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Molecular mechanism of Bai-Bo Formula for treatment of vitiligo based on network pharmacology and molecular docking

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ABSTRACT

Using network pharmacology and molecular docking, this study aims to evaluate the pharmacological mechanism of Bai-Bo Formula in the prevention and treatment of vitiligo. The chemical composition collection and anticipated targets for Bai-Bo Formula for vitiligo were compiled using TCMSP and BATMAN-TCM databases. GeneCards, OMIM, and DisGeNET databases were used to extract targets associated with vitiligo. The acquired drug-component targets were intersected with the illness targets to identify common genes, and a drug-component-target network was created using Cytoscape 3.7.2. Protein-protein interactions of Bai-Bo Formula were examined using String, and function enrichment analyses were performed on the primary targets using gene ontology and the Kyoto encyclopaedia of genes and genomes. Finally, molecular docking technologies were used to validate the combination of primary components and key targets. A study evaluated 167 active ingredients and 1425 prediction targets in 12 traditional Chinese remedies known as Bai-Bo Formula for treating vitiligo. 169 target genes were found to interact with the medicine. The protein-protein interaction network analysis identified 91 important proteins, with the top 5 targets being IL6, TNF, AKT1, IL1, and STAT. Bai-Bo Formula regulates immune inflammation, apoptosis, and autophagy via various pathways, including AGE-RAGE, PI3K-Akt, and TNF signaling. Molecular docking indicated that the primary components could attach effectively to the target protein. Based on network pharmacology and molecular docking, the mechanism of Bai-Bo Formula in the treatment of vitiligo via multicomponent, multitarget, and multichannel was investigated in depth.

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Introduction

Vitiligo is an acquired pigment condition of the skin and/or mucous membranes, characterised by the absence of epidermal melanocytes and the development of leukoplakia. The incidence rate in children and adults ranges from 0.5% to 2% (1). The demise of melanocytes is one of the features of vitiligo. Additionally, the pathophysiology and aetiology of vitiligo are various, which medical study has not yet verified. The majority of individuals believe that the aetiology of vitiligo comprises the oxidative stress theory, the autoimmune hypothesis, the neural theory, the inherent theory, and the melanocyte bleeding hypothesis, among others (2). Clinically, the most frequent first-line treatments are glucocorticoids, synthetic -melanocyte-stimulating hormone, calcineurin inhibitors, vitamin D3, phototherapy, surgery, traditional Chinese medicine (TCM), and others. The short-term efficacy of Western medicine in the treatment of this condition is adequate, however, the long-term treatment has undesirable side effects, such as local skin irritation, atrophy, telangiectasia, hormone-dependent dermatitis, etc. Modern medicine has steadily established the mechanism underlying TCM's low incidence of side effects, high compliance, and stable therapeutic outcomes. TCM is widely utilised due to its low incidence of adverse effects, high compliance, and stable curative effects.

Traditional Chinese medicine (TCM), a complete medical system, plays a crucial role in the health maintenance of the Asian population (3). TCM has accumulated popularity in Western nations due to its potent therapeutic benefits and comparatively minimal adverse effects. According to the philosophy of traditional Chinese herbal medicine, TCM is regarded as a viable preventative and therapeutic source for complex illnesses such as vitiligo (4).

Chinese medicine's Bai-Bo Formula has the functions of "Tonifying the liver and kidneys" and "nourishing blood and expelling wind." The following are the major components of the medication: Polygonum multiflorum, Tribulus terrestris, Angelica sinensis, Rehmannia glutinosa, Clematis chinensis, Ligustrum lucidum, Astragalus complanatus, Eclipta prostrata, Saposhnikovia divaricata, Xanthium sibiricum, Lemna minor, Glycyrrhiza uralensis. Angelica sinensis and Rehmannia glutinosa could replenish Qi and blood; Polygonum multiflorum, Tribulus terrestris, Ligustrum lucidum, Eclipta prostrata and Astragalus complanatus could "Tonify the liver and kidneys"; Clematis chinensis, Saposhnikovia divaricata, Xanthium sibiricum and Lemna minor could "disp The combination of the aforementioned medications has the effect of "tonifying the liver and kidneys" as well as "removing blood clots and expelling wind." Bai-Bo Formula is suggested

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by specialists in TCM diagnosis and therapy for the treatment of vitiligo, and its clinical efficacy has been proven in several cases. Yet, the method through which Bai-Bo Formula treats vitiligo is unclear. Using systems biology, bioinformatics, network science, and other fields, network pharmacology may systematically expose the internal mechanism of pharmacology from numerous levels and perspectives in order to have a stronger leading strategy for medical assessment and treatment (5).

Materials and Methods

Chemical composition collection and target prediction of Bai-Bo Formula

Oral bioavailability (OB) 30% and drug-likeness (DL) 0.18 were utilised as conditions using the Traditional Chinese Medicine Data base and Analytical Platform (TCMSP,https://tcmsp-e.com/). The active constituents of Polygonum multiflorum, Tribulus terrestris, Angelica sinensis, Rehmannia glutinosa, Clematis chinensis, Ligustrum lucidum, Astragalus complanatus, Eclipta prostrata, Saposhnikovia divaricata, Xanthium sibiricum, Lemna minor, and Glycyrrhiza uralensis. The gathered targets were calibrated using Uniprot (https://uniprot.org/) data, non-human genes were omitted, and incorrect duplicate targets were eliminated to produce standard gene names. For medications that could not be recovered from TCMSP, candidate genes were acquired via logging into the BATMAN-TCM database (http://bionet.ncpsb.org.cn/batman-tcm/) in order to get high-confidence proteins. The gathered targets were calibrated using Uniprot (https://uniprot.org/) data, non-human genes were omitted, incorrect duplicate targets were eliminated, and standard gene names were obtained. Not retrievable from TCMSP; acquired as candidate genes by logging into the BATMAN-TCM database (http://bionet.ncpsb.org.cn/batman-tcm/). The gathered targets were calibrated using Uniprot (https://uniprot.org/) data, non-human genes were omitted, incorrect duplicate targets were eliminated, and standard gene names were obtained.

Acquisition of vitiligo-related targets

Entering the keywords "Vitiligo" was searched to obtain disease-related targets in GeneCards (https://www.genecards.org/), OMIM (https://www.omim.org/), DisGeNET (https://www.disgenet.org/), and all targets in the three databases were combined in an excel, with duplicate genes removed and corrected by the Uniprot database to obtain the disease target gene information.

Drug-disease target prediction results

The obtained drug-component targets were mapped against the disease targets and then Veen plots were made to obtain the intersecting genes. The drug-component-target network was then constructed using Cytoscape 3.7.2 software.

Target protein interaction network construction

To further investigate the protein-protein interactions of Bai-Bo Formula for vitiligo, the drug-interacting genes were uploaded to the interaction database String (https://string-db.org/) for protein interaction network construction (PPI) database; the species was set to "Homosapiens ", the minimum interaction score was set to 0.7 to ensure confidence in this study, and the remaining parameters were left at their default settings. The results were stored in TSV format, the TSV files were imported into Cytoscape 3.7.2, and the networks were analysed (Cytoscape→Tools→Network analyzer→ Networkanalysis→Analyzenetwork), save the network analysis results, using the node size and colour reaction to reflect the size of Degree, the larger the node the larger the Degree value; the thickness of the edge is used to reflect the size of Combinescore, the thicker the edge the larger the Combinescore, select the core targets to make the protein Interaction network diagram.

GO enrichment analysis and KEGG pathway analysis

The drug-disease intersection genes were uploaded to the DAVID database (https://david.ncifcrf.gov/summary.jsp), the gene identifier was selected as OFFICIAL_GENE_SYMBOL and the species was set as Homo Sapiens. The role of target proteins in gene function was noted in three aspects: Biological Process (BP), Cellular Component (CC) and Molecular Function (MF). To elucidate the targets of xxx treatment of vitiligo in the signaling pathway KEGG pathway enrichment analysis was performed. The top 10 GO functions in BP, CC, MF ranking and 20 pathways in KEGG pathway entries associated with vitiligo ($P < 0.01$) were selected as the main gene function enrichment processes and signalling pathways for Bai-Bo Formula treatment of vitiligo to predict the mechanism of action of Bai-Bo Formula treatment of vitiligo.

Molecular docking

In general, the higher the Degree value in a network, the more important it is. This means that the protein with the higher Degree value in the protein interaction network plays an important role in the drug treatment of vitiligo. The top five active ingredients of Bai-Bo Formula and the top five key targets were molecularly docked using AutoDock Vina (1.1.2) to verify their interaction activity. The method is as follows: (1) download the compound in mol2 format from the TCMSP website, pour it into Chembio3D for energy minimisation, then import AutodockTools-1.5.6 to add hydrogen, calculate the charge, assign charge, set rotatable keys and save it as "pdbqt" (2) Download key target proteins from the PDB (http://www.rcsb.org/) database (human-derived proteins preferred, high structural similarity between the original ligand and the active ingredient to be docked preferred, high resolution selected); (3) Pour the proteins into PyMoL (2.3.0) to remove the original ligand and water molecules, then import the proteins into AutoDocktools (v1.5.6) for hydrogenation, charge calculation, charge assignment, specifying the atom type and saving in "pdbqt" format; (4) use the original protein-ligand as the centre of the docking box or, if no original ligand is present, the docking region near the reported key amino acid residues. The size of the lattice box was set to 50 × 50 × 50 (the spacing of each lattice point was 0.375 Å), and the rest of the parameters were set as default. (5) Interaction pattern analysis was performed using PyMOL and Ligplot.

Results

Prediction of active ingredients and targets of Bai-Bo Formula

After searching through TCMSP with OB ≥ 30 %, DL

≥ 0.18 and eliminating invalid ingredients, 5 ingredients were collected by screening for Lemna minor, 10 ingredients for eclipta prostrata, 9 ingredients for ligustrum lucidum, 7 ingredients for xanthium sibiricum, 18 ingredients for saposhnikovia divaricata, 88 ingredients for glycyrrhiza uralensis, 9 ingredients for astragalus complanatus, 2 ingredients for angelica sinensis, and 3 ingredients for clematis chinensis. The BATMAN-TCM database was used to obtain 3, 21 and 16 components under the condition of Scorecutoff > 20 for Rehmannia glutinosa, tribulus terrestris, and polygonum multiflorum, which could not be included after screening by the TCMSP. A total of 167 components were obtained.

Vitiligo-related targets

After merging the GeneCards, OMIM and DisGeNET databases, 1270, 10 and 395 targets were obtained respectively. A total of 1425 vitiligo-related targets were obtained after de-duplication, and the obtained genes were corrected by the UniProt database. After taking the intersection of the drug's target of action with the target genes of vitiligo, 169 intersecting target genes of vitiligo and the drug were obtained as the interactive target genes for drug treatment of vitiligo (Figure 1).

Results of drug-component-target prediction

Using the drug-component-target data, a "network.xlsx" file and a "type.xlsx" file were constructed and imported into Cytoscape 3.7.2 for graphing. The network has 331 nodes and 1497 edges, and the top five components of the Degree are MOL000098, MOL000006, MOL000422, MOL000358, and Gamma-Aminobutyric Acid (Figure 2).

Core targets and network interactions

The 91 intersecting target genes of vitiligo and drugs were obtained after taking the intersection of all drug targets with the target genes of vitiligo as the interaction target genes of drugs for vitiligo treatment. The 91 intersecting target genes obtained were imported into String (https://string-db.org/) database for protein-protein interaction prediction, with species set to: HomoSapiens and confidence set to: 0.7. The network file was saved as TSV format and the TSV file was imported into Cytoscape 3.7.2 software The protein interaction network was plotted, and points with Degree > 5 were selected for the graph, which had 99 nodes and 1864 edges. The topological analysis of the network was carried out and the degree value was used to reflect the size and colour of the targets, and the combined score value was used to reflect the thickness of the edges to construct the protein-protein interaction network.

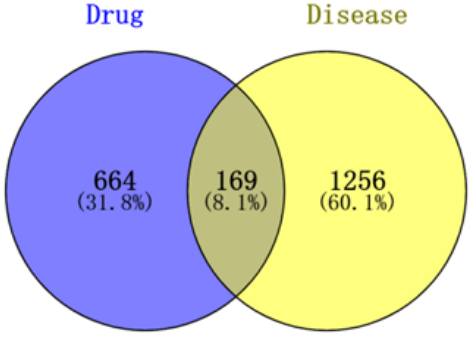

Figure 1. Intersecting target genes in vitiligo and drugs.

The top five targets in terms of degree value: IL6, TNF, AKT1, IL1β, and STAT3 (Figure 3).

Biofunctional enrichment analysis

GO enrichment analysis

Take the drug-disease intersection genes and use the DAVID database for GO gene function enrichment analysis, a total of 537 GO entries were screened, with $p <$

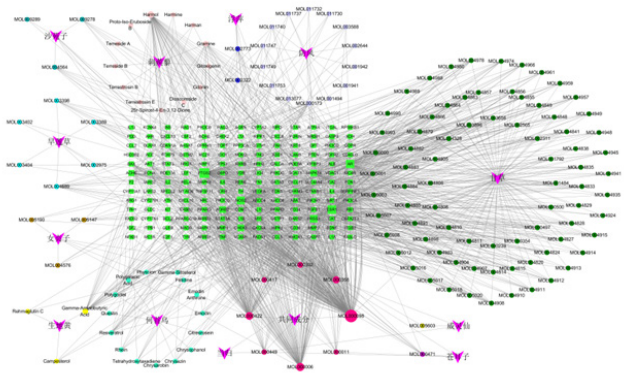

Figure 2. Drug-component-target prediction results. Note: Rectangles indicate targets, ovals indicate drug components and diamond-shaped nodes indicate herbal medicines.

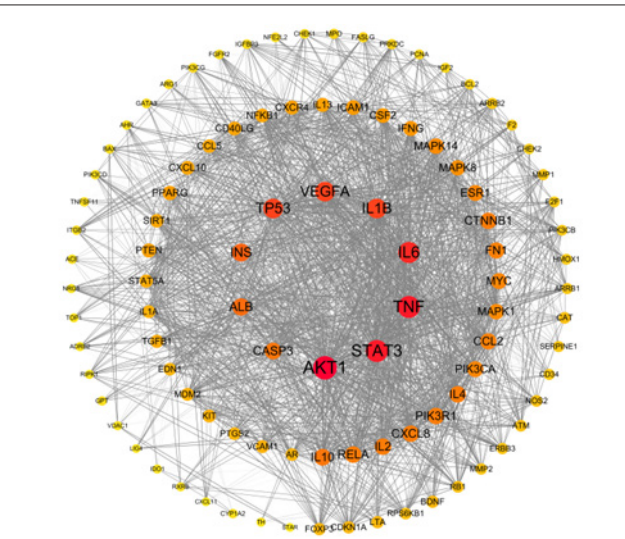

Figure 3. Network diagram of Bai-Bo Formula active ingredient–vitiligo disease target."

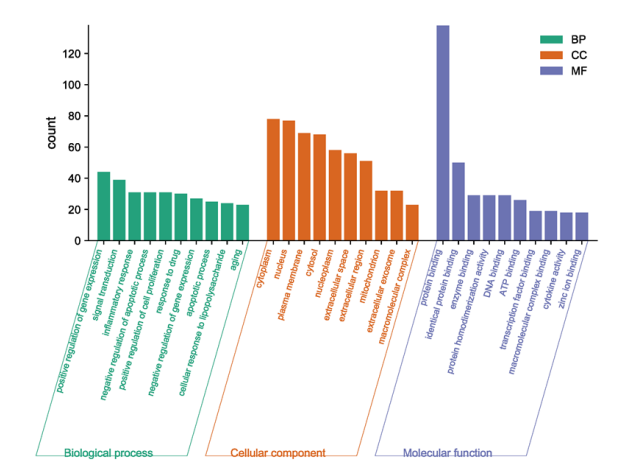

Figure 4. GO function enrichment analysis.

0.01 as the criterion to screen 429 major entries of significantly enriched biological functions for Bai-Bo Formula treatment of vitiligo, (Figure 4), mainly involving positive regulation of gene expression, signal transduction, inflammatory response, negative regulation of apoptotic process, positive regulation of cell proliferation, response to drugs, negative regulation of gene expression, apoptotic process, cellular response to lipopolysaccharides, ageing; 43 entries related to cellular components (CC), involving cytoplasm, nucleus, plasma membrane, cytoplasm, nucleoplasm, extracellular gap, extracellular zone, mitochondria, extracellular exosomes, macromolecular complexes; 65 entries related to molecular functions, involving protein binding, identical protein binding, enzyme Binding, protein homodimerisation activity, DNA binding, ATP binding, transcription factor binding, macromolecular complex binding, cytokine activity, zinc ion binding.

KEGG pathway enrichment analysis

Pathway enrichment analysis was performed using the DAVID database, and a total of 177 pathways related to the Bai-Bo Formula for vitiligo were enriched. 143 pathways were screened according to $P < 0.01$ for Bai-Bo Formula and vitiligo, and the pathways related to vitiligo were screened as: AGE-RAGE signaling pathway in diabetic complications, chemoattractant-receptor activation, PI3K-Akt signalling pathway, TNF signalling pathway, MAPK signalling pathway, FoxO signalling pathway, hepatitis C, cytokine-cytokine receptor interactions, HIF-1 signalling pathway, C-type lectin receptor signalling pathway, apoptosis, chemokine signalling pathway, IL-17 signalling pathway, Toll-like receptor signalling pathway, T cell receptor signalling pathway, Th17 cell differentiation, osteoblast differentiation, relaxin signalling pathway, prolactin signalling pathway, JAK-STAT signalling pathway and other pathways (Figure 5).

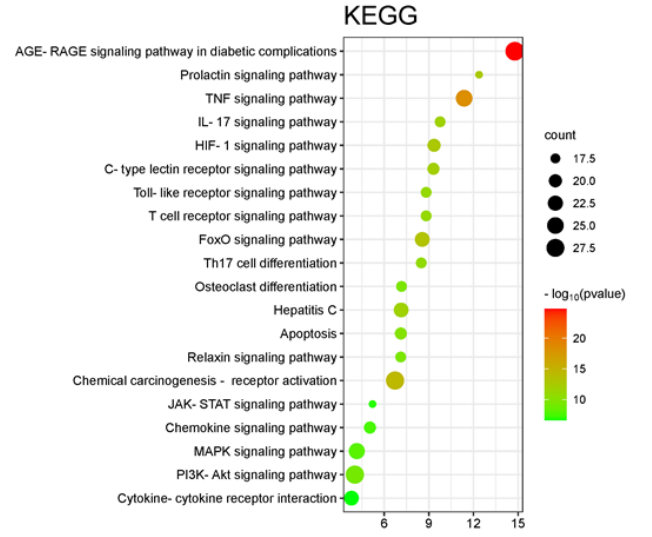

Figure 5. KEGG enrichment analysis. Note: The size of the circles represents the number of genes enriched in the corresponding pathway, and from green to red represents a progressively smaller *P*-value. The top 20 KEGG metabolic pathways will be filtered according to the *P*-value, and the bubble plot will be drawn according to the *P*-value. The horizontal axis is represented by the number of genes enriched in the pathway, the size of the bubble represents the number of genes enriched in the corresponding pathway, and the shade of the colour represents the significance, so that the significant enrichment information can be observed visually.

Molecular docking results

Based on the previous analysis, the top 5 important targets were selected for semi-flexible docking with the compounds having a high degree of binding energy (affinity) to indicate how well the small molecule binds to the target protein.

The docking results showed that the small molecules could enter the active centre of the target proteins and the small molecules that docked best with each protein were selected for display. Lys65, Tyr68 of IL1β, with hydrogen bond lengths of 3.00Å, 2.75Å, 2.95Å, 3.14Å, respectively. luteolin forms hydrogen bonds with Asp34, Arg30, Arg179, Gln175 of IL6, with hydrogen bond lengths of 2.70Å, 2.82Å, 3.01Å, 3.08Å, 3.08Å, respectively. 2.95Å, 2.96Å; luteolin formed hydrogen bonds with Gln24, Glu324, Gln326 of STAT3, with hydrogen bond lengths

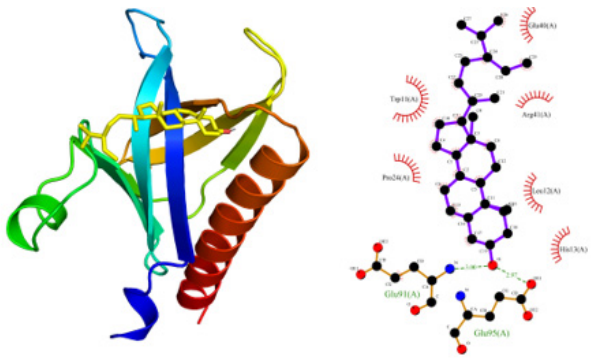

Figure 6. Analysis of the interaction pattern between beta-sitosterol and AKT1 protein.

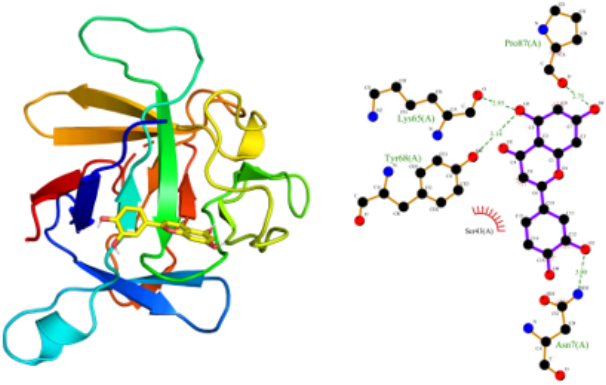

Figure 7. Analysis of the interaction pattern between luteolin and IL1β protein.

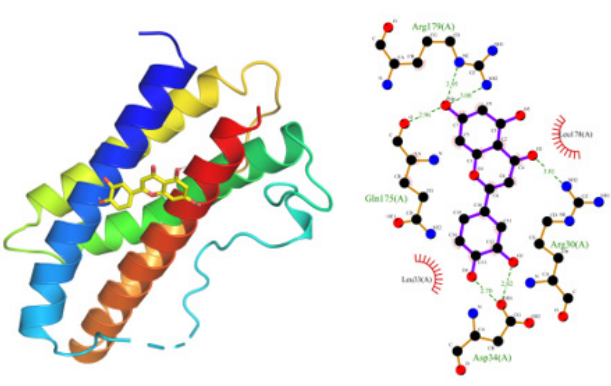

Figure 8. Analysis of the interaction pattern between luteolin and IL6 protein.

of 2.98Å, 3.07Å, 2.98Å, 2.80Å, 3.25Å; beta-sitosterol formed hydrogen bonds with Glu116, Pro100 of TNF, with hydrogen bond lengths of 2.73Å and 2.81Å respectively; all these small molecules formed strong hydrophobic interactions with the surrounding amino acid residues (Figures 6–10), and the docking results are detailed in Table 1.

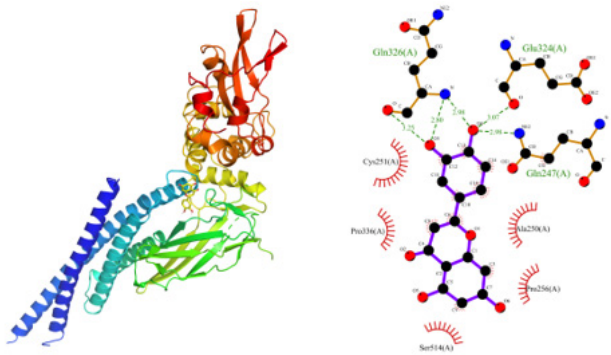

Figure 9. Analysis of the interaction pattern between luteolin and STAT3 protein.

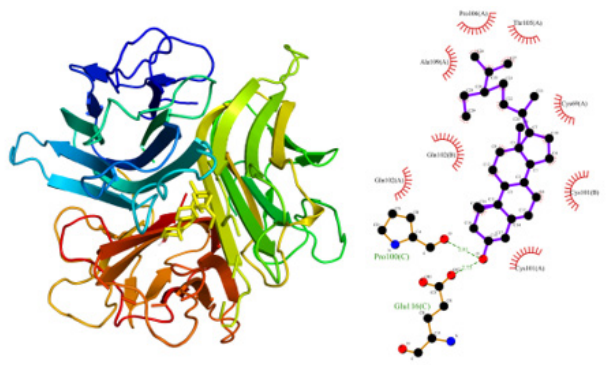

Figure 10. Analysis of the interaction pattern of beta-sitosterol with TNF protein.

Discussion

Vitiligo is a frequently acquired, localized, or generalised condition of skin depigmentation that can affect anyone at any age and on any portion of the body. According to TCM physicians, "blood insufficiency and wind dryness" are the primary causes and pathophysiology of vitiligo. The major goals of the treatment are to nourish the blood, activate blood, tonify the liver and kidney, and expel wind. In this paper, the "traditional Chinese medicine compound target" network, PPI network, GO, KEGG enrichment, molecular docking, and other technologies were used to investigate the mechanism of bailing tablets in the treatment of vitiligo from a variety of perspectives and levels. The major active ingredients of Bai-Bo Formula, which acts synergistically on numerous targets to exhibit curative effects, are quercetin, luteolin, kaempferol, beta-sitosterol, and gamma-aminobutyric acid, according to the "traditional Chinese medicine compound target" network. A chemical frequently corresponds to many targets based on the node characteristics of candidate compounds, indicating the multitarget treatment of vitiligo with the Bai-Bo Formula. Moreover, one chemical might originate from a range of herbs, demonstrating the benefits of TCM's compatibility. One of the flavonols is quercetin. It was shown that H2O2 might cause the endoplasmic reticulum to expand and could prevent melanocytes' endoplasmic reticulums from producing functioning tyrosinase, which would lead to the pathophysiology of vitiligo. By reducing the number of H2O2-mediated oxidation reaction pathways, quercetin can eventually lower the prevalence of vitiligo (6). Natural flavonoid luteolin has numerous pharmacological properties, including anti-inflammatory, antiallergenic, antioxidant, and others. The pathogenesis of vitiligo should be heavily influenced by oxidation and inflammation. An important chemokine in inflammatory skin conditions is IL-8. The research also demonstrated that pathogenesis occurs as a result of increased IL-8 gene expression in the skin of vitiligo patients. Lutein's potent anti-inflammatory and antioxidant properties can stop melanocytes from expressing and releasing the IL-8 gene, which lowers the incidence of vitiligo (1). It has been established that the flavonoid molecule kaempferol is a melanopoietin (2), which has anti-inflammatory and antioxidant properties. The 12 herbs in the Bai-Bo Formula contain a number of chemicals that work at several levels, including cellular, factor, protein production, etc. These findings support the

Table 1. Docking results of core small molecules to core target proteins.

Target	PDB ID	Compound	Affinity(kcal/mol)	Target	PDB ID	Compound	Affinity(kcal/mol)
IL6	1ALU	MOL000006	-7	TNF	2E7A	MOL000006	-8.9
		MOL000173	-6.9			MOL000173	-9
		MOL000228	-6.8			MOL000228	-9.1
		MOL000358	-4			MOL000358	-4.4
		MOL000449	-6.8			MOL000449	-9
AKT1	1UNQ	MOL000006	-6.4	IL1β	6I8Y	MOL000006	-6.7
		MOL000173	-6			MOL000173	-6.3
		MOL000228	-7.4			MOL000228	-6.6
		MOL000358	-4.3			MOL000358	-4
		MOL000449	-5.9			MOL000449	-6.2
STAT3	6NJS	MOL000006	-7.6				
		MOL000173	-7.3				
		MOL000228	-7				
		MOL000358	-3.8				
		MOL000449	-7.1				

study's predictions and possibly attest to the accuracy of the findings.

The greatest degree value of IL-6, TNF and other proteins, which may be a key target for its therapeutic action, is indicated by the PPI network of the Bai-Bo Formula. IL-6 transcript levels were markedly elevated in vitiligo patient PBMCs and skin tissues. Studies conducted in vitro indicate that IL-6 may function as an "intracrine" molecule by controlling melanogenesis and melanocyte proliferation. Additionally, increased IL-6 expression may trigger IL6R expression in target melanocytes, activating downstream signalling that ultimately affects melanocyte viability and homeostasis. It is crucial to the biology of melanocytes (7). The major pathophysiology of vitiligo is oxidative stress, and TNF can cause oxidative stress by activating the enzymes that produce ROS and RNS. Inflammation and oxidative damage brought on by TNF interact (8). Due to the frequent observation of aberrant immune responses in vitiligo patients, TNF is crucial in the pathogenesis of several autoimmune disorders, including vitiligo. Moreover, patients with vitiligo had greater lesion levels of TNF-α (9). These findings are consistent with earlier research. According to KEGG enrichment analysis, the treatment for vitiligo involves negative regulation of gene expression, apoptotic processes, signal transduction, inflammatory responses, positive regulation of cell proliferation, response to drugs, negative regulation of apoptotic processes, and other related pathways. Among these, Bai-Bo Formula controls the key melanogenesis pathways, including AGE-RAGE, PI3K-Akt, and JAK-STAT. The combination between AGE and RAGE may stimulate ERK and CREB signalling, boost MITF expression and tyrosinase activity, and eventually increase melanocyte melanin synthesis. Furthermore, contrary to what has previously been claimed (10), RAGE regulates melanogenesis rather than generating free radicals and triggering proinflammatory cascades. Tyrosinase, MITF, and transient receptor potential expression are all downregulated by the PI3K/Akt signalling pathway, which prevents melanogenesis (11). Interferon-gamma (IFN-γ) and chemokines released by keratinocytes through the Janus kinase (JAK)/signal transducer and activator of transcription (STAT)-1 signalling pathway cause activated CXCR3+, CD8+ T cells to promote melanocyte detachment and apoptosis. This results in the recruitment of CXCR3+, and CD8+ T cells and the creation of a positive feedback loop. JAK inhibitors, which now have FDA approval for the treatment of numerous immune-related disorders, target the JAK/STAT system. JAK inhibitors, such as ruxolitinib, baricitinib, and tofacitinib, are successful in treating vitiligo, confirming the notion that the IFN-γ-chemokine signalling axis is involved in the disease's pathogenesis (12). By managing the differentiation of IL-17 and Th17 and controlling the secretion of inflammatory substances such tumour necrosis factor-, which can lessen melanocyte injury, Bai-Bo Formula may also have an impact on immunological inflammation (13).

According to the results of protein topology analysis and previous research support, we selected core proteins, including IL-6, TNF, AKT1, IL1β and STAT3 for molecular docking. The results revealed that the main compounds could bind well with core proteins, which further verifies that they may be the main protein targets of the Bai-Bo Formula. Our study has some limitations. On the one hand, network pharmacology is a bioinformatics analysis based on databases. Due to the limitations of the databases, there may be deviations in the prediction target, and improving the database construction will enhance the credibility of the results. On the other hand, we analyzed the potential mechanisms of Bai-Bo Formul in the treatment of vitiligo, and the results need to be further verified by cell/animal experiments or clinical experiments.

The anti-inflammatory, antioxidant, and melanocyte death-inhibiting properties of Bai-Bo Formula are important. While treating vitiligo, a substance may act on a variety of targets, each of which may be connected to a number of different pathways. Clinically, the Bai-Bo Formula is successful in treating vitiligo, although it's unclear how it works. This paper investigated the multicomponent, multitarget, and multichannel mechanism of the Bai-Bo Formula in the treatment of vitiligo using TCM network pharmacology and molecular docking technologies. It emphasises the validity and efficacy of the Bai-Bo Formula, offers clinical practitioners guidance in the treatment of vitiligo, and offers fresh perspectives on how the Bai-Bo Formula works.

Ethics Approval
Not applicable.

Data Availability
The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Acknowledgments
Not applicable.

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This study did not receive any funding in any form.

Conflicts of Interest
The authors declare no conflicts of interest.

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