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Structure-Based Identification of Sanketan Heliotropium indicum as RANK- RANKL Inhibitor: A Bioinformatics Approach for Osteoporosis Therapy

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Abstract

Heliotropium indicum, a traditional medicinal plant, contains various bioactive compounds with potential therapeutic effects. This study employed a structure- based virtual screening approach to evaluate selected compounds from H. indicum against RANKL (Receptor Activator of Nuclear Factor κB Ligand), a key regulator in osteoclastogenesis and osteoporosis. Docking simulations using AutoDockTools and PyRx targeted the RANKL structure (PDB ID: 3QBQ). The binding free energy (ΔG) values were used to assess ligand affinity, with Indicine (–5.6 kcal/mol), Heliotrine (–6.0 kcal/mol), and 24-Methylenecholesterol (–6.6 kcal/mol) demonstrating the strongest binding affinities. Interaction analyses revealed stable hydrogen bonding and hydrophobic contacts with key residues such as SER296, ARG222, and GLY96. Further ADME-Tox profiling and Lipinski's Rule of Five filtration showed that most compounds had high gastrointestinal absorption, were non-substrates for P-glycoprotein, had minimal CYP450 inhibition, and low toxicity risks. Indicine and Heliotrine exhibited superior drug-likeness and safety profiles. 24-Methylenecholesterol also emerged as a promising lead. These findings suggest that specific H. indicum compounds could serve as potential RANKL inhibitors for osteoporosis therapy. The computational evaluations provide a strong basis for future in vitro and in vivo validation to support the development of phytopharmaceuticals targeting bone resorption.

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Introduction

Osteoporosis is a major degenerative disease affecting over 200 million people globally and is among WHO's top ten health concerns. In 2010, Europe recorded 22 million women and 5.5 million men aged 50– 84 diagnosed with osteoporosis. In Indonesia, Perosi reported a 19.7% prevalence, while the Ministry of Health estimated a national average of 10.3%, indicating that two in five Indonesians are at risk. Although its mortality rate is lower than cancer or cardiovascular diseases, osteoporosis severely impacts the elderly's quality of life.

Osteoporosis is classified into primary and secondary types. Primary osteoporosis occurs mainly in postmenopausal women and in older adults over 70 years, while secondary osteoporosis results from diseases, medical treatments, or idiopathic factors. Common causes include systemic and endocrine disorders, malignancies, long-term glucocorticoid use, lifestyle factors, and major depression (Marcucci, 2015).

Several therapeutic approaches have been used to manage osteoporosis, including hormone replacement therapy (HRT), calcitonin, and bisphosphonates. Although effective, these therapies pose serious side effects such as increased risk of breast and endometrial cancer, gastrointestinal disturbances, osteonecrosis of the jaw, and hypocalcemia. Despite their clinical effectiveness, bisphosphonates and other conventional anti-resorptive drugs are associated with adverse effects such as atypical femoral fractures and osteonecrosis of the jaw" (Khosla & Hofbauer, 2017). These limitations underscore the urgent need for safer and more selective agents that can effectively inhibit osteoclast activity.

The equilibrium between osteoblasts and osteoclasts regulates bone homeostasis. Osteoprotegerin (OPG), a decoy receptor, inhibits the mechanism by which RANKL, a cytokine released by osteoblasts, binds to its receptor RANK on precursor cells, promoting osteoclast development. Osteoclast-specific genes like TRAP and other enzymes involved in precursor fusion and bone matrix breakdown are expressed when RANKL binds to RANK, activating signaling pathways like NF-κB and MAPK. The interaction of RANKL with RANK also activates key regulators such as NFATc1 and cathepsin K (CTSK), which further induce the transcription of osteoclast-specific genes (Attia, 2010; Sihombing, 2012; Rachner, 2011). Osteoporosis treatment approaches that control osteoclastogenesis are becoming more and more promising.

A widespread medicinal plant in tropical Asia, particularly Indonesia, Heliotropium indicum L. (Sangketan) has long been used to treat wounds, fractures, infections, and other conditions. Heliotropium indicum is widely used in traditional medicine across Asia and Africa for wound healing, inflammation, and bone-related disorders, highlighting its ethnopharmacological importance" (Khare, 2008). Despite being widely used, it is still unknown what molecular foundation underlies its effects on bone resorption and healing. This study screens bioactive compounds from H. indicum for possible interactions with RANKL and designs inhibitors based on natural products using bioinformatics and molecular docking. Both RANKL and macrophage colony-stimulating factor (M-CSF) are necessary for the differentiation of osteoclasts, which are specialized bone-resorbing cells that are abundant in mitochondria. As shown in postmenopausal osteoporosis and its subsequent forms, dysregulation of the osteoblast–osteoclast balance can result in aberrant bone remodeling (Drake, 2008; Takayanagi, 2007; Kawatani, 2009).

H. indicum is a traditional medicinal plant whose name is derived from the Greek words helios (sun) and trope (turning), although it does not exhibit heliotropic behavior. This annual herb, reaching 15–50 cm in height, grows in waste and residential areas and flowers year-round. All parts of the plant have medicinal value: the leaves treat eye disorders and sore throat, the roots act as astringent, expectorant, and antipyretic, and aqueous leaf extracts show anti-leukemic activity. The plant contains diverse phytochemicals including alkaloids (heliotrine, indicine N-oxide, europine N-oxide, cynoglossine), triterpenes (β-amyrin, lupeol, rapone), sterols (β-sitosterol, campesterol, stigmasterol), amines (putrescine, spermidine, spermine), and volatile oils (β-linalool, phytol) (Sarkar, et al., 2021). These bioactive compounds have been associated with various pharmacological properties that may contribute to bone health.

Although H. indicum has been widely used in ethnopharmacology, its molecular mechanisms in regulating bone remodeling remain poorly understood, particularly in relation to the RANK/RANKL signaling axis. Therefore, this study aims to investigate the interaction of bioactive compounds from H. indicum with RANKL using bioinformatics and molecular docking approaches. Natural products have historically provided a rich source of lead compounds for drug discovery, and remain crucial in the search for safer anti-osteoporotic agents" (Harvey et al., 2015). The expected benefit is to provide a scientific basis for the development of safer and more selective natural inhibitors of RANKL. In the long term, these findings may support the discovery of phytopharmaceuticals from H. indicum as alternative therapies for osteoporosis, contributing both to public health improvement and to advancing knowledge in pharmacognosy and drug discovery.

Method

Ligand Preparation

The bioactive compounds analyzed in this study were obtained from the KNApSAcK family compound database. The corresponding three-dimensional structures were retrieved in Structure Data File (SDF) format and converted to Protein Data Bank (PDB) format using Open Babel. The ligands were then prepared for docking by optimizing geometry, adding polar hydrogens, and converting to PDBQT format using AutoDockTools 1.5.6. A total of 20 bioactive compounds reported in Heliotropium indicum were used as ligand samples in this study.

Receptor Preparation

The target macromolecule, Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL), was obtained from the Protein Data Bank (https://www.rcsb.org/) with the PDB ID: 3QBQ. The receptor structure was prepared by removing water molecules, heteroatoms, and non-essential ligands using PyMOL, and polar hydrogens were added using AutoDockTools 1.5.6. The resulting receptor was saved in PDB format for docking.

Active Site Prediction

The active site of the RANKL receptor (PDB ID: 3QBQ) was predicted to identify potential binding pockets for bioactive compounds from Heliotropium indicum. Initially, the three-dimensional structure of the protein was analyzed using PyMOL to visualize surface topology and identify potential ligand-binding cavities. For more accurate and automated pocket detection, the structure was then submitted to PrankWeb, which are web-based tools used to predict and rank possible binding sites based on pocket volume, surface area, and residue accessibility.

The most probable active site was selected based on the largest pocket score and the presence of biologically relevant residues previously reported in literature. The coordinates of this predicted binding site were extracted and used to define the docking grid box for molecular docking simulations in AutoDockTools. This ensured that the docking protocol focused on the functionally significant region of the receptor likely involved in ligand.

Molecular Docking Simulation

The molecular docking simulation was carried out using AutoDock 4.2.6 integrated within the PyRx software platform. The primary objective of the docking process was to evaluate the binding affinity and molecular interactions between the selected bioactive ligands from Heliotropium indicum and the target receptor RANKL. Binding affinity was assessed based on the calculated binding free energy (ΔG) values, which indicate the strength and stability of the ligand–receptor complexes.

Post-docking, the ligand–receptor interactions were visualized and analyzed using PyMOL software to examine key interacting residues, hydrogen bonds, hydrophobic contacts, and overall binding conformation within the active site of the receptor.

Pharmacokinetics and Pharmacodynamics Prediction

The final step involved the prediction of pharmacokinetic and pharmacodynamic profiles, encompassing absorption, distribution, metabolism, and toxicity (ADMET). This was conducted using the web-based server admetSAR.

The evaluation of absorption included parameters such as human intestinal absorption (HIA) and Caco-2 cell permeability. For distribution, parameters such as plasma protein binding (PPB) and the ability to penetrate the blood–brain barrier (BBB) were assessed. Metabolic profiling focused on the potential of the compounds to act as substrates or inhibitors of cytochrome P450 (CYP) enzymes. Additionally, toxicity profiles were predicted by evaluating carcinogenicity and mutagenicity of the compounds

Results and Discussion

Identification of Bioactive Compounds from *Heliotropium indicum* Using KNApSAcK

The identification of potential bioactive compounds from *Heliotropium indicum* (locally known as Sangketan) was performed using the KNApSAcK phytochemical database, which catalogs natural product information linked to plant species. A total of 22 bioactive compounds were predicted and retrieved, reflecting the chemical diversity of this medicinal herb.

These compounds belong to several major phytochemical classes, including pyrrolizidine alkaloids,

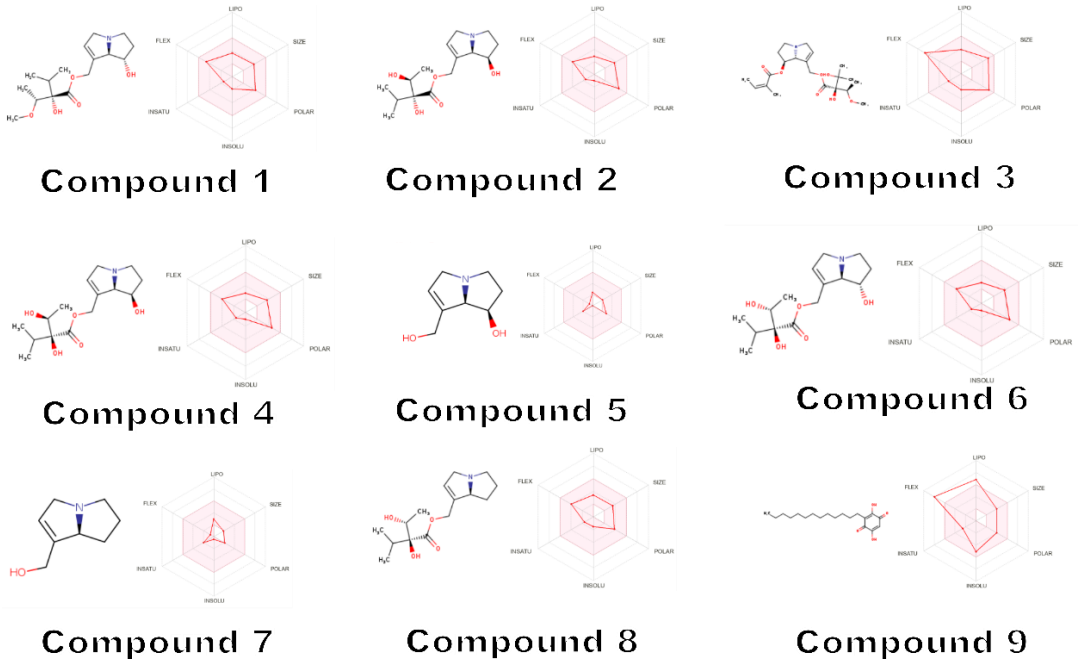
polyamines, sterols, terpenoids, and phenolic derivatives. The list includes structurally diverse molecules, many of which have been previously reported to exhibit antimicrobial, anticancer, and anti-inflammatory activities—properties relevant to bone health and osteoclast regulation.

Identified bioactive compounds include: Alkaloids & Pyrrolizidine Derivatives: Helindicine, Acetylindicine, Lindelofidine, Trachelanthamine, Indicinine, Indicine, Indicine N-oxide, Heliotrine, Supinine, Supinidine, Rinderine, Retronecine, Lasiocarpine, Lycopsamine, Echinatine, Heleurine. Polyamines: Homospermidine. Sterols & Terpenoids: 24-Methylene cholesterol, β-Stigmasterol, (–)-β-Sitosterol, Campesterol Phenolic Compound: Rapanone.

These compounds were selected for further in silico screening and docking against the RANKL receptor to investigate their potential as natural inhibitors in the treatment of osteoporosis. Their 3D structures were retrieved in .sdf and .pdb formats from public compound databases such as PubChem and prepared for virtual screening (Table 1).

Table 1. Bioactive compound in *Heliotropium indicum*

C_ID	CAS ID	Metabolite	Molecular formula	Mw	Organism or InChIKey etc.
C00064599	883902-09-8	Helindicine	C15H23NO4	281.16270823	Heliotropium indicum
C00064407	55529-84-5	Acetylindicine	C17H27NO6	341.1838376	Heliotropium indicum
C00064352	488-06-2	Lindelofidine	C8H15NO	141.11536411	Heliotropium indicum
C00063851	14140-18-2	Trachelanthamine	C15H27NO4	285.19400836	Heliotropium indicum
C00063647	11014-14-5	Indicinine		0.	Heliotropium indicum
C00051748	4427-76-3	Homospermidine	C8H21N3	159.17354769	Heliotropium indicum
C00026205	41708-76-3	Indicine N-oxide	C15H25NO6	315.16818754	Heliotropium indicum
C00026197	488-00-6	Heleurine	C16H27NO4	297.19400836	Heliotropium indicum
C00026189	480-83-1	Echinatine	C15H25NO5	299.17327292	Heliotropium indicum
C00007271	474-63-5	24-Methylene cholesterol	C28H46O	398.35486609	Heliotropium indicum
C00003674	83-48-7	beta-Stigmasterol	C29H48O	412.37051615	Heliotropium indicum
C00003672	83-46-5	(-)-beta-Sitosterol	C29H50O	414.38616622	Heliotropium indicum
C00003647	474-62-4	Campesterol	C28H48O	400.37051615	Heliotropium indicum
C00002860	573-40-0	Rapanone	C19H30O4	322.21440945	Heliotropium indicum
C00002121	551-58-6	Supinine	C15H25NO4	283.17835829	Heliotropium indicum
C00002120	551-59-7	Supinidine	C8H13NO	139.09971404	Heliotropium indicum
C00002111	6029-84-1	Rinderine	C15H25NO5	299.17327292	Heliotropium indicum
C00002108	480-85-3	Retronecine	C8H13NO2	155.09462867	Heliotropium indicum
C00002099	10285-07-1	Lycopsamine	C15H25NO5	299.17327292	Heliotropium indicum
C00002097	303-34-4	Lasiocarpine	C21H33NO7	411.22570242	Heliotropium indicum
C00002091	480-82-0	Indicine	C15H25NO5	299.17327292	Heliotropium indicum
C00002090	303-33-3	Heliotrine	C16H27NO5	313.18892298	Heliotropium indicum



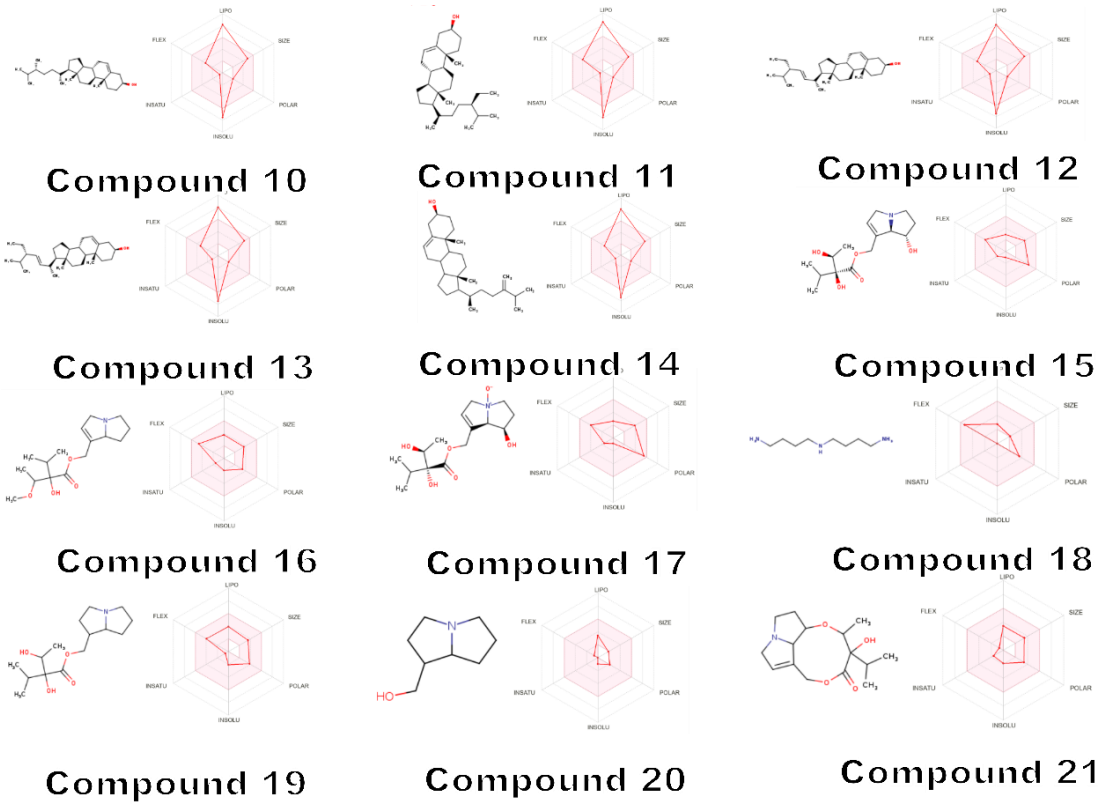


Figure 1. Chemical structure and psycochemical space for oral bioavailability

Structure-Based Drug Design

The crystal structure of the receptor activator of nuclear factor kappa-B ligand (RANKL) used in this study was retrieved from the Protein Data Bank (PDB ID: 3QBQ). This structure was resolved at a high resolution of 2.4 Å, providing detailed insight into the molecular architecture essential for understanding ligand interactions (Figure 1A).

To validate the structural integrity of the protein model prior to computational docking studies, the Ramachandran plot analysis was performed using the PROCHECK server. The Ramachandran plot evaluates the backbone dihedral angles phi (φ) and psi (ψ) of amino acid residues to assess conformational quality. The Ramachandran plot statistics for 3QBQ showed Residues in favored regions: 92.3%, Residues in allowed regions: 6.8%, Residues in disallowed regions: 0.9%. The overall stereochemical quality indicates that the structure is reliable for downstream computational studies (Figure 1B).

The Ramachandran plot results demonstrate that the vast majority of residues in the 3QBQ structure occupy energetically favorable conformations, indicative of a well-resolved and properly folded protein model. The small fraction of residues in disallowed regions is within acceptable limits for crystallographic structures and may correspond to flexible loop regions or functionally important sites. This high-quality structural model ensures that the active site geometry is accurately represented, which is critical for precise molecular docking and dynamics simulations. The reliability of the structure supports confidence in subsequent analyses of ligand binding and interaction patterns, ultimately aiding in the design of effective inhibitors targeting the RANKL–RANK interaction implicated in osteoclastogenesis and osteoporosis.

Active Site Prediction Using PrankWeb

Active site prediction was performed using PrankWeb, a web server that utilizes machine learning for ligand-binding site detection. The input receptor used was the three-dimensional structure of RANKL with PDB ID: 3QBQ. Based on the analysis, PrankWeb successfully identified multiple potential ligand- binding pockets.

The top-ranked binding pocket was selected based on the highest score provided by PrankWeb, which reflects the predicted likelihood of ligand interaction. The predicted active site included the following key residues: Top Pocket Score: 3.28 and predicted binding pocket residues: SER296, SER227, GLY96, ARG222, HIS223, LEU297, HIS223, ILE174, and PRO300. These residues are located on the receptor surface and are likely involved in the natural interaction between RANKL and its ligand RANK. This validates the selection

of the site as a biologically relevant target for inhibitor screening.
The location and conformation of the pocket also support favorable ligand accessibility, making it a viable site for the docking of bioactive compounds from Heliotropium indicum. These predictions provided the spatial coordinates and residues used in the grid box setting for molecular docking simulations.

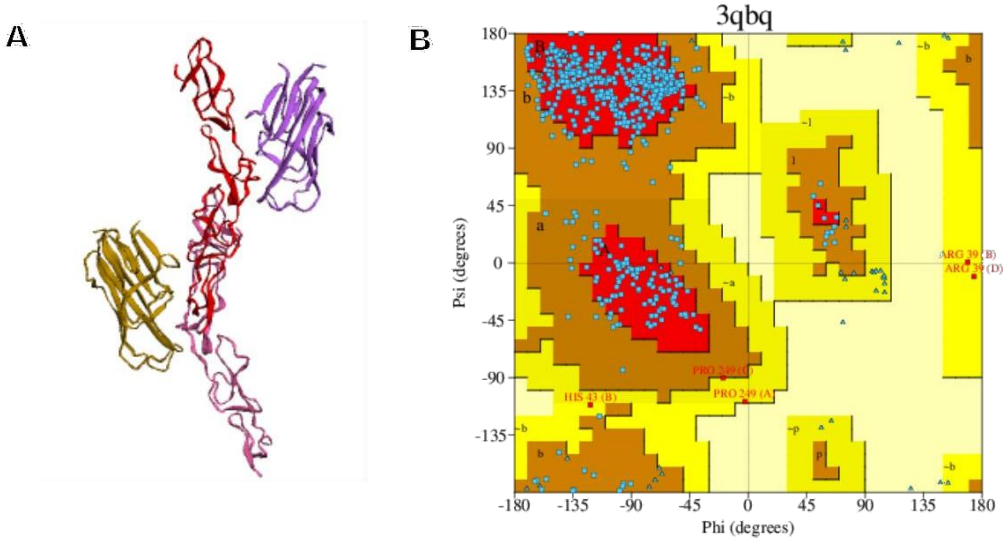


Figure 2. Structural analysis of the RANKL receptor (PDB ID: 3QBQ)
2A. Three-dimensional ribbon representation of the RANKL protein structure showing its overall fold and secondary structural elements. **2B.** Ramachandran plot of the RANKL structure illustrating the distribution of backbone dihedral angles (phi and psi) of amino acid residues. The majority of residues fall within favored and allowed regions, indicating good stereochemical quality of the protein model.

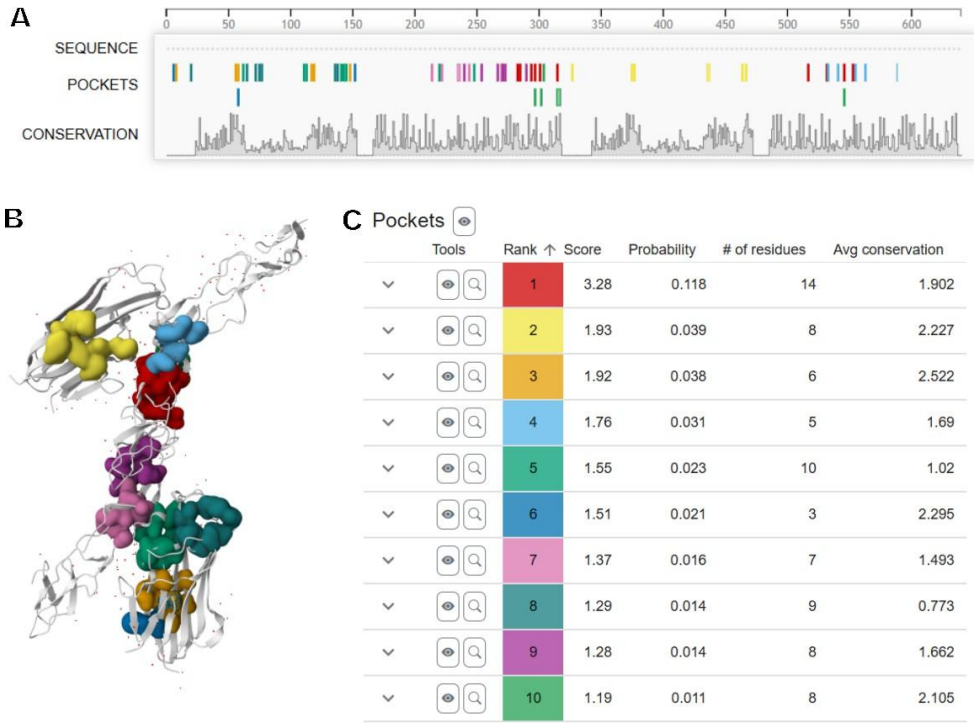


Figure 3. Active site prediction of RANKL (PDB ID: 3QBQ) using PrankWeb server
3A. Sequence-based analysis showing predicted binding pockets along the amino acid sequence, with conservation scores indicating evolutionary conservation of residues. **3B.** Visualization of the predicted active site pockets on the full RANKL structure: pockets are highlighted in different colors on the protein surface, while the protein backbone is shown in ribbon representation. **3C.** Pocket scores representing the confidence and likelihood of each predicted binding pocket as a functional active site.

Molecular Docking Analysis

Table 1 presents the results of molecular docking investigations involving various bioactive compounds and their interactions with the target protein residues. Most compounds in this study demonstrated favorable binding energies ranging from -4.1 to -6.6 kcal/mol. Lower binding energy values indicate stronger affinity toward the target protein.

Key amino acid residues involved in these interactions include SER296, SER227, GLY96, ARG222, and HIS223, which predominantly contribute to hydrogen bond formation with the ligands. Additionally, hydrophobic interactions play a significant role, where several compounds engage in alkyl and pi-alkyl interactions with residues such as LEU297, HIS223, ILE174, and PRO300.

Among the tested compounds, those with the lowest binding energies—namely 24-methylenecholesterol (-6.6 kcal/mol), (-)-beta-sitosterol (-6.2 kcal/mol), and heliotrine (-6.0 kcal/mol)—exhibit the highest potential as inhibitors of the target receptor.

Overall, these molecular docking results suggest that the analyzed bioactive compounds have a strong likelihood of stable interaction with the RANKL receptor, laying a promising foundation for further research in drug development targeting osteoclastogenesis inhibition.

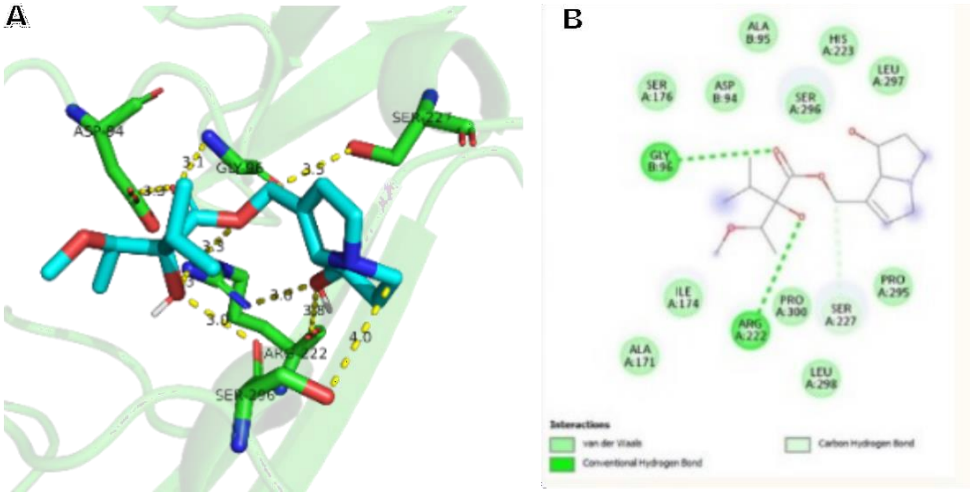


Figure 4. Visualization of the interaction between Indicine and RANKL (PDB ID: 3QBQ). Panel **(A)** shows the 3D representation of the binding interaction, while panel **(B)** illustrates the 2D interaction map between Indicine and RANKL

Lipinsky Rule of 5 Filtration

The purpose of this selection process was to identify Heliotropium indicum derivatives with optimal characteristics for drug administration based on Lipinski's Rule of Five. A summary of each compound's compliance with these criteria is presented in Table 2. The screening results show that most of the analyzed compounds conform to Lipinski's rules, indicating favorable drug-likeness properties. However, (-)-beta-sitosterol and beta-stigmaterol exhibited violations related to the LogP parameter (>5), suggesting potential issues with bioavailability due to high lipophilicity.

On the other hand, compounds such as Indicine, Lasiocarpine, Lycopasamine, Supinine, Campesterol, Echinatine, Acetylindicine, and several others demonstrated full compliance with Lipinski's Rule of Five. These compounds represent promising candidates for oral drug development due to their favorable pharmacokinetic profiles.

Table 2. Psycochemical properties of sangketan and filtration based on lipinksky rule of 5

Compound	Physicochemical properties									
	Formula	Mw (g/mol)	Num. heavy atoms	Num. arom. heavy atoms	Fraction Csp3	Num. rotatable bonds	Num. H-bond acceptors	Num. H-bond donors	Molar Refractivity	TPSA (Å²)
1	C16H27NO5	313.39	22	0	0.81	7	6	2	85.87	79.23
2	C15H25NO5	299.36	21	0	0.80	6	6	3	81.14	90.23
3	C21H33NO7	411.49	29	0	0.71	10	8	2	110.75	105.53
4	C15H25NO5	299.36	21	0	0.80	6	6	3	81.14	90.23
5	C8H13NO2	155.19	11	0	0.75	1	3	2	45.00	43.70
6	C15H25NO5	299.36	21	0	0.80	6	6	3	81.14	90.23
7	C8H13NO	139.19	10	0	0.75	1	2	1	43.84	23.47
8	C15H25NO4	283.36	20	0	0.80	6	5	2	79.97	70.00
9	C19H30O4	322.44	23	0	0.68	12	4	2	93.93	74.60
10	C28H48O	400.68	29	0	0.93	5	1	1	128.42	20.23
11	C29H50O	414.71	30	0	0.93	6	1	1	133.23	20.23
12	C29H48O	412.69	30	0	0.86	5	1	1	132.75	20.23
13	C29H48O	412.69	30	0	0.86	5	1	1	132.75	20.23
14	C28H46O	398.66	29	0	0.86	5	1	1	127.95	20.23
15	C15H25NO5	299.36	21	0	0.80	6	6	3	81.14	90.23
16	C16H27NO4	297.39	21	0	0.81	7	5	1	84.70	59.00
17	C15H25NO6	315.36	22	0	0.80	6	6	3	84.50	116.42
18	C8H21N3	159.27	11	0	1.00	8	3	3	48.79	64.07
19	C15H27NO4	285.38	20	0	0.93	6	5	2	80.45	70.00
20	C8H15NO	141.21	10	0	1.00	1	2	1	44.31	23.47
21	C15H23NO4	281.35	20	0	0.80	1	5	1	77.78	59.00

Pharmacokinetics profile and toxixity prediction

A comprehensive pharmacokinetic profile of the tested compounds is summarized in Table 3. This profile includes key ADME parameters such as gastrointestinal (GI) absorption, blood-brain barrier (BBB) permeability, substrate status for P-glycoprotein (P-gp), and inhibitory effects on cytochrome P450 enzymes (CYP450).

The results indicate that most compounds, including Heliotrine, Indicine, Lasiocarpine, Lycopasamine, and Supinine, exhibit high intestinal absorption but do not penetrate the blood-brain barrier, which may reduce central nervous system side effects. Several compounds, such as Indicine, Lasiocarpine, and Lycopasamine, function as P-gp substrates, potentially influencing their bioavailability and efflux mechanisms.

Beta-stigmaterol was identified as a potential inhibitor of CYP2C9, suggesting possible drug-drug interaction risks through cytochrome P450 metabolism pathways. Overall, these findings highlight that compounds with favorable ADME profiles, combined with low predicted toxicity, hold promise for further pharmaceutical development and optimization.

Table 3. Lipophilicity of *Heliotropium indicum*

Compound	Lipophilicity					
	Log $P_{o/w}$ (iLOGP)	Log $P_{o/w}$ (XLOGP3)	Log $P_{o/w}$ (WLOGP)	Log $P_{o/w}$ (MLOGP)	Log $P_{o/w}$ (SILICOS-IT)	Consensus Log $P_{o/w}$
1	1.81	0.91	-0.05	0.32	0.86	0.97
2	3.54	-0.44	-0.71	0.07	0.32	0.56
3	3.84	1.28	0.58	0.52	1.56	1.56
4	2.41	-0.44	-0.71	0.07	0.32	0.33
5	1.46	-1.29	-1.03	-0.12	-0.06	-0.21
6	2.44	-0.44	-0.71	0.07	0.32	0.34
7	1.46	-0.32	0.00	0.76	0.88	0.56
8	2.70	0.54	0.32	0.88	1.19	1.13
9	3.88	6.50	5.09	1.68	4.99	4.43
10	4.97	8.80	7.63	6.54	6.63	6.92
11	5.05	9.34	8.02	6.73	7.04	7.24
12	5.08	8.56	7.90	6.62	6.86	6.98
13	5.08	8.56	7.80	6.62	6.86	6.98
14	4.83	8.62	7.55	6.43	6.64	6.82

Compound	Lipophilicity					
	Log $P_{o/w}$ (iLOGP)	Log $P_{o/w}$ (XLOGP3)	Log $P_{o/w}$ (WLOGP)	Log $P_{o/w}$ (MLOGP)	Log $P_{o/w}$ (SILICOS-IT)	Consensus Log $P_{o/w}$
15	2.53	-0.44	-0.71	0.07	0.32	0.35
16	3.08	1.08	0.98	1.13	1.74	1.60
17	1.11	-0.91	-0.70	-1.76	-3.02	-1.06
18	2.05	-0.66	0.05	0.41	0.45	0.46
19	2.92	1.38	0.40	0.99	1.20	1.38
20	1.85	0.52	0.08	0.90	0.89	0.85
21	2.69	0.56	0.34	0.88	1.06	1.11

Table 4. ADMET prediction

Compound	GI absorption	BBB permeant	Log kp (cm/s)	P-gp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
Heliotrine	High	No	-7.57	No	No	No	No	No	No
Indicine	High	No	-8.44	Yes	No	No	No	No	No
Lasiocarpine	High	No	-7.90	Yes	No	No	No	No	No
Lycopsamine	High	No	-8.44	Yes	No	No	No	No	No
Retronecine	High	No	-8.16	No	No	No	No	No	No
Rinderine	High	No	-8.44	Yes	No	No	No	No	No
Supinidine	High	No	-7.83	No	No	No	No	No	No
Supinine	High	No	-7.65	No	No	No	No	No	No
Rapanone	High	No	-3.65	No	No	Yes	Yes	Yes	No
Campesterol	Low	No	-2.50	No	No	No	No	No	No
(-)-beta-Sitosterol	Low	No	-2.20	No	No	No	No	No	No
beta-Stigmasterol	Low	No	-2.74	No	No	No	Yes	No	No
24-Methylene cholesterol	Low	No	-2.61	No	No	No	Yes	No	No
Echinatine	High	No	-8.44	Yes	No	No	No	No	No
Heleurine	High	Yes	-7.35	No	No	No	No	No	No
Indicine N-oxide	High	No	-8.87	Yes	No	No	No	No	No
Homospermidine	High	No	-7.74	No	No	No	No	No	No
Indicinine	High								
Trachelanthamine	High	No	-7.06	No	No	No	No	No	No
Lindelofidine	High	No	-6.79	No	No	No	No	No	No
Acetylindicine	High	No	-8.28	Yes	No	No	No	No	No
Helindicine	High	No	-7.62	No	No	No	No	No	No

Binding Affinity of Bioactive Compounds to RANKL

Molecular docking simulations were successfully performed to evaluate the potential of bioactive compounds from Heliotropium indicum as inhibitors of the target protein, RANKL (Receptor Activator of Nuclear Factor κB Ligand). The docking process was conducted using AutoDockTools 4.2.6 integrated within the PyRx software platform to model the interaction between small molecules and the active site of the target protein.

The three-dimensional structure of the target protein was retrieved from the Protein Data Bank with the ID 3QBQ, which represents the crystal structure of human RANKL with high resolution. This structure was

selected to ensure accurate prediction of interaction sites and ligand affinity. Validation of the structure using a Ramachandran plot confirmed a high percentage of residues within allowed regions, supporting the reliability of the simulation.

In this study, binding free energy (ΔG) was used as an indicator of ligand-receptor affinity. More negative ΔG values indicate stronger and more stable interactions, which may translate into greater biological activity. Several compounds showed promising binding energies, including: 24-Methylene cholesterol: –6.6 kcal/mol. (–)-β-Sitosterol: –6.2 kcal/mol. Heliotrine: –6.0 kcal/mol.

Key amino acid residues involved in hydrogen bonding and hydrophobic interactions included SER296, ARG222, SER227, GLY96, HIS223, SER178, LEU297, ILE174, and PRO300. These interactions encompassed hydrogen bonds, π-alkyl interactions, and van der Waals forces that stabilize the ligand-receptor complex.

These findings suggest that the tested compounds may serve as promising candidates for the development of therapeutic agents targeting the RANK/RANKL signaling pathway, which is critical in diseases such as osteoporosis. However, successful drug development also requires favorable pharmacokinetic and toxicity profiles, which must be further evaluated.

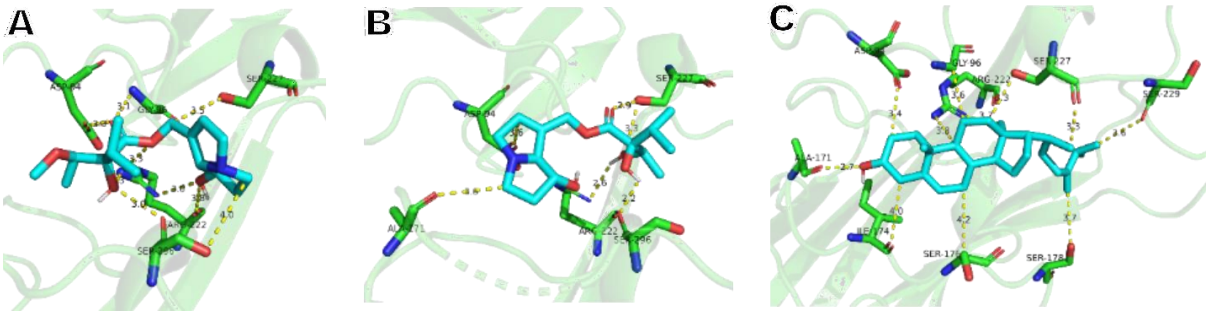


Figure 5. 3D Visualization of the interactions between Heliotrine **(A)**, Indicine **(B)**, and 24-Methylenecholesterol **(C)** with RANKL (PDB ID: 3QBQ)

Table 5. Interaction Profiles of Selected Compounds with RANKL

Compound	DG (kcal/mol)	Amino acid residue		
		Hydrogen bond	Hydrophobic	Other
Heliotrine	-6.0	GLY96, ARG222, SER227		
indicine	-5.6	SER296, SER227, ALA171		
Lasiocarpine	-5.6	SER296, GLY96, ARG222	Alkyl: LEU297, LEU235, VAL 230	
Lycopsamine	-5.6	SER296, SER227, GLY96, ALA171		
Retronecine	-4.9	SER296, ARG222, PRO295, ASP94		
Rinderine	-5.7	SER296, LEU298, ASP94		
Supindine	-4.6	SER178, SER296, ARG222	Pi-Sigma: HIS223	
Supinine	-5.7	SER296, SER229, SER227, HIS223	Pi-Sigma: HIS223	
Rapanone	-5.2	SER296, SER227	Alkyl: LEU297, ILE174, PRO300	
Campesterol	-5.7	SER227, GLY94	Alkyl: ILE174, PRO234	
(-)-beta-sitosterol	-6.2	ALA171,	Alkyl: LEU297, HIS223, ILE174, PRO231	
Beta-stigmasterol	-5.9		Alkyl: VAL92, ALA95, ALA1172, ALA171, ILE174,	
24 - methylenecholesterol	-6.6		PRO300	
Echinatine	-5.6	SER178, SER296, SER227, GLY96	Alkyl: LEU297, HIS223, ILE174	

Compound	DG (kcal/mol)	Amino acid residue		
		Hydrogen bond	Hydrophobic	Other
Heleurine	-5.8	SER227, GLY96		
Indicine N-oxide	-5.4	SER227, SER296, ARG222, GLY96	Pi-Sigma: HIS223	
Homospermidine	-4.1	PRO295, GLY96	Pi-alkyl: HIS223	
Trachelanthamine	-5.8	SER296, SER178		
Lindelofidine	-4.5	PRO295, ARG222		
Acetylindicine	-5.8	SER174, SER296, ARG222, HIS223		

Drug-Likeness Profiling of Heliotropium indicum Compounds

Since oral drugs are preferred in pharmacological therapy due to patient convenience, Lipinski's Rule of Five was applied to evaluate the pharmacokinetic properties of organic compounds, particularly their oral bioavailability and permeability. This rule assesses several physicochemical parameters, including molecular weight, hydrogen bond donors and acceptors, and the LogP value (a measure of lipophilicity), all of which influence solubility and absorption in the human body.

Based on the analysis of the compounds tested in this study, most molecules complied with Lipinski's criteria, indicating good potential as orally administrable drug candidates. However, a few compounds violated the LogP threshold, reflecting high lipophilicity. While lipophilicity can enhance membrane permeability, excessive LogP values may impair aqueous solubility and reduce overall bioavailability within systemic circulation.

These findings support the drug-likeness of the majority of the tested compounds, although those with high lipophilicity may require structural modifications or formulation strategies to improve their pharmacokinetic profiles.

ADMET Profiling: Absorption, Distribution, Metabolism, and Toxicity Analysis

Regarding distribution, most compounds were predicted not to act as substrates of P-glycoprotein (P- gp), implying they are less likely to be effluxed out of cells by this transporter protein. This characteristic can contribute to improved systemic bioavailability. In terms of metabolism, none of the compounds were predicted to inhibit major cytochrome P450 enzymes, including CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4. This suggests a low potential for drug–drug interactions, making them favorable candidates for further drug development.

For toxicity, the majority of the compounds did not exhibit carcinogenic or mutagenic properties, indicating a relatively favorable safety profile for pharmaceutical applications. These findings underscore the importance of further evaluation and optimization of these compounds to ensure their efficacy and safety in potential therapeutic formulations.

Candidate Selection Based on Multi-Parameter Analysis Conclusion

Considering the comprehensive results of molecular docking, compliance with Lipinski's Rule of Five, and the predicted pharmacokinetic and toxicity profiles, only a subset of compounds from *Heliotropium indicum*—namely Indicine, Lasiocarpine, Lycopsamine, Supinine, Campesterol, Echinatine, Heliotrine, Acetylindicine, and 24-Methylenecholesterol—fulfilled all established acceptance criteria.

Among these, Indicine and Heliotrine consistently exhibited the lowest binding free energies (ΔG), indicating a higher binding affinity toward the target protein RANKL (PDB ID: 3QBQ). These two compounds also demonstrated compatible interaction modes as RANKL inhibitors, along with superior safety profiles compared to other bioactive molecules in the *Heliotropium* genus.

While 24-Methylenecholesterol also met all pharmacokinetic and toxicity criteria, it showed a slightly lower binding affinity than Indicine and Heliotrine. Nevertheless, molecular interaction analysis confirmed that 24-Methylenecholesterol was still able to bind to the RANKL protein with acceptable interaction strength, supporting its pharmacological potential in modulating osteoclastogenesis. Thus, it ranks as a promising third candidate for further development in osteoporosis therapy.

Interaction profiling further revealed that Indicine and Heliotrine engaged in stronger hydrophobic interactions with key RANKL residues such as SER296, ARG222, and GLY96, contributing significantly to the stability of the ligand–receptor complex. Although 24-Methylenecholesterol displayed weaker interactions, its consistent engagement with RANKL underscores its value as a viable inhibitor candidate.

In summary, despite having a lower binding affinity, the favorable pharmacokinetic profile and compliance with Lipinski's criteria position 24-Methylenecholesterol as a valuable compound for further investigation. These observations highlight Indicine, Heliotrine, and 24-Methylenecholesterol as promising inhibitors of RANKL, with Indicine and Heliotrine emerging as the most stable and effective candidates for potential anti-osteoporotic drug development.

Conclusions

Computational investigations revealed the significant potential of bioactive compounds from *Heliotropium indicum* as effective inhibitors of RANKL. Among the tested compounds, Indicine and Heliotrine stood out by demonstrating high binding affinity to RANKL, accompanied by favorable pharmacokinetic properties and low toxicity profiles. 24-Methylenecholesterol also showed promise as an inhibitor, albeit with a lower binding affinity. Therefore, Indicine, Heliotrine, and 24-Methylenecholesterol represent promising candidates for further research and development in osteoporosis therapy.

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