In silico Analysis of Several Bioactive Compounds for Representative in Propolis from South Kazakhstan

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ABSTRACT

Introduction: Honey bees (*Apis mellifera* L.) create propolis, a complex natural material, by combining plant resins with bee secretions and beeswax.

Methods: The aim was to evaluate the bioavailability of the important bioactive compounds identified usually in many propolis samples through *in silico* analyses. Computational analysis revealed a substantial overlap between these compounds' targets and some important proteins and enzymes. Molecular docking studies revealed that naringin, rhamnazin, and rhamnetin exhibited high affinity for the active sites of CYP19A1 and NOX4 enzymes. Thermodynamic binding energies of all complexes ranged from -8.2 to -8.6 kcal/mol, above the threshold values for stable protein-ligand interaction.

Results: Rhamnetin emerged as a potential inhibitor candidate with the highest binding affinity against NOX4 at -8.6 kcal/mol, followed by rhamnazin at -8.5 kcal/mol. Naringin showed remarkable potential for biological activity by interacting with CYP19A1 at -8.2 kcal/mol. When drug likeness properties were compared, naringin exhibited violations of multiple filters with a bioavailability score of 0.17, while rhamnazin and rhamnetin satisfied all evaluated criteria with scores of 0.55.

Conclusion: This study is intended as a hypothesis-generating preliminary investigation. The interaction of naringin, rhamnazin, and rhamnetin found in propolis with disease-related proteins such as CYP19A1, and NOX4 supports the hormonal balancing, anti-diabetic, anti-oxidant, and anti-inflammatory therapeutic capacity of propolis.

Keywords: ADME, Bioactive compounds, Drug likeness, Immune modulator, Propolis.

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1. Introduction

Beyond honey production, bee hives yield valuable products such as propolis, royal jelly, pollen, and beeswax, which have gained increasing attention for their medicinal and therapeutic properties [1]. Among these, propolis stands out as one of the most chemically complex and biologically active hive products [2]. Bees use their salivary enzymes, pollen, and beeswax to break down these plant compounds inside the hive. Numerous pharmacological characteristics of propolis include immunomodulatory, antiviral, antifungal, antibacterial, anti-inflammatory, and anticancer effects. These activities are primarily due to its abundance of flavonoid and phenolic substances, which act as potent free radical scavengers and modulators of cellular signalling pathways [3,4].

Propolis's biological activity and chemical makeup are inextricably tied to its botanical and geographical origins [2,5]. This geographic variability necessitates region-specific investigations to completely comprehend the possible uses of propolis from each particular region [4,5].

The flora of Kazakhstan is particularly rich, with widespread distribution of alfalfa, sun-flower, buckwheat, acacia, sagebrush, clover, and numerous medicinal and aromatic plants [6]. Computational approaches including target prediction, molecular docking, and ADME profiling enable the evaluation of bioactive compounds' pharmacokinetic properties and their interactions with disease-relevant protein targets [7-10]

We selected three representative bioactive compounds commonly reported in propolis from diverse geographic regions: naringin, rhamnazin, and rhamnetin [9]. Using *in silico* tools, we evaluated their drug-likeness, pharmacokinetic properties, and potential interactions with human protein targets. Molecular docking studies were performed to assess binding affinities with identified targets, while ADME profiling provided insights into oral bioavailability and compliance with drug-likeness filters. The findings presented here are intended to establish a scientific rationale for subsequent chemical and biological analyses of propolis samples gathered from Kazakhstan's various ecological zones, ultimately supporting the strategic development of high-value bee products for apitherapeutic applications.

2. Materials and Methods

Three compounds, naringin, rhamnazin, and rhamnetin, were chosen for this *in silico* investigation. The selection of the phenolic compounds was based on their consistent detection

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across multiple propolis samples obtained from different geographical locations in the world [10].

2.1. Extraction of propolis samples

Samples of propolis were obtained from various locations around Kazakhstan (Table 1).

Table 1. Propolis samples collected from Kazakhstan

Sample Code	Location
PP1	Station 1: Kelintobe village, Kyzylorda region
PP2	Station 2: Zhanakorgan village, Kyzylorda region
PP3	Station 3: Kogam village, Turkestan region
PP4	Station 4: Shieli district, Kyzylorda region

2.2. Extraction of propolis samples

Extraction was performed by mixing five grams of each sample with 200 mL of 70% methanol, followed by incubation in a shaker incubator at room temperature for 72 hours. The extracts were clarified by filtration (whatman No.1) and dried under vacuum at 40 °C.

2.3. Determination of total flavonoid content

The extract (0.5 mL) was mixed with aluminium chloride and left for 30 min at room temperature. Absorbance was read at 415 nm (Spectro Nano, BM LabTech). Quercetin was used as standard, and results were expressed as mg Quercetin Equivalent (QE)/g extract [11].

2.4. Determination of total phenolics content

Folin-Ciocalteu method was used [12], adapted for microplate reading. Absorbance was recorded at 765 nm using a microplate spectrophotometer (Spectro Nano, BM LabTech). Gallic acid served as the reference standard, and values were expressed as mg Gallic Acid Equivalent (GAE)/g extract.

2.5. DPPH radical scavenging assay

Antioxidant activity was evaluated via DPPH assay [13]. Various extract concentrations (10-500 μg/mL) were reacted with 0.1 mM DPPH for 30 min at room temperature in darkness.

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Absorbance was read at 517 nm. Scavenging activity was calculated as percent inhibition relative to control, and Inhibition Concentration (IC_{50}) values were derived.

2.6. Retrieval of compound structures

The three-dimensional compound structures were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), in March and April 2025. The following PubChem Compound Identifiers (CIDs) were used: naringin (CID 442428), rhamnazin (CID 5321954), and rhamnetin (CID 5281691). The SDF files were converted to PDB format using open Babel (v2.4.1). Energy minimization was performed using the MMFF94 force field to obtain stable conformations for molecular docking studies.

2.7. Target profiling

In silico target predication was conducted using SwissTargetPrediction (http://www.swisstargetprediction.ch/) in April 2025 [14]. The platform predicts macromolecular targets using 2D/3D similarity to known ligands. The analysis was conducted specifically for *Homo sapiens* proteins. SMILES strings for each compound were retrieved from PubChem and used as input. The prediction results were obtained as probability scores and presented in tabular format and pie charts showing the distribution of potential protein classes.

2.8. ADME and drug likeness profiling

The SwissADME web tool (http://www.swissadme.ch/), accessible in April 2025, was used to assess the pharmacokinetic characteristics and drug similarity of the chosen drugs [15]. The compounds' SMILES strings were uploaded to the site for in-depth examination.

Drug likeness was evaluated according to five established filters: Lipinski (Pfizer), Ghose (Amgen), Veber (GSK), Egan (Pharmacia), and Muegge (Bayer) rules. Gastrointestinal absorption and BBB permeability were predicted using the BOILED-Egg model [16].

2.9. Molecular docking studies

Molecular docking was conducted to evaluate binding modes and affinities toward predicted targets. Based on target prediction results, CYP19A1 (cytochrome P450 19A1) was selected for naringin, while NOX4 (NADPH oxidase 4) was selected for both rhamnazin and rhamnetin.

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2.10. Protein preparation

Crystal structures of target proteins were obtained from the RCSB Protein Data Bank (https://www.rcsb.org/) [17]. The following PDB entries were used: CYP19A1 (PDB ID: 3EQM) and NOX4 (PDB ID: 7DPZ). Polar hydrogen and Gasteiger charges were added using AutoDock Tools (v1.5.7), and proteins were saved in PDBQT format for docking.

2.11. Docking protocol

Molecular docking was carried out using CB-Dock2 (https://cadd.labshare.cn/cb-dock2/) in May 2025 [18]. The protein structures in PDB format and ligand structures in MOL2 format were uploaded to the server. Docking calculations were performed using the AutoDock Vina algorithm integrated into the CB-Dock2 platform. For every protein-ligand combination, the best five binding postures were produced after the binding sites were predicted using cavity detection.

2.12. Docking validation

Docking validation was performed by re-docking co-crystallized ligands, and RMSD values between predicted and experimental poses were calculated. For the docking approach, RMSD values less than 2.0 Å were deemed acceptable.

2.13. Visualization and analysis

Docking results were analyzed via binding free energies (ΔG) and interaction patterns. The best poses (lowest ΔG) were selected for each complex. Interaction diagrams (2D/3D) were generated using BIOVIA Discovery Studio Visualizer (v2021). Non-covalent interactions (hydrogen bonds, hydrophobic contacts, and π–π stacking) were identified and recorded using BIOVIA Discovery Studio Visualizer 2025 (Dassault Systèmes, San Diego, CA, USA).

2.14. Statistical Analysis

All computational predictions were performed with default parameters of the respective online tools. Molecular docking results were obtained from three independent docking runs to ensure reproducibility. Binding energy values were reported as mean values. The interaction patterns were manually verified for consistency across multiple poses.

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3. Results

3.1. Extraction Yield of Propolis Samples

The yields of the obtained extracts were calculated as percentages (Table 2).

Table 2. Percentage yields of propolis extracts

Sample Code	Yield %
PP1	7.3 %
PP2	7.1 %
PP3	6.3 %
PP4	6.9 %

3.2. Biological activities and Phenolics and Flavonoid Contents

The total flavonoid and phenolics contents and DPPH radical scavenging activities of all extracts are given in Table 3. It was observed that the highest total phenolic content was in sample PP4 (65,3 ± 9,3 mg GAE/g) and the lowest was in sample PP1 (32,9 ± 4,7 mg Gallic Acid Equivalent (GAE)/g). The highest total flavonoid content was observed in sample PP4 (6,5 ± 0,6 mg Qurcetin Equivalent (QE)/g) and the lowest in sample PP2 (3,9 ± 0,4 mg QE/g). According to the Table 3, the Inhibition Concentration (IC_{50}) values of the propolis extracts range from 4.2 to 7.1 mg/mL.

Table 3. Total flavonoid- phenolics content and DPPH• radical scavenging activity of ethanolic extracts of propolis samples

Sample Code	Total Flavonoid Content (mg QE/g extract ±SD)	Total Phenolic Content (mg GAE/g extract ±SD)	DPPH• radical scavenging activity IC_{50} (mg/mL)
PP1	4.3 ± 0.4	32.9 ± 4.7	7.1 ± 0.7
PP2	3.9 ± 0.4	37.3 ± 5.9	6.3 ± 0.5
PP3	5.7 ± 0.7	64.9 ± 8.8	6.6 ± 0.6
PP4	6.5 ± 0.6	65.3 ± 9.3	4.2 ± 0.3
Ascorbic acid	-	-	0.5 ± 0.1

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3.3. Target Prediction Analysis

The analysis revealed distinct target profiles for each compound, with predictions spanning multiple protein classes including enzymes, family A G protein-coupled receptors, and nuclear receptors (Figure 1).

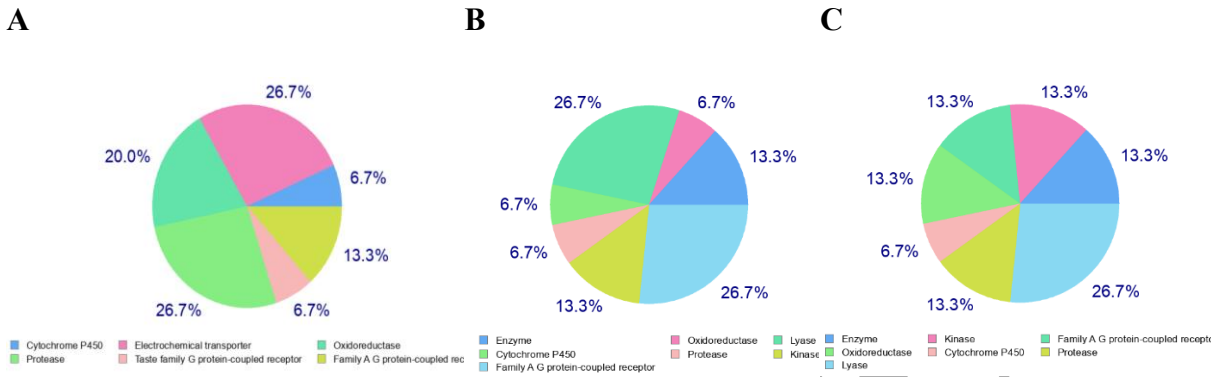


Figure 1. All protein classes with the potential for interaction with the naringin (A), rhamnazin (B), and rhamnetin (C) molecules for *Homo sapiens*

CYP19A1 (aromatase) was identified as a high probability target for naringin among cytochrome P450 enzymes. For both rhamnazin and rhamnetin, the target prediction analysis identified NOX4 (NADPH oxidase 4) as a high probability target. Table 4 summarizes the three target proteins showing the highest correlation with each bioactive compound based on the SwissTargetPrediction analysis.

Table 4. The proteins with high interaction with naringin, rhamnetin, and rhamnazin molecules

Bioactive Compounds	Target
Naringin	CYP19A1 (Cytochrome P450 19A1)
Rhamnazin	NOX4 (NADPH oxidase 4)
Rhamnetin	NOX4 (NADPH oxidase 4)

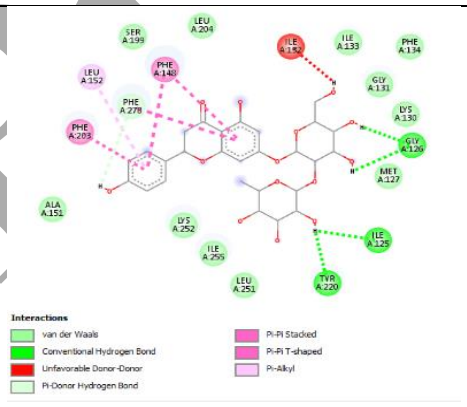
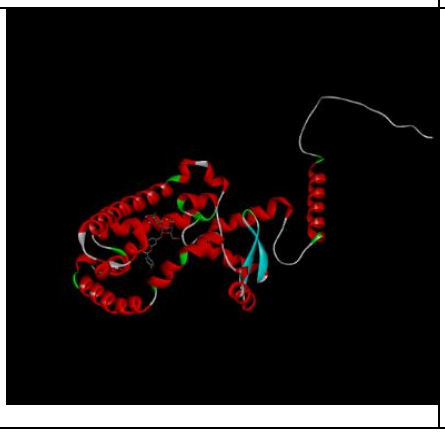
CYP19A1 and NOX4 targeting aligns with propolis's known hormonal and antioxidant activities [4]. NOX4 targeting by rhamnazin and rhamnetin is significant, as NOX4 is implicated in oxidative stress and fibrosis-related diseases [19-21].

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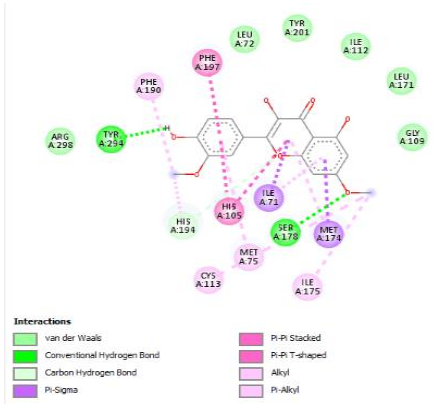
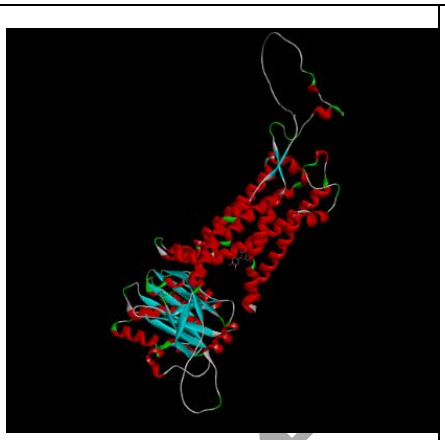
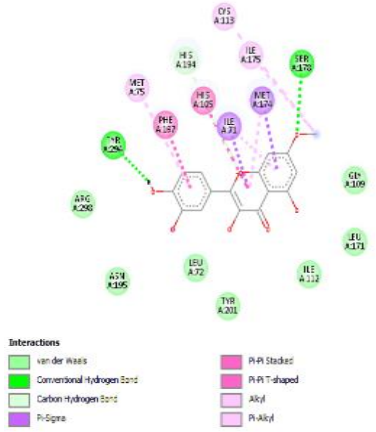
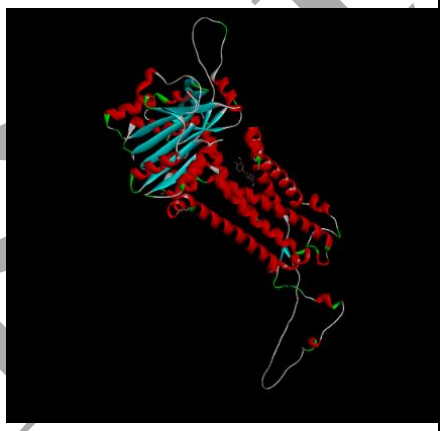
3.4. Molecular Docking Studies

The binding energies and interaction patterns were analyzed to assess the potential of these compounds as modulators of the target proteins. Table 5 presents the molecular docking results including binding energies and key interactions for each compound-target complex. Docking validation was attempted through the redocking of co-crystallized ligands into their respective protein binding sites. The purpose of this procedure was to assess the ability of the applied docking protocol to reproduce experimentally determined ligand-binding orientations. The predicted and experimentally determined binding poses were intended to be compared using root-mean-square deviation (RMSD), with values below 2.0 Å generally considered indicative of acceptable pose reproduction. Although the redocking procedure was performed, the RMSD values could not be successfully retrieved due to technical limitations encountered during the validation process. Consequently, quantitative RMSD-based validation results were not available for inclusion in the present study. The docking findings should therefore be interpreted as preliminary computational predictions requiring further methodological and experimental verification.

Table 5. Molecular docking results for naringin, rhamnazin, and rhamnetin with their respective target proteins

Compounds/ Taget	2D	3D	ΔG (kcal/mol)
Naringin/ CYP19A1			-8.2

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Rhamnazin/ NOX4			-8.5
Rhamnetin/ NOX4			-8.6

Two-dimensional and three-dimensional representations of molecular interactions between the selected compounds and their target proteins. (A) Naringin with CYP19A1 showing hydrogen bonds with Arg115, Met374, Ser478, and Glu483. (B) Rhamnazin with NOX4 showing hydrogen bonds with His94 and His222 and hydrophobic interactions in the binding pocket. (C) Rhamnetin and NOX4 exhibit hydrophobic interactions and many hydrogen bonds with important active site residues. All compounds exhibited strong binding affinities (ΔG < -6.0 kcal/mol): rhamnetin (-8.6 kcal/mol) > rhamnazin (-8.5 kcal/mol) for NOX4, and naringin (-8.2 kcal/mol) for CYP19A1 (Figure 2).

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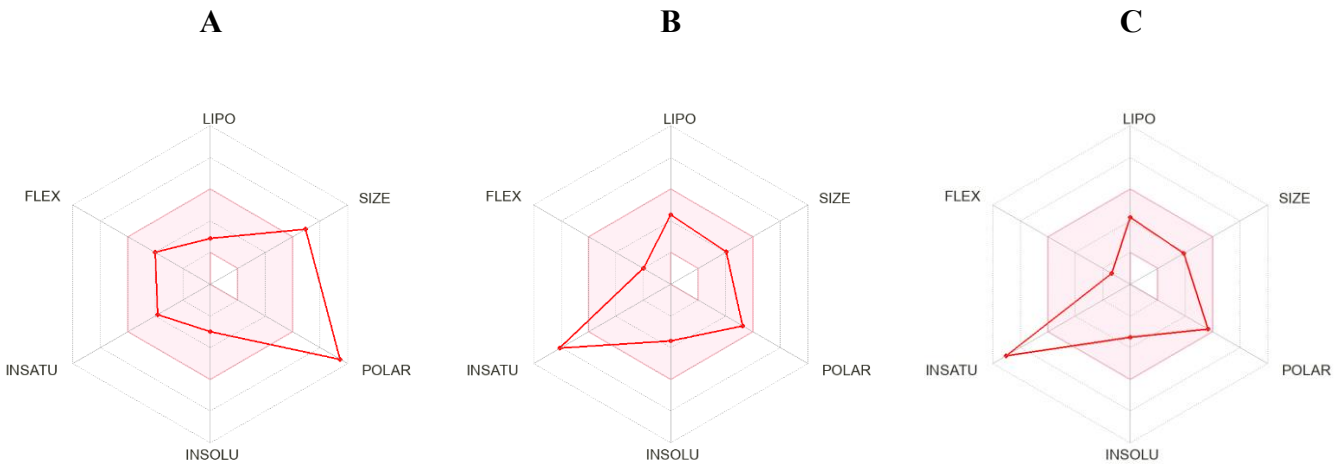


Figure 2. Bioavailability radar charts of naringin (A), rhamnazin (B), and rhamnetin (C) compounds. LIPO: lipophilicity (-0.7 < XLOGP3 < +5.0), SIZE: molecular weight (150-500 g/mol), POLAR: polarity (20-130 Å² TPSA), INSOLU: insolubility (0 < Log S < 6), INSATU: unsaturation (0.25-1.00 fraction Csp3), and FLEX: flexibility (0-9 rotatable bonds) represents the optimal physicochemical range for oral bioavailability

Naringin showed strong binding to CYP19A1 (-8.2 kcal/mol), comparable to other flavonoids. Naringin and naringenin inhibit aromatase, potentially modulating estrogen levels [18]. Binding to active site residues (Arg115, Met374, Ser478) suggests competitive inhibition, relevant to hormone-dependent cancers and metabolic disorders [19-21]. For the NOX4 interactions, both rhamnazin and rhamnetin showed binding energies exceeding -8.5 kcal/mol, indicating strong potential as NOX4 inhibitors. Previous research has identified flavonoids as promising NOX4 inhibitors, with several studies demonstrating that flavonoid-rich extracts can reduce NOX4 expression and activity in cellular models of oxidative stress. The binding energies measured for rhamnazin and rhamnetin are equivalent to or better than those reported for synthetic NOX4 inhibitors currently under research [19]. The quantity and location of methoxy groups in rhamnetin (7-methoxy-3,3',4',5-tetrahydroxyflavone) and rhamnazin (3,5,4'-trihydroxy-7,3'-dimethoxyflavone) vary, which may affect the binding pocket's ability to form hydrogen bonds and hydrophobic interactions. The extra hydroxyl group in rhamnetin may enable stronger hydrogen bonding with active site residues, accounting for its enhanced binding affinity (Figure 2-3).

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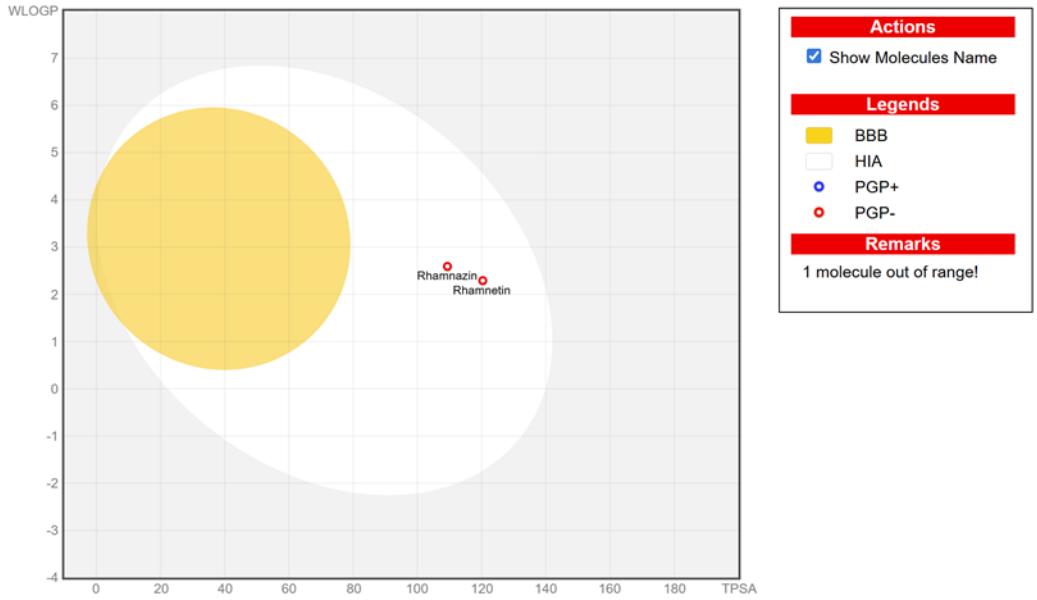


Figure 3. BOILED-Egg model of compounds

The bioavailability radar for naringin showed that this compound falls outside the optimal range for multiple parameters, particularly SIZE (molecular weight) and POLAR (polarity) (Figure 2-3, Table 6).

Table 6. Drug likeness properties of naringin, rhamnazin, and rhamnetin compounds.

	Lipinski	Ghose	Veber	Egan	Muegge	Bioavailability Score
Naringin*	No	No	No	No	No	0.17
Rhamnazin	Yes	Yes	Yes	Yes	Yes	0.55
Rhamnetin	Yes	Yes	Yes	Yes	Yes	0.55

(Naringin*; Lipinski; 3 violations: MW>500, NorO>10, NHorOH>5, Ghose; 4 violations: MW>480, WLOGP<-0.4, MR>130, #atoms>70, Veber; 1 violation: TPSA>140, Egan; 1 violation: TPSA>131.6, Muegge; 3 violations: TPSA>150, H-acc>10, H-don>5)

Naringin violated multiple drug-likeness filters due to its high molecular weight and hydrogen bond count, typical of flavonoid glycosides that reduce passive membrane permeability.

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4. Discussion

The experimental findings obtained from the Kazakhstan propolis extracts provide a complementary context for the *in silico* results. As shown in Table 3, the extracts differed considerably in their total phenolic and flavonoid contents and DPPH radical-scavenging activities. PP3 and PP4 exhibited the highest total phenolic contents (64.9 ± 8.8 and 65.3 ± 9.3 mg GAE/g extract, respectively), whereas PP4 also showed the highest total flavonoid content (6.5 ± 0.6 mg QE/g extract) and the lowest DPPH IC_{50} value (4.2 ± 0.3 mg/mL), indicating the strongest radical-scavenging activity among the tested propolis extracts. In contrast, PP1, which had the lowest total phenolic content (32.9 ± 4.7 mg GAE/g extract), showed the highest DPPH IC_{50} value (7.1 ± 0.7 mg/mL). Overall, this pattern is qualitatively consistent with the recognized contribution of phenolic and flavonoid constituents to the antioxidant capacity of propolis. The relatively lower IC_{50} values observed in extracts with higher phenolic/flavonoid contents suggest that these compound classes may contribute substantially to the measured radical-scavenging activity, although the present data do not establish a direct causal relationship. These experimental observations also provide a biological context for the molecular docking results obtained for rhamnazin and rhamnetin against NOX4. Both compounds are flavonoid-type phenolic constituents and showed favorable predicted interactions with NOX4, a protein involved in the generation of reactive oxygen species. Therefore, the antioxidant activity observed at the extract level and the predicted interaction of selected phenolic compounds with NOX4 can be considered complementary findings that support a hypothesis for the biological activity of propolis. However, this relationship should be interpreted cautiously because TPC, TFC, and DPPH assays measure the collective activity of complex propolis extracts, whereas docking evaluates individual compounds against a specific molecular target. Since the concentrations of naringin, rhamnazin, and rhamnetin were not determined in the tested extracts, the present results cannot be used to attribute the measured antioxidant activity specifically to any one of these compounds. Chemical profiling and compound-specific antioxidant and NOX4 inhibition assays will therefore be required to experimentally establish the contribution of these molecules to the biological activity of the Kazakhstan propolis samples.

Naringin's interaction with CYP19A1 suggests potential in estrogen-related conditions, including hormone receptor-positive breast cancer. Naringin and its aglycone naringenin reduce estrogen biosynthesis and show protective effects against mammary carcinogenesis

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[20]. Naringin is also studied in metabolic disorders involving estrogen metabolism and oxidative stress [21].

NOX4 is involved in hypertension, atherosclerosis, cardiac fibrosis, diabetic nephropathy, and pulmonary fibrosis [19-21]. Flavonoids with similar structures show antifibrotic effects via NOX4 modulation [14,19]. The bioavailability scores of 0.55 are comparable to those of many approved drugs and suggest that these compounds warrant further investigation in preclinical models. The SwissADME analysis further indicated differences in the predicted drug-likeness of the selected compounds. Rhamnazin and rhamnetin satisfied the evaluated drug-likeness filters and had predicted bioavailability scores of 0.55, whereas naringin showed multiple violations and a lower predicted bioavailability score of 0.17. These results suggest that the physicochemical properties of the selected compounds may influence their pharmacokinetic behavior. Nevertheless, these values are computational predictions and should not be interpreted as direct evidence of oral bioavailability or therapeutic efficacy. Experimental pharmacokinetic studies will be required to determine whether the predicted properties are maintained under biological conditions.

Initially, the substances chosen for examination, while commonly reported in propolis, may not fully represent the chemical complexity of propolis samples from Kazakhstan. Future studies should include chemical characterization of actual propolis samples collected from different regions of Kazakhstan to determine the specific compounds present and their concentrations.

Second, Computational predictions require experimental validation through *in vitro* binding and activity assays to confirm CYP19A1 and NOX4 inhibition. A limitation of the present study is that, although a redocking-based validation procedure was attempted, quantitative RMSD values could not be retrieved due to technical limitations. Therefore, the reliability of the predicted binding poses cannot be conclusively established through RMSD-based validation in the current analysis. Further studies incorporating reproducible validation protocols and experimental assays are warranted to verify the predicted protein–ligand interactions. Third, ADME predictions require experimental validation, and naringin's metabolism to naringenin may influence its *in vivo* activity. Fourth, synergistic interactions among propolis compounds were not addressed and warrant future investigation.

5. Conclusions

Naringin showed promising interaction with CYP19A1 (-8.2 kcal/mol), while rhamnazin and rhamnetin demonstrated strong affinities for NOX4 (-8.5 and -8.6 kcal/mol respectively). This

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binding profile is consistent with a computationally inferred, hypothesis-generating mechanistic contribution to therapeutic potential of propolis. ADME profiling revealed that while naringin showed multiple violations of drug likeness filters with a bioavailability score of 0.17, both rhamnazin and rhamnetin satisfied all evaluated criteria with favorable scores of 0.55, indicating their potential for oral administration. Future work should include chemical characterization of propolis from different regions of Kazakhstan and experimental validation of the predicted interactions through *in vitro* and *in vivo* studies.

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Writing – review and editing: S.D.D., Z.S., S.K., A.O., M.Y.N.

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Conflicts of Interest: The authors declare no conflicts of interest.

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