Mechanistic Evaluation of the Antidiabetic Potential of Kazakhstan Honey through *in vitro* and *in silico* Approaches

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ABSTRACT

Introduction: This study evaluated the antioxidant, antidiabetic, and antiglycation properties of honey samples from southern Kazakhstan and investigated their underlying molecular mechanisms using integrated in vitro and in silico approaches.

Methods: Honey samples were evaluated for total phenolic and flavonoid contents, DPPH radical scavenging, α-glucosidase inhibition, and bovine serum albumin glycation inhibition. Representative phenolic compounds were further analyzed by network pharmacology, protein–protein interaction analysis, Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway enrichment, and molecular docking.

Results: DPPH radical scavenging IC_{50} values ranged from 157.9 to 337.8 μg/mL. Total phenolic and flavonoid contents ranged from 98.3–116.7 mg GAE/g and 5.3–6.9 mg QE/g extract, respectively. BSA glycation and α-glucosidase inhibition IC_{50} values ranged from 318–443 and 36.7–65.3 μg/mL, respectively. HC showed the antioxidant activity (IC_{50} = 157.9 μg/mL) and α-glucosidase inhibitory (IC_{50} = 37.9 μg/mL) activities, whereas H13 exhibited the highest antiglycation activity (IC_{50} = 318 μg/mL). Computational analyses identified STAT3, EGFR, ALB, and SRC as hub proteins, highlighted the PI3K–Akt pathway, and demonstrated favorable docking interactions and pharmacokinetic properties of the investigated phenolic compounds.

Conclusion: This study provides the first integrated experimental and computational evidence supporting the multitarget antidiabetic potential of Kazakhstan honey, highlighting its promise as a functional food and a source of natural lead compounds for nutraceutical and pharmaceutical applications.

Keywords: α-glucosidase inhibition, BSA glycation inhibition, In silico, Kazakhstan Honey, T2DM.

1. Introduction

Honey is a biologically active natural product composed of carbohydrates, phenolic acids, flavonoids, enzymes, organic acids, amino acids, vitamins, and minerals [1,2]. Among these constituents, phenolic compounds and flavonoids are the primary contributors to its antioxidant capacity, whereas glucose oxidase-derived hydrogen peroxide and its naturally acidic pH account for its antimicrobial activity [1,2]. Owing to this unique composition, honey is increasingly recognized as a functional food with antioxidant, antimicrobial, anti-inflammatory, antiproliferative, and immunomodulatory properties, which may contribute to the prevention of chronic diseases, including cardiovascular disorders, cancer, neurological diseases, and diabetes mellitus [2,3].

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by insulin resistance, progressive pancreatic β-cell dysfunction, and persistent hyperglycemia, leading to severe vascular complications [4]. Because oxidative stress, chronic inflammation, and postprandial hyperglycemia are key drivers of disease progression, natural products capable of targeting these interconnected mechanisms have attracted increasing interest. Honey has been reported to improve glycemic control and oxidative status; however, the molecular mechanisms underlying these effects remain incompletely understood. Its biological activity is largely determined by its phenolic composition, which varies according to botanical and geographical origin. Kazakhstan, with its rich floral diversity and favorable conditions for apiculture, represents a valuable yet underexplored source of bioactive honey. Nevertheless, studies integrating antioxidant, α-glucosidase inhibitory, and antiglycation activities with computational approaches to elucidate the molecular mechanisms of Kazakhstan honey remain scarce.

Recent advances in network pharmacology and molecular docking have facilitated the investigation of the multitarget mechanisms of natural products [5]. Accordingly, this study evaluated the antidiabetic potential of honey samples collected from fifteen localities in southern Kazakhstan by integrating antioxidant, α-glucosidase inhibitory, and bovine serum albumin (BSA) glycation inhibition assays with network pharmacology, protein–protein interaction analysis, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses, and molecular docking. This integrated approach provides

mechanistic insight into the multifunctional antidiabetic properties of Kazakhstan honey and highlights its potential as a functional food and a source of bioactive compounds.

2. Materials and Methods

2.1. The Collection of Honey Samples

Honey samples were collected from 15 distinct localities in southern Kazakhstan (Table 1).

Table 1. Honey samples collected from Kazakhstan

Sample	Location
H2	Station 1, Shauldir, Kogam village
H3	Station 1, Kelintobe village, Zhanakorgan
HC	Station 1, Shymkent
H4	Station 2, Kelintobe village, Kyzylorda region
H5	Station 3, Zhanakorgan district, Kyzylorda region
H6	Station 4, Kyzylorda region
H7	Station 5, Kelintobe village
H8	Station 2, Shauldir, Kogam village
H9	Station 3, Otrar district, Turkestan region
H10	Station 1, Shieli district, Kyzylorda region
H11	Station 4, Shauldir, Turkestan region
H12	Station 5, Shauldir, Otrar district
H13	Station 6, Kogam village, Otrar district
H14	Station 7, Kogam village, Turkestan region
H15	Station 8, Turkestan region

2.2. Extraction of Honey Samples

Honey samples were obtained from certified local beekeepers in Southern Kazakhstan. The collected samples were stored at 4°C until analysis. For extraction, 5 g of each sample was mixed with 100 mL of methanol and incubated in a shaker incubator at room temperature for

24 h. The mixtures were filtered using Whatman No.1 filter paper and concentrated under reduced pressure using a rotary evaporator at 40°C. The obtained crude extracts were lyophilized and stored at −20°C until further analyses. Stock solutions were prepared in dimethyl sulfoxide (DMSO) before biological assays.

2.3. DPPH Radical Scavenging Assay

The antioxidant activity of the extracts was evaluated using the DPPH free radical scavenging assay [6]. Different concentrations of extracts (10–500 µg/mL) were prepared and mixed with 0.1 mM DPPH solution. The reaction mixtures were incubated in the dark for 30 min at room temperature. Absorbance was measured at 517 nm using a microplate reader. Radical scavenging activity was calculated as percentage inhibition relative to the control group, and IC50 values were determined.

2.4. Total phenolic contents (TPC)

To determine the total phenolic content of the honey samples extracts, the spectrophotometric Folin-Ciocalteu method was used as previously described with slight modification by Clarke et al. [6]. Total phenolic content (TPC) was calculated as milligram gallic acid equivalents per gram of dry extract.

2.5. Total flavonoid content (TFC)

TFC was determined using the aluminum chloride colorimetric assay. Briefly, 0.5 mL of extract was mixed with 0.5 mL of 2% aluminum chloride solution and incubated for 30 min at room temperature. Absorbance was measured at 415 nm. Quercetin was used for calibration and the results were expressed as mg quercetin equivalents (QE)/g extract [7].

2.6. Alpha- Glucosidase inhibition test

The α-glucosidase activity of the extracts was evaluated as previously described in literature with minor modification [8]. Acarbose and phosphate buffer were utilized as positive and negative control standards, respectively.

2.7. BSA glycation inhibition assay

Advanced Glycation End Products (AGEs) formation was assessed using a Bovine Serum Albumin (BSA; Sigma-Aldrich)-glucose glycation model with fluorescence detection (Ex 330 nm/Em 440 nm), as previously described with minor modifications [9].

2.8. Target Prediction for Bioactive Compounds

The three-dimensional structures of fumaric acid, p-hydroxybenzoic acid, p-coumaric acid, trans-2-hydroxycinnamic acid, and chrysin, reported as constituents of Kazakhstan honey

samples, were retrieved from the PubChem database on February 15, 2026 [10,11]. Putative gene targets for these compounds were obtained using the SwissTargetPrediction database, accessed on February 15, 2026 [12].

2.9. T2DM-Associated Target Identification

Disease-associated genes for T2DM were retrieved from the GeneCards database on February 15, 2026. The search term "Type 2 Diabetes Mellitus" was entered, and genes with a relevance score of 30 or above were included in the analysis [13]. The relevance score threshold of ≥30 was used to prioritize genes showing a stronger association with T2DM and to reduce noise potentially arising from genes with low association levels. This approach is consistent with filtering strategies frequently employed in network pharmacology studies.

2.10. PPI Network Construction

The jvenn tool was used to identify overlapping targets between the honey-derived bioactive compounds and T2DM [14]. To characterize the protein interaction network of overlapping genes, data were imported into the STRING database on February 17, 2026, with species set to *Homo sapien*s and interactions filtered at medium confidence or higher (score >= 0.400) [15]. The resulting protein interaction data were imported into Cytoscape version 3.10.4 for network visualization and analysis [16]. SwissTargetPrediction and GeneCards are widely used and validated bioinformatics databases in the literature. To enhance reliability, only the common targets found at the intersection of compound targets and T2DM-associated genes were included in subsequent analyses.

2.11. GO Enrichment and KEGG Pathway Analysis

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed using ShinyGO 0.82, with species set to *Homo sapiens* and significance threshold of $P < 0.05$. The database was accessed on February 18, 2026 [17].

2.12. Molecular Docking

Following PPI analysis, the target protein with the highest degree value was selected. The three-dimensional crystal structure of the identified target protein was retrieved from the RCSB Protein Data Bank on February 19, 2026 [18]. The three-dimensional molecular structures of the ligands were obtained from the PubChem database [11]. Ligand binding mode and interaction analyses were performed using AutoDock 4.2 software with the integrated AutoDockTools (MGLTools 1.5.7) interface [19]. Optimal binding conformations and

interaction profiles of receptor-ligand complexes were visualized using BIOVIA Discovery Studio Visualizer (Version 25.1) [20,21].

2.13. Statistical Analysis

All experiments were performed in triplicate and results were expressed as mean ± standard deviation (SD). Statistical analyses were carried out using GraphPad Prism software. Statistical significance was considered at $P< 0.05$.

3. Results

3.1. Extraction Yield of Bee Products

The extraction yields of the bee product samples are summarized in Table 2. Methanolic extracts were used for total phenolic content analysis due to the well-established association between phenolic compounds and biological activity. Variations among samples may be attributed to differences in geographical origin and seasonal conditions.

Table 2. Percentage yields, DPPH activity, TPC and TFC content of honey extracts

Samples	Yield %	DPPH• radical scavenging activity IC50 (µg/mL)	Total Phenolic Content (mg GAE/g extract ±SD)	Total Flavonoid Content (mg QE/g extract ±SD)	BSA glycation inhibition assay AGE inhibition (IC50; µg/mL)	Alpha-Glucosidase inhibition activity (IC50; µg/mL)
H2	11%	337.8 ± 15.9	110.3 ± 10.6	5.3 ± 0.5	385 ± 34	57.8 ± 6.9
H3	10%	253.7 ± 13.2	111.3 ± 9.6	5.6 ± 1.1	340 ± 25	39.6 ± 6.1
HC	9%	157.9 ± 14.1	105.6 ± 13.9	5.9 ± 1.1	321 ± 23	37.9 ± 6.1
H4	10%	243.8 ± 16.5	104.3 ± 16.9	6.3 ± 1.3	390 ± 40	55.8 ± 3.9
H5	11%	278.6 ± 20.5	110.9 ± 13.6	5.9 ± 0.9	425 ± 45	63.1 ± 9.5
H6	9%	288.3 ± 20.3	114.9 ± 13.6	5.5 ± 0.7	373 ± 30	58.3 ± 6.9
H7	10%	235.4 ± 19.5	99.6 ± 11.3	6.9 ± 1.4	443 ± 51	65.3 ± 9.5
H8	12%	264.2 ± 18.4	113.6 ± 12.9	6.7 ± 1.0	360 ± 39	44.9 ± 6.3
H9	10%	289.9 ± 19.6	101.7 ± 13.9	6.9 ± 1.1	350 ± 30	39.6 ± 5.6
H10	9%	276.6 ± 21.7	113.9 ± 11.6	5.5 ± 0.7	400 ± 44	56.3 ± 9.1
H11	11%	228.4 ± 20.8	116.7 ± 8.9	6.4 ± 1.2	420 ± 42	58.4 ± 3.6
H12	11%	258.13 ± 22.4	98.3 ± 8.6	6.3 ± 1.0	393 ± 40	55.3 ± 6.9
H13	10%	233.7 ± 17.5	99.6 ± 9.3	5.9 ± 0.9	318 ± 22	36.7 ± 9.5
H14	10%	244.9 ± 18.5	106.3 ± 10.9	5.5 ± 0.7	370 ± 33	44.9 ± 6.3
H15	9%	199.1 ± 18.7	109.7 ± 13.6	6.9 ± 0.9	387 ± 30	49.3 ± 7.6
BHA: Reference substance for DPPH	-	8.6 ± 1.9	-	-	--	
Quercetin	-	10.1 ± 1.95	-	-	20	-
Chrysin (Sigma)	-	18.9 ± 3.76	-	-	45	6.5 ± 1.0
Acarbose (Glucobay, Bayer)	-	-	-	-	-	144.9 ± 6.3
Aminoguanidine (Positive control)	-	-	-	-	50	-

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3.2. DPPH radical scavenging activity

The antioxidant activities of the methanolic honey extracts are presented in Table 2. The extracts exhibited DPPH radical scavenging activity with IC_{50} values ranging from 157.9 to 337.8 μg/mL. Lower IC_{50} values indicate stronger antioxidant capacity. Although all extracts showed lower activity than the reference antioxidants (quercetin and BHA), they demonstrated moderate radical scavenging potential.

3.3. Total Phenolic and Flavonoid Contents

The methanolic honey extracts contained moderate amounts of total phenolics and flavonoids (Table 2). Total phenolic content ranged from 98.3 to 116.7 mg GAE/g extract, whereas total flavonoid content varied between 5.3 and 6.9 mg QE/g extract.

3.4. α-Glucosidase Inhibitory Activity

The α-glucosidase inhibitory activities of the methanolic honey extracts are summarized in Table 2. IC_{50} values ranged from 36.7 to 65.3 μg/mL, indicating moderate enzyme inhibitory activity. Acarbose (Glucobay®, Bayer) was used as the positive control for comparison.

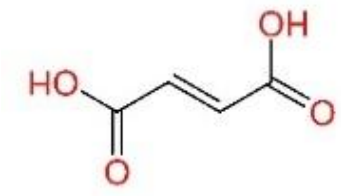
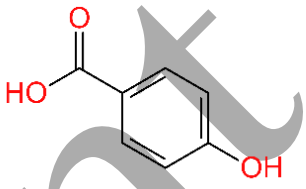
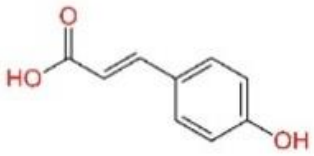
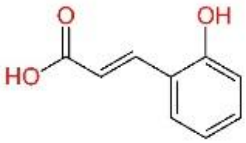
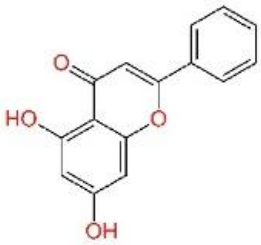
3.5. BSA Glycation Inhibitory Activity

The antiglycation activities of the methanolic honey extracts were evaluated using the BSA glycation inhibition assay, and the results are presented in Table 2. All extracts exhibited inhibitory activity against protein glycation, suggesting their potential to reduce the formation of advanced glycation end products (AGEs).

3.6. Principal Bioactive Compounds

Five representative bioactive compounds were selected based on the phytochemical profile reported by Ongalbek et al. (2024) [22]. Their molecular structures and PubChem CIDs are presented in Table 3. These compounds represent the major phenolic constituents commonly identified in Kazakhstan honey samples [22].

Table 3. Bioactive compounds identified in honey samples.

Compound Name - PubChem CID	Molecular Structure
Fumaric Acid (444972)	
p-Hydroxybenzoic acid (135)	
p-Coumaric acid (637542)	
trans-2-Hydroxycinnamic acid (637540)	
Chrysin (5281607)	

3.7. PPI Network Construction

Genes associated with type 2 diabetes mellitus (T2DM), the five bioactive honey compounds, and their interactions were analyzed using a protein-protein interaction (PPI) network. A total of 125 overlapping target genes were identified (Figure 1). After removing duplicate entries, the overlapping targets were imported into the STRING database (confidence score = 0.4, Homo sapiens), and proteins without interactions were excluded. The PPI network was constructed in Cytoscape (version 3.10.4) (Figures 1 and 2). Based on degree centrality, the top 10 hub proteins were identified as ALB, EGFR, STAT3, ESR1, SRC, ERBB2, PTGS2, PPARG, GSK3B, and MMP9 (Table 4).

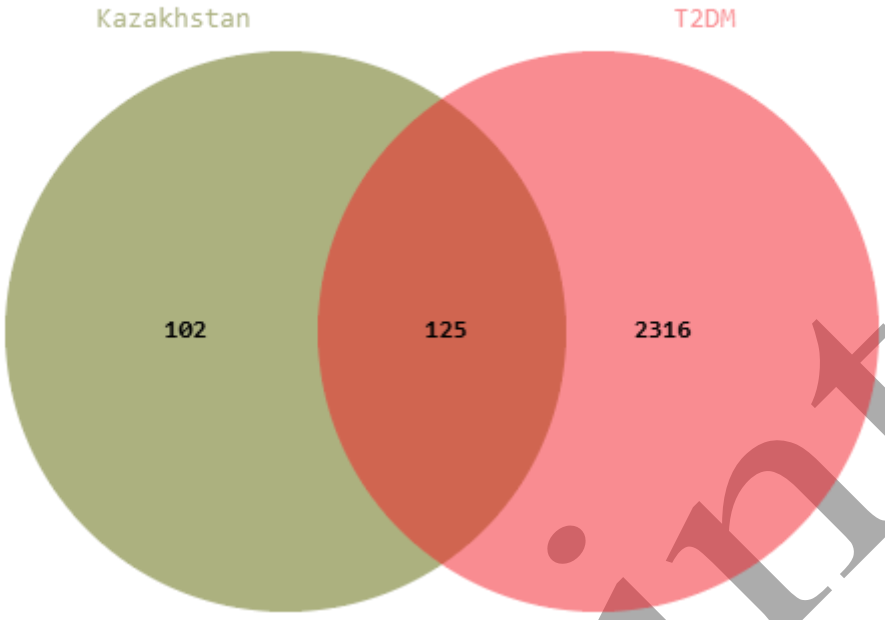


Figure 1. Venn diagram illustrating overlapping genes between T2DM and five bioactive compounds from Kazakhstan honey

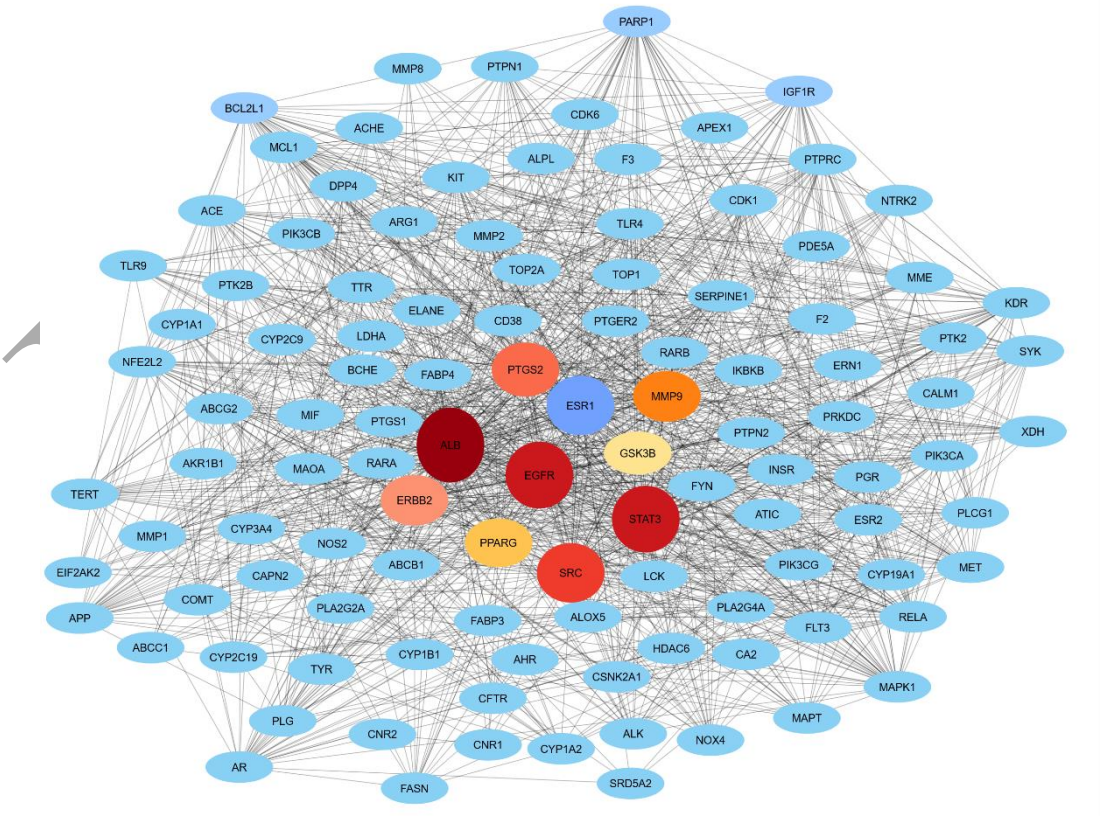


Figure 2. PPI network analysis. Node color intensity reflects degree centrality

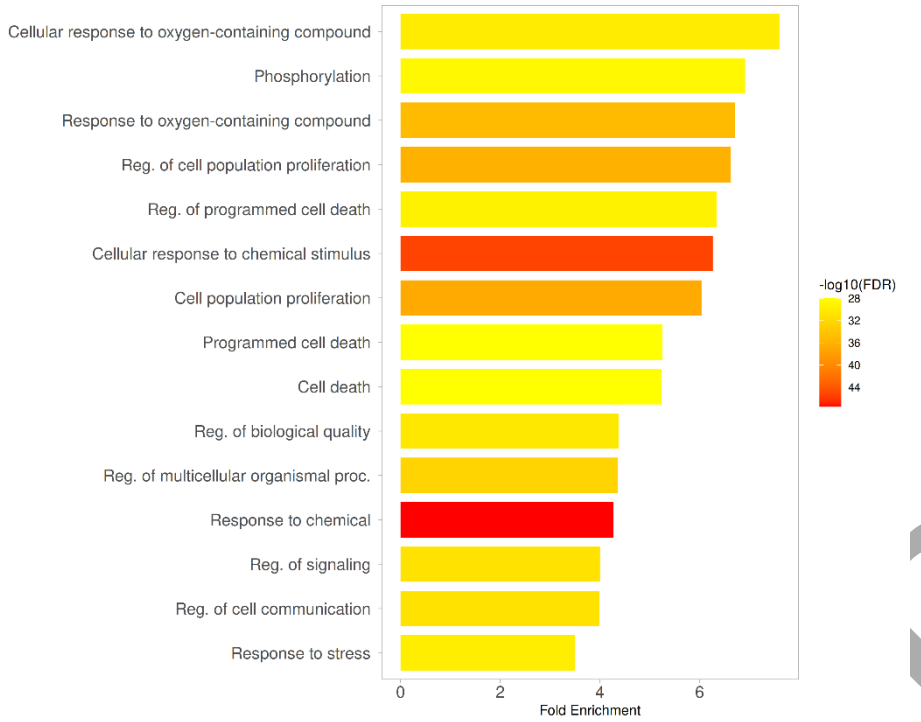
ALB was identified as the node with the highest degree value in the PPI network analysis. ALB is not a classical therapeutic target for T2DM. Therefore, the aim of the docking analysis is not to demonstrate that ALB is a direct antidiabetic target, but rather to evaluate the potential of the compounds to interact with one of the hub proteins highlighted in the network analysis.

Table 4. Top 10 targets ranked by degree value.

Name	Degree	Betweenness Centrality	Closeness Centrality
ALB	83	0.11795	0.80292
EGFR	74	0.06493	0.75342
STAT3	74	0.06792	0.75342
ESR1	66	0.05725	0.71429
SRC	66	0.06323	0.71429
PTGS2	60	0.04133	0.68750
ERBB2	56	0.02338	0.67073
MMP9	56	0.02623	0.67073
PPARG	54	0.03156	0.66265
GSK3B	48	0.01442	0.63953

3.8. GO Enrichment Analysis

GO and KEGG enrichment analyses are presented in Figures 3 and 4, where the p-value indicates statistical significance, fold enrichment reflects the magnitude of enrichment, and gene count represents the number of genes involved in each pathway. GO analysis showed that cellular response to oxygen-containing compound was the most significantly enriched biological process, followed by response to oxygen-containing compound and phosphorylation, highlighting pathways associated with oxidative stress, antioxidant defense, and inflammation (Figure 3a). Among cellular components, membrane-related structures, including membrane microdomains and receptor complexes, were significantly enriched (Figure 3b). Molecular function analysis identified significant enrichment in protein tyrosine kinase activity, protein kinase activity, and phosphotransferase activity, indicating their roles in cell signaling and regulation (Figure 3c).



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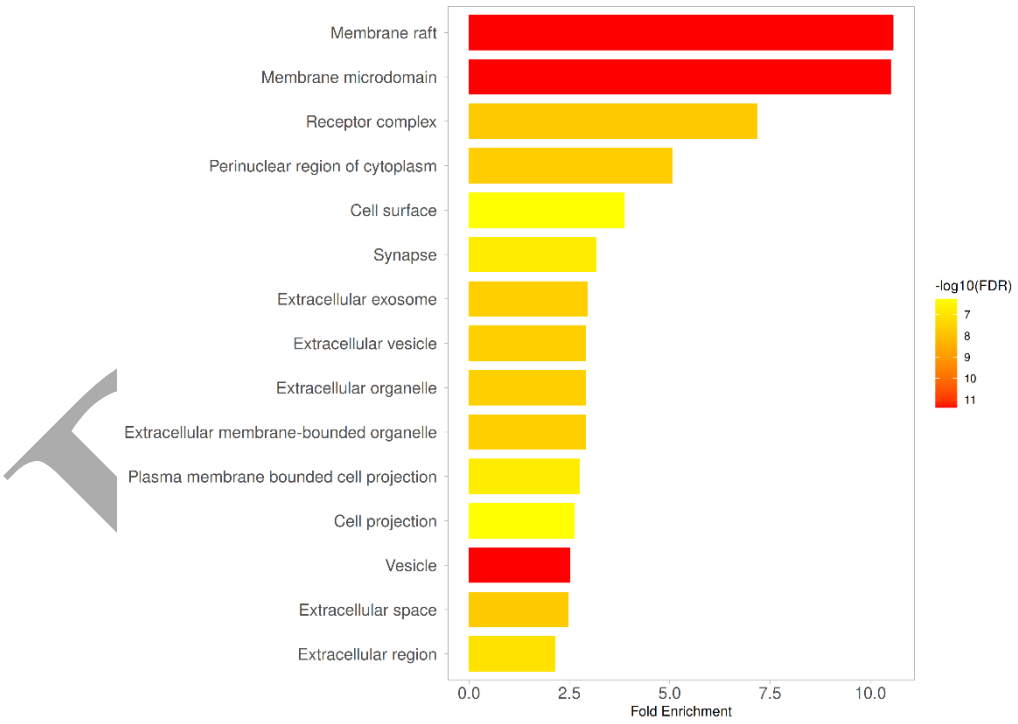
260 **Figure 3a.** GO Biological Process enrichment analysis



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262 **Figure 3b.** GO Cellular Component enrichment analysis

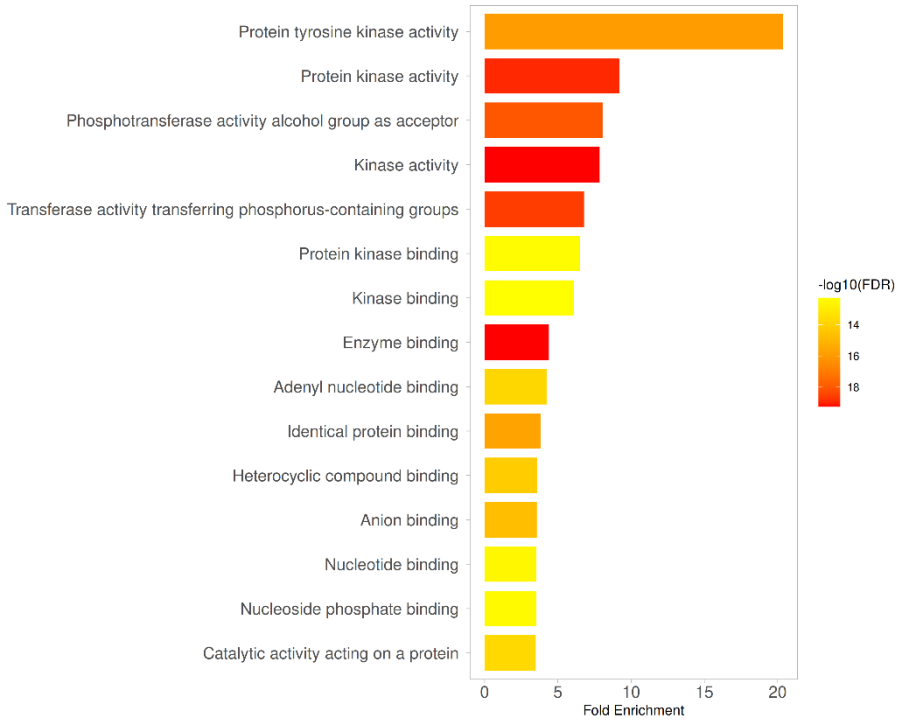


Figure 3c. GO Molecular Function Enrichment analysis

3.9. KEGG Pathway Analysis

The top 25 enriched KEGG pathways are presented in Figures 4 and 5. The resistance to EGFR tyrosine kinase inhibitors pathway showed the highest fold enrichment, while cancer-related signaling pathways exhibited the greatest statistical significance. Significant enrichment was also observed for the PD-L1 expression and PD-1 checkpoint and PI3K-Akt signaling pathways, highlighting their potential roles in antioxidant and anti-inflammatory mechanisms.

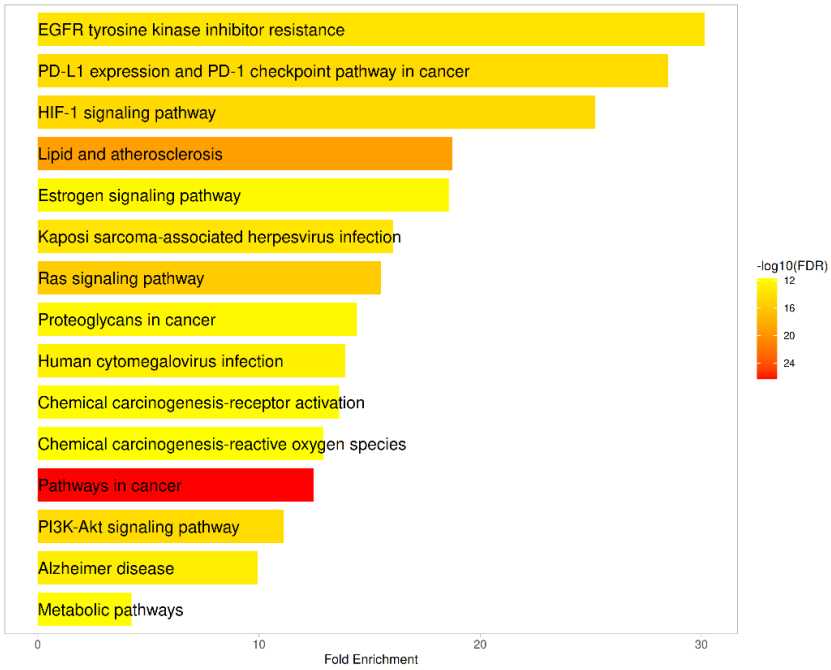


Figure 4. KEGG pathway enrichment analysis

The PI3K-Akt signaling pathway, illustrated in Figure 5, plays a central role in inflammation, diabetes mellitus, and related pathological conditions. Activation of receptor tyrosine kinases (RTKs) and G-protein-coupled receptors (GPCRs) stimulates PI3K, generating phosphatidylinositol-3,4,5-trisphosphate (PIP3) and activating Akt. Akt regulates cell survival, protein synthesis, apoptosis, and cell-cycle progression. Under oxidative stress, Akt promotes anti-apoptotic proteins such as *Bcl-2* and *Bcl-xL*, supporting mitochondrial protection and cell survival. However, excessive oxidative stress may cause DNA damage and cell-cycle arrest, emphasizing the role of cyclin-dependent kinases (CDKs) in cellular integrity (Figure 5).

The *PI3K-Akt* pathway is also a major mediator of insulin signaling and glucose homeostasis. Insulin receptor activation triggers IRS phosphorylation, *PI3K/Akt* activation, GLUT4 translocation, glucose uptake, glycogen synthesis, and suppression of gluconeogenesis. In T2DM, impaired IRS-mediated signaling reduces Akt phosphorylation and GLUT4 translocation, contributing to hyperglycemia, β-cell dysfunction, and vascular complications through impaired eNOS activation. Thus, modulation of PI3K-Akt signaling represents a promising strategy for improving insulin sensitivity and reducing diabetic complications [23,24] (Figure 5).

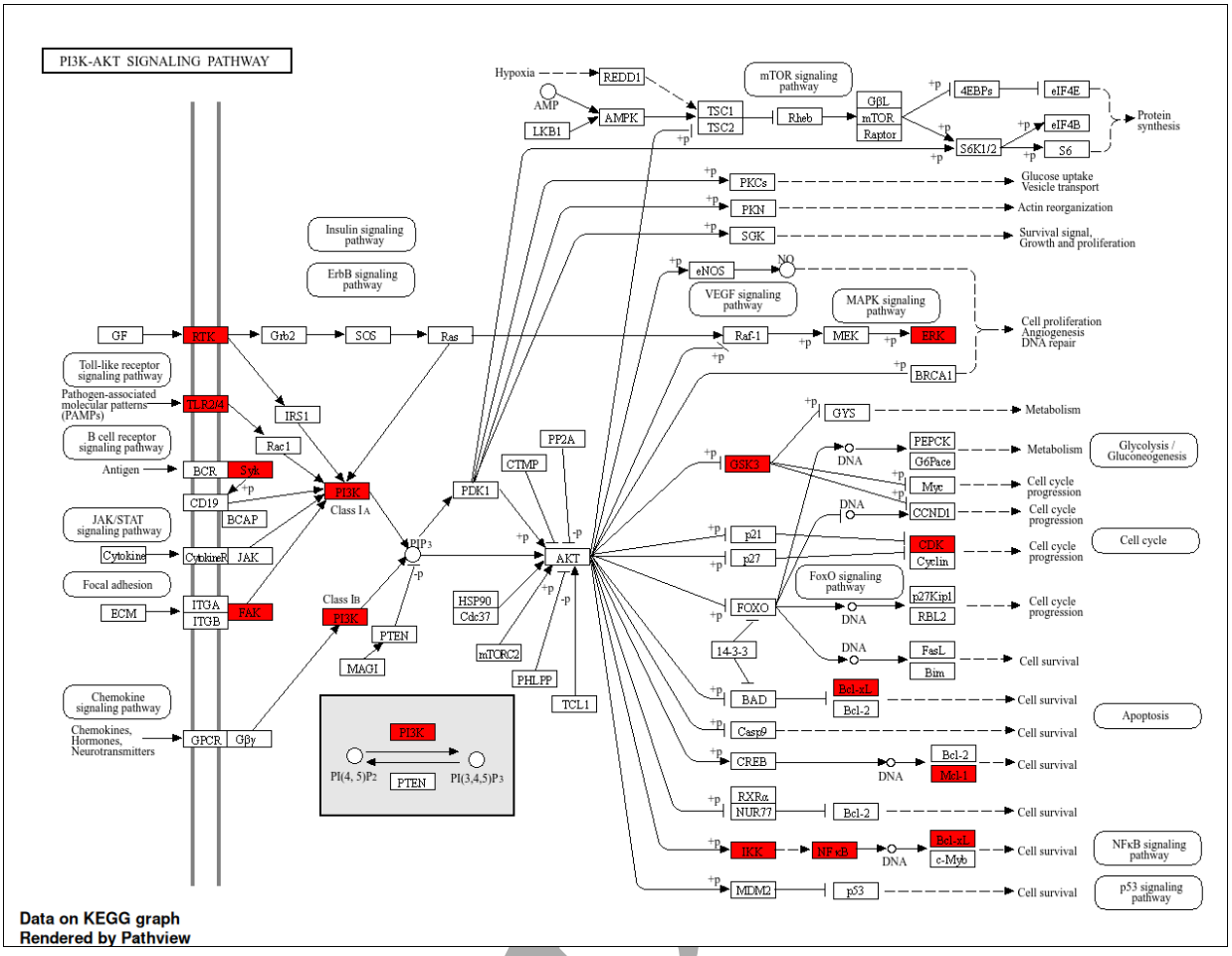
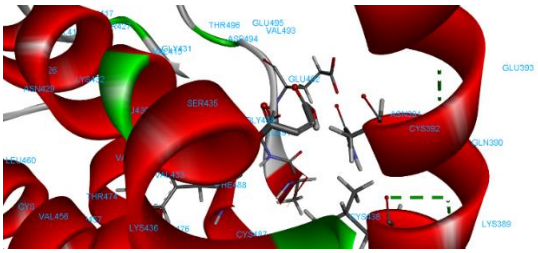
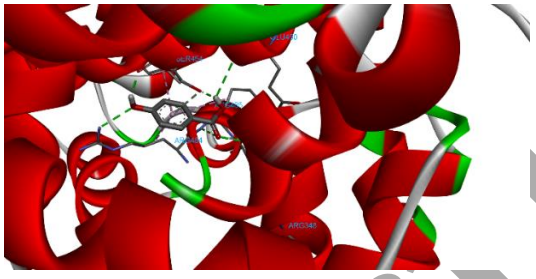
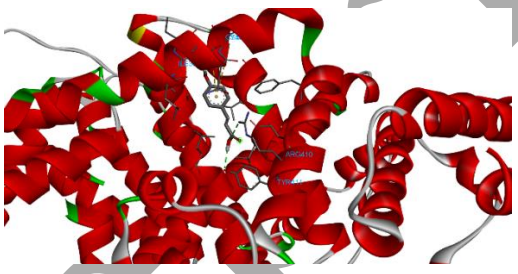
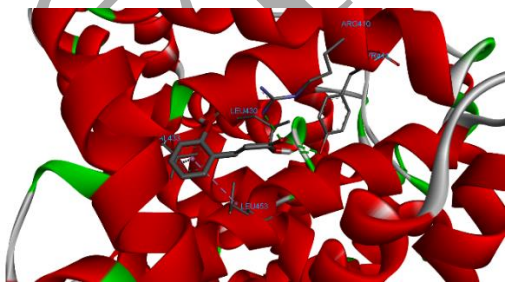
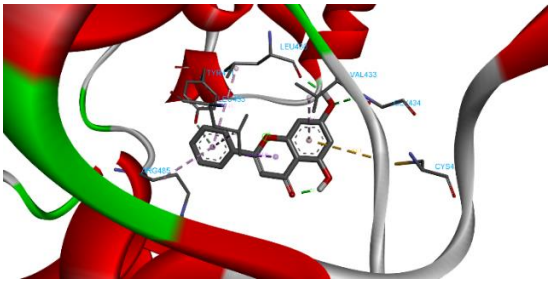


Figure 5. PI3K-AKT signaling pathway with highlighted target genes

3.10. Molecular Docking

Based on the PPI results, the top protein yielding the highest degree values were included in the molecular docking study (Table 5).

Table 5. ALB (1E7A) docking scores.

Compound	2D Structure	Docking Score (kcal/mol)
Fumaric Acid		-3.95
p-Hydroxybenzoic acid		-6.1
p-Coumaric acid		-4.98
trans-2-Hydroxycinnamic acid		-5.2
Chrysin		-7.45

Molecular docking analysis was performed to validate the interactions between the identified bioactive compounds and the core T2DM target protein selected based on the highest degree value in the PPI network. Binding affinity was evaluated using docking scores (kcal/mol), with lower (more negative) values indicating stronger interactions. Among the tested compounds, chrysin exhibited the strongest binding affinity (-7.45 kcal/mol), followed by p-hydroxybenzoic acid (-6.10 kcal/mol), trans-2-hydroxycinnamic acid (-5.20 kcal/mol), and p-coumaric acid (-4.98 kcal/mol). These phenolic compounds established hydrogen-bond and hydrophobic interactions that contributed to binding stability. In contrast, fumaric acid showed the weakest binding affinity (-3.95 kcal/mol), likely due to its smaller molecular structure and limited interaction capacity (Table 5).

4. Discussion

The present study demonstrates that Kazakhstan honey possesses a coordinated multitarget biological profile against key mechanisms involved in type 2 diabetes mellitus (T2DM). Rather than acting solely as a radical scavenger, the investigated honey samples exhibited antioxidant, α-glucosidase inhibitory, and antiglycation activities, indicating their potential to simultaneously modulate oxidative stress, postprandial glucose metabolism, and advanced glycation processes. The integration of biochemical assays with network pharmacology, molecular docking, and pharmacokinetic prediction further provides mechanistic support for these observations. Collectively, the findings suggest that the antidiabetic potential of Kazakhstan honey is associated with the complementary actions of multiple phenolic constituents rather than a single dominant compound.

Oxidative stress contributes substantially to insulin resistance, pancreatic β-cell dysfunction, and diabetic complications through excessive reactive oxygen species (ROS) production and persistent inflammatory signaling. Consistent with this mechanism, all honey samples demonstrated measurable radical-scavenging activity together with appreciable total phenolic and flavonoid contents. Variations among samples may reflect differences in botanical origin and environmental conditions that influence phenolic composition. Thus, the antioxidant capacity of Kazakhstan honey should be interpreted as the collective effect of its complex phenolic matrix, supporting the contribution of synergistic interactions among bioactive phytochemicals [23,24].

Beyond antioxidant activity, Kazakhstan honey exhibited measurable α-glucosidase inhibition, suggesting an additional mechanism relevant to glycemic control. Because α-glucosidase catalyzes the final step of carbohydrate digestion, its inhibition delays glucose absorption and attenuates postprandial hyperglycemia. Although the honey extracts were less active than the reference inhibitor, their activity remains biologically relevant because multiple phenolic constituents may exert additive or synergistic effects within this complex natural matrix [23,24].

The honey samples also demonstrated significant antiglycation activity, indicating their potential to interfere with another process implicated in diabetic complications. Advanced glycation end products (AGEs), oxidative stress, and chronic hyperglycemia are interconnected mechanisms contributing to nephropathy, retinopathy, neuropathy, and cardiovascular disorders. Therefore, the simultaneous antioxidant, α-glucosidase inhibitory, and antiglycation activities observed in Kazakhstan honey suggest potential modulation of multiple stages of T2DM progression. The variability among samples despite relatively similar total phenolic contents further suggests that qualitative differences in phenolic composition may be more influential than total phenolic concentration, highlighting the need for future metabolomic investigations.

To further elucidate the molecular basis of these findings, network pharmacology, enrichment analysis, molecular docking, and pharmacokinetic prediction were employed as complementary computational approaches. Unlike the biochemical assays evaluating whole honey extracts, these analyses focused on representative phenolic constituents and should therefore be interpreted as mechanistic hypotheses rather than direct evidence. Nevertheless, the computational findings were consistent with the experimental observations, providing systems-level insight into the multitarget potential of Kazakhstan honey.

GO and KEGG enrichment analyses indicated that the predicted targets were predominantly associated with oxidative stress responses, inflammatory signaling, glucose metabolism, protein phosphorylation, and cellular responses to chemical stress. Among the enriched pathways, the PI3K–Akt signaling pathway was particularly noteworthy because of its central role in insulin sensitivity, glucose uptake, cell survival, and metabolic homeostasis. Dysregulation of this pathway is a characteristic feature of insulin resistance and contributes to T2DM progression. Thus, enrichment of PI3K–Akt and related pathways provides a biologically plausible explanation for the coordinated antioxidant, α-glucosidase inhibitory, and

antiglycation activities observed experimentally, supporting the involvement of interconnected molecular pathways rather than a single target [21,23,24].

Protein–protein interaction analysis identified *ALB, EGFR, STAT3,* and *SRC* as principal hub proteins within the predicted network. Rather than representing definitive pharmacological targets, these proteins should be regarded as central regulatory nodes involved in glucose metabolism, oxidative stress, inflammatory signaling, and cellular homeostasis. Their identification provides a useful framework for prioritizing future experimental validation while emphasizing the hypothesis-generating nature of network pharmacology.

Molecular docking further supported these findings by demonstrating favorable binding affinities between the selected phenolic compounds and proteins associated with metabolic regulation. Chrysin exhibited the strongest binding affinity, consistent with its reported antioxidant, anti-inflammatory, and antidiabetic properties.

A major strength of the present study is the integration of complementary experimental and computational approaches. Nevertheless, the computational findings require validation through enzyme kinetics, cell-based mechanistic studies, molecular dynamics simulations, and diabetic animal models. Moreover, the cell-free assays cannot fully reproduce the complexity of glucose metabolism and intracellular signaling under physiological conditions. Future studies integrating metabolomic profiling with advanced cellular and in vivo models will be essential to identify the principal bioactive constituents and clarify the synergistic mechanisms underlying these multitarget effects [21,23,24].

Overall, the findings demonstrate that Kazakhstan honey exhibits a coordinated multitarget biological profile involving several interconnected mechanisms implicated in T2DM. Its combined antioxidant, α-glucosidase inhibitory, and antiglycation activities, together with complementary computational evidence, underscore its potential as a functional food and source of naturally derived bioactive compounds. Rather than attributing these effects to a single constituent, the results emphasize the collective contribution of structurally diverse phenolic compounds acting through complementary molecular mechanisms. These findings provide a scientific basis for future translational studies evaluating the potential of Kazakhstan honey in diabetes and other oxidative stress-related metabolic disorders.

5. Conclusion

Kazakhstan honey represents more than a natural sweetener; it is a complex functional matrix enriched with phenolic compounds capable of simultaneously modulating oxidative stress, carbohydrate metabolism, and protein glycation. By integrating in vitro bioactivity assays with network pharmacology, molecular docking, and pharmacokinetic prediction, this study provides a scientifically grounded framework for understanding the molecular basis of its antidiabetic potential. These findings support further investigation of Kazakhstan honey as a promising functional food and as a sustainable source of bioactive molecules for future nutraceutical and pharmaceutical development. The present work exhibits the first integrated experimental and computational evidence supporting the multitarget antidiabetic potential of Kazakhstan honey, emphasising its promise as a source for nutraceutical and pharmaceutical applications.

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Authors' Contribution

Conceptualization: ZS, SDD. Data curation: ZS, SDD, SK, FU. Formal analysis: ZS, SK, ESG. Methodology: ZS, SDD, SK, ESG, AO. Software: SK, ESG, NS, TD. Validation: ZS, SDD, SK, TD. Investigation: ZS, SDD, SK, ESG, FU. Writing - original draft: SDD, SK, ESG, NS. Writing - review and editing: ZS, SDD, SK, AO.

Ethics

Ethics committee approval is not required for this study.

Conflict of Interest

No potential conflicts of interest relevant to this article were reported.

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Data Availability

The data that support the findings of this study are available on request from the corresponding author.

Declaration on the Use of AI

During the preparation of this manuscript, the authors used generative AI tools (Claude Opus 4.6 Extended, Anthropic) to prepare the initial draft of the introduction section and to improve linguistic clarity, grammar, and readability of the English text. All scientific content, data analysis, interpretation, and conclusions were subsequently rewritten, verified, and approved by the authors.

References

[1] Wilczyńska A, Żak N. Polyphenols as the main compounds influencing the antioxidant effect of honey: A review. *Int J Mol Sci*. 2024; 25(19):10606. [DOI:10.3390/ijms251910606]

[2] Zammit Young GW, Blundell R. A review on the phytochemical composition and health applications of honey. *Heliyon.* 2023; 9(2):e12507. [DOI:10.1016/j.heliyon.2022.e12507]

[3] Rahmani AH, Babiker AY. Review on role of honey in disease prevention and treatment through modulation of biological activities. *Open Life Sci*. 2025; 20(1):20251069. [DOI:10.1515/biol-2025-1069]

[4] Lu X, Xie Q, Pan X, Zhang R, Zhang X, Peng G, et al. Type 2 diabetes mellitus in adults: Pathogenesis, prevention and therapy. *Signal Transduct Target Ther*. 2024; 9(1):262. [DOI:10.1038/s41392-024-01951-9]

[5] Sadybekov AV, Katritch V. Computational approaches streamlining drug discovery. *Nature*. 2023; 616(7958):673-85. [DOI:10.1038/s41586-023-05905-z]

[6] Clarke G, Ting KN, Wiart C, Fry J. High correlation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric reducing activity potential and total phenolics content indicates redundancy in use of all three assays to screen for antioxidant activity of extracts of plants from the Malaysian rainforest. *Antioxidants* (Basel). 2013; 2(1):1-10. [DOI:10.3390/antiox2010001]

[7] Bag GC, Grihanjali Devi P, Bhaigyabati T. Assessment of total flavonoid content and antioxidant activity of methanolic rhizome extract of three Hedychium species of Manipur valley. *Int J Pharm Sci Rev Res*. 2015; 30(1):154-9. http://globalresearchonline.net/journalcontents/v30-1/28.pdf

[8] Kumar D, Kumar H, Vedasiromoni JR, Pal BC. Bio-assay guided isolation of α-glucosidase inhibitory constituents from Hibiscus mutabilis leaves. *Phytochem Anal*. 2012; 23(5):421-5. [DOI:10.1002/pca.1375]

[9] Wu CH, Yen GC. Inhibitory effect of naturally occurring flavonoids on the formation of advanced glycation endproducts. *J Agric Food Chem*. 2005; 53(8):3167-73. [DOI:10.1021/jf048550u]

[10] Simoben CV, Babiaka SB, Moumbock AFA, Namba-Nzanguim CT, Eni DB, Medina-Franco JL, et al. Challenges in natural product-based drug discovery assisted with in silico-based methods. *RSC Adv*. 2023; 13(45):31578-94. [DOI:10.1039/d3ra06831e]

[11] Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, et al. PubChem 2025 update. *Nucleic Acids Res*. 2025; 53(D1):D1380-D1388. [DOI:10.1093/nar/gkae1059]

[12] Daina A, Michielin O, Zoete V. SwissTargetPrediction: Updated data and new features. *Nucleic Acids Res*. 2019; 47(W1):W93-W98. [DOI:10.1093/nar/gkz382]

[13] Stelzer G, Rosen N, Plaschkes I, Zimmerman S, Twik M, Fishilevich S, et al. The GeneCards Suite: From gene data mining to disease genome sequence analyses. *Curr Protoc Bioinformatics*. 2016; 54:1.30.1-1.30.33. [DOI:10.1002/cpbi.5]

[14] Bardou P, Mariette J, Escudié F, Djemiel C, Klopp C. jvenn: An interactive Venn diagram viewer. *BMC Bioinformatics*. 2014; 15:293. [DOI:10.1186/1471-2105-15-293]

[15] Szklarczyk D, Kirsch R, Koutrouli M, Nastou K, Mehryary F, Hachilif R, et al. The STRING database in 2023: Protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Res*. 2023; 51(D1):D638-D646. [DOI:10.1093/nar/gkac1000]

[16] Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: A software environment for integrated models of biomolecular interaction networks. *Genome Res.* 2003; 13(11):2498-504. [DOI:10.1101/gr.1239303]

[17] Ge SX, Jung D, Yao R. ShinyGO: A graphical gene-set enrichment tool for animals and plants. *Bioinformatics.* 2020; 36(8):2628-9. [DOI:10.1093/bioinformatics/btz931]

[18] Burley SK, Bhikadiya C, Bi C, Bittrich S, Chen L, Crichlow GV, et al. RCSB Protein Data Bank: Powerful new tools for exploring 3D structures of biological macromolecules. *Nucleic Acids Res.* 2021; 49(D1):D437-D451. [DOI:10.1093/nar/gkaa1038]

[19] Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, et al. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J Comput Chem*. 2009; 30(16):2785-91. [DOI:10.1002/jcc.21256]

[20] Dassault Systèmes BIOVIA. Discovery Studio Visualizer, Release 2025, San Diego: Dassault Systèmes, 2025.

[21] Ricci CG, Netz PA. Docking studies on DNA-ligand interactions. *J Chem Inf Model*. 2009; 49(8):1925-35. [DOI:10.1021/ci9001537]

[22] Ongalbek D, Tokul-Ölmez O, Şahin B, Küçükaydın S, Aydoğmuş-Öztürk F, Sıcak Y, et al. Classification of buckwheat honey produced in Kazakhstan according to their biochemical ingredients and bioactivities by chemometric approach. *Food Chem.* 2024; 451:139409. [DOI:10.1016/j.foodchem.2024.139409]

[23] Taheri R, Mokhtari Y, Yousefi AM, Bashash D. The PI3K/Akt signaling axis and type 2 diabetes mellitus (T2DM): From mechanistic insights into possible therapeutic targets. *Cell Biol Int.* 2024; 48(8):1049-68. [DOI:10.1002/cbin.12189]

[24] Huang X, Liu G, Guo J, Su Z. The PI3K/AKT pathway in obesity and type 2 diabetes. *Int J Biol Sci.* 2018; 14(11):1483-96. [DOI:10.7150/ijbs.27173]