (Sharan et al., 2009). In addition, bisphosphonates, selective estrogen receptor modulators (SERMs), CTSK inhibitors (Odanacatib), and anabolic agents (Parathyroid hormone and analogues of its related proteins) are used for the treatment of osteoporosis. Unfortunately, conventional treatment methods induce side effects such as cancer, stroke, toxicities, and cardiovascular problems and are incapable of restoring normal bone metabolism (Noh et al., 2020; Wang et al., 2019). Considering the effects of osteoporosis, the economic burden that can be levied upon the global population and the side effects caused by the conventional treatment methods, there is an urgent need for alternate and effective therapeutics to treat osteoporosis. Various studies have reported that the protective effects conferred by the phytochemicals of natural sources can be an effective alternative option for the treatment of osteoporosis (Suvarna et al., 2018).

Oldenlandia umbellata (OU) Linn, also called *Hedyotis umbellata* Linn, is widely found in India, Burma, and tropical areas. It is an annual plant that finds its use as an expectorant. It has reported pharmacological properties such as anti-inflammatory (Mahibalan et al., 2008) and anti-pyretic (Padhy & Endale, 2014), anti-nociceptive (Saraswathy et al., 2008), anti-bacterial (Arun et al., 2010), anti-helminthic (Somnath et al., 2014), antioxidant, and hepatoprotective activities (Sivapraksham et al., 2014). In Siddha medicine, OU is used to treat asthma, bronchitis, and tuberculosis

(Yoganarasimhan, 2000). Even though OU possesses potential pharmacological properties, it remains unexplored for its therapeutic potential against osteoporosis. Thus, the study aimed to computationally screen the reported phytoconstituents of OU against the potential therapeutic targets of osteoporosis to identify the potential therapeutic lead for osteoporosis treatment. The study also aims to validate the *in silico* results using molecular dynamics (MD) simulations.

Materials and Methods

Target Preparation

The three-dimensional (3D) structures of 10 molecular target proteins of osteoporosis, including GSK3 β – PDB: 1UV5, CTSK – PDB: 4X6H, TRAP – PDB: 1QFC, TRAF6 – PDB: 3HCT, AP-1 – PDB: 2H7H, NF- κ B (p65 and p50 – PDB: 1NFI, RANK – PDB: 3ME2, c-fms – PDB: 6N33, α v β ₃ integrin – PDB: 1L5G are retrieved from The Research Collaboratory for Structural Bioinformatics – Protein Data Bank (RCSB-PDB, <http://rcsb.org/>). The protein was prepared using AutoDock 4.2.6–AutoDock Tools (ADT) 1.5.6 (Morris et al., 2009) and exported as a Pdbqt file.

Ligand Preparation

The 3D structures of five of the eight phytochemical compounds identified from OU Linn. (Mahibalan et al., 2016), such as asperulosidic acid (PubChem CID: 11968867), cedrelopsin (PubChem CID: 12302592), deacetylasperuloside (PubChem CID: 44593378), scandoside (PubChem CID: 21602023), and scandoside methyl ester (PubChem CID: 442433) have been retrieved from the PubChem Database in SDF format (<https://pubchem.ncbi.nlm.nih.gov/>). As the 3D chemical structures of Oledicoumarin, Hedyotis cone B, and Pheophorbide A methyl ester (Mahibalan et al., 2016) were unavailable, they were generated using Marvin Sketch software (<https://www.chemaxon.com>). The two-dimensional representation of the selected eight phytoconstituents of OU is shown in Figure 1. The ligands were prepared for molecular docking using AutoDock 4.2.6–AutoDock Tools 1.5.6, and the output was saved in “PDBQT” format.

Molecular Docking Analysis

The molecular docking (rigid protein–flexible ligand) analysis was performed between the eight selected phytoconstituents of OU against the potential molecular therapeutic targets of osteoporosis using AutoDock 4.2.6–AutoDock Tools 1.5.6 (Morris et al., 2009). The native ligand-bound residues and residues predicted using CASTp 3.0 (<http://sts.bioe.uic.edu/castp/>) online tool (Tian et al., 2018) of the target proteins were selected as the binding site to perform docking. A grid box of dimension of 40 × 40 × 40

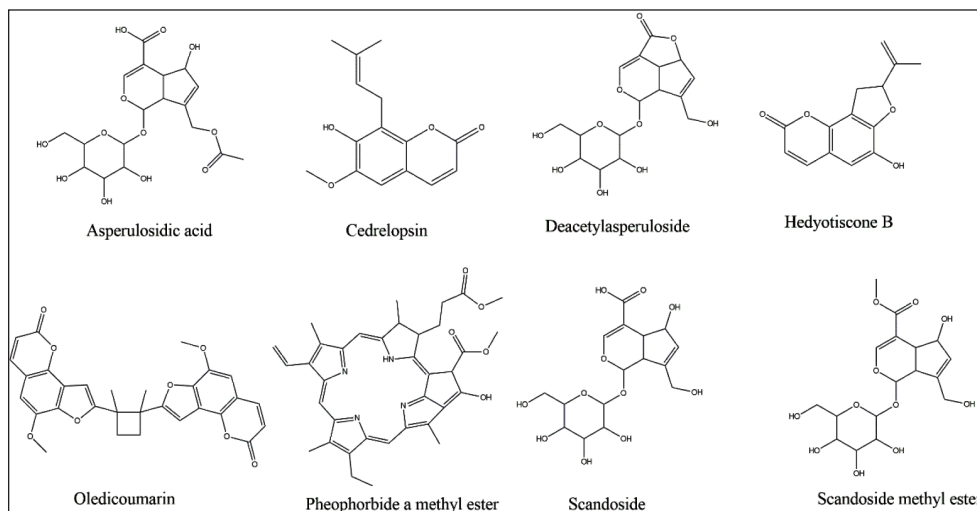


Figure 1. 2D-Chemical Structures of Phytoconstituents of *Oldenlandia umbellata*.

with a spacing of 0.375 Å was generated around the active site of the target proteins. The docking was performed using the run module utilizing autogrid4 and autodock4 programs. The ligands with binding energies below the -8.0 kcal/mol threshold were selected. The binding poses of the ligands in the protein–ligand complexes were visualized using UCSF Chimera molecular visualization program (Pettersen et al., 2004) and LigPlot⁺ program (Laskowski & Swindells, 2011).

Evaluation of Drug-likeness and ADME Properties

The physicochemical properties and drug-likeness of the phytochemical compounds from OU were assessed using Molinspiration (<https://www.molinspiration.com/>) online tool. The server Molinspiration Cheminformatics offers a number of tools for processing and manipulating molecules, including the conversion of SDfiles and SMILES to QSAR, molecular modeling, and drug development. Additionally, it provides data visualization, bioactivity prediction, and fragment-based virtual screening. The physicochemical properties such as Molecular weight, partition coefficient (Log P), topological polar surface area (TPSA), number of hydrogen bond acceptors (HA), number of hydrogen bond donors (HD), and Lipinski rule violations (LRV) were computed for all eight phytochemicals. In addition, the eight phytochemical constituents of OU were evaluated for ADME and toxicity parameters. The ADME properties were analyzed using the SwissADME server (<http://www.swissadme.ch/>) (Daina et al., 2017). SwissADME is a web-based tool that provides predictive models for physicochemical qualities, pharmacokinetics, drug-likeness, and medicinal chemistry friendliness, including proprietary approaches such as the BOILED-Egg, iLOGP, and Bioavailability Radar (Daina et al., 2017).

The ADME parameters were assessed using ADMETLAB 2.0 (<https://admetmesh.scbdd.com/>), which includes solubility (Log S), hERG potassium ion channel inhibitor (hERG), plasma protein binding (PPB), gastrointestinal absorption (G_i), blood-brain permeation (BBB), substrate for permeability glycoprotein (P-gp), cytochrome P450 inhibitors (CYP), and skin permeation co-efficient (Log Kp) (cm/s) (Xiong et al., 2021).

Bioactivity and Toxicity Prediction Analysis

The bioactivity scores of the selected phytoconstituents against G-protein-coupled receptor ligand (GPCR), ion channel modulator (ICM), kinase inhibitor (KI), nuclear receptor ligand (NR), protease inhibitor (PI), and enzyme inhibitor (EI) were also assessed using Molinspiration online tool and toxicity parameters were predicted using ProTox-II online tool (https://tox-new.charite.de/protox_II/) and Orisis DataWarrior tool (<https://openmolecules.org/datawarrior/>) (Sander et al., 2015).

Molecular Dynamics Simulation

MD simulation of the protein–ligand complexes were done using the UAMS WebGRO server for Macromolecular Simulations (<https://simlab.uams.edu/>). The topology files of the best complexes were prepared through PRODRG (van Aalten et al., 1996) server using the GROMOS96 43a1 force field. Proteins bereft of ligands were subjected to protein in water simulation. GROMOS96 43a1ff was applied to a Triclinic system with an SPC water model containing protein–ligand complexes for MD simulation. The system was 0.15 M NaCl neutralized based on its ionic charges. The energy minimization was done for every 50,000 steps using the steep descent algorithm. The NVT/NPT equilibration was done at 310 K and 1 Bar pressure. Using the Leap-frog integrator,

the MD was performed for 100 ns with 1000 frames per simulation. Further, 1000 snapshots (100 ps intervals) were taken during the 100 ns simulation. The respective trajectories of complexes were analyzed from root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), radius of gyration (Rg), and hydrogen bonds (HBs). Finally, the 3D and 2D interaction after the MD simulation were generated using UCSF Chimera and BIOVIA Discovery Studio Visualizer tool.

Results and Discussion

Molecular Docking Analysis

The molecular docking analysis between the target proteins and phytoconstituents was performed using AutoDock 4.2.6–AutoDock Tools 1.5.6. AutoDock offers multiple advantages in molecular docking of small ligands to target proteins due to receptor flexibility, blind-docking, precalculated grid maps on a binding site, free-energy scoring function based on linear regression analysis, good correlation between predicted inhibition constants and experimental data, whereas still it has a limitation that the unreliability of the results when the small molecules are docked to the flexible docking sites (Amjadi-Moheb & Akhavan-Niaki, 2019). The binding free energies of the docked target–ligand complexes are shown in Table 1. The phytoconstituents that exhibited higher binding affinity with the lowest binding energies below -8.00 kcal/mol against the targets were only considered significant in the present study. Among the eight selected phytoconstituents of OU, four compounds, including cedrelapsin, deacetylasperuloside, oledicoumarin, and pheophorbide a methyl ester exhibited the lowest binding energies below -8.00 kcal/mol, indicating higher binding affinity towards the molecular target proteins.

GSK3 β , a serine/threonine kinase, is a central molecule of wnt/ β -catenin signaling and has a crucial role in glycogen metabolism. GSK3 β negatively regulates osteoblast differentiation and bone formation. Thus, overexpression of GSK3 β in wnt/ β -catenin signaling causes proteasomal degradation of β -catenin, preventing its nuclear translocation and activation of transcription factors, such as T-cell factor/lymphoid enhancer factor (TCF/LEF), Runx-2, and AP-1, which are essential for osteoblast formation (Amjadi-Moheb & Akhavan-Niaki, 2019). Thus, targeting GSK3 β could be a potential therapeutic target for treating osteoporosis *via* stimulating osteoblast formation. Molecular docking showed that deacetylasperuloside, oledicoumarin, and pheophorbide a methyl ester exhibited the lowest binding energies of about -8.84 kcal/mol, -10.21 kcal/mol, and -10.91 kcal/mol, respectively, against GSK3 β (Figure 2A). Among them, pheophorbide A methyl ester exerted the lowest binding energy of -10.91 kcal/mol towards GSK3 β forming a strong hydrogen bond interaction (2.91 Å) with ARG141 (Figure

2B). On the other hand, oledicoumarin formed a hydrogen bond with the ASP200 (2.95 Å) residue of GSK3 β with the binding energy of -10.21 kcal/mol (Figure 2C). Deacetylasperuloside formed 4H bonds against the residues of GSK3 β , that is, LYS85 (2.91 Å and 2.98 Å), VAL135 (2.83 Å), and ASP200 (2.87 Å) exhibiting the binding energy of about -8.84 kcal/mol (Figure 2D).

CTSK, a lysosomal protease enzyme abundant in osteoclasts, resorbs bone. RANK-RANKL signaling regulates CTSK expression and degrades bone matrix, primarily Type I collagen, and activates MMPs (matrix metalloproteases), which accelerate bone resorption. CTSK from mature osteoclasts induces actin ring formation, a sealing zone with an acidified milieu that dissolves the mineralized bone matrix, making it an attractive target for osteoporosis treatment (Dai et al., 2020). The molecular docking results showed that oledicoumarin exhibited the lowest binding energy of about -9.94 kcal/mol against CTSK. Subsequently, followed by pheophorbide a methyl ester (-9.05 kcal/mol) and deacetylasperuloside (-8.68 kcal/mol), as shown in Figure 3A. Pheophorbide A methyl ester formed an H-bonding with the residue of HIS162 (Figure 3B). In contrast, oledicoumarin did not show any hydrogen bond formation, which indicated that the higher binding affinity exhibited was due to the hydrophobic interactions towards the residues of target proteins (Figure 3C). At last, deacetylasperuloside formed two H-bonding with the HIS162 (2.84 Å) and GLY66 (3.07 Å) residues of CTSK (Figure 3D) exhibiting effective binding between the CTSK–ligand complexes.

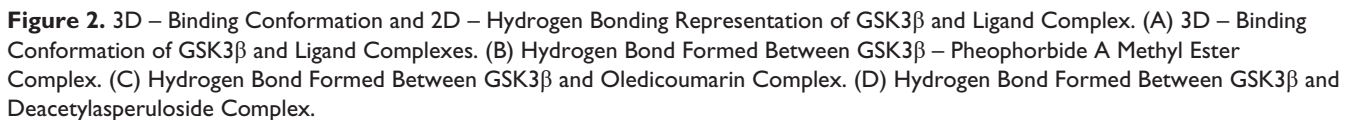
NF- κ B is a transcription factor and a well-known master regulator of various physiological processes and pathways. Usually, in the inactive state, NF- κ B exists in a hetero-dimer p65/p50 form. Upon activation through RANK-RANKL signaling, it is translocated to the nucleus leading to the activation of multiple transcription factors, including the nuclear factor of activated T-cells, cytoplasmic 1 (NFATc1), a master regulator of osteoclastogenesis facilitating bone resorption (Cheng et al., 2018). Thus, NF- κ B is a potential molecular target of therapeutic value for osteoporosis treatment. The phytochemical constituents of OU were docked against NF- κ Bp65 and NF- κ Bp50, and they exhibited more binding affinity towards NF- κ Bp65. Among the phytochemical constituents, oledicoumarin and deacetylasperuloside exhibited higher binding affinity towards NF- κ Bp65 with the lowest binding energies of -8.49 kcal/mol and -8.05 kcal/mol, respectively, as shown in Figure 4A. Oledicoumarin formed five H-bonding with the NF- κ Bp65 residues, including ASN155 (3.07 Å), ASP185 (3.10 Å), THR191 (2.78 Å), and two H-bonding with LYS79 (2.87 and 2.90 Å) (Figure 4B,) whereas deacetylasperuloside formed six H-bonding with ARG30 (2.62, 2.72 and 2.84 Å), LYS79 (3.35 Å), ASN190 (3.14 Å), and THR191 (3.26 Å) residues of NF- κ Bp65 (Figure 4C).

Table 1. Binding Free Energies of the Target-Ligand Complexes.

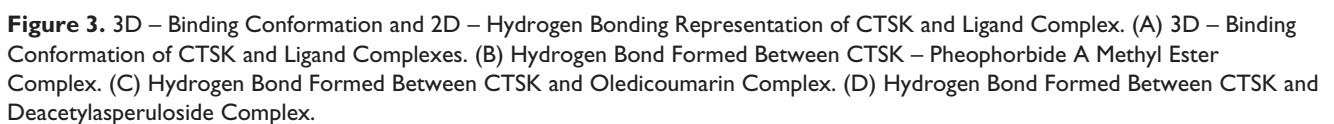
Ligands	GSK3 β (IUV5)	CTSK (4X6H)	TRAP5b (IQFC)	TRAF-6 (3HCT)	AP-1 (2H7H)	NF- κ Bp65 (INFI)	NF- κ Bp50 (INFI)	RANK (3ME2)	c-fms (6N33)	$\alpha\beta_3$ integrin (1L5G)
	Binding Energy (kcal/mol)									
Asperulosidic acid	-6.54	-5.94	-3.89	-3.33	-6.39	-5.93	-4.03	-5.63	-6.26	-7.03
Cedrelopsin	-7.00	-6.92	-4.16	-5.07	-5.55	-6.66	-6.03	-6.34	-8.28	-6.22
Deacetylasperuloside	-8.84	-8.68	-5.18	-5.75	-7.13	-8.05	-7.40	-9.39	-7.28	-7.98
Hedyotis cone B	-7.35	-6.66	-3.02	-5.00	-5.73	-7.24	-5.88	-6.32	-7.37	-6.43
Oledicoumarin	-10.21	-9.94	>0	-6.92	-7.49	-8.49	-2.78	-7.61	>0	-9.30
Pheophorbide a methyl ester	-10.97	-9.05	>0	-6.33	-7.24	-5.67	>0	-5.23	>0	-7.76
Scandoside	-6.03	-4.61	-3.62	-4.32	-4.13	-5.06	-4.89	-4.51	-6.25	-3.56
Scandoside methyl ester	-5.65	-5.83	-2.62	-4.06	-3.72	-5.05	-4.09	-5.25	-6.41	-6.16

Note: * >0 represents that the ligand is nonspecific to the targeted protein.

Abbreviations: GSK3 β , glycogen synthase kinase 3 β ; CTSK, cathepsin K; TRAF-6, TNF receptor-associated factor 6; AP-1, activator protein 1; RANK, receptor activator of nuclear factor-kappa-B; NF- κ Bp65, nuclear factor kappa-light-chain-enhancer of activated B cells.



Abbreviation: GSK3 β , glycogen synthase kinase 3 β .



Abbreviation: CTSK, cathepsin K.

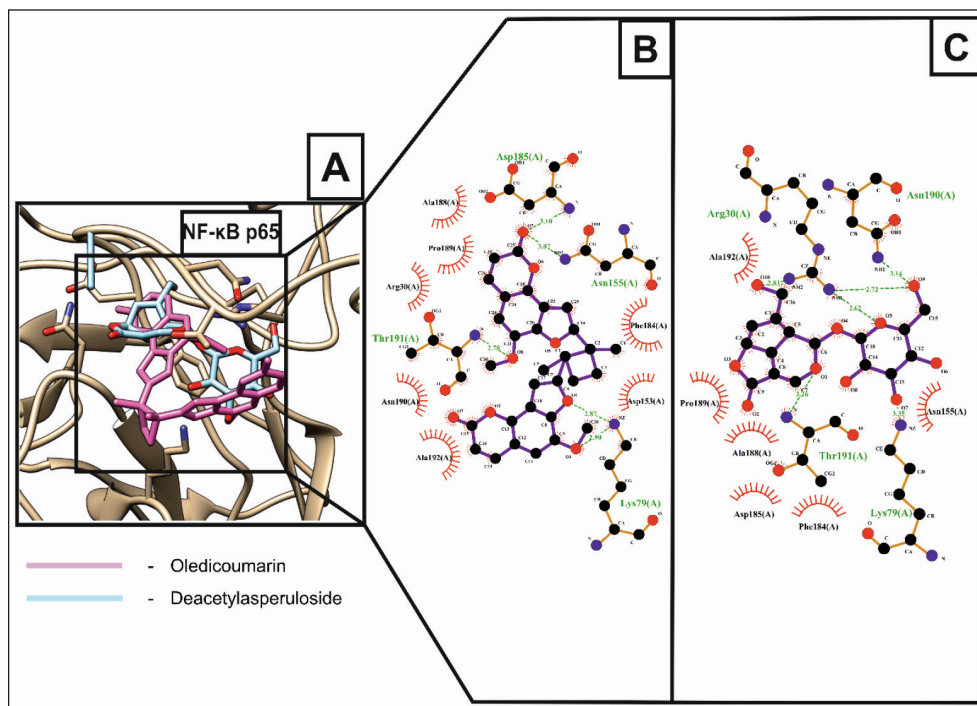


Figure 4. 3D – Binding Conformation and 2D – Hydrogen Bonding Representation of NF-κBp65 and Ligand Complex. (A) 3D – Binding Conformation of NF-κBp65 and Ligand Complexes. (B) Hydrogen Bond Formed Between NF-κBp65 and Oledicoumarin Complex. (C) Hydrogen Bond Formed Between NF-κBp65 and Deacetylasperuloside Complex.

Abbreviation: NF-κBp65, nuclear factor kappa-light-chain-enhancer of activated B cells.

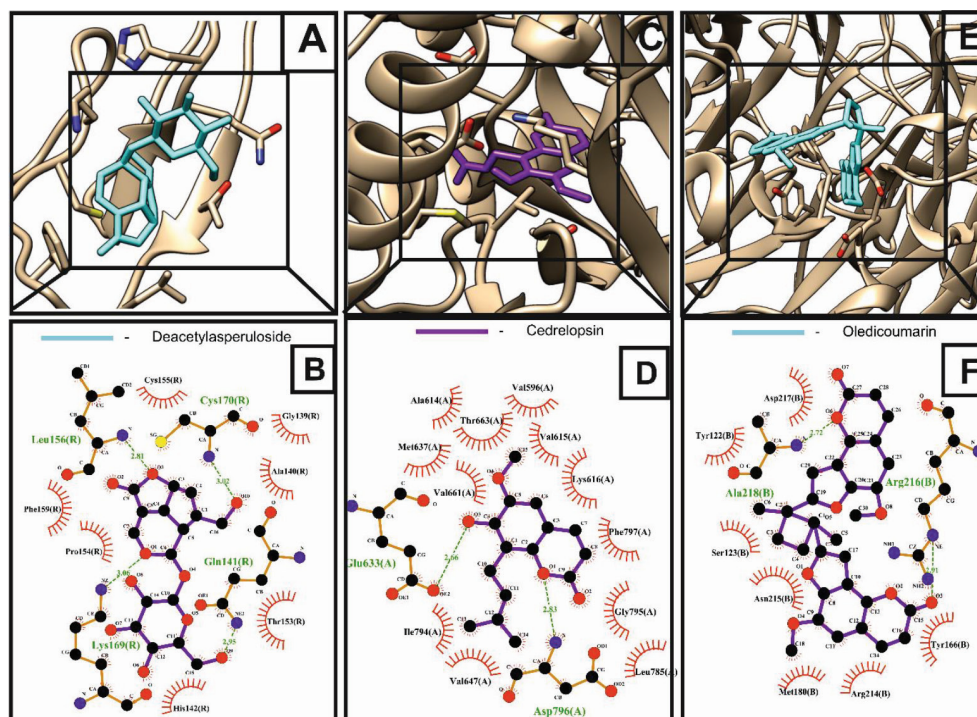


Figure 5. 3D – Binding Conformation and 2D – Hydrogen Bonding Representation of RANK, c-fms, $\alpha\beta 3$ Integrin, and Ligand Complex. (A) Binding Pose of RANK and Deacetylasperuloside Complex. (B) Hydrogen Bond Formed Between RANK and Deacetylasperuloside Complex. (C) Binding Pose of c-fms and Cedrellopsin Complex. (D) Hydrogen Bond Formed Between c-fms and Cedrellopsin Complex. (E) Binding Pose of $\alpha\beta 3$ Integrin and Oledicoumarin Complex. (F) Hydrogen Bond Formed Between $\alpha\beta 3$ Integrin and Oledicoumarin Complex.

Abbreviation: RANK, receptor activator of nuclear factor-kappa-B.

The RANK belongs to the TNF receptor superfamily, which governs RANK-RANKL-OPG signaling that regulates osteoclast differentiation, survival, and function. On RANKL activation, RANK stimulates multiple downstream effectors such as MAPK, NF- κ B, AP-1, c-FOS, and NFATc1, thereby leading to the pathogenesis of osteoporosis by inducing osteoclast differentiation.²⁸ Thus, RANK is a potential target of osteoporosis. Our result showed that deacetylasperuloside has a higher binding affinity towards the molecular target RANK with a binding energy of -9.39 kcal/mol forming four H-bonding with target residues including GLU141 (2.95 Å), LEU156 (2.83 Å), LYS169 (3.06 Å), and CYS170 (3.02 Å) in the RANK-deacetylasperuloside complex (Figure 5A and B).

CSF1R (c-fms) is type III receptor tyrosine kinase (RTK) implicated in various diseases, from inflammation to cancer, with crucial physiological functions regulating cellular processes such as survival and differentiation. Until now, CSF1 and IL-34 are the known ligands of c-fms, and both tend to induce osteoclasts differentiation leading to osteoporosis. CSF1 plays a vital role in the differentiation of osteoclasts from its precursor implicating its crucial role in osteoporosis. In addition, C-fms mediated signaling tends to induce increased RANK expression in the osteoclast. Thus, making it a potential target for osteoporosis (Mun et al., 2020). Molecular docking analysis results implicated that cedrelopsin showed the lowest binding energy of -8.28 kcal/mol forming two H-bonding with c-fms residues, that is, GLU633 (2.66 Å) and ASP796 (2.83 Å) (Figure 5C and D).

$\alpha\beta3$ integrin is a heterodimeric adhesion receptor mediating cell–cell and cell–matrix interaction, where osteoclasts exhibit the highest $\alpha\beta3$ integrin expression. $\alpha\beta3$ integrin aids in the actin ring formation, forming a sealing zone around the bone matrix and where osteoclast erodes the bone matrix, thereby increasing the bone resorption. It also has been proved that inhibiting the $\alpha\beta3$ integrin could reduce osteoporosis without affecting the number of osteoclast or osteoblast cells (Nakamura et al., 2007). Our results implicated that oledicoumarin, with the lowest binding energy of -9.30 kcal/mol, had the highest binding affinity towards the molecular target $\alpha\beta3$ integrin. In addition, it also formed two H-bonding with the ARG216 (2.91 Å) and ALA218 (2.72 Å) residues of $\alpha\beta3$ integrin (Figure 5E and F). From the molecular docking results, cedrelopsin, deacetylasperuloside, oledicoumarin, and pheophorbide a methyl ester, were considered for further evaluation.

Drug-likeness and ADME Analysis

In general, the drug-likeness of the compounds was assessed based on Lipinski's-rule-of-five (Ghose et al., 1998). Lipinski rule states that a drug candidate should have an MW < 500, Log P < 5, HA < 10, and HD < 5 to be orally effective (Lipinski et al., 2001; Ali et al., 2012). The drug-likeness

and absorption, distribution, metabolism, and excretion (ADME) scores of the selected four ligands are shown in Table 2. The data suggested that cedrelopsin and deacetylasperuloside obeyed the Lipinski rules with 0 violations.

The ADME properties of the selected ligands are shown in Table 2. TPSA is an ideal parameter for characterizing the drug transport properties and correlating the hydrogen bonding, indicating the molecule's bioavailability. The optimal limit of TPSA is suggested to be less than 160 Å² (Husain et al. (2016)). The TPSA of four selected phytoconstituents were in the range of 59.67 – 155.15 , Å², which is below the limit indicating good bioavailability. Besides, the solubility (Log S) of a drug greatly confers the facilitation of drug absorption, making it a crucial property to be evaluated. Compounds exhibiting aqueous solubility of about -4.0 to 0.5 log mol/L are considered to have considerable aqueous solubility. Thus, cedrelopsin and deacetylasperuloside exhibited solubility of about -3.814 and -0.539 log mol/L, signifying adequate aqueous solubility. The human Ether-à-go-go-Related Gene (hERG) encodes for the voltage-gated potassium channel capable of regulating the voltage potential in cardiac action. Thus, the inhibition of the hERG could cause repolarization of cardiac potential; thereby, it could induce sudden death (Jing et al., 2015). Thus, results indicated that pheophorbide a methyl ester is capable of inducing cardiac toxicity, as shown in Table 2.

Among the ADME parameters, Gi is a vital parameter to assess the extent of absorption of the drug in the gastrointestinal tract, as it directly affects the bioavailability of the drug. Results exhibited that cedrelopsin and pheophorbide a methyl ester showed the highest probability of gastrointestinal absorption. Distribution properties were assessed based on PPB, their ability to cross the BBB and to be a substrate for P-gp. PPB is an essential pharmacokinetic parameter as it influences the ADME properties and plays a crucial role in drug–drug interaction (Sun et al., 2018). Hence, the phytoconstituents exhibiting below 65% of PPB were considered significant. Results showed that deacetylasperuloside exhibited about 20.98% of PPB. Thus, the unbound deacetylasperuloside (70%) is available for exerting its bioactivity. Cedrelopsin and pheophorbide A methyl ester were shown to have the ability to cross the blood-brain barrier, whereas other constituents could not permeate through BBB. Hence, deacetylasperuloside and oledicoumarin will not induce any adverse effects affecting CNS. It is interesting to note that none of the selected phytoconstituents was a substrate for P-gp. The distribution properties of the phytoconstituents are represented in Table 2.

Biotransformation of the absorbed drugs occurs via metabolism involving various biochemical pathways and enzymes structurally modifying them to different molecules (Zhang & Tang, 2018). Among the multiple enzymes regulating metabolism, CYP 450 enzymes (Subfamilies: 1A2, 2C19, 2C9, 2D6 and 3A4) play a crucial role in metabolizing most of the drugs by oxidation. In addition, they can be inhibited or induced

Table 2. Drug Likelihood Scores and ADME Analysis of Selected Phytoconstituents of *Oldenlandia umbellata*.

Ligands	Drug-likeness					ADME								
	MW	Log P	HA	HD	LRV	TPSA	Log S	hERG	PPB	G _i	BBB	P-gp	CYP*	Log Kp
Cedrelopsin	260.29	3.34	4	1	0	59.67	-3.814	-	81.74%	High	+	-	+	-5.44
Deacetylasperu- loside	372.33	-3.35	10	5	0	155.15	-0.539	-	20.98%	Low	-	-	-	-10.69
Oledicoumarin	512.51	6.31	8	0	2	105.7	-6.875	-	68.02%	Low	-	-	+	-5.1
Pheophorbide a methyl ester	606.72	6.25	9	2	2	127.05	-5.735	-	97.41	High	+	-	+	-11.75

Abbreviations: MW, molecular weight (<500); TPSA, topological polar surface area; HA, number of hydrogen bond acceptors (≤10); HD, number of hydrogen bond donors (≤5); LRV, Lipinski rule violations; Log P, (octanol/water) partition co-efficient (≤5); Log S, solubility (-4~-0.5 log mol/L); hERG, hERG potassium ion channel inhibitor (- ~ non-toxic; + ~ toxic); PPB, plasma protein binding; G_i, gastrointestinal absorption; BBB, blood-brain permeation; P-gp, substrate for permeability glycoprotein; CYP*, cytochrome P450 inhibitors; Log K_p, skin permeation co-efficient (cm/s) - ("+" substrate; "-" not a substrate).

by drugs that can lead to adverse effects like toxicity or render the drug ineffective (Durán-Iturbide et al., 2020). CYP1A2 is a vital enzyme for metabolization in the liver, whereas CYP2C19 involves metabolizing several drugs and detoxifying potential carcinogens. On the other hand, CYP2C9 highly contributes to the metabolism, predominantly in the clearance of constituents with a narrow therapeutic index. The CYP2D6 isoform is vital in the hepatic metabolism of the drugs; inhibiting the CYP2D6 enzymes renders the drugs ineffective and leads to adverse effects (Durán-Iturbide et al., 2020). Cedrelopsin, oledicoumarin, and pheophorbide a methyl ester tend to be a substrate for CYP1A2. In addition, cedrelopsin tends to inhibit CYP2C19, whereas oledicoumarin is a substrate for CYP2C9. None of the phytoconstituents were substrates for CYP2D6 and CYP3A4. Fortunately, deacetylasperuloside is not a substrate for any CYP450 enzymes.

Permeability is an important parameter as it affects the ADME of the drug. Skin permeability (Kp) is the ability of the drug to permeate the skin. The more negative the Log Kp (cm/s) value, the drug is impermeable through the skin (Daina et al., 2017). The skin permeability (Log Kp) of the phytoconstituents ranged from -5.1 to -11 , indicating that oledicoumarin is shown to have higher permeability, whereas pheophorbide A methyl ester shown to have low permeability.

Bioactivity and Toxicity Prediction

The bioactivity scores of the phytoconstituents were assessed using the Molinspiration tool and are represented in Table 3. Constituents with bioactivity scores higher than 0.00 are presumed to have significant bioactivity, whereas scores ranging from -0.50 to 0.00 and lesser than -0.50 are considered moderately active and inactive, respectively (Husain et al., 2016). Deacetylasperuloside showed bioactivity scores >0 , that is, GPCR (0.34), ICM (0.11), KI (0.09), NR (0.21), PI (0.27), and EI (0.57), indicating inhibition of various receptors, ion channels, and enzymes involving multiple pathways and has significant bioactivities.

Toxicity is a critical parameter that should be considered in the drug development process as it confers to failure at

the later stage of drug development. Acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, immunotoxicity, adverse outcome pathways (Tox21), and toxicity targets can all be predicted with the help of the free server ProTox-II, which uses molecular similarity, pharmacophores, fragment propensities, and machine-learning models (Banerjee et al. 2018). Table 3 depicts the toxicity assessment of the selected phytoconstituents of OU. Results showed that deacetylasperuloside did not show any hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, cytotoxicity, and reproductive toxicity compared to other phytoconstituents. Overall results suggested that deacetylasperuloside did not violate the Lipinski rule and possess good ADME properties with good bioactive scores without significant toxicity. In this regard, deacetylasperuloside could be a good therapeutic lead for treating osteoporosis. Hence, the deacetylasperuloside complex with the lowest binding energies (≤ 8.0 kcal/mol) was subjected to MD simulation analysis.

Molecular Dynamics Simulation

The protein–ligand complexes including deacetylasperuloside and GSK3 β , CTSK, NF- κ Bp65, and RANK were subjected to 100 ns MD simulation to assess the stability of the complex.

RMSD is used to assess the stability of the complex via assessing the trajectories of the protein backbone coordinates, which is calculated by rooting the average value of the square of the atomic coordinates. Thus, the lower the fluctuation, the higher the stability of the complex (Abraham et al., 2015). Figure 6A shows that the GSK3 β -DA complex has an average RMSD of 4 Å encompassing rigid structure attaining equilibrium after 60 ns. Besides, the CTSK-DA complex formed a rigid, stable complex with the average RMSD of 2 Å attaining equilibrium at 30 ns and maintained throughout the 100 ns simulation as shown in Figure 6B. The NF κ Bp65 and DA complex showed average RMSD >5 Å but equilibrating after 60 ns (Figure 6C). Compared to forementioned complexes NF κ Bp65-DA complex showed higher fluctuations but

Table 3. Bioactivity Scores and Toxicity Prediction Analysis of Selected Phytoconstituents of *Oldenlandia umbellata*.

Ligands	Bioactivity Prediction							Toxicity Analysis				
	GPCR	ICM	KI	NR	PI	EI	HT	CG	IT	MG	CT	RE
Cedrelopsin	−0.38	−0.47	−0.52	0.04	−0.70	0.21	−	−	+	+	−	−
Deacetylasperuloside	0.34	0.11	0.09	0.21	0.27	0.57	−	−	−	−	−	−
Oledicoumarin	−0.15	−0.45	−0.17	−0.07	−0.24	−0.04	−	+	−	−	−	−
Pheophorbide a methyl ester	0.17	−0.09	−0.21	−0.38	−0.07	−0.24	−	−	+	−	−	−

Note: Bioactivity scores greater than 0 are active, scores $-0.5 \leq 0$ are considerably bioactive, whereas scores less than -0.5 are considered inactive (“+” indicates active, “−” indicates inactive).

Abbreviations: GPCR, G-protein-coupled receptor ligand; ICM, ion channel modulator; KI, kinase inhibitor; NR, nuclear receptor ligand; PI, protease inhibitor; EI, enzyme inhibitor; HT, hepatotoxicity; CG, carcinogenicity; IT, immunotoxicity; MG, mutagenicity; CT, cytotoxicity; RE, reproductive effect.

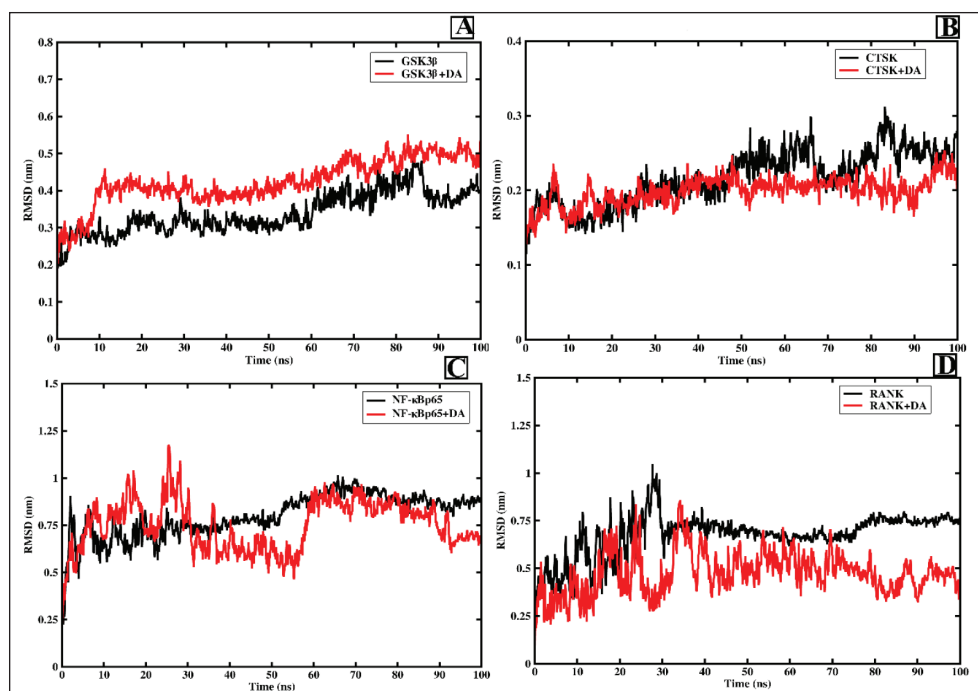


Figure 6. RMSD Plot for the MD Simulation of Protein–Ligand Complexes. (A) GSK3 β -DA, (B) CTSK-DA, (C) NF- κ Bp65-DA, and (D) RANK-DA.

Abbreviations: GSK3 β , glycogen synthase kinase 3 β ; CTSK, cathepsin K; NF- κ Bp, nuclear factor kappa-light-chain-enhancer of activated B cells; RANK, receptor activator of nuclear factor-kappa-B.

equilibration after 60 ns depicted the stability of the complex during simulation. From Figure 6C, it can be observed that from 50 ns, the protein–ligand complex has achieved equilibrium with the RMSD ranging from 3 to 5 Å, but a significant fluctuation in the RANK-DA complex indicates lesser stability of the complex. Hence, the protein–ligand complexes, that is, GSK3 β and CTSK, were intact through the MD simulation, exhibiting stable conformations of the complexes. The experimental data for RMSF, Rg, and HBs are provided in the supplementary information.

In conclusion, ligand complexed with GSK3 β and CTSK has shown good stability, compactness, and rigidity compared to NF- κ Bp65 and RANK. Though pharmacokinetic and toxicity parameters of the phytoconstituents are computationally analyzed, it is imperative to analyze its safety and efficacy via *in vitro* and *in vivo* experimentation to assess its correlation with computational analysis. Further, *in vitro* evaluation in appropriate cell models and *in vivo* evaluation in animal models are needed to assess the therapeutic efficacy of the OU for the treatment of osteoporosis.

Conclusion

Conclusively, from the results, deacetylasperuloside exhibited significant binding affinity towards the molecular targets of

osteoporosis, that is, GSK3 β , CTSK, NF- κ Bp65, and RANK. Further, MD simulation for 100 ns revealed that GSK3 β and CTSK complexes were highly stable and maintained their conformation till the end of the simulation. Hence, it can be anticipated that deacetylasperuloside could be a potential therapeutic molecule, which may inhibit the potential therapeutic targets.

Since all the phytoconstituents of OU showed good and considerable binding affinity towards the therapeutic targets of osteoporosis; further, *in vitro* and *in vivo* studies are needed to assess the therapeutic efficacy and safety profile of OU for the treatment of osteoporosis.

Author Contributions

Data curation, analysis, and preparation of the manuscript were done by Sivasakthi Paramasivam. Conceptualization, supervision of the study, and critical revision of the manuscript were done by Senthamil Selvan Perumal.

Declaration of Conflicting Interests

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References

- Abraham, M. J., Murtola, T., Schulz, R., Páll, S., Smith, J. C., Hess, B., & Lindahl, E. (2015). GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX*, 1–2, 19–25. <https://doi.org/10.1016/j.softx.2015.06.001>
- Ali, P., Meshram, J., Sheikh, J., Tiwari, V., Dongre, R., & Hadda, T. B. (2012). Predictions and correlations of structure activity relationship of some aminoantipyrine derivatives on the basis of theoretical and experimental ground. *Medicinal Chemistry Research*, 21(2), 157–164. <https://doi.org/10.1007/s00044-010-9505-0>
- Amjadi-Moheb, F., & Akhavan-Niaki, H. (2019). Wnt signaling pathway in osteoporosis: Epigenetic regulation, interaction with other signaling pathways, and therapeutic promises. *Journal of Cellular Physiology*, 234(9), 14641–14650. <https://doi.org/10.1002/jcp.28207>
- Arun, P., Purushotham, K. G., Johnsy Jayarani, J., et al. (2010). In vitro antibacterial activity of *Oldenlandia umbellata* an Indian medicinal Plant. *Journal of Pharmaceutical Science and Technology*, 2, 198–201.
- Banerjee, P., Eckert, A. O., Schrey, A. K., & Preissner, R. (2018). ProTox-II: A webserver for the prediction of toxicity of chemicals. *Nucleic acids research*, 46(W1), W257–W263. <https://doi.org/10.1093/nar/gky318>
- Chau, J. F. L., Leong, W. F., & Li, B. (2009). Signaling pathways governing osteoblast proliferation, differentiation and function. *Histology and Histopathology*, 24(12), 1593–1606. <https://doi.org/10.14670/HH-24.1593>
- Cheng, L., Zhou, S., Zhao, Y., Sun, Y., Xu, Z., Yuan, B., & Chen, X. (2018). Tanshinone IIA attenuates osteoclastogenesis in ovariectomized mice by inactivating NF- κ B and Akt signaling pathways. *American Journal of Translational Research*, 10(5), 1457–1468.
- Dai, R., Wu, Z., Chu, H. Y., Lu, J., Lyu, A., Liu, J., & Zhang, G. (2020). Cathepsin K: The action in and beyond bone. *Frontiers in Cell and Developmental Biology*, 8, 433. <https://doi.org/10.3389/fcell.2020.00433>
- Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*, 7, 42717. <https://doi.org/10.1038/srep42717>
- Durán-Iturbide, N. A., Díaz-Eufracio, B. I., & Medina-Franco, J. L. (2020). In silico ADME/Tox profiling of natural products: A focus on BIOFACQUIM. *ACS Omega*, 5(26), 16076–16084. <https://doi.org/10.1021/acsomega.0c01581>
- Ghose, A. K., Viswanadhan, V. N., & Wendoloski, J. J. (1998). Prediction of hydrophobic (lipophilic) properties of small organic molecules using fragmental methods: An analysis of ALOGP and CLOGP methods. *Journal of Physical Chemistry A*, 102(21), 3762–3772. <https://doi.org/10.1021/jp980230o>
- Györi, D. S., & Mócsai, A. (2020). Osteoclast signal transduction during bone metastasis formation. *Frontiers in Cell and Developmental Biology*, 8, 507. <https://doi.org/10.3389/fcell.2020.00507>
- Husain, A., Ahmad, A., Khan, S. A., Asif, M., Bhutani, R., & Al-Abbasi, F. A. (2016). Synthesis, molecular properties, toxicity and biological evaluation of some new substituted imidazolidine derivatives in search of potent anti-inflammatory agents. *Saudi Pharmaceutical Journal*, 24(1), 104–114. <https://doi.org/10.1016/j.jsps.2015.02.008>
- Jing, Y., Easter, A., Peters, D., Kim, N., & Enyedy, I. J. (2015). In silico prediction of hERG inhibition. *Future Medicinal Chemistry*, 7(5), 571–586. <https://doi.org/10.4155/fmc.15.18>
- Laskowski, R. A., & Swindells, M. B. (2011). LigPlot+: Multiple ligand-protein interaction diagrams for drug discovery. *Journal of Chemical Information and Modeling*, 51(10), 2778–2786. <https://doi.org/10.1021/ci200227u>
- Lipinski, C. A., Lombardo, F., Dominy, B. W., Feeney, P. J. (2001). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews*, 46(1–3), 3–26.
- Mahibalan, S., Gopal, N., Shanmuga Pandian, P., Jasmine, S. (2008). Anti inflammatory activity of various extracts of *Hedyotis umbellata* linn. *Natural Products: An Indian Journal*, 4, 184–186.
- Mahibalan, S., Rao, P. C., Khan, R., Basha, A., Siddareddy, R., Masubuti, H., Fujimoto, Y., & Begum, A. S. (2016). Cytotoxic constituents of *Oldenlandia umbellata* and isolation of a new symmetrical coumarin dimer. *Medicinal Chemistry Research*, 25(3), 466–472. <https://doi.org/10.1007/s00044-015-1500-z>
- Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of Computational Chemistry*, 30(16), 2785–2791. <https://doi.org/10.1002/jcc.21256>
- Mun, S. H., Park, P. S. U., & Park-Min, K. H. (2020). The M-CSF receptor in osteoclasts and beyond. *Experimental and Molecular Medicine*, 52(8), 1239–1254. <https://doi.org/10.1038/s12276-020-0484-z>
- Nakamura, I., Duong, L. T., Rodan, S. B., & Rodan, G. A. (2007). Involvement of α v β 3 integrins in osteoclast function. *Journal of Bone and Mineral Metabolism*, 25(6), 337–344. <https://doi.org/10.1007/s00774-007-0773-9>
- Noh, J.-Y. Y., Yang, Y., & Jung, H. (2020). Molecular mechanisms and emerging therapeutics for osteoporosis. *International Journal of Molecular Sciences*, 21(20), 7623. <https://doi.org/10.3390/ijms21207623>
- Padhy, I., & Endale, A. (2014). Evaluation of anti-inflammatory and antipyretic activity of *Oldenlandia umbellata* Linn. roots. *International journal of Pharmaceuticals and Health care research*, 2, 12–14.
- Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., & Ferrin, T. E. (2004). UCSF Chimera—A visualization system for exploratory research and analysis. *Journal of Computational Chemistry*, 25(13), 1605–1612. <https://doi.org/10.1002/jcc.20084>
- Saad, F. A. (2020). Novel insights into the complex architecture of osteoporosis molecular genetics. *Annals of the New York Academy of Sciences*, 1462(1), 37–52. <https://doi.org/10.1111/nyas.14231>
- Sander, T., Frey, J., von Korff, M., & Rufener, C. (2015). DataWarrior: An open-source program for chemistry aware data visualization and analysis. *Journal of Chemical Information and Modeling*, 55(2), 460–473. <https://doi.org/10.1021/ci500588j>

- Saraswathy, G., Maheswari, E., Saravanan, K., Asirvatham, R. (2008). Antitussive activity of *Oldenlandia umbellata* on cough reflex induced by sulfur dioxide in mice. *Pharmacologyonline*, 3, 253–262.
- Sharan, K., Siddiqui, J. A., Swarnkar, G., Maurya, R., & Chattopadhyay, N. (2009). Role of phytochemicals in the prevention of menopausal bone loss: Evidence from in vitro and in vivo, human interventional and pharmacokinetic studies. *Current Medicinal Chemistry*, 16(9), 1138–1157. <https://doi.org/10.2174/092986709787581806>
- Sivapraksham, S. S. K., Karunakaran, K., S. U., Maheswari, S. U., Kuppusamy, S. (2014). A review on phytochemical and pharmacological profile of *Hedyotis corymbosa* linn. *International Journal of Pharmaceutical Sciences Review and Research*, 26, 320–324.
- Somnath, D. E., Akalanka, D., Sudhakar Babu, A. M. S., Aneela, S. (2014). In vitro anthelmintic activity of aqueous and methanolic extracts of *Oldenlandia umbellata*. *International Journal of Pharmacy and Pharmaceutical Sciences*, 6, 94–95.
- Sun, L., Yang, H., Li, J., Wang, T., Li, W., Liu, G., & Tang, Y. (2018). In silico prediction of compounds binding to human plasma proteins by QSAR models. *ChemMedChem*, 13(6), 572–581. <https://doi.org/10.1002/cmdc.201700582>
- Sun, P., Yang, Q., Wang, Y., Peng, J., Zhao, K., Jia, Y., Zhang, T., Lu, X., Han, W., & Qian, Y. (2020). Pristimerin inhibits osteoclast differentiation and bone resorption in vitro and prevents ovariectomy-induced bone loss in vivo. *Drug Design, Development and Therapy*, 14, 4189–4203. <https://doi.org/10.2147/DDDT.S275128>
- Suvarna, V., Sarkar, M., Chaubey, P., Khan, T., Sherje, A., Patel, K., & Dravyakar, B. (2018). Bone health and natural products- an insight. *Frontiers in Pharmacology*, 9, 981. <https://doi.org/10.3389/fphar.2018.00981>
- Tian, W., Chen, C., Lei, X., Zhao, J., & Liang, J. (2018). CASTp 3.0: Computed atlas of surface topography of proteins. *Nucleic Acids Research*, 46(W1), W363–W367. <https://doi.org/10.1093/nar/gky473>
- van Aalten, D. M. F., Bywater, R., Findlay, J. B. C., Hendlich, M., Hooft, R. W., & Vriend, G. (1996). PRODRG, a program for generating molecular topologies and unique molecular descriptors from coordinates of small molecules. *Journal of Computer-Aided Molecular Design*, 10(3), 255–262. <https://doi.org/10.1007/BF00355047>
- Walsh, M. C., & Choi, Y. (2014). Biology of the RANKL–RANK–OPG system in immunity, bone, and beyond. *Frontiers in Immunology*, 5, 511. <https://doi.org/10.3389/fimmu.2014.00511>
- Wang, X., Liang, T., Zhu, Y., Qiu, J., Qiu, X., Lian, C., Gao, B., Peng, Y., Liang, A., Zhou, H., Yang, X., Liao, Z., Li, Y., Xu, C., Su, P., & Huang, D. (2019). Melatonin prevents bone destruction in mice with retinoic acid-induced osteoporosis. *Molecular Medicine*, 25(1), 43. <https://doi.org/10.1186/s10020-019-0107-0>
- Xiong, G., Wu, Z., Yi, J., Fu, L., Yang, Z., Hsieh, C., Yin, M., Zeng, X., Wu, C., Lu, A., Chen, X., Hou, T., & Cao, D. (2021). ADMETlab 2.0: An integrated online platform for accurate and comprehensive predictions of ADMET properties. *Nucleic Acids Research*, 49(W1), W5–W14. <https://doi.org/10.1093/nar/gkab255>
- Yoganarasimhan, S. N. (2000). *Medicinal plants of India: Tamil Nadu*. Vedams Books, Bangalore, India.
- Zhang, Z., & Tang, W. (2018). Drug metabolism in drug discovery and development. *Acta Pharmaceutica Sinica. B*, 8(5), 721–732. <https://doi.org/10.1016/j.apsb.2018.04.003>