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ANALYSIS OF THE ANTIBACTERIAL TARGETS OF MAACKIAIN BASED ON NETWORK PHARMACOLOGY AND MOLECULAR DOCKING

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Abstract

Background: Growing antimicrobial resistance represents a major challenge to global health, as infectious agents such as bacteria, fungi, and viruses are increasingly able to withstand the effects of standard antibiotics. This highlights the urgent need for new antimicrobial agents. Maackiain, a bioactive compound from Sophora species, has demonstrated promising antimicrobial potential. The present study aimed to elucidate the antibacterial pathways and potential targets of maackiain through an integrated network pharmacology–molecular docking strategy.

Methodology: Database mining was conducted to screen for potential targets of maackiain and genes involved in antibacterial activity. Common targets were determined, followed by protein–protein interaction (PPI) network construction. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were performed to elucidate biological functions and pathways. Molecular docking was conducted to assess binding affinities between maackiain and key target proteins.

Results: A total of 50 overlapping targets between maackiain and antibacterial-related genes were identified. Core targets included NFKB1, ESR1, MTOR, ACE, and PARP1. GO enrichment revealed involvement in inflammatory response, protein phosphorylation, and innate immune regulation. KEGG analysis indicated associations with HIF-1 signaling, AGE-RAGE signaling in diabetic complications, and arginine and proline metabolism. Molecular docking showed strong binding affinities (binding energies < –6.9 kcal/mol) between maackiain and the identified core targets.

Conclusion: Network pharmacology and molecular docking suggest that maackiain exerts antibacterial activity through multi-target and multi-pathway mechanisms involving inflammation and immune modulation. Further experimental validation is warranted to confirm its therapeutic potential.

Keywords: Maackiain; Network Pharmacology; Molecular Docking

1. Introduction

Network pharmacology represents a newly-developed discipline founded on the systems biology concept. It examines the networks within biological systems and picks out particular signal points (Nodes) to formulate multi-target drug molecules[1]. Pharmacology attaches great importance to the multi-pathway modulation of signaling pathways to boost the treatment outcomes of drugs and mitigate toxic and side effects, consequently elevating the success ratio of new drug clinical trials and cutting down the expenditure of pharmaceutical discovery and innovation. Due to the complexity of the signal networks of diseases, multi-target and combination drug therapies offer a novel network-centered method for drug exploration [2]. The network can enhance the curative effects of medications, predict adverse adverse effects, and offer a more extensive selection of disease targets, which has completely changed the concept and remedy of diseases.

Maackiain is the main component of some herbs. It is extracted from the root, seed, and heartwood of Sophora flavescens [3], Maackia amurensis Rupr [4], Sophora tonkinensis Gagnep [5], Trifolium pretense L. [6], and Ononis spinosa L. [7]. The molecular formula of maackiain is $C_{16}H_{12}O_5$, and its molar mass is 284.26 g/mol. The molecular structure is shown in Figure 1. The structure of maackiain contains a pterocarpan skeleton. Pterocarpan is a kind of bioactive isoflavone compound [8]. In nature, maackiain has two optical isomers, namely (+) maackiain and (-) maackiain, and the most common isomer is (-) maackiain [9]. Analyses have suggested that maackiain has many physiological functions, like anti-inflammatory [10], and antibacterial effects [11], protecting nerve cells [12], treating cervical cancer [13] and so on. No literature report has been published on analyzing the antibacterial effect of maackiain using network pharmacology. Because of this, this study intends to predict the targets of maackiain's antibacterial effect with network pharmacology's aid, hoping to open up more ideas for the comprehensive and in-depth study of maackiain.

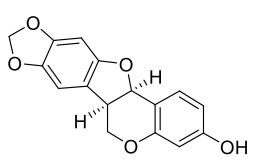

Figure 1: Chemical structure of maackiain

2. Methodology

2.1 Collection of chemical constituents and prediction of targets

The targets of maackiain were all retrieved via SwissTargetprediction (http://www.swisstargetprediction.ch/) and SuperPred (https://prediction.charite.de/). Upon calibrating the obtained targets with the data from Uniprot (https://www.uniprot.org/), human genes were eliminated, and the invalid and duplicate targets were excluded, thereby attaining the standardized gene names.

2.2 Attainment of antibacterial-related targets

By inputting the keyword "antibacterial" in the GeneCards (https://www.genecards.org/) and PharmGKB (https://www.pharmgkb.org/) databases respectively for retrieval, the objects associated with the disease were obtained. All the targets among the two databases were merged into Excel, and the copy genes were removed. Then, the genetic data of the disease targets was calibrated via the Uniprot database.

2.3 Prediction of maackiain-disease targets

The obtained drug component targets and the targets linked to the disease were put in contrast with each other. Thereafter, a Venn diagram was made to get the intersection genes. Then, the "maackiain-target" network was built by means of the Cytoscape 3.8.2 software.

2.4 Building of the interaction of target proteins framework

For the purpose of conducting a more in-depth study on the protein–protein interactions during the antibacterial treatment using maackiain, the drug–disease intersection genes were fed into the STRING interaction database (https://string-db.org/) to generate the PPI network. The species was configured as Homo sapiens, and the remaining parameters were left at their preset configurations. The data were saved in TSV format. Next, the TSV file was incorporated into Cytoscape 3.8.2 to conduct network analysis (Cytoscape → Tools → Network Analyzer → Network Analysis → Analyze

Network). The outcomes from the network analysis were stored, and the size and color of the node were adopted to denote the degree value. In general, the larger the magnitude of the node, the higher its degree value. In the end, the network diagram for protein interaction was generated. A medium-to-high confidence score threshold (≥ 0.7) was applied in the STRING database to ensure the reliability of the protein–protein interactions included in the network.

2.5 GO-term enrichment assessment and analysis of KEGG pathways

The maackiain–disease intersection genes were uploaded to the DAVID 6.8 database (https://david.ncifcrf.gov/summary.jsp) for functional enrichment analysis. The gene identifier was set as OFFICIAL_GENE_SYMBOL, and the species was restricted to Homo sapiens. GO enrichment analysis was performed to annotate the target proteins of maackiain in antibacterial treatment from three aspects: biological process (BP), cellular component (CC), and molecular function (MF). KEGG pathway enrichment analysis was also conducted to elucidate the signaling pathways potentially involved in the antibacterial mechanism of maackiain.

The top 10 GO terms in BP, CC, and MF, as well as the top 20 KEGG pathways, were selected based on statistical significance (lowest adjusted p-values) and biological relevance to antibacterial effects. Enriched terms and pathways with $P < 0.01$ were considered for further analysis, and statistical significance was determined using the Benjamini–Hochberg false discovery rate (FDR) method, with adjusted $P < 0.05$ regarded as significant. This combined criterion ensured that the selected GO terms and KEGG pathways were both statistically robust and biologically meaningful in reflecting the potential antibacterial mechanisms of maackiain.

2.6 Molecular Docking Validation

The two-dimensional structure of maackiain was first drawn using ChemDraw, then imported into Chem3D for energy minimization using the MM2 module to obtain the most stable conformation. The optimized structure was saved in.mol2 format. The crystal structures of the human target proteins (ESR1, ACE, MTOR, NFKB1, and PARP1) were downloaded from the Protein Data Bank (PDB) and visualized using PyMOL. Before docking, the receptor proteins were preprocessed using AutoDockTools (MGLTools 1.5.6) by removing water molecules, adding hydrogens, calculating Gasteiger charges, and merging nonpolar hydrogens. The processed ligand and receptor structures were then converted to.pdbqt format. Molecular docking was performed using AutoDock Vina 1.1.2 with default parameters, and the docking poses were ranked according to the binding affinity scores. The top-scoring conformations were further visualized and analyzed in PyMOL and Discovery Studio to identify key amino acid residues and interaction types between maackiain and the target proteins.

3. Results

3.1 Prediction of maackiain targets

Using a probability > 0 as the criterion for including targets in SwissTargetPrediction, 76 targets were identified. Through SuperPred, 121 targets of Maackiain were retrieved. Following the removal of duplicates, a total of 191distinct targets were acquired.

3.2 Antibacterial-related target exploration

Via the GeneCards and PharmGKB databases, 1150 and 195 antibacterial targets were respectively pinpointed. Upon computing the union, a total of 1242 antibacterial-correlated targets were obtained. The identified genes were calibrated with the assistance of the UniProt database. By intersecting the drug's active targets and the antibacterial-related target genes, 50 overlapping target genes were discerned (refer to Figure 2). These are the genes that function as interacting targets in the drug's antibacterial treatment process.

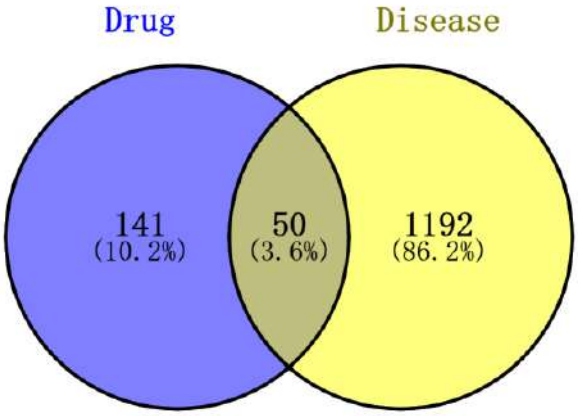

Figure 2: Intersection and distribution of drug and disease targets.

3.3 Prediction results of drug-component-targets

Using the drug-target dataset, the files "network.xlsx" and "type.xlsx" were created to represent the relationships and classifications within the dataset. These files were then introduced into Cytoscape 3.7.2 for visual analysis. The resulting network consists of 51 nodes, representing drugs, components, and targets, and 49 edges, illustrating the interactions between them. The results are shown in Figure 3.

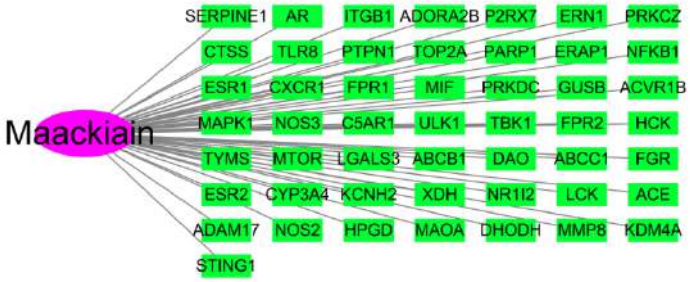

Figure 3: Network diagram of the relationship between maackiain and related target genes. Rectangles represent targets, and ellipses represent drug components.

3.4 Core targets and network interactions

By finding the intersection between all drugs' action targets and antibacterial target genes, 50 shared target genes were identified. These genes represent the interactive targets for the drug's antibacterial effects. The 50 intersection target genes were fed into the STRING database (https://string-db.org/) for PPI prediction. The species was set to Homo sapiens, and the confidence threshold was set to 0.4. The file of the network was stored in TSV-style format and then introduced into Cytoscape 3.8.2 software for the construction of the protein interaction network. This network included 47 nodes and 340 edges, as depicted in Figure 4. Topological analysis was carried out on the network. The size and color of the nodes reflected their degree value, while the depth of the edges represented the combined score quantity. This process constructed the protein-protein interaction network. Within the network, NFKB1, ESR1, MTOR, ACE, PARP1, and MAPK1 were identified as the core targets.

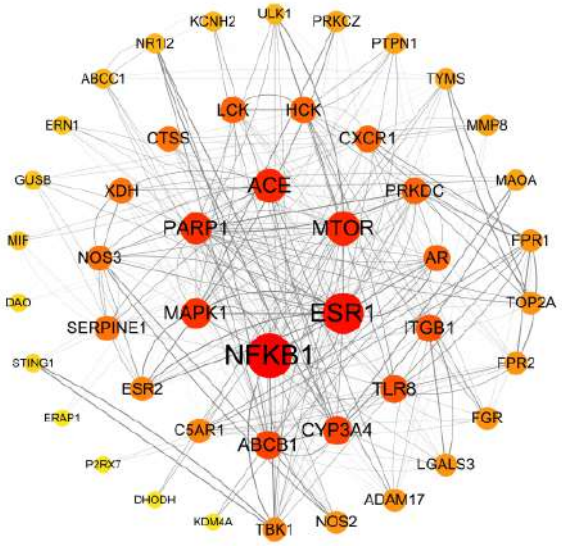

Figure 4: Protein-protein interaction network.

3.5 Biological function enrichment analysis

3.5.1 Enrichment analysis of Gene Ontology

The genes at the intersection of drugs and diseases were chosen. Moreover, the GO gene functional enrichment analysis was executed by utilizing the DAVID database. Altogether 169 GO terms were picked out. Taking $P < 0.05$ as the standard, 98 main terms of significantly enriched BP for maackiain in treating antibacterial were screened. These mainly included inflammatory response, protein phosphorylation, innate immune response, signal transduction, inhibition of gene expression, stimulation of gene expression, reaction to external stimulus, peptidyl-threonine phosphorylation, response to lipopolysaccharide, and protein autophosphorylation. There were 28

terms related to CC, involving plasma membrane, cytosol, cytoplasm, integral component of membrane, nucleoplasm, membrane, extracellular exosome, extracellular region, mitochondrion, and chromatin. Among them, 43 terms related to MF were involved, including protein binding, identical protein binding, ATP binding, enzyme binding, zinc ion binding, protein homodimerization activity, receptor binding, protein kinase binding, oxidoreductase activity, and ubiquitin protein ligase binding (refer to Figure 5).

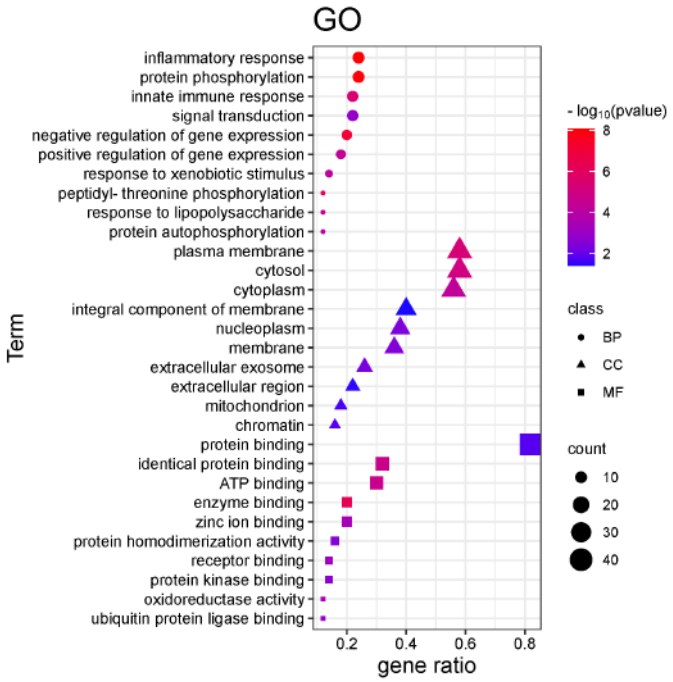

Figure 5: GO enrichment analysis results.

3.5.2 KEGG pathway analysis for enrichment

The DAVID database was utilized to conduct pathway enrichment analysis. Altogether, 46 pathways related to antibacterial treatment were enriched. According to $P < 0.05$, 90 pathways for antibacterial treatment were filtered out, and the pathways related to antibacterial were screened out, including the HIF-1 signalling pathway, chemical carcinogenesis-receptor activation, AGE-RAGE signalling pathway in diabetic complications, arginine and proline metabolism, insulin resistance, chemokine signalling pathway, sphingolipid signalling pathway, diabetic cardiomyopathy, relaxin signalling pathway, apoptosis, Yersinia infection, Apelin signalling pathway, prolactin signalling pathway, antifolate resistance, research on PD- L1 expression in tumours and PD-1 checkpoint pathway.

endocrine resistance, NOD-like receptor signalling pathway, cancer pathways, Kaposi sarcoma-associated herpesvirus infection, Th17 cell differentiation and other pathways. The results are shown in Figure 6.

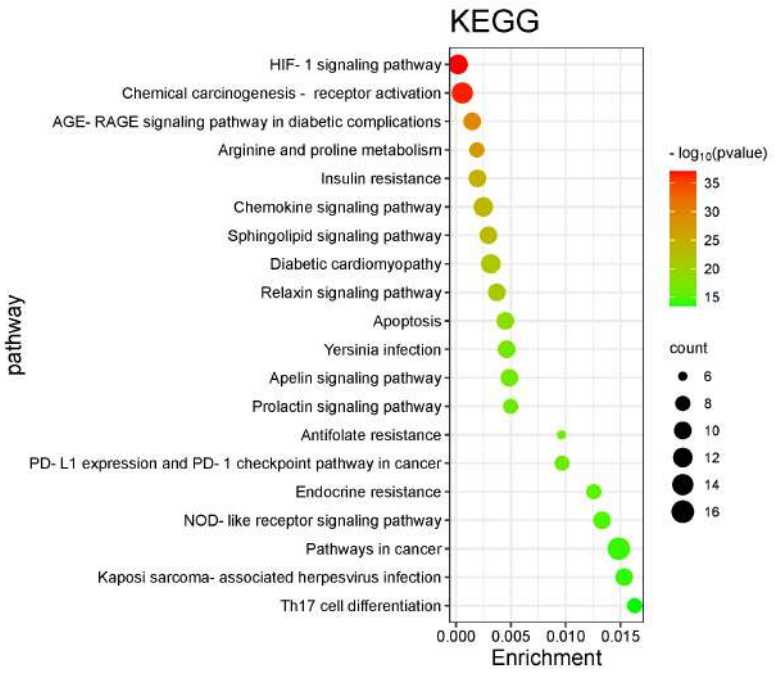

Figure 6: Outcomes derived from KEGG-based pathway enrichment analysis. The magnitude of the rings denotes the quantity of genes enriched in the corresponding pathways. Whereas the color variation from green to red signifies that the P-value is gradually going down. According to the P-value, the 20 top-ranked KEGG metabolic pathways were identified. A bubble graph was created to visualize these results, with the horizontal axis denoting the number of genes rich within the pathways. The magnitude of the bubbles corresponds to the gene count enriched within the respective pathways, and the color intensity reflects the importance level, enabling an intuitive observation of the significantly enriched pathways.

3.6 Results of molecular docking

In light of the previous analysis, the five top significant targets were chosen for semi-flexible combination with compounds which had relatively high degree values. Binding energy (affinity) was employed to denote how well the small molecules bind to the target proteins. When the interaction energy is under 0, it indicates that the small molecules are able to bind freely to the target proteins. Moreover, the smaller the value of the binding force, the higher the binding affinity. It was shown by the docking results that the small molecules could make their way into the active centers of the target proteins without a hitch. Then, the small molecules that had the best docking results

for every protein were selected to be presented graphically. Maackiain forms hydrogen bonds with GLN-259, HIS-491, and HIS-331 of ACE, with bond lengths of 2.4 Å, 2.0 Å, and 2.5 Å, separately. Maackiain forms a hydrogen bond with ARG-515 of ESR1, with a bond length of 2.6 Å. Maackiain forms hydrogen bonds with TYR-2144, THR-2143, and GLY-2142 of MTOR, with bond distances of 2.1 Å, 2.1 Å, and 2.5 Å, separately. Maackiain forms hydrogen bonds with ALA-62, LYS-149, and ARG-157 of NFKB1, with bond distances of 2.79 Å, 2.5 Å, and 2.7 Å, respectively. Maackiain forms a hydrogen bond with GLY-863 of PARP1, with a bond distance of 2.3 Å. Table 1 and Figures 6–11 summarize the outcomes of the molecular docking analysis.

Table 1: Binding affinity evaluation of maackiain toward core targets

Targets	PDB ID	Active ingredient	Binding energy(kcal/mol)
ESR1	6SBO		-8.5
ACE	5AMB		-9.2
MTOR	8ERA	Maackiain	-7.3
NFKB1	1SVC		-6.9
PARP1	7AAC		-9.5

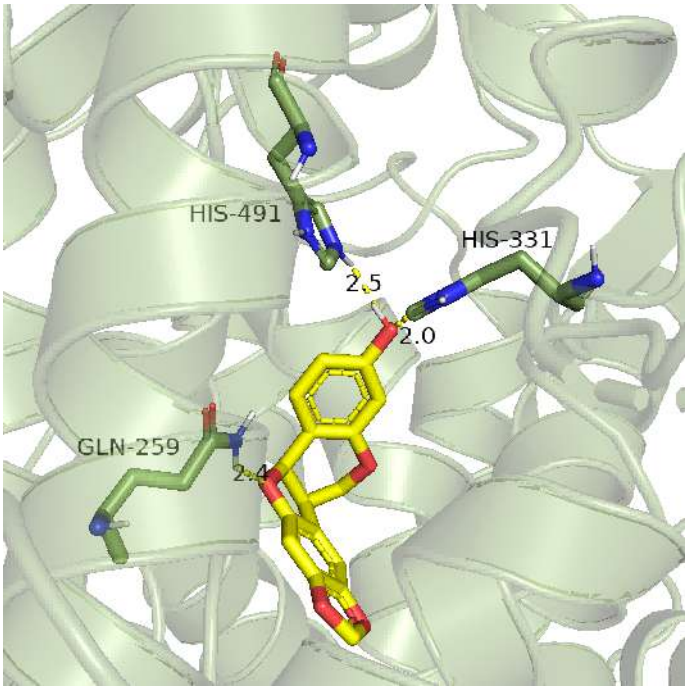

Figure 6: Docking outcomes. Interaction pattern between maackiain and ACE protein.

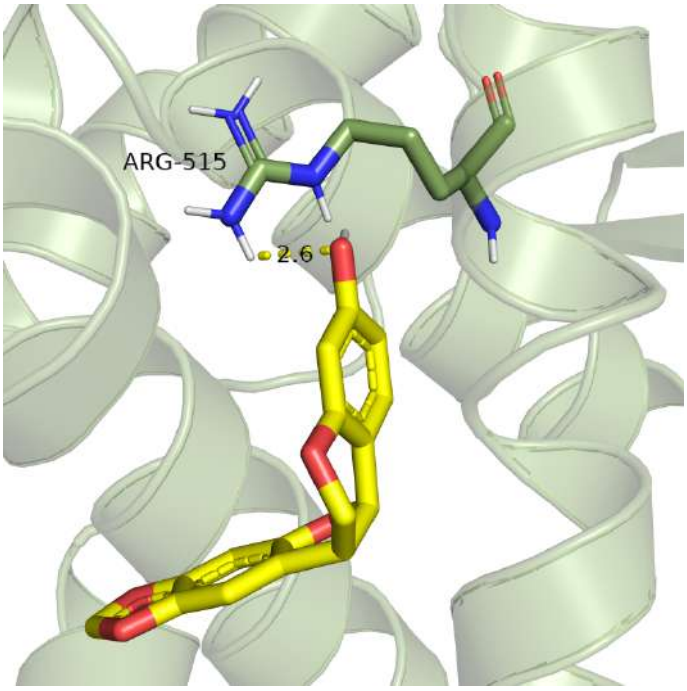

Figure 7: Docking outcomes. Interaction pattern between maackiain and ESR1 protein.

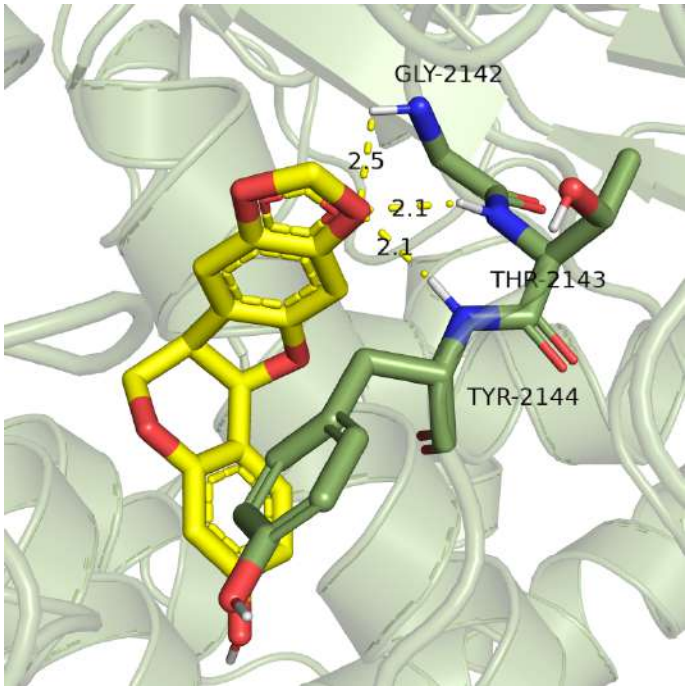

Figure 8: Docking outcomes. Interaction pattern between maackiain and MTOR protein.

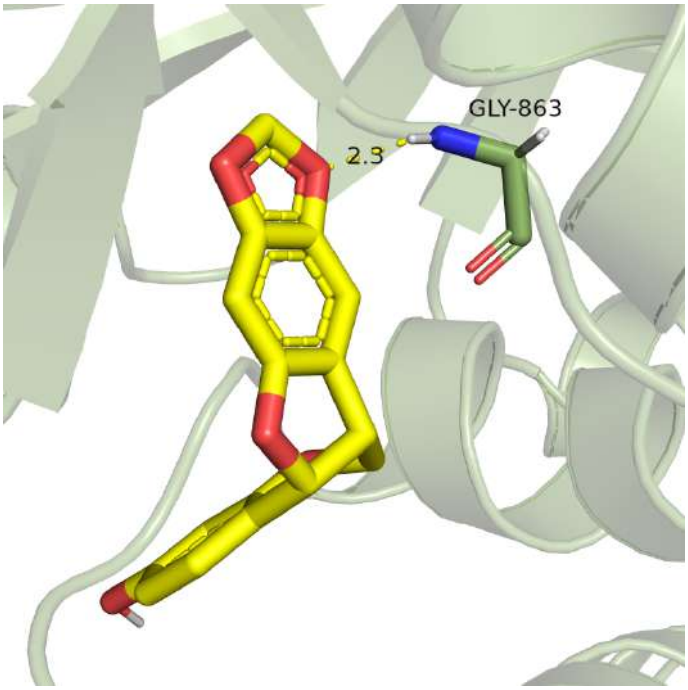

Figure 10: Docking outcomes. Interaction pattern between maackiain and PARP1 protein.

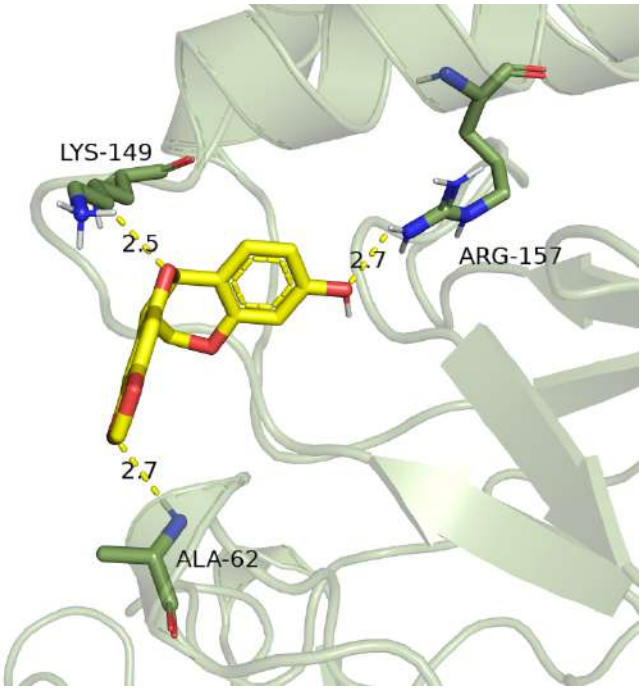

Figure 11: Docking outcomes. Interaction pattern between maackiain and NFKB1 protein.

4. Discussion

This study found five main target proteins of maackiain acting on bacteria, namely NFKB1, ESR1, MTOR, ACE, and PARP1. Meanwhile, the pathways related to antibacterial were screened out, including the HIF-1 signalling pathway, the activation of receptors in chemical carcinogenesis pathway, the age-signaling pathway of diabetic complications, the arginine and proline metabolism pathway, and the insulin resistance pathway. The molecular docking results indicated that maackiain has the least binding energy (-9.5 kcal/mol) with PARP1 and the maximum binding energy (-6.9 kcal/mol) with NFKB1, indicating that maackiain has a relatively high binding possibility with NFKB1, ESR1, MTOR, ACE, and PARP1. Maackiain exhibits antibacterial effects [14; 15]. Studies have shown that (-)-maackiain demonstrates significant antibacterial activity against Acinetobacter baumannii, with MIC95 with a range spanning from 256 to 512 μg/mL [16]. Additionally, (-)-maackiain also inhibits the glutathione transferase (GST) activity of the white-rot fungus Trametes versicolor [17].

NFkB1 protein is a critical component of the highly efficient regulatory factor NF-kB, which is widely present in various cell types. As a core mediator, NF-kB participates in signal transduction pathways related to multiple biological processes, including inflammation and immunity. NFkB1 can form homodimeric or heterodimeric complexes, with the p65-p50 heterodimer being the most common. These dimers selectively bind to kB sites in the DNA of target genes, exhibiting different binding preferences and affinities. The NFkB1 gene codes for the p50 and p105 proteins, which are components of the regulatory factors NF-kB and IkB-γ, respectively, and influence NF-kB-involved signal transduction [18]. NF-kB1 is involved in inflammatory responses, and its reduction is capable of promoting the regulation of pro-inflammatory cytokines such as IL-12 and TNF-α [19]. Additionally, inflammasomes are closely related to NF-kB, and they collaborate and interact with each other during the initiation. It has been shown that maackiain has an anti-inflammatory effect by the mechanism that acacia globulin inhibits Pseudomonas aeruginosa-mediated activation processes of NF-kB and activator vesicles, thereby reducing IL-1beta production [20]. Meanwhile, (-)-maackiain markedly potentiated the role of caspase-1 in macrophages against Nigerian mycobacteria and significantly promoted Nigerian mycobacteria-mediated caspase-1. These outcomes indicate that (-)-maackiain promotes the generation of IL-1beta by activating blunted vesicles/caspase-1 molecules, thus exerting an immunostimulatory effect [3]. Maackiain significantly lessens LPS-induced pulmonary damage and inflammation via the NF-kB/Nrf2/AQPs signalling pathway modulation [10].

PARP1 is capable of sensing breaks within DNA molecules and subsequently catalyzing the poly(ADP-ribosyl) ation of itself and associated proteins [21]. This alteration results in the liberation of PARP1 from the DNA end and triggers a range of reactions such as DNA repair [21]. PARP1, together with DNA ligase III and XRCC1, participates in DSB repair [21]. The main function of PARP1 is to bind to SSB as part of the BER pathway [22]. Organisms and cell systems lacking functional PARP1 will exhibit drastically damaged base excision repair and genomic instability [23]. PARP1 is widely expressed in all stages of mouse development and adult tissues [24]. Many PARP1s are activated in gastric epithelial cells infected by *H. pylori*. PARP1 is capable of directly interacting with NF-kB and promoting the interaction of NF-kB with other proteins and DNA, thereby promoting the manifestation of pro-inflammatory cytokines and the functioning of nitric oxide synthase [25].

ESR1 is the gene for estrogen receptor alpha, and it plays a vital function in hormone-dependent tumors such as breast cancer. The chloroform-extracted portion from P. betle presented remarkable antibacterial activity, having an inhibition zone diameter of 7 mm and a MIC of 100 $mg.mL^{-1}$ against Bacillus gaemokensis. It is worth noting that this strain was the most common biofilm producer among the separated bacterial strains. Via in silico network pharmacology analysis, it was found that proteins like ESR1 and IL-6 were mainly involved in the biological processes relevant to dental caries. Additionally, these proteins were shown to influence the protective role of P. betle [26]. Increased expression of ESR1 correlates with adverse clinical outcomes and is regarded as a potential indicator of prognosis in *H. pylori*–induced gastric carcinoma [27].

There is no mTOR protein in bacteria, but there is an indirect relationship between bacteria and the mTOR signalling pathway. Specifically, bacteria regulate host cell function by affecting the host's

mTOR signalling pathway or through metabolites, toxins, etc. mTOR inhibits autophagy by sensing the intracellular metabolic state (such as amino acid sufficiency). The amino acid starvation state caused by bacterial infection can decrease mTOR activity, thereby triggering xenophagy to degrade intracellular bacteria [28]. Rhein treatment is able to notably suppress the PI3K/Akt/mTOR signalling pathway by decreasing the phosphorylation levels of PI3K, Akt, mTOR, and p70S6K1 proteins [29]. The PI3K/Akt/mTOR signal transduction route activates the HIF-1 signalling pathway through multiple mechanisms, mainly reflected in the stability, translation, and transcriptional regulation of HIF-1α, and HIF-1αis orchestrated via the PI3K/Akt/mTOR signalling pathway [30]. The relationship between mTOR and Helicobacter pylori is mainly reflected in the activation effect of Helicobacter pylori infection on the mTOR signalling pathway. After *H. pylori* infects the body, it may activate the intracellular PI3K/Akt/mTOR signalling pathway through toxins such as CagA it produces, thereby promoting cell hyperplasia and epithelial-mesenchymal transition and increasing the probability of canceration. *H. pylori* infection in gastric epithelial cells triggers the PI3K/AKT/mTOR and MAPK cascade responses, leading epithelial cells to transform into tumor cells via alterations like apoptosis, proliferation, and differentiation [31]. Research has found that maackiain induces autophagy within cervical cancer cells by activating AMPK and suppressing the mTOR regulatory pathway [13].

Angiotensin-Converting Enzyme (ACE). ACE is a protease expressed by mammals (including humans) and mainly exists in tissues such as the kidneys, lungs, and heart. It transforms angiotensin I into angiotensin II, thereby modulating blood pressure and fluid balance [32]. Although no ACE exists in bacteria, certain bacterial infections may indirectly affect the host's ACE, or Renin-Angiotensin System (RAS), through the following mechanisms: For example, the inflammatory response triggered by bacterial infections may regulate the expression or activity of the host's ACE through cytokines; proteases secreted by some bacteria may degrade angiotensin or bradykinin, thus affecting the host's vascular function; some pathogenic bacteria may change the dynamic balance of the RAS system by influencing the signal pathways of host cells. ACEI exposure diminished the capacity of murine neutrophils to eliminate MRSA, Pseudomonas aeruginosa, and Klebsiella pneumoniae *in vitro* [33]. Sini decoction can significantly reduce the expression of ACE, AngII, and AT1R induced by Escherichia coli. The MKN28, N87, and MKN45 gastric cell lines all display ACE. The expression of ACE mRNA is jointly regulated by H. pylori and cytokines (IL-1b, IL-6, IL-8, TNF-a, and TGFb1). In the antrum and pylorus areas, mucus-secreting cells exhibit ACE in a membranous form on the apical cell surface. Near gastric ulcers in the mucosa, ACE is conveyed by both chief cells and mucus-secreting cells within the antral and pyloric glands. ACE has been present in endothelial cells, stromal cells, and macrophages in granulation tissue [34]. It has been reported that the response of ACE to lipoproteins is upregulated, which might be associated with the combination of *H. pylori* LPS and toll - like receptors together with the activation of NF-kB [35].

This study found that one of the antibacterial targets of maackiain is TLR8, and TLR8 is a member of the TLRs family. TLRs are a family of pattern recognition receptors present on various immune and epithelial cells, which play a role in maintaining intestinal immune homeostasis [36]. TLRs are capable of sensing molecular motifs characteristic of microorganisms, detecting MAMPs originating from normal microbiota as well as PAMPs released by pathogenic microbes [37]. Clindamycin reduced the expression of TLR8 receptors [38]. TLR8 signaling can increase the production of TNF-α and IL-1β [39]. Bacitracin and neomycin increase the expression of TLR8 [40]. Evidence indicates that purified *H. pylori* RNA can be sensed by TLR7 and TLR8, and activation of TLR8 subsequently promotes pro-inflammatory cytokine expression via the MyD88 signaling cascade [41].

An oral dose of maackiain as high as 2000 mg/kg has been shown to be safe, with no fatalities observed in mice within 24 hours of the study [42]. At 100 ng/mL, maackiain significantly reduced

LDH release, indicating slight cytotoxic impacts on A549 cells [3]. Current evidence suggests that maackiain is a generally safe compound with low toxicity to most cell types. However, comprehensive long-term toxicological studies in animals remain insufficient and require further investigation.

Network pharmacology, as a burgeoning discipline, has been established and developed based on principles such as systems biology, the analysis of biological system networks, and the identification of specific signal nodes for multi-target drug design [1]. This field holds significant importance as it investigates the mechanisms of drug actions in disease treatment at the levels of biological targets and pathways [43]. Moreover, the network pharmacology approach facilitates the construction of bioinformatics networks. Uncovering the connections between disease-related genes and drug-target genes elucidates drug-disease correlations, offering valuable insights for innovative drug research, development, and application [43]. With the increasing knowledge of the mechanisms behind the therapeutic effects of approved drugs, many effective drugs have been found to act on multiple targets rather than a single one. For instance, the antiepileptic drugs felbamate and topiramate exemplify this multi-target mechanism [44]. Despite its advantages, network pharmacy faces several challenges in its application. These include limitations related to database selection, algorithm variability, inconsistent research quality, and the lack of standardization. Additionally, network pharmacology faces challenges in comprehensively mapping complex interactions. This limitation restricts its capacity to analyze the relationships among compounds, gut microbiota, and the host. Moreover, the majority of network pharmacology methods concentrate on building interaction networks between compounds and targets, frequently overlooking information related to concentration.

Although molecular docking in this study was conducted on human proteins (ESR1, ACE, MTOR, NFKB1, and PARP1) rather than bacterial targets, the results provide meaningful insights into the host-mediated antibacterial mechanisms of maackiain. Previous research has demonstrated that maackiain enhances nigericin-mediated inflammasome activation and promotes IL-1β production, indicating its capacity to modulate immune and inflammatory responses [3]. Binding to these human targets may similarly regulate host immune and cellular pathways that indirectly facilitate bacterial clearance. Moreover, several of these proteins possess structural or functional homologs in bacteria, suggesting that maackiain may also exert direct antimicrobial effects. While this study does not fully exclude their roles in host immune regulation, it integrates network pharmacology, molecular docking, and literature evidence to propose a dual function of maackiain in host–pathogen interactions.

Network pharmacology serves as an integrative framework that facilitates the elucidation of multi-target and multi-pathway interactions at the systems level. However, its application to antibacterial studies remains limited, as most databases are primarily based on human or mammalian proteins, leading to potential bias and underrepresentation of microbial targets. Consequently, the predicted interactions mainly reflect host-mediated pathways, while direct bacterial mechanisms may be overlooked. Integrating bacterial genomics, proteomics, and experimental validation with network pharmacology will therefore be essential to comprehensively elucidate maackia's antibacterial actions.

5. Conclusion

In conclusion, maackiain potentially exerts antibacterial effects through a dual mechanism involving modulation of host defense pathways and possible direct bacterial inhibition. Future studies should incorporate microbial protein structures and biological validation to further substantiate these findings.

Ethics Approval and Consent of Participants

The present work did not include any studies involving human participants or animal subjects. All information was derived from publicly accessible databases, eliminating the need for ethical clearance or participant consent.

Human and Animal Rights

This research did not include any experiments or procedures involving human or animal subjects.

Author Contribution

Qin Honghan performed data analysis and prepared the initial draft of the manuscript.

Dr. Sher Zaman Safi and Dr. Shalini Vellasamy critically reviewed the paper and offered valuable feedback for improvement.

All contributors approved the final manuscript and take joint responsibility for the integrity of the work.

Declaration of AI and AI-Assisted Technologies in the Writing Process

ChatGPT was employed to enhance the clarity and fluency of the English language in this manuscript. The author subsequently revised and edited all sections to ensure accuracy and accepts complete responsibility for the published content.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article. The datasets used in this research were obtained from publicly available databases as cited in the Methods section.

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None

Conflict Of Interest

All authors state that they have no competing interests related to this study.

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