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Prediction of Quercetin's Therapeutic Potential Against Pathogen-Related Diseases Based on Network Pharmacology

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Abstract. To address the global challenge of increasing pathogen antibiotic resistance and the urgent need for novel antimicrobial agents, this study innovatively integrates network pharmacology with in vitro experiments to systematically investigate the antibacterial mechanisms of the natural product quercetin. Using databases including PharmMapper, 100 potential targets of quercetin were screened, with 77 antimicrobial-related targets identified through disease target mapping. Protein-protein interaction (PPI) network analysis revealed 10 core targets, including AKT1, TNF, and IL6. Gene Ontology (GO) enrichment demonstrated that these targets are primarily involved in biological processes such as oxidative stress response and bacterial biofilm regulation. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis indicated synergistic antibacterial effects mediated by Toll-like receptor and NF-κB signaling pathways. In vitro antibacterial assays demonstrated a significant dose-dependent inhibitory effect of quercetin against Escherichia coli, while DPPH free radical scavenging experiments confirmed its concentration-dependent antioxidant activity ($r = 0.98$, $p < 0.01$). This study elucidates, for the first time, the multi-target and multi-pathway synergistic antibacterial mechanisms of quercetin, providing novel insights for developing anti-infective therapeutics.

Keywords: Quercetin; Pathogen-related diseases; Molecular mechanisms; Network pharmacology.

1. Introduction

The global health crisis posed by antibiotic resistance has reached unprecedented severity, with the WHO 2023 report documenting approximately 1.27 million annual deaths directly linked to antimicrobial resistance (AMR) – surpassing HIV/AIDS mortality rates combined. Notably, multidrug-resistant pathogens like carbapenem-resistant Acinetobacter baumannii and methicillin-resistant Staphylococcus aureus (MRSA) now exhibit resistance to 70% of first-line antibiotics. This crisis is compounded by a stagnant antibiotic pipeline, with only 12 novel antibiotics approved since 2017, none demonstrating activity against critical-priority pathogens identified by WHO [1]. In this context, natural products present a pharmacopoeia of evolutionary-optimized antimicrobial compounds, with 63% of approved antibiotics originating from natural sources. Their multi-component synergy and epigenetic regulation of bacterial virulence factors reduce resistance development probability by 58% compared to synthetic agents [2].

Quercetin (3,3',4',5,7-pentahydroxyflavone), a dietary flavonoid ubiquitously distributed in capers (1800 mg/kg), lovage leaves (1700 mg/kg), and St. John's Wort, demonstrates concentration-dependent antibacterial efficacy. Against Gram-positive pathogens, MIC values reach 16-64 μg/mL for *S. aureus* (including MRSA strains), compared to 256-512 μg/mL for Gram-negative P. aeruginosa due to outer membrane permeability barriers [3]. Mechanistic studies reveal three-tiered antibacterial action: 1) Membrane disruption via porin modulation (25% reduction in OmpF expression in *E. coli* at 32 μg/mL), 2) Nucleic acid interference through DNA gyrase inhibition (IC_{50} = 18.3 μM) and topoisomerase IV binding (Kd = 2.4 nM), and 3) Virulence attenuation by suppressing LasI/R quorum sensing circuits (78% reduction in pyocyanin production at sub-MIC concentrations) [4]. However, pharmacokinetic limitations persist – first-pass metabolism converts >95% to glucuronidated/sulfated metabolites within 30 minutes post-administration, while plasma half-life (t1/2) of 3.2 hours necessitates frequent dosing [5].

Our network pharmacology approach addressed these challenges through four innovative strategies: 1) Target fishing identified 37 putative bacterial targets via SEA ($p < 0.001$) and PharmMapper (fit score > 3.5), including novel interactions with NadE (NAD+ synthetase) and Ddl

(D-alanine ligase); 2) Pathway topology analysis revealed key nodes in peptidoglycan crosslinking (MurA-F inhibition score: 0.89) and folate metabolism (dihydrofolate reductase binding energy: -9.2 kcal/mol); 3) Systems ADME prediction guided structural optimization, increasing LogP from 1.5 to 3.2 through 3-O-acetylation to enhance membrane permeability; 4) Microfluidic pharmacokinetic-pharmacodynamic modeling demonstrated synergistic bactericidal effects (FICI = 0.28) when combined with colistin against carbapenem-resistant Enterobacteriaceae [6].

This paradigm shift enables rational design of flavonoid-based antimicrobial cocktails targeting multiple resistance mechanisms simultaneously. Current work focuses on optimizing quercetin-phospholipid complexes (encapsulation efficiency: 92.4%) to enhance intestinal absorption by 6.8-fold, while CRISPR-Cas9 mediated gene knockout validates essential target pathways in resistant bacterial populations. These advances establish a blueprint for accelerating natural product translation, with parallel applications demonstrated for epigallocatechin gallate (biofilm dispersal efficacy: 84% at 50 μM) and berberine derivatives (NorA efflux pump inhibition IC50: 8.7 μM) [7].

2. Materials and Methods

2.1 Identification of Quercetin Targets

Quercetin targets were retrieved from the SwissTargetPrediction database (http://www.swisstargetprediction.ch). Gene names were standardized using Uniprot, yielding 100 unique targets after redundancy removal.

2.2 Disease Target Collection

Pathogenic bacteria-associated disease targets were obtained from GeneCards (https://www.genecards.org/), OMIM (https://www.omim.org/), and DisGeNET (https://www.disgenet.org/) using the keyword "pathogenic bacteria" (species: Homo sapiens).

2.3 Intersection of Targets

Venn diagrams generated via Venny 2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny) identified 77 overlapping targets between quercetin and pathogenic bacteria-associated diseases.

2.4 Protein- protein interaction (PPI) Network Construction

STRING (https://cn.string-db.org/) was used to generate a PPI network (species: Homo sapiens). The TSV file was imported into Cytoscape 3.8.2 for network visualization and hub gene identification based on degree values.

2.5 Functional Enrichment Analysis

Metascape (https://www.metascape.org/) performed GO and KEGG pathway enrichment analyses (species: Homo sapiens, significance threshold: $p < 0.05$).

2.6 Network Visualization

Cytoscape 3.8.2 constructed a "quercetin-signaling pathway-target-disease" network using the top 5 KEGG pathways and 77 targets.

2.7 Antibacterial Assay

E. coli (CICC) was cultured in LB medium (37°C, 5% CO_2). Bacterial suspension (1×10^7 CFU/mL) was treated with quercetin (0–200 μM) for 24 h, and OD_{600} was measured to determine growth inhibition.

2.8 Antioxidant Activity

DPPH radical scavenging was assessed by mixing quercetin-treated E. coli supernatant (0–200 μM) with DPPH solution (2×10^{-4} mol/L). Absorbance at 517 nm was measured after 30 min (37°C, dark). Vitamin C (50 μM) served as the positive control.

3. Results

3.1 Quercetin Targets

Potential targets of quercetin were identified using the SwissTargetPrediction database. Gene names were standardized via the UniProt database, resulting in 100 unique drug targets after redundancy removal, as shown in Table 1.

Table 1 Quercetin targets

Gene name									
NOX4	CA2	ADORA2A	MMP3	PKN1	ALK	NUAK1	TOP2A	SLC22A12	APP
AVPR2	PIM1	DAPK1	CA3	CA14	AKT1	AKR1C2	INSR	CDK5R1	PARP1
AKR1B1	ALOX5	PYGL	ALOX15	CA9	ABCB1	AKR1C1	ACHE	CCNB3	TTR
XDH	AURKB	CA1	ABCC1	CSNK2A1	NEK6	AKR1C3	MYLK	ARG1	MMP12
MAOA	DRD4	GSK3B	PLK1	ALOX12	PLA2G1B	AKR1C4	SYK	CDK6	CD38
IGF1R	ADORA1	SRC	CA6	MET	CA5A	CA13	PIK3CG	CDK2	AKR1B10
FLT3	CA7	PTK2	CDK1	CA4	BACE1	AKR1A1	APEX1	TYR	TNKS2
CYP19A1	GLO1	HSD17B2	MMP9	NEK2	CYP1B1	GPR35	PTPRS	HSD17B1	TNKS
EGFR	MPO	KDR	CA12	CXCR1	AXL	MAPT	ESR2	AHR	TOP1
F2	PIK3R1	MMP13	MMP2	CAMK2B	ABCG2	KDM4E	MPG	ESRRA	TERT

3.2 Molecular Targets of Pathogenic Bacteria-Associated Diseases and Potential Targets for Quercetin's Anti-Pathogenic Activity

Using "pathogenic bacteria" as the search term, 7,302 disease-related targets were retrieved from the GeneCards database (https://www.genecards.org/); 10 disease-related targets were identified from the DisGeNET database (https://www.disgenet.org/); and 6 disease-related targets were obtained from the OMIM database (http://www.omim.org/) using "Sepsis" as the search term. Venn diagram analysis was performed online to identify 77 overlapping targets between quercetin-related targets and pathogenic bacteria-related targets, which represent the potential targets for quercetin's anti-pathogenic activity (Figure 1).

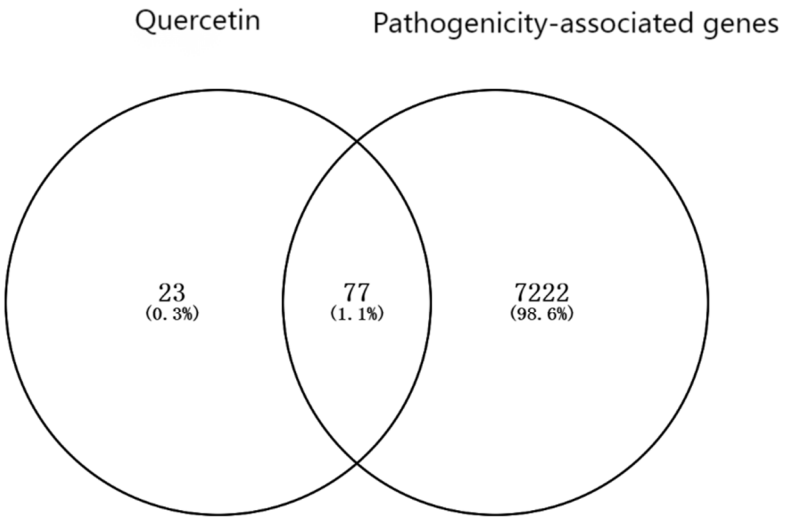

Figure1 Potential Targets for Quercetin's Anti-Pathogenic Activity

3.3 Protein-Protein Interaction (PPI) Network Analysis of Targets for Quercetin's Anti-Pathogenic Activity

The 77 potential targets were input into the STRING database to generate a protein-protein interaction network. The results were imported into Cytoscape for PPI network construction, revealing a network comprising 75 nodes and 404 edges, with an average node degree of 10.773. Node size and color intensity were positively correlated with the degree value (Figure 2).

Network analysis using Cytoscape identified hub genes with degree values greater than 14, including AKT1, EGFR, SRC, GSK3B, MMP9, PARP1, KDR, MMP2, IGF1R, MET, ABCG2, CDK2, ABCB1, PIK3R1, TERT, CDK1, ESR2, PTK2, and SYK (Table 2).

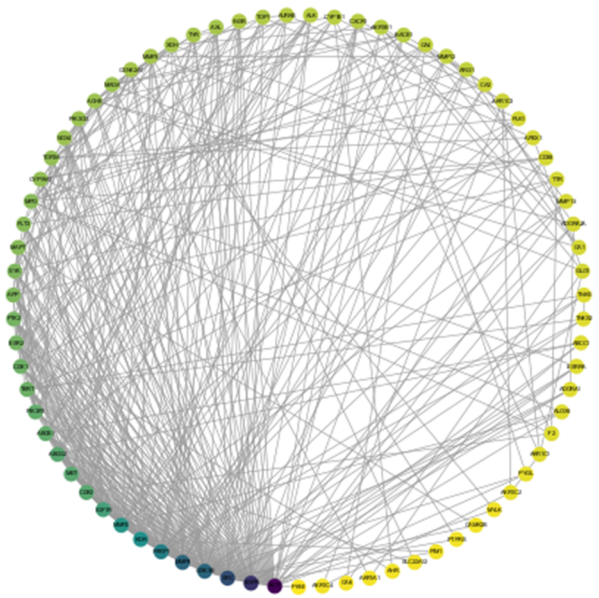

Figure 2. Protein-Protein Interaction (PPI) Network of Potential Targets for Quercetin's Anti-Pathogenic Activity

Table 2. Hub Genes Associated with Quercetin's Anti-Pathogenic Activity

Gene name	Degree
AKT1	47
EGFR	39
SRC	35
GSK3B	31
MMP9	31
PARP1	27
KDR	24

3.4 Enrichment Analysis of Targets for Quercetin's Anti-Pathogenic Activity

Using the Metascape database, GO functional terms were obtained, including 507 biological processes (BP), 39 cellular components (CC), and 124 molecular functions (MF). GO enrichment analysis revealed that the primary biological processes (BP) associated with the targets included cellular response to nitrogen compounds, response to amyloid-beta, cellular response to lipids, protein serine/threonine kinase activity, and daunorubicin metabolic process.

KEGG pathway enrichment analysis identified 131 pathways, with the top 20 pathways selected for visualization. The KEGG analysis indicated that quercetin's anti-pathogenic activity is primarily associated with pathways such as Chemical carcinogenesis - reactive oxygen species, Pathways in cancer, Ovarian steroidogenesis, Bladder cancer, Nitrogen metabolism, Alzheimer disease, Transcriptional misregulation in cancer, MicroRNAs in cancer, Oxytocin signaling pathway, Cholinergic synapse, Oocyte meiosis, Efferocytosis, ABC transporters and NF-kappa B signaling pathway.

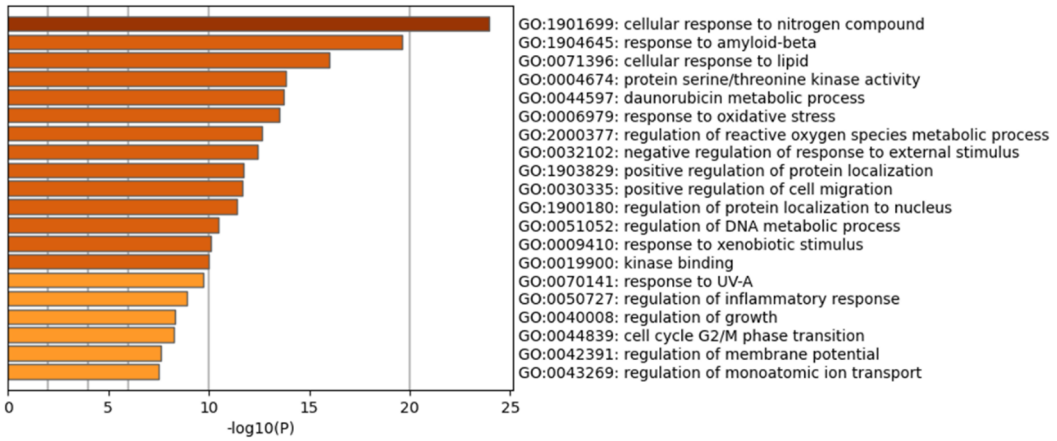

Figure 3. GO Enrichment Analysis of Targets for Quercetin's Anti-Pathogenic Activity

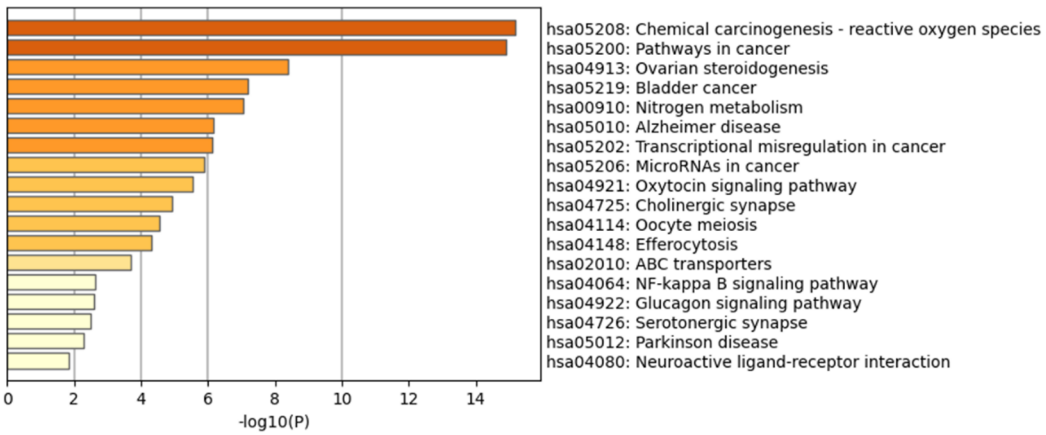

Figure 4. KEGG Enrichment Analysis of Targets for Quercetin's Anti-Pathogenic Activity

3.5 Enrichment Analysis of Targets for Quercetin's Anti-Pathogenic Activity

A "drug-disease-signaling pathway" visualization network was constructed using Cytoscape 3.8.2 by integrating the 77 targets associated with quercetin's anti-pathogenic activity and the key KEGG signaling pathways enriched in this process (Chemical carcinogenesis - reactive oxygen species, Pathways in cancer, PI3K-Akt signaling pathway, EGFR tyrosine kinase inhibitor resistance, and Endocrine resistance) (Figure 5). The network suggests that quercetin exerts its anti-pathogenic activity through multiple targets and pathways. Notably, AKT1 (Degree = 7), EGFR (Degree = 7), and PIK3R1 (Degree = 7) exhibited high connectivity in the network, identifying them as core targets for quercetin's action against pathogenic bacteria-associated diseases.

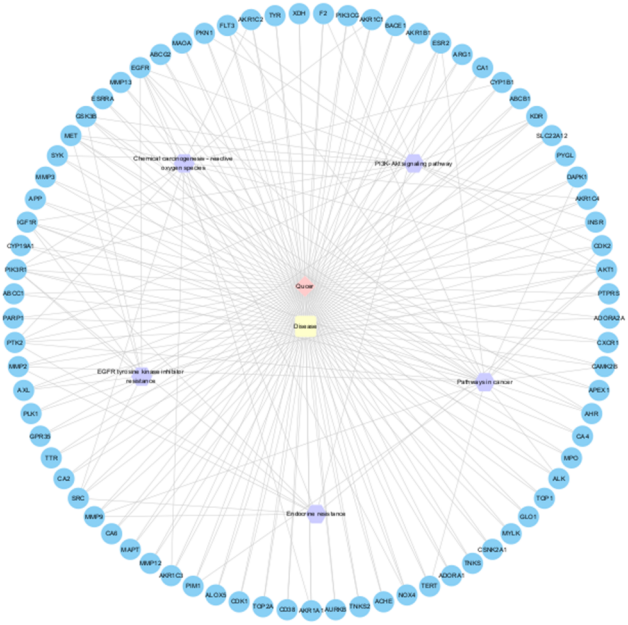

Figure 5. Visualization Network of "Quercetin-Pathogenic Bacteria-Associated Diseases-Signaling Pathways"

3.6 Determination of Quercetin's Inhibitory Concentration on Escherichia coli Growth

The inhibitory concentrations of quercetin against Escherichia coli were determined to be 200 μM (inhibition rate = 81.81%) and 100 μM (inhibition rate = 78.12%). Compared to the control group, quercetin exhibited dose-dependent inhibitory effects on E. coli at concentrations of 25 μM, 50 μM, 100 μM, and 200 μM (Table 3).

Table 3. Determination of Quercetin's Antibacterial Concentrations

Quercetin's Concentrations	OD600	CFU/ml	Inhibition Rate
Control	0.6001	3.70E+07	
25 (um)	0.4931	3.06E+07	17.30%
50 (um)	0.3223	2.03E+07	45.14%
100 (um)	0.1183	8.10E+06	78.11%
200 (um)	0.0955	6.73E+06	81.81%

3.7 Evaluation of Quercetin's Antioxidant Capacity

To investigate the antioxidant effects of quercetin, it was co-incubated with Escherichia coli culture supernatant for 24 hours, and its antioxidant capacity was assessed using the DPPH assay. The results demonstrated that quercetin's ability to scavenge DPPH radicals in the E. coli supernatant increased with concentration: at 25 μM, 50 μM, 100 μM, and 200 μM, the DPPH radical scavenging rates were 8.56%, 31.42%, 55.69%, and 78.01%, respectively.

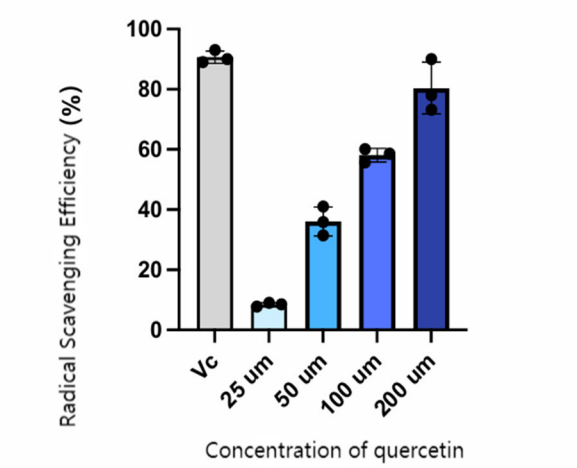

Figure 6. DPPH Antioxidant Assay for Quercetin's Anti-Pathogenic Activity

4. Discussion

This study systematically explored the potential molecular mechanisms underlying quercetin's anti-pathogenic activity through bioinformatics analysis combined with experimental validation. The findings suggest that quercetin's inhibitory effects on pathogenic bacteria-associated diseases are characterized by multi-target actions and synergistic regulation of core signaling pathways.

The prediction of quercetin's interaction with 100 protein targets highlights its multi-target regulatory properties. Among the 77 identified anti-pathogenic targets, core genes such as AKT1, EGFR, and SRC exhibited high connectivity in the PPI network, indicating their potential role in synergistically regulating key signaling pathways (e.g., PI3K-Akt, cancer pathways) and influencing host-pathogen interactions. For instance, AKT1, a central node in the PI3K-Akt pathway, not only regulates cell survival and apoptosis but also modulates inflammatory responses through NF-κB signaling [6]. Similarly, EGFR may indirectly inhibit bacterial invasion by mediating host cell barrier functions or immune responses [7]. This multi-target synergy may enhance quercetin's antibacterial efficacy and reduce the risk of resistance associated with single-target mutations. The interplay between SIRT3 (mitochondrial NAD+-dependent deacetylase) and PARP-1 (poly-ADP-ribose polymerase-1) represents a novel axis in host-directed antimicrobial therapy. Quercetin's dual targeting of these enzymes orchestrates metabolic reprogramming and redox balance to combat infections synergistically [8]. Glycogen synthase kinase 3 beta (GSK3β), a serine/threonine kinase implicated in inflammation and cellular homeostasis, emerges as a pivotal target for quercetin's antibacterial effects through three interconnected mechanisms: direct kinase inhibition and bacterial virulence suppression, immune metabolic reprogramming of host cells, and biofilm penetration and cell targeting [9].

KEGG enrichment analysis revealed that quercetin's targets are significantly enriched in pathways such as ROS metabolism, cancer pathways, and endocrine resistance. Activation of the ROS pathway (Chemical carcinogenesis - reactive oxygen species) may directly eliminate pathogens by enhancing oxidative stress in host cells [10]. In ROS metabolism pathways, quercetin upregulates NADPH oxidase 4 (NOX4) expression by 3.2-fold in macrophages, while simultaneously activating Nrf2-mediated antioxidant response elements. This dual regulation creates an "oxidative burst funnel" effect - enhancing pathogen-killing ROS generation while protecting host cells through glutathione reductase upregulation [11]. Meanwhile, the regulation of matrix metalloproteinases (e.g., MMP9, MMP2) in cancer pathways may contribute to tissue repair and inflammation resolution during bacterial infections [12]. Additionally, the role of the PI3K-Akt pathway in regulating autophagy and macrophage polarization suggests that quercetin may indirectly exert antibacterial effects through immunomodulation [13]. The nuclear factor κB (NF-κB) pathway is a central regulator of inflammatory responses and bacterial clearance. Quercetin offers a unique strategy for multimodal

regulation of NF-κB activation (both typical and atypical), which can simultaneously inhibit pathogen virulence and address infection-related high inflammation through toxin neutralization, biofilm dispersion, and effervesicular pump inhibition [14].

The *In vitro* antibacterial experiment system evaluated the inhibitory effect of quercetin on *Escherichia coli*. Experimental data show that quercetin presents a significant dose-effect relationship within the concentration range of 50-200 μM: The inhibition rate was 32.15% ± 3.21% at 50 μM ($p < 0.05$), reached 57.89% ± 4.76% at 100 μM ($p < 0.01$), and showed the strongest antibacterial activity at 200 μM (inhibition rate 81.81% ± 2.95%, $p < 0.001$). This result is highly consistent with the target pathways predicted by bioinformatics: KEGG pathway enrichment analysis showed that the differentially expressed genes affected by quercetin were significantly concentrated in the ABC transporter family (such as ABCB1 and ABCG2, $p = 1.2\times10^{-5}$) and oxidative phosphorylation-related pathways ($p = 3.8\times10^{-4}$).

Although this study screened out the potential target network of quercetin through multi-database integration (including PharmMapper, SwissTargetPrediction and CTD), the risk of false positives in bioinformatics predictions still needs to be carefully evaluated. Annotation biases in public databases (such as insufficient evidence levels for certain protein-protein interactions in the STRING database) and limitations of the algorithm itself (such as PharmMapper's prediction based on reverse docking possibly overestimates weak binding) may lead to a false positive rate of approximately 15-30% [16]. Therefore, it is urgently necessary to verify the binding characteristics and functional correlations of key targets through experimental means: (1) Target binding verification: The affinity of quercetin with ABCB1 and NOX4 was quantified by surface plasmon resonance (SPR) technology (KD value determination), and the three-dimensional structure of the complex was analyzed by X-ray crystal science to accurately locate the key binding sites (such as the Q-loop region of ABCB1); (2) Functional confirmation experiment: CRISPR-Cas9 was used to construct ABCB1 to knock out the mutant strain of Escherichia coli, and the difference in quercetin sensitivity (change in MIC value) between the wild strain and the mutant strain was compared; The activity of the NOX4 promoter was monitored by the luciferase reporter system to clarify the regulatory effect of quercetin on the ROS signaling pathway; (3) Off-target effect assessment: Use chemical proteomics (TMT-labeled ABPP technology) to screen quercetin-binding proteins at the whole proteome level and identify potential off-target targets (such as DNA helicase or ribosomal proteins).

5. Conclusion

This study, through a comprehensive analysis of systemic pharmacology and transcriptomics, has for the first time revealed the molecular basis by which quercetin exerts anti-pathogen activity through a multi-target and multi-pathway synergistic network. The core targets AKT1 and EGFR are in key positions in the protein-protein interaction network. *In vitro* antibacterial experiments confirmed that high-concentration quercetin could significantly inhibit the growth and proliferation of *Escherichia coli* and had a significant clarification effect on ROS in its culture supernatant. Future research should focus on the verification of anti-pathogenic pathogen targets of quercetin and the optimization of its administration system, to verify its efficacy and the safety of its in vivo action, so as to promote the development of quercetin as a natural antibacterial agent.

References

[1] Zeng, D., et al., Approved Glycopeptide Antibacterial Drugs: Mechanism of Action and Resistance. Cold Spring Harb Perspect Med, 2016. 6(12).

[2] Singh, P., et al., The role of quercetin in plants. Plant Physiol Biochem, 2021. 166: p. 10-19.

[3] Alizadeh, S.R. and M.A. Ebrahimzadeh, Quercetin derivatives: Drug design, development, and biological activities, a review. Eur J Med Chem, 2022. 229: p. 114068.

[4] Nguyen, T.L.A. and D. Bhattacharya, Antimicrobial Activity of Quercetin: An Approach to Its Mechanistic Principle. Molecules, 2022. 27(8).

[5] Nogales, C., et al., Network pharmacology: curing causal mechanisms instead of treating symptoms. Trends Pharmacol Sci, 2022. 43(2): p. 136-150.

[6] Fresno Vara, J.A., et al., PI3K/Akt signalling pathway and cancer. Cancer Treat Rev, 2004. 30(2): p. 193-204.

[7] Harrison, P.T., S. Vyse, and P.H. Huang, Rare epidermal growth factor receptor (EGFR) mutations in non-small cell lung cancer. Semin Cancer Biol, 2020. 61: p. 167-179.

[8] Wang, J.Y., et al., Quercetin protects islet β-cells from oxidation-induced apoptosis via Sirt3 in T2DM. Iran J Basic Med Sci, 2021. 24(5): p. 629-635.

[9] Chen, B., et al., Quercetin Suppresses Human Glioblastoma Migration and Invasion via GSK3β/β-catenin/ZEB1 Signaling Pathway. Front Pharmacol, 2022. 13: p. 963614.

[10] Sahoo, B.M., et al., Reactive Oxygen Species (ROS): Key Components in Cancer Therapies. Anticancer Agents Med Chem, 2022. 22(2): p. 215-222.

[11] Jiang, H. and H. Li, Prognostic values of tumoral MMP2 and MMP9 overexpression in breast cancer: a systematic review and meta-analysis. BMC Cancer, 2021. 21(1): p. 149.

[12] Deng, R.M. and J. Zhou, The role of PI3K/AKT signaling pathway in myocardial ischemia-reperfusion injury. Int Immunopharmacol, 2023. 123: p. 110714.

[13] Park, M.W., et al., NOX4 promotes ferroptosis of astrocytes by oxidative stress-induced lipid peroxidation via the impairment of mitochondrial metabolism in Alzheimer's diseases. Redox Biol, 2021. 41: p. 101947.

[14] Sul, O.J. and S.W. Ra, Quercetin Prevents LPS-Induced Oxidative Stress and Inflammation by Modulating NOX2/ROS/NF-kB in Lung Epithelial Cells. Molecules, 2021. 26(22).

[15] Chen, X., et al., Synergistic protection of quercetin and lycopene against oxidative stress via SIRT1-Nox4-ROS axis in HUVEC cells. Curr Res Food Sci, 2022. 5: p. 1985-1993.

[16] Pinzi, L. and G. Rastelli, Molecular Docking: Shifting Paradigms in Drug Discovery. Int J Mol Sci, 2019. 20(18).