Integrated In Silico and Experimental Evaluation of the Antioxidant and Immunomodulatory Potential of Myricetin Associated with Bee-Derived Products

Zeliha Selamoglu[1,2,3*], Şeyda Kaya[4], Sevgi Durna Daştan[5,6], Furkat Ubaev[7], Taner Dastan[8], Nurdana Salybekova[1]

[1]Department of Biology, Faculty of Sciences, Khoja Akhmet Yassawi International Kazakh- Turkish University, Turkestan, Kazakhstan
[2]Department of Medical Biology, Medicine Faculty, Nigde Omer Halisdemir University, Nigde, Türkiye
[3]Western Caspian University, Baku, Azerbaijan

ORCID: https://orcid.org/0000-0001-9056-6435
*Corresponding Author's E-mail: zselamoglu@ohu.edu.tr

[4]Department of Medical Services and Techniques, Medical Laboratory Techniques Program, Vocational School of Health Services, Gaziantep Islam Science and Technology University, Gaziantep / Türkiye
ORCID: https://orcid.org/0000-0001-8489-8687
E -mail: sydkaya58@gmail.com

[5]Department of Biology, Faculty of Science, Sivas Cumhuriyet University, Sivas, Türkiye
[6]Beekeeping Development, Application and Research Center, Sivas Cumhuriyet University, Sivas, Türkiye
ORCID: https://orcid.org/0000-0003-4946-5602
E-mail: sdurna@cumhuiyet.edu.tr

[7]Center for Sustainable Development and Scientific Research, Khoja Akhmet Yassawi International Kazakh-Turkish University, Turkestan, Kazakhstan
ORCID: https://orcid.org/0009-0000-6180-1985

E-Mail: furkhat.ubaev@mail.ru

[8]Department of Biochemistry, Faculty of Science, Sivas Cumhuriyet University, 58140 Sivas, Turkiye

ORCID: https://orcid.org/0000-0003-0296-6979

E-Mail: tdastan@cumhuriyet.edu.tr

[1]Department of Biology, Faculty of Sciences, Khoja Akhmet Yassawi International Kazakh- Turkish University, Turkestan, Kazakhstan
ORCID: https://orcid.org/0000-0002-3750-1023
E-mail: nurdana.salybekova@ayu.edu.kz

ABSTRACT

Introduction: The detection of myricetin as a major flavonoid content in some Kazakhstan bee products suggests that this flavonoid may be primarily responsible for the bioactive properties coming from bee products. In this study, therefore, it is worth investigating the necessity of planning *in silico* and *in vitro* analysis related to myricetin and bee products.

Material and Methods: The intersection of myricetin and oxidant targets was determined using jvenn tool. Subsequently, the intersection of oxidant targets and myricetin was transferred to the STRING database to construct a protein-protein interaction (PPI) network. Furthermore, GO and KEGG pathway enrichment analyses were performed on the selected core targets using the ShinyGO tool. On the other hand, the in vitro DPPH analysis and anti-inflammation tests were applied.

Result: According to molecular docking scores, affinity values and low binding energies were obtained between myricetin and target proteins that are almost identical to those of trolox, the standard active ingredient. Bee-derived products showed notable antioxidant activity and moderate anti-inflammatory potential, whereas myricetin exhibited significantly stronger antioxidant and anti-inflammatory properties.

Conclusion: The present in silico analyses suggest that myricetin may interact with multiple oxidative stress- and inflammation-related molecular targets. Network pharmacology, GO/KEGG enrichment, and molecular docking analyses collectively indicate the potential involvement of myricetin in ROS regulation and inflammatory signalling pathways. Supporting these computational findings, our experimental DPPH radical scavenging and anti-inflammatory assay results demonstrated that myricetin exhibited strong antioxidant and notable anti-inflammatory activities compared with bee-derived product extracts.

Keywords: Anti-inflammation, Antioxidant, Bee products, Kazakhstan, Molecular docking, Myricetin, Network pharmacology

1. Introduction

Oxidants are present within cells as free radicals, including hydroxyl radicals and superoxide anions, as well as reactive oxygen and nitrogen species. These molecules induce oxidative damage to proteins, lipids, and DNA, leading to cellular dysfunction, chronic inflammation, and the development of age-related neurodegenerative and cardiometabolic diseases. Excessive ROS (Reactive Oxygen Species) production can trigger mitochondrial dysfunction, lipid peroxidation, and activation of pro-inflammatory signaling pathways, processes that play a central role in the pathogenesis of many chronic diseases [1]. Living systems possess defense mechanisms against oxidative stress, including catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD), glutathione, vitamins C and E, and flavonoids. These systems regulate ROS levels to limit oxidative damage; however, when endogenous defenses are insufficient, antioxidant-rich dietary supplements, such as flavonoid derivatives, may provide additional protection. Therefore, the antioxidant potential of dietary flavonoids and phenolic compounds derived from bee products is of considerable interest [2].

Myricetin is a flavonol widely distributed in plants and plant-derived bee products [3]. Numerous in vitro and in vivo studies have demonstrated its potent antioxidant activity through free radical scavenging, metal chelation, inhibition of lipid peroxidation, and modulation of endogenous antioxidant enzymes. Increasing evidence also supports its anti-inflammatory, neuroprotective, antimicrobial, and metabolic regulatory properties [4-5]. Myricetin has been identified in plant families such as Primulaceae, Myricaceae, Anacardiaceae, Polygonaceae, and Pinaceae, and is frequently investigated because of its antioxidant, anticancer, immunomodulatory, and anti-inflammatory activities [4-5]. If myricetin is present in floral pollen, honeybees can transfer it to bee pollen and other hive products [2-5]. Along with quercetin and kaempferol, myricetin is among the major flavonoids identified in bee products [6]. Several studies have reported strong correlations between phenolic content and antioxidant or anti-inflammatory capacity [7]. Among these compounds, flavonols such as myricetin have attracted particular attention, highlighting the need for further in silico and in vitro investigations [8]. Myricetin has been reported as a major flavonol in certain regional honeys from Kazakhstan [3]. Its occurrence in bee products depends on floral composition, vegetation, and seasonal factors. Therefore, given the presence of

suitable plant species in the flora of southern Kazakhstan, the occurrence of myricetin in regional bee products is highly plausible [8].

The aim of this study was to evaluate the antioxidant and anti-inflammatory potential of myricetin, a flavonoid commonly found in bee products, and to investigate its interactions with relevant molecular targets using in silico approaches. In addition, in vitro DPPH and albumin denaturation assays were performed using myricetin and bee products from Kazakhstan. The findings are intended to serve as a preliminary basis for future experimental validation studies. The limited regional evidence further emphasizes the significance of this study for both the phenolic inventory of Kazakhstani bee products and the preclinical evaluation of the potential health benefits of myricetin.

2. Materials and Methods

2.1. Data Acquisition

PubChem is an open-access database containing the chemical structures of numerous molecules affiliated with the National Institutes of Health (NIH). The 3D structure of the myricetin compound was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/).

2.2. Identification of Predicted Targets and Myricetin

The GeneCards database provides researchers with comprehensive information on all annotated and predicted human genes. Oxidative stress-related targets were retrieved from the GeneCards database using the keywords ‘oxidative stress’ and ‘reactive oxygen species (ROS) (https://www.genecards.org/) and selecting those with a relevance score ≥10. Preliminary sensitivity comparisons using different relevance score thresholds (≥5, ≥10, and ≥15) demonstrated that the ≥10 cutoff provided an optimal balance between the number of retrieved targets and biological specificity. Lower thresholds substantially increased low-confidence targets, whereas higher thresholds markedly reduced pathway coverage. The 326 genes associated with oxidative stress and ROS targets were identified (Supplementary 1). SwissTargetPrediction, an online tool, is used to predict the targets of bioactive small molecules [9]. One hundred target genes for myricetin were obtained from the SwissTargetPrediction tool (http://www.swisstargetprediction.ch/) (Supplementary 2).

The jvenn tool (https://jvenn.toulouse.inrae.fr/app/index.html) was used to find the intersection of targets between oxidant targets and the myricetin compound. Jvenn is an integrated tool used to compare lists with Venn diagrams [10]. Cytoscape is a network biology visualisation and analysis application that visualises molecular connections and biological processes Cytoscape 3.10.4). In the compound-target network, each compound or target is represented by a node, and the relationship between the compound and the target is shown by an edge [11].

2.3. PPI Network

The intersecting genes were transferred to the STRING (https://string-db.org/) database to obtain information about the protein interaction network. The STRING database is a repository that performs protein-protein interaction networks and functional enrichment analyses for any sequenced genome of interest [12]. Compound-target intersecting genes were transferred to the STRING database and the species was specified as '*Homo sapiens'*. Protein-protein interaction (PPI) data were obtained based on interactions with a medium confidence level or higher (score ≥ 0.400). Using Cytoscape 3.10.3, 10 potential core targets were identified by selecting target nodes with DC (Degree Centrality), BC (Betweenness Centrality), and CC (Closeness Centrality) degree values. Degree Centrality was considered the primary parameter for hub target prioritization due to its ability to identify highly connected proteins with potential biological significance within the interaction network.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was performed using the ShinyGO 0.82 tool (https://bioinformatics.sdstate.edu/go/). ShinyGO is an online data tool that utilises data from Ensembl and STRING to obtain enriched GO terms and other pathways for a large number of species [13]. Core target genes were selected using HUGO Gene Nomenclature Committee (HGNC) gene symbols for the species '*Homo sapiens*', with a p-value <0.05 set for significant differences, and visualisation was performed.

2.4. Molecular Docking

Molecular docking is a theoretical simulation method used in drug discovery to study the interactions between receptors and ligands. The Protein Data Bank (PDB) (http://www.rcsb.org/) is a database that provides information about the three-

dimensional structures of molecules such as proteins and nucleic acids [14]. Docking targets were selected according to degree centrality values in the PPI network, biological relevance to oxidative stress pathways, and literature evidence regarding ROS production and inflammatory signalling. The 3D structure of the target proteins was obtained from the PDB database, and the 3D structure of the ligand molecule was obtained from the PubChem database. AutoDock 4.2 software and the integrated AutoDockTools (MGLTools 1.5.7) interface were used [15-16].

2.5. Extraction of Bee Products

Bee product samples including pollen, propolis and honey 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 70% ethanol and incubated in a shaker incubator at room temperature for 48 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.

2.6. Determination of Total Flavonoid Content

Total flavonoid content (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 [17].

2.7. DPPH Radical Scavenging Assay

The antioxidant activity of the extracts was evaluated using the DPPH free radical scavenging assay [18]. 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.

The commercially available standard myricetin (Sigma-Aldrich) was used as a reference compound in all activity assays for comparative analysis.

2.8. Albumin Denaturation Assay (Anti-inflammatory Activity)

The anti-inflammatory potential of the alcoholic extracts of the tested bee product samples was evaluated based on their ability to inhibit heat-induced denaturation of bovine serum albumin (BSA). The reduction in protein denaturation indicates anti-inflammatory activity. Briefly, 0.5 mL of 1% BSA solution was mixed with 0.1 mL of the test samples at different concentrations. The pH of the reaction mixture was adjusted to 6.3 using PBS. The mixtures were incubated at 37°C for 20 minutes, followed by heating at 70°C for 5–10 minutes to induce protein denaturation. After incubation, the mixtures were cooled to room temperature. The resulting turbidity was measured spectrophotometrically at 560 nm [19]. The commercially available standard myricetin (Sigma-Aldrich) was used for comparative analysis as a standard pure drug molecule. The higher percentage of inhibition indicates stronger anti-inflammatory activity of the tested compounds. The percentage inhibition of protein denaturation was calculated using the following formula:

Inhibition%= [(Absorbance of control –Absorbance of sample): Absorbance of control] ×100

2.9. 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. Myricetin

The chemical structure of myricetin is shown in Figure 1.

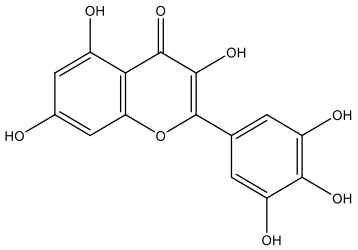


Figure 1. Myricetin chemical structure

3.2. Compound-Target Network and Analysis

There are 12 common targets between myricetin targets and oxidative stress related targets according to the Venn diagram results (Figure 2).

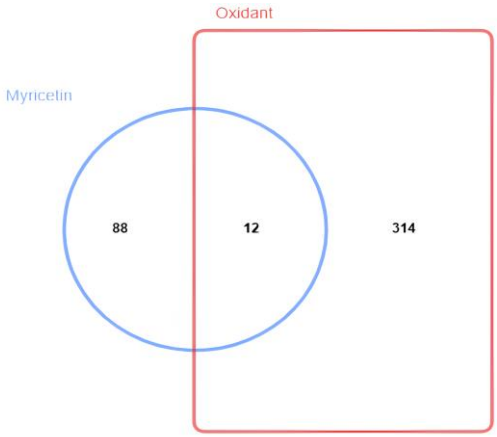


Figure 2. Myricetin and oxidative stress related intersection targets

The relationship between the component and the target is shown by connecting lines. There are 416 nodes and 426 edges in the active compound and target network. The light blue octagon represents the active compound myricetin, while the dark blue ellipses represent the genes associated with myricetin. The yellow hexagon represents oxidant targets, while the orange ellipses represent oxidant target genes. The dark green V-shaped genes represent the intersection genes in myricetin and oxidant targets (Figure 3).

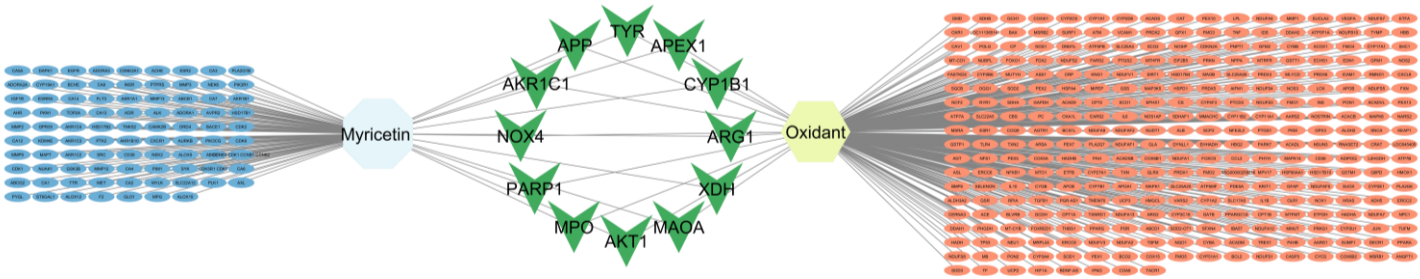


Figure 3. Network between myricetin and oxidant targets

3.3. PPI Network Construction and Analysis

Following the loading of compound target intersection genes into the STRING database for the protein-protein interaction (PPI) network, a total of 12 nodes and 19 edges were generated in the network (Figure 4).

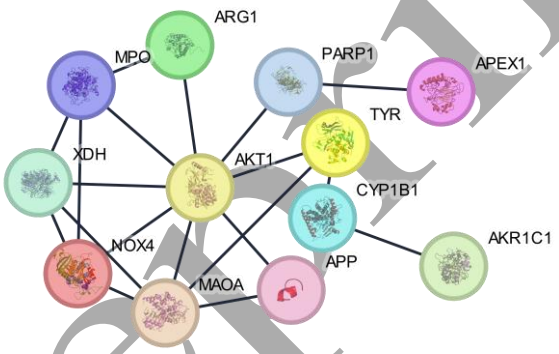


Figure 4. PPI analysis

As shown in Table 1, the top 10 hub proteins (AKT1, MAOA, NOX4, MPO, XDH, TYR, APP, PARP1, CYP1B1, and ARG1) were identified based on PPI network topology parameters, including DC, BC, and CC. Among these parameters, DC was primarily prioritized because it reflects the number of direct interactions associated with each protein and is commonly used to identify biologically influential hub targets in network pharmacology analyses.

Table 1. The top 10 targets according to the degree value

Name	Degree Centrality	Betweenness Centrality	Closeness Centrality
AKT1	8	0.6152	0.7333
MAOA	5	0.1000	0.5789
NOX4	4	0.0061	0.5
MPO	4	0.0182	0.5
XDH	4	0.0061	0.5
TYR	3	0.3273	0.55
APP	2	0.0	0.4583
PARP1	2	0.1818	0.4783
CYP1B1	2	0.1818	0.3929
ARG1	2	0.0	0.4583

3.4. GO enrichment and KEGG pathway analysis

The 12 common targets obtained were analysed using the ShinyGO 0.82 tool. The biological processes given in Figure 5(a) were evaluated in terms of oxidative stress. Biological Process: Positive regulation of reactive oxygen species metabolic process shows that enrichment is high. Although the "positive regulation of reactive oxygen species metabolic process" term demonstrated enrichment within the GO analysis, its statistical support was comparatively weaker based on the corresponding FDR-adjusted p-value. Reactive oxygen species metabolic process enrichment is high and statistically significant. Response to oxidative stress indicates a process with high enrichment and statistical significance. Cellular response to oxidative stress has moderate enrichment and statistical significance. The Cellular Component shown in Figure 5(b) was evaluated in terms of oxidative stress. Mitochondrion is the most statistically significant component. The Molecular Function shown in Figure 5(c) was evaluated in terms of oxidative stress. Monooxygenase activity, Heme binding, and Oxidoreductase activity are functions that can generally be associated with oxidative stress. The KEGG pathway analysis given in Figure 5(d) has been evaluated in terms of oxidative stress. Chemical carcinogenesis-reactive oxygen species is a pathway that

is both statistically significant and highly enriched (Enrichment score = 4.1 and FDR = 0.0033).

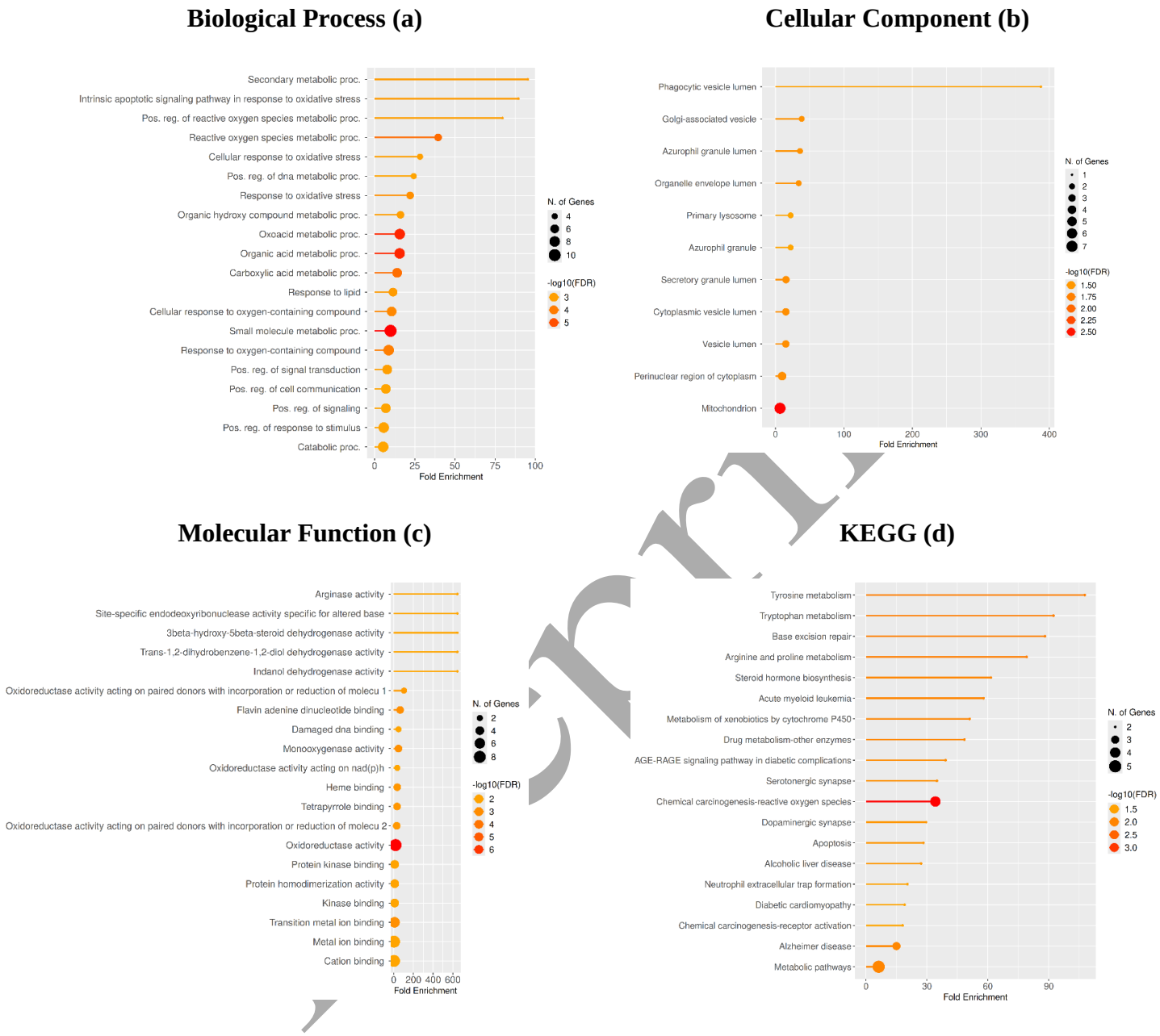
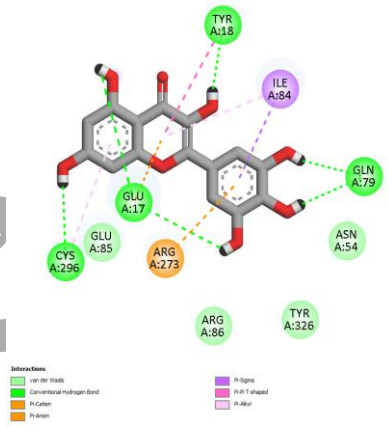


Figure 5. GO and KEGG Analysis (GO enrichment analysis of oxidative stress-related targets. Statistical significance was evaluated using FDR-adjusted p-values generated by ShinyGO analysis)

3.5. Molecular Docking

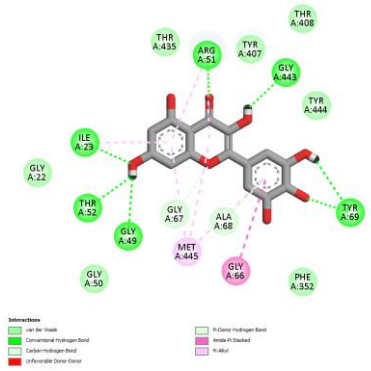
According to the PPI network analysis, the two proteins with the highest degree centrality values and strongest relevance to oxidative stress-related pathways were selected for molecular docking analyses. Molecular docking results have demonstrated that myricetin can interact well with the AKT1 protein. Myricetin had two hydrogen bonds with the AKT1 protein at GLU A:17 and GLN A:79, and one hydrogen bond at CYS A:296 and TYR A:18. In addition, it revealed a binding energy of -6.82 kcal/mol with pi-based interactions and van der Waals bonds present in the structure. The docking score was determined to be -8.56 kcal/mol for capivasertib (Table 2).

Table 2. AKT1, Myricetin, Capivasertib Docking Score and Structure

AKT1 - (6HHF)	2D Structure	Docking Score
Myricetin		**-6.82**
Capivasertib (Referance inhibitor for AKT1)		-8.56

Molecular docking results have demonstrated that myricetin can interact well with the MAOA protein. Myricetin formed two hydrogen bonds with TYR A:69 and one hydrogen bond with GLY A:443, ARG A:51, ILE A:23, THR A:52, and GLY A:49 of the MAOA protein. Furthermore, the pi-based interactions and van der Waals bonds present in the structure yielded a binding energy of -8.18 kcal/mol. Furthermore, the pi-based interactions and van der Waals bonds present in the structure revealed a binding energy of -9.15 kcal/mol for Clorgyline (Referance inhibitor) (Table 3).

Table 3. MAOA, Myricetin, Clorgyline Docking Score and Structure

MAOA - (2BXS)	2D Structure	Docking Score
Myricetin		**-8.18**
Clorgyline (Referance inhibitor for MAOA)		**-9.15**

Although network pharmacology analysis identified multiple potential target proteins, molecular docking studies were selectively conducted on representative hub targets with higher biological relevance and adequate structural suitability. The selection criteria primarily included topological significance within the PPI network, particularly degree centrality values, functional association with the investigated signaling pathways, and the availability of high-resolution crystallographic structures in the PDB. Accordingly, docking analyses were focused on the most representative targets to provide a mechanistically robust and biologically meaningful interpretation rather than extending the analysis to all predicted proteins.

Additionally; RMSD analysis between the reference ligand and the docked complex was planned; however, a reliable RMSD value could not be obtained due to inconsistencies in atom mapping during the structure preparation and alignment processes. Therefore, the validation process was comprehensively evaluated based on the binding orientations of the ligands within the active site and their protein–ligand interaction profiles.

The docking scores obtained for myricetin against AKT1 (−6.82 kcal/mol) and MAOA (−8.18 kcal/mol) suggest moderate to strong binding affinity according to commonly accepted molecular docking evaluation criteria. Molecular Docking scores should not be interpreted as direct evidence of biological activity but rather as supportive computational predictions. Binding affinities lower than −6.0 kcal/mol are generally

considered indicative of potentially stable ligand–protein interactions in molecular docking studies.

3.6. Extraction Field of Bee Products

Extractions of bee products, including pollen, propolis, and honey samples, were prepared using a 70% ethanol solvent. The yields of the obtained extracts were calculated as percentages (Table 4).

Table 4. Percentage yields of bee product extracts

Samples	Code	Locality	Field %
Pollen	N1	Koğam village	9.8
	N2	Shymkent	7.6
	N3	Kelintobe village	7.4
Propolis	P1	Kelintobe village	10.8
	P2	Zhanakorgan province	9.9
	P3	Koğam village	7.6
Honey	B4	Kelintobe village	5.9
	B5	Zhanakorgan province	6.3
	B6	Koğam village	7.1

3.7. Total Flavonoid Content

The total flavonoid content of all extracts is given in Table 5. It has been determined that the total flavonoid content varies depending on the sample type.

Table 5. Total flavonoid content of bee product extracts (mg QE/g extract)

Samples	Code	Total Flavonoid Content (mg QE/g extract)
Pollen	N1	5.6 ± 0.5
	N2	6.3 ± 0.4
	N3	7.3 ± 0.5
Propolis	P1	6.2 ± 0.3
	P2	6.9 ± 0.7
	P3	5.5 ± 0.6
Honey	B4	4.8 ± 0.5
	B5	5.7 ± 0.5
	B6	6.4 ± 0.8

3.8. Anti-inflammatory Activity of Bee Products

The albumin denaturation inhibition activities of ethanol extracts of pollen, propolis, and honey samples collected from different localities in Kazakhstan are presented in Table 6.

Table 6. Percentage of albumin denaturation inhibition of ethanolic extracts of bee products from Kazakhstan

Samples	**Code**	**100 mg/mL (%)**	**10 mg/mL (%)**	**1 mg/mL (%)**
Diclofenac Sodium*	Standard inhibitor. Positive control	80.03 ± 0.70	66.86 ± 1.28	60.66 ± 0.32
Pollen	N1	41.9 ± 2.5	26.6 ± 3.8	20.1 ± 2.3
	N2	40.7 ± 4.4	29.0 ± 4.7	24.5 ± 3.7
	N3	32.4 ± 1.8	25.3 ± 4.6	18.0 ± 2.4
Propolis	P1	33.8 ± 3.6	25.0 ± 4.5	17.2 ± 3.4
	P2	31.3 ± 2.5	26.6 ± 3.9	20.0 ± 2.7
	P3	33.8 ± 4.4	27.3 ± 2.4	24.5 ± 2.6
Honey	B4	21.1 ± 2.6	35.5 ± 3.6	25.4± 3.4
	B5	25.2 ± 3.5	26.3 ± 3.8	17.0 ± 1.2
	B6	29.7 ± 4.3	24.6 ± 3.9	17.3 ± 2.3

The albumin denaturation inhibition test is a reliable and widely used in vitro method for preliminary screening of anti-inflammatory activity. Protein denaturation plays a significant role in inflammatory processes and constitutes one of the mechanisms of action of anti-inflammatory drugs. Therefore, it is thought that extracts showing high activity in this test may also have the potential for anti-inflammatory effects under in vivo conditions. In conclusion, ethanol extracts of bee products collected from Kazakhstan exhibited significant anti-inflammatory activity in the albumin denaturation inhibition test. Further studies are recommended to isolate and characterize the active compounds, apply different in vitro anti-inflammatory tests, and evaluate their in vivo activity.

4. Discussion

The main objective of this study was to evaluate the antioxidant and anti-inflammatory potential of myricetin and bee products from Kazakhstan using integrated in silico and in vitro approaches. Myricetin is among the major bioactive flavonoids identified in

bee products, and reports of high myricetin levels in plant species native to Kazakhstan suggest that regional bee products may constitute an important source of this compound. Previous studies have demonstrated that myricetin protects cells against oxidative stress through direct free radical scavenging and indirect modulation of endogenous antioxidant enzymes, including SOD, CAT, and GPx [4,20]. The present findings demonstrated that bee-derived products collected from different regions of Kazakhstan contain considerable flavonoid levels and exhibit moderate anti-inflammatory activity in the albumin denaturation assay. Pollen and propolis extracts generally showed higher flavonoid contents and stronger biological activities than honey samples. Although the anti-inflammatory effects were lower than those of diclofenac sodium, the results support the hypothesis that flavonoid-rich bee products represent valuable natural sources of antioxidant and anti-inflammatory compounds. These findings were consistent with network pharmacology and molecular docking analyses, which identified targets and pathways associated with oxidative stress and inflammation. The potential interactions of myricetin with oxidative stress- and inflammation-related molecular targets were investigated through GO/KEGG pathway enrichment, network analysis, protein-protein interaction (PPI) mapping, and molecular docking. The combined in silico and in vitro findings suggest that myricetin, together with pollen, honey, and propolis extracts, may reduce oxidative damage and modulate inflammatory responses. Twelve common targets were identified between myricetin and oxidative stress-related genes retrieved from the GeneCards database. Functional analyses indicated that these targets are primarily involved in ROS production, redox regulation, inflammatory signaling, and mitochondrial metabolism. The network analysis, comprising 416 nodes and 426 edges, highlighted the multi-target nature of myricetin, suggesting that its biological effects arise from coordinated modulation of interconnected signaling pathways rather than a single molecular target. PPI analysis identified AKT1, MAOA, NOX4, MPO, XDH, TYR, APP, PARP1, CYP1B1, and ARG1 as key targets, with AKT1, MAOA, NOX4, and MPO showing the highest centrality scores. AKT1 is a major regulator of the PI3K/AKT/NF-κB axis and plays a critical role in oxidative stress and inflammation. Myricetin interaction with AKT1 suggests potential suppression of inflammatory signaling. MAOA contributes to mitochondrial ROS generation through hydrogen peroxide production during neurotransmitter metabolism. The strong

binding affinity of myricetin for MAOA (−8.18 kcal/mol) indicates a potential role in reducing mitochondrial oxidative stress, which may be relevant to neurodegenerative disorders [21,22].

NOX4 and MPO are major contributors to ROS production and inflammatory signaling [23,24]. Their identification as central network nodes suggests that myricetin may modulate oxidative stress-associated pathways. However, direct inhibitory effects on these enzymes require experimental confirmation. GO and KEGG enrichment analyses revealed significant associations with the "reactive oxygen species metabolic process," "response to oxidative stress," mitochondrial components, and the "chemical carcinogenesis-reactive oxygen species" pathway. These findings indicate that myricetin may influence mitochondrial function, redox homeostasis, and ROS-mediated DNA damage. Enrichment of monooxygenase and oxidoreductase activities further supports the capacity of myricetin to regulate oxidative enzyme systems alongside its direct free radical scavenging effects. Molecular docking demonstrated favorable binding affinities of myricetin toward AKT1 (−6.82 kcal/mol) and MAOA (−8.18 kcal/mol), providing a molecular basis for its anti-inflammatory and antioxidant activities. These interactions suggest potential relevance in conditions characterized by chronic inflammation, mitochondrial dysfunction, and oxidative stress.

Overall, the present findings indicate that myricetin is a multi-target natural compound capable of modulating redox balance, mitochondrial function, and inflammatory signaling pathways. These results are consistent with previous reports describing its antioxidant, cytoprotective, and anti-inflammatory effects [25]. Consequently, myricetin may represent a promising candidate for further investigation in oxidative stress- and inflammation-related disorders, including neurodegenerative, cardiovascular, and metabolic diseases.

5. Conclusion

The present study employed an integrated in silico and in vitro approach to evaluate the antioxidant and anti-inflammatory potential of myricetin and Kazakhstan-derived bee products. Total flavonoid content was determined alongside biological activity

assessments, providing a comprehensive framework for investigating the health-promoting properties of myricetin and flavonoid-rich bee products. Network pharmacology, GO/KEGG enrichment, and molecular docking analyses indicated that myricetin may exert multi-target effects through the modulation of pathways associated with ROS regulation, redox homeostasis, mitochondrial function, and inflammatory signaling. These findings support the potential relevance of myricetin in oxidative stress-related disorders. The in vitro results demonstrated that Kazakhstan bee products contain considerable levels of flavonoids and exhibit measurable antioxidant and anti-inflammatory activities, highlighting their potential as natural sources of bioactive compounds. The concordance between experimental findings and computational predictions provides preliminary evidence supporting the biological significance of myricetin and related phenolics. However, the present findings should be interpreted with caution, as the study relied substantially on computational approaches, including network pharmacology and molecular docking, which are constrained by the completeness and accuracy of currently available databases. In addition, comprehensive phytochemical characterization using HPLC or LC-MS is required to confirm and quantify myricetin and other bioactive flavonoids in the investigated samples. As an exploratory study, the integration of total flavonoid analysis, in vitro bioactivity assays, and in silico predictions provides an initial basis for understanding the mechanisms underlying the observed biological effects. Nevertheless, further phytochemical, molecular, and in vivo investigations are necessary to validate the proposed targets, elucidate the underlying mechanisms, and establish causal relationships between phytochemical composition and biological activity. Overall, these findings identify myricetin as a promising natural candidate for future research aimed at the prevention or adjunctive management of oxidative stress- and inflammation-related diseases.

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Authors' Contribution

Conceptualization: ZS, SDD, NS

Data curation: ŞK, SDD, ZS, FU

Formal analysis: ŞK, ZS, TD

Methodology: ŞK, SDD, ZS

Software: ŞK,TD,FU

Validation: ŞK, SDD, ZS, TD

Investigation: ŞK, SDD, ZS, TD

Writing - original draft: SDD, ŞK, ZS, NS

Writing -review& editing: ŞK, SDD, ZS, TD

Ethics

Ethics committee approval is not required for this study.

Declaration on the Use of AI

During the preparation of this manuscript, the authors used generative AI (ChatGPT, GPT-5.1) solely to prepare the initial draft of the introduction section and to improve linguistic clarity, grammar, and readability; all scientific content was subsequently rewritten, verified, and approved by the authors.

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 authors.

References

[1] Liu S, Liu J, Wang Y, Deng F, Deng Z. Oxidative Stress: Signaling Pathways, Biological Functions, and Disease. Med. Comm. 2025;6(7). DOI: 10.1002/mco2.70268.

[2] Rudrapal M, Khairnar SJ, Khan J, Dukhyil AB, Ansari MA, Alomary MN, Alshabrmi FM, Palai S, Deb PK and Devi R. Dietary Polyphenols and Their Role in Oxidative Stress-Induced Human Diseases: Insights Into Protective Effects, Antioxidant Potentials and Mechanism(s) of Action. Front. Pharmacol. 2022;13. DOI: 10.3389/fphar.2022.806470.

[3] Ongalbek D, Tokul-Ölmez Ö, 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. 2026;451:148733. DOI: 10.1016/j.foodchem.2024.139409.

[4] Nadalin P, Kim JK, Park SU. Recent studies on myricetin and its biological and pharmacological activities. Excli. J. 2023;22:1223-1231. DOI: 10.17179/excli2023-6571.

[5] Agraharam G, Girigoswami A, Girigoswami K. Myricetin: a Multifunctional Flavonol in Biomedicine. Curr. Pharmacol. Rep. 2022 Feb;8:48-61. DOI: 10.1007/s40495-021-00269-2.

[6] Eşerler S, Vardarlı S, Savaş G, Mutlu C. Some physical, functional and chemical properties and biological effects of bee pollen. Bee J. 2023;23(2):280-95. DOI: 10.31467/uluaricilik.1319365.

[7] El Ghouizi A, Bakour M, Laaroussi H, Ousaaid D, El Menyiy N, Hano C, Lyoussi B. Bee Pollen as Functional Food: Insights into Its Composition and Therapeutic Properties. Antioxidants. 2023 Mar;12(3):557. DOI: 10.3390/antiox12030557.

[8] Nazarova V, Salikova N, Bektemissova A. Isolation and Identification of Flavonoids from Linosyris villosa Plant. Chem. J. Kazakhstan. 2020;3:236-46.

[9] Daina A, Michielin O, Zoete V. SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules. Nucleic Acids Res. 2019;47(W1):W357-64. DOI: 10.1093/nar/gkz382.

[10] Bardou P, Mariette J, Escudié F, Djemiel C, Klopp C. Jvenn: an interactive Venn diagram viewer. BMC Bioinform. 2014;15(1):293. DOI: 10.1186/1471-2105-15-293.

[11] Otasek D, Morris JH, Bouças J, Pico AR, Demchak B. Cytoscape Automation: empowering workflow-based network analysis. Genome Biol. 2019;20(1):185. DOI: 10.1186/s13059-019-1758-4.

[12] Szklarczyk D, Kirsch R, Koutrouli M, Nastou K, Mehryary F, Hachilif R, Gable AL, Fang T, Doncheva NT, Pyysalo S, Bork P, Jensen LJ, von Mering C. The STRING database in 2023: protein–protein association networks and functional enrichment analyses for any sequenced genome of interest. Nucleic Acids Res. 2023;6:51(D1):D638-646. DOI: 10.1093/nar/gkac1000.

[13] Ge SX, Jung D, Yao R. ShinyGO: a graphical gene-set enrichment tool for animals and plants. Bioinformatics. 2020 Apr;36(8):2628-2629. DOI: 10.1093/bioinformatics/btz931.

[14] Burley SK, Bhikadiya C, Bi C, Bittrich S, Chen L, Crichlow GV, Christie CH, Dalenberg K, Di Costanzo L, Duarte JM, Dutta S, Feng Z, Ganesan S, Goodsell DS, Ghosh S, Green RK, Guranović V, Guzenko D, Hudson BP, Lawson CL, Liang Y, Lowe R, Namkoong H, Peisach E, Persikova I, Randle C, Rose A, Rose Y, Sali A, Segura J, Sekharan M, Shao C, Tao YP, Voigt M, Westbrook JD, Young JY, Zardecki C, Zhuravleva M. RCSB Protein Data Bank: powerful new tools for exploring 3D structures of biological macromolecules for basic and applied research and education in fundamental biology, biomedicine, biotechnology, bioengineering and energy sciences. Nucleic Acids Res. 2021;8:49(D1):D437-451. DOI: 10.1093/nar/gkaa1038.

[15] Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J. Comput. Chem. 2009;30(16):2785-2791. DOI: 10.1002/jcc.21256.

[16] Ricci CG, Netz PA. Docking Studies on DNA-Ligand Interactions: Building and Application of a Protocol To Identify the Binding Mode. J. Chem. Inf. Model. 2009;49(8):1925-1935. DOI: 10.1021/ci9001537.

554 [17] Bag GC, Grihanjali Devi P, Bhaigyaba T. Assessment of total flavonoid content
555 and antioxidant activity of methanolic rhizome extract of three Hedychium species of
556 Manipur valley. Int. J. Pharm. Sci. Rev. Res. 2015;30(1):154-159.

557 [18] Clarke G, Ting K, Wiart C, Fry J. High Correlation of 2,2-diphenyl-1-
558 picrylhydrazyl (DPPH) Radical Scavenging, Ferric Reducing Activity Potential and
559 Total Phenolics Content Indicates Redundancy in Use of All Three Assays to Screen
560 for Antioxidant Activity of Extracts of Plants. Antioxidants. 2013;2(1):1-10.
561 DOI:10.3390/antiox2010001

562 [19] Dharmadeva S, Galgamuwa LS, Prasadinie C, Kumarasinghe N. In vitro anti-
563 inflammatory activity of *Ficus racemosa* L. bark using albumin denaturation method.
564 Ayu. 2018 Oct-Dec;39(4):239-242. DOI: 10.4103/ayu.AYU_27_18.

565 [20] Chobot V, Hadacek F. Exploration of pro-oxidant and antioxidant activities of the
566 flavonoid myricetin. Redox Rep. 2011;16(6):242-247. DOI:
567 10.1179/1351000211Y.0000000015.

568 [21] Herraiz T, Guillén H. Monoamine Oxidase-A Inhibition and Associated
569 Antioxidant Activity in Plant Extracts with Potential Antidepressant Actions. BioMed.
570 Res. Int. 2018;4810394. DOI: 10.1155/2018/4810394.

571 [22] Li X, Wei S, Niu S, Ma X, Li H, Jing M, Zhao Y. Network pharmacology
572 prediction and molecular docking-based strategy to explore the potential mechanism
573 of Huanglian Jiedu Decoction against sepsis. Comput. Biol. Med. 2022;144:105389.
574 DOI: 10.1016/j.compbiomed.2022.105389.

575 [23] Yang Q, Wu FR, Wang JN, Gao L, Jiang L, Li HD, et al. Nox4 in renal diseases:
576 An update. Free Radic. Biol. Med. 2018;124:466-472. DOI:
577 10.1016/j.freeradbiomed.2018.06.042.

578 [24] Lin W, Chen H, Chen X, Guo C. The Roles of Neutrophil-Derived
579 Myeloperoxidase (MPO) in Diseases: The New Progress. Antioxidants (Basel).
580 2024;13(1):132. DOI: 10.3390/antiox13010132.

581 [25] Geiger K, Muendlein A, Leiherer A, Gaenger S, Brandtner EM, Wabitsch M,
582 Fraunberger P, Drexel H, Heinzle C. Myricetin attenuates hypoxia-induced

583 inflammation in human adipocytes. Mol. Biol. Rep. 2023;50(12):9833-9843. DOI:
584 10.1007/s11033-023-08865-9.