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Original Research Article

Investigation of potential targets and mechanisms of action of Bailing tablets on vitiligo based on network pharmacology and molecular docking

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Abstract

Purpose: *To investigate the potential molecular mechanism underlying the therapeutic effect of Bailing Tablet (BLT) on vitiligo.*
Methods: *The targets of BLT and vitiligo-related genes were obtained from public databases. The approach of network pharmacology was utilized to explore the potential mechanisms and candidate targets. Molecular docking was applied to assess the binding strength between BLT's compounds and relevant targets.*
Results: *A total of 144 active compounds and 275 corresponding targets were identified in BLT, as well as 1342 genes associated with vitiligo. A total of Forty-three intersected genes were considered as candidate targets of BLT against vitiligo. Protein-protein interaction (PPI) network showed that ALB, AKT1, and IL6 were the top 3 genes with higher interactions, playing more crucial roles in this network. In addition, it was found that the candidate targets of BLT on vitiligo were significantly associated with a variety of biological processes (apoptosis, cell proliferation, and gene expression regulation) and pathways (signal transduction pathway, apoptosis, and necroptosis). The topological analysis of the herb-compound-target-pathway network highlighted the important roles of AKT1, CDK2, and NOS2 in the therapeutic effects of BLT on vitiligo. Molecular docking analysis revealed a good binding force among the 3 genes and corresponding targets.*
Conclusion: *The underlying mechanisms of action of BLT against vitiligo have been systematically elucidated, thus affording an effective strategy for unraveling the pharmacological mechanisms of action of TCM. The findings also provide a deeper understanding of BLT and its use in the treatment of vitiligo.*

Keywords: *Bailing tablet, Vitiligo, Network pharmacology, Molecular docking, Protein-protein interaction*

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INTRODUCTION

Vitiligo is a progressive and acquired depigmenting skin disorder that is clinically characterized by the presence of typical non-scaly and chalky-white macules on the skin owing to selective loss of melanocytes. It affects 1-4 % of the world's population [1]. Although vitiligo has no effects on the life expectancy of patients, it negatively influences their quality of

life as it damages their self-esteem, which can be psychologically devastating. Multiple environmental and genetic factors have been proven to be associated with vitiligo. It has been proposed that oxidative stress, impaired melanocyte migration, and abnormal metabolism and cell detachment contribute to the loss of pigmentation in vitiligo [2]. Major progress has been made in revealing the pathogenesis of vitiligo, which highlights the essential role of the immune system in this disease. Previous studies have shown a strong association between vitiligo and several autoimmune disorders, including type I diabetes and alopecia areata [3].

At present, available treatments for vitiligo are limited, and relevant clinical therapeutic drugs for vitiligo mainly focus on enhancing melanogenesis and restoring the skin color. Other therapies for restoring color include vitamin D analogs, excimer laser, and steroid therapy. However, it is worth noting that these anti-vitiligo treatments have limitations and adverse effects, such as erythema, blisters, and perilesional pigmentation [4].

In the process of seeking effective therapy for vitiligo, traditional Chinese medicine (TCM) has attracted worldwide attention, because it serves as a promising alternative treatment for the condition, considering its better therapeutic effects and less adverse effects. In addition, some TCM physical therapies such as acupuncture and Chinese cupping have also shown superior efficacy in the treatment of vitiligo [5]. Nonetheless, the prolonged procedure and slow therapeutic outcomes of single TCM treatment may lead to patients' emotional uneasiness and voluntary termination of treatment. Therefore, priorities should be taken in the future by combining TCM with Western medicine in the treatment of vitiligo, taking full account of the quick and effective efficacy of Western medicine.

Bailing tablet (BLT), a Traditional Chinese medicine preparation, which is composed of *Panax notoginseng* (Burk.) F. H. Chen (Sanqi), *Carthamus tinctorius* L. (Honghua), *Paeonia suffruticosa* Andr. (Mudanpi), *Prunus persica* (L.) Batsch (Taoren), *Saposhnikovia divaricata* (Turcz.) Schischk. (Fangfeng), *Atractylodes lancea* (Thunb.) DC. (Cangshu), *Portulacea oleracea* L. (Machixian), *Paeonia lactiflora* Pall. (Chishao), *Angelica sinensis* (Oliv.) Diels (Danggui), *Angelica dahurica* (Fisch.ex Hoffm.) Benth. Et Hook.f. (Baizhi), and *Astragalus membranaceus* (Fisch.) Bge. (Huangqi). The use of BLT in treating vitiligo has gained widespread adoption in Chinese clinics owing to its favorable effects. Modern pharmacological studies have shown that Danggui, Sanqi, and Huangqi enhances immune function, and Machixian slows the progress of aging. The above herbs up-regulate the activity of tyrosinase [6]. Nonetheless, the active components as well as the molecular mechanism of BLT against vitiligo remains unclear, and requires further investigations. The application of network pharmacology is a systematic approach to decipher the molecular mechanisms underlying traditional Chinese medicine (TCM) in disease treatment [7]. In the present study, we employed network pharmacology to comprehensively explore the underlying mechanisms of BLT in the treatment of vitiligo.

METHODS

Identification of active components and targets of BLT

The BLT components were obtained from Traditional Chinese Medicine Systems Pharmacology (TCMSP) (https://lsp.nwu.edu.cn/tcmsp.php) database and analysis platform. Only those components with an oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.17 were considered as bioactive. Thereafter, the targets of the active compounds were also obtained from the database. Finally, the target name was uninformed based on UniProt (www.uniprot.org/) and non-human targets were excluded.

Searching for vitiligo-related targets

Vitiligo targets were retrieved from the DisGeNet database (https://www.disgenet.org/) and GeneCards database (www.genecards.org/), with the search term as “vitiligo”. After the removal of duplicated genes, the remaining genes were identified as vitiligo-related genes.

Network construction and analysis

The Cytoscape software (version 3.7.2) was adopted to carry out network analysis. Venn diagram was generated to identify the potential targets of BLT in the treatment of vitiligo. The protein-protein interaction (PPI) information was obtained from the STRING (https://string-db.org/) database.

Functional enrichment analysis

To determine the functional significance of the identified targets, functional enrichment analyses were performed on the DAVID platform (version 6.8, https://david.ncifcrf.gov/). The pathway terms with a p-value of ≤ 0.05 were regarded as

significant and interesting terms. The top 20 enriched non-disease-related pathways were selected for the construction of the herb-compound-target-pathway network.

Molecular Docking Analysis

The protein structure of the following targets: AKT1 (PDB ID: 6ccy), CDK2 (PDB ID: 2a4l), and NOS2 (PDB ID: 3e7g), were downloaded from the RCSB Protein Database Bank (PDB, https://www.rcsb.org/). The water modules were removed from the pdb file of proteins, and pdbqt file of each protein was prepared using AutoDock Tools 1.5.6 software. The chemical structure of compounds were also downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Docking analysis was conducted using the Autodock Vina with a grid box containing the active sites of the proteins. The conformation with the lowest binding energy was exported, and the 2D and 3D diagrams of the ligand-protein conformation were visualized using LigPlus and PyMOL, respectively.

RESULTS

Bioactive components and relevant targets of BLT

In this study, a total of 144 compounds from 11 herbs were recognized as active compounds of BLT (Figure 1). To be specific, the number of active compounds in Danggui, Sanqi, Honghua, Mudanpi, Taoren, Fangfeng, Cangshu, Baizhi, Machixian, Chishao, and Huangqi were 2, 9, 23, 11, 23, 21, 11, 23, 10, 29, and 21, respectively. 275 potential targets were collected from the 144 active compounds (*Supplementary material*: Table S1, Figure 1). The number of potential targets linked with Danggui, Sanqi, Honghua, Mudanpi, Taoren, Fangfeng, Cangshu, Baizhi, Machixian, Chishao, and Huangqi were 52, 187, 215, 170, 51, 84, 62, 61, 219, 98, and 210, respectively. Notably, common overlaps of the targets were observed in the 11 herbs, indicating a target-specific synergistic mechanism of action among the different Chinese herbs in the BLT.

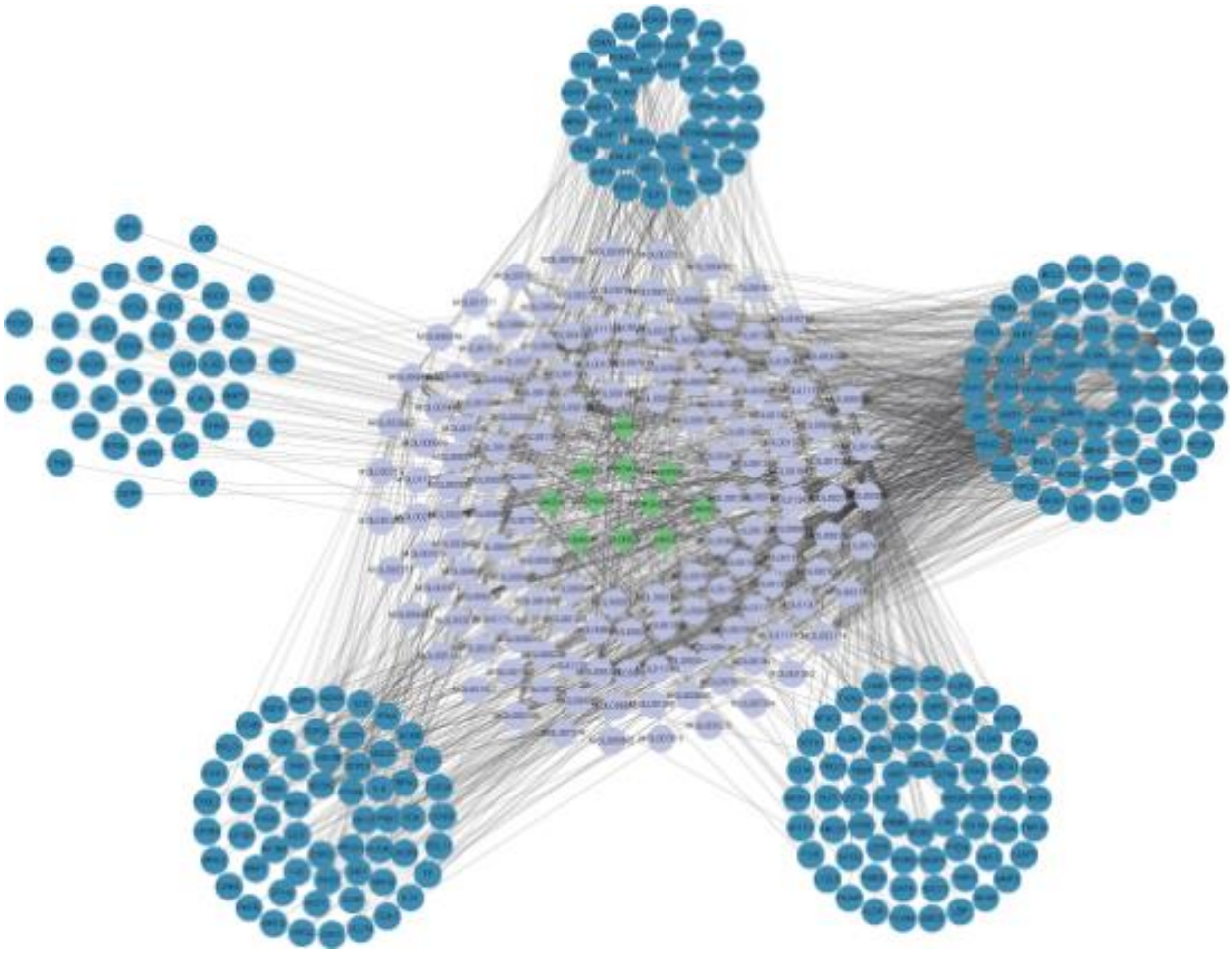

Figure 1: Herb-compound-target network of BLT. Green diamonds, purple and blue circles represent herbs, components and relevant targets, respectively

Potential targets of BLT against vitiligo

After excluding the overlapping genes, a total of 1342 vitiligo-related genes were obtained, including 1194 from the GeneCards and 395 from the DisGeNet database (*Supplementary material*: Table S2). To further identify the candidate targets of BLT against vitiligo, a Venn diagram with vitiligo targets and BLT targets was constructed, and 43 intersected targets were identified as the candidate targets accordingly (Figure 2 A). The candidate targets were then submitted to the STRING database to obtain the PPI information (confidence shub >0.4), and a PPI network of these candidate targets consisted of 306 edges was constructed (Figure 2 B). The albumin (ALB), serine-threonine protein kinase (AKT1), interleukin-6 (IL-6), MYC proto-oncogene, bHLH transcription factor (MYC), peroxisome proliferator activated receptor gamma (PPARG), interleukin 1 beta (IL1B), and caspase 3 (CASP3) are target proteins with higher degree values when compared with other targets, indicating their important roles in the treatment of vitiligo.

Enrichment analysis data and identified hub targets

To gain a deeper understanding of the biological functions associated with the 43 candidate targets, DAVID was employed to carry out enrichment analyses. The data demonstrated that 253 GO terms were significantly associated with the candidate targets, with 211 biological processes (BP), 16 cellular components (CC), and 26 molecular functions (MF) (*Supplementary material*: Table S3). Figure 3 **A** shows the top 10 terms of BP, CC, and MF**,** which presented a significant association with apoptotic processes, cell proliferation, gene expression regulation, and response to hypoxia. Furthermore, the results demonstrated that the 43 candidate targets were mainly enriched in 88 KEGG pathways (Supplementary material: Table S4). Meanwhile, the top 20 non-disease-related pathways with a lower p value were shown in Figure 3 B, which were specifically associated with signal transduction, apoptosis, and necroptosis. These results demonstrated the vital molecular mechanism of BLT therapy against vitiligo, and provided new insights that would facilitate the drug development of vitiligo. In order to find the hub targets of BLT against vitiligo, the herb-compound-target-pathway network was built, and topological analysis revealed that the top 3 targets were AKT1, cyclin dependent kinase 2 (CDK2), and nitric oxide synthase 2 (NOS2). They were at a higher degree, and thus were considered to play crucial anti-vitiligo roles. As a result, they were regarded as hub genes for further analysis (Figure 4). Furthermore, the top five hub compounds, namely, quercetin, kaempferol, luteolin, wogonin, and beta-sitosterol, might exert more vital roles in the anti-vitiligo effects of BLT on account of their higher degrees.

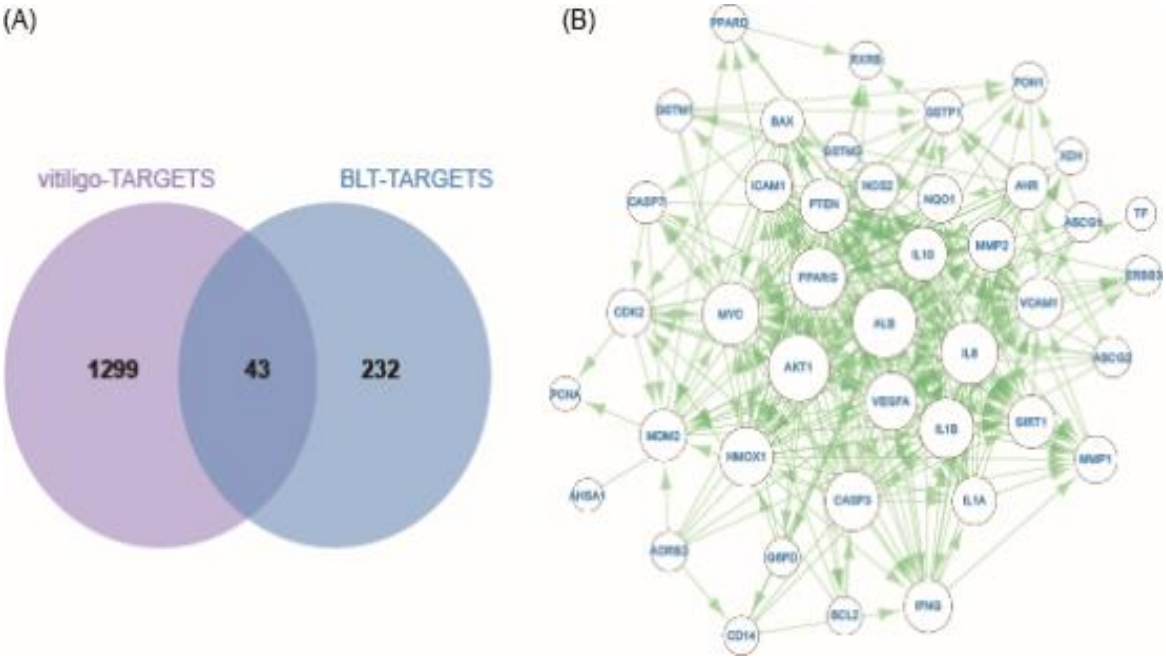

Figure 2: Candidate targets of BLT in treating vitiligo. (A): Venn diagram of BLT targets and vitiligo targets with 43 intersected genes as candidate targets of BLT against vitiligo; (B): The PPI network of the 43 candidate genes of BLT.

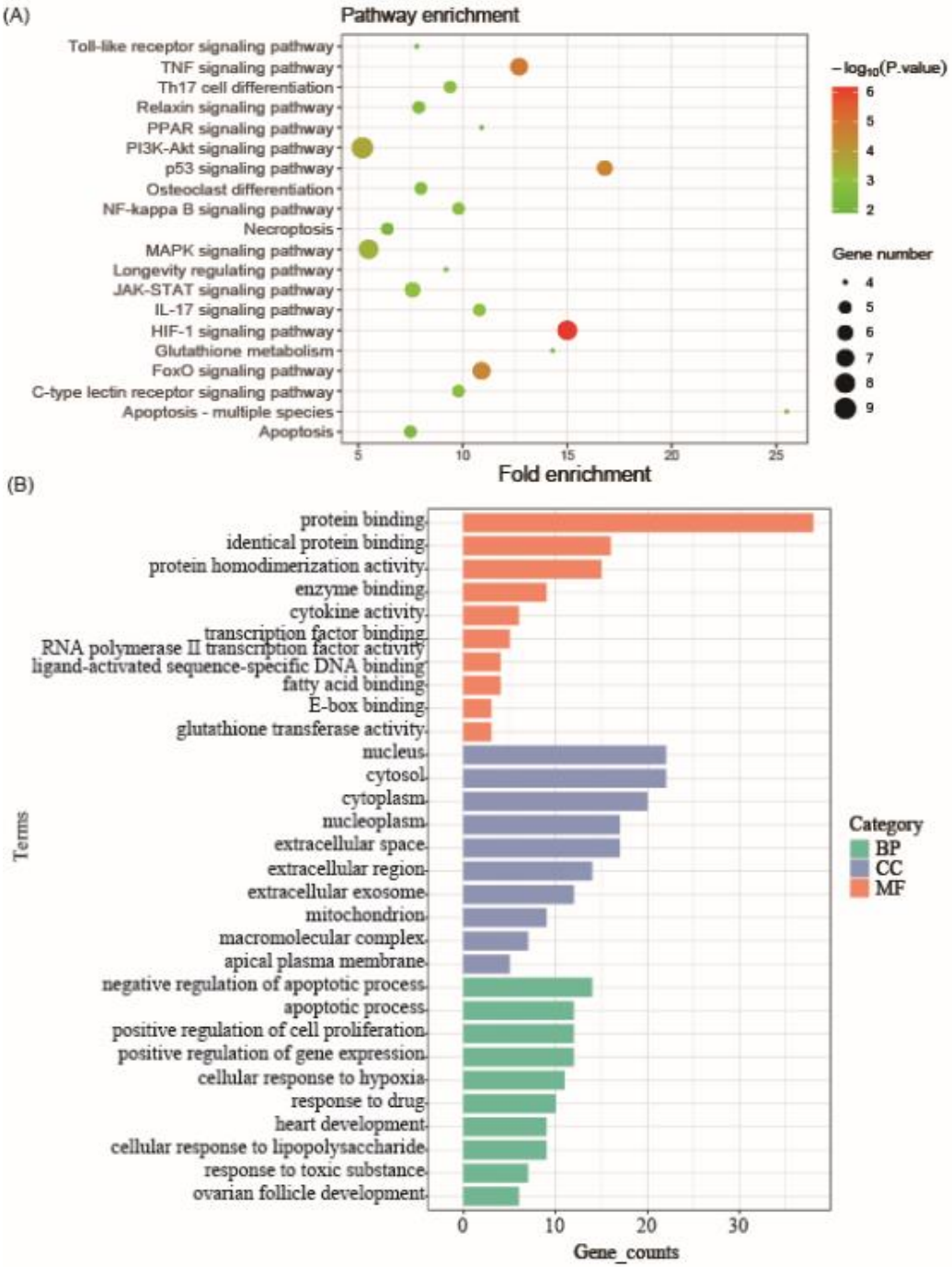

Figure 3: Diagrams of GO and KEGG pathway-enrichment analysis. (A): The dot plot of the top 20 KEGG pathways significantly associated with the candidate targets; (B): Top 10 terms with a higher gene counts in BP, CC, and MF that significantly associated with the candidate targets

Molecular docking

Docking analysis was performed to examine the binding interactions between three hub genes, namely AKT1, CDK2, and NOS2, and the respective compounds present in BLT. As shown in **Figure 5**, the 3D complex showed the binding poses of the 3 hub proteins with the corresponding compounds in the ligand-binding pockets. Moreover, hydrogen bonding and van der Waals forces were observed in the protein-ligand complexes. It was suggested that a ligand molecule spontaneously bound strongly to a receptor protein with a binding energy <−5. The binding affinities of CDK2-wogonin, AKT1-quercetin, and NOS2-kaempferol pairs were -7.34, -7.3, and -7.68, indicating that these protein-ligand pairs formed stable complexes, and may eventually influence the biological functions of these proteins.

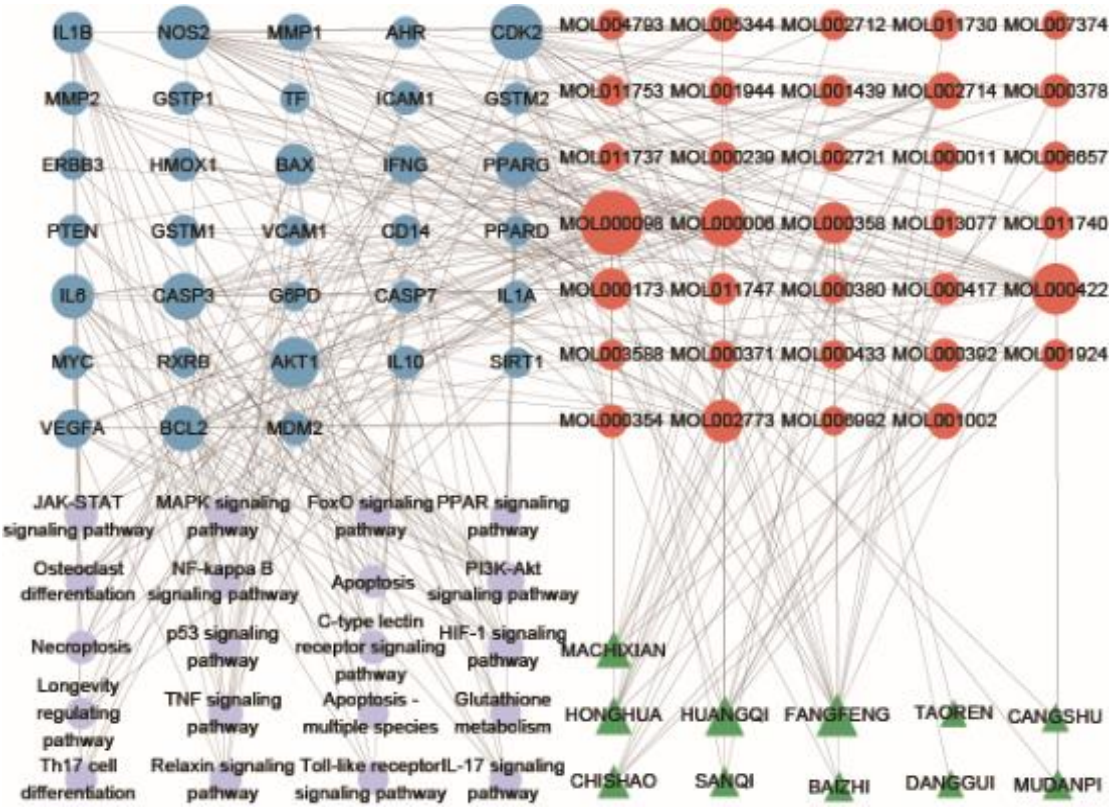

Figure 4: The network of herbs, components, targets, and pathways involved in the action of BLT against vitiligo. Green triangles, red circles, blue circles, and purple circles represent medicinal materials, components, targets, and pathways, respectively. The size of a node in the network is directly proportional to its degree value

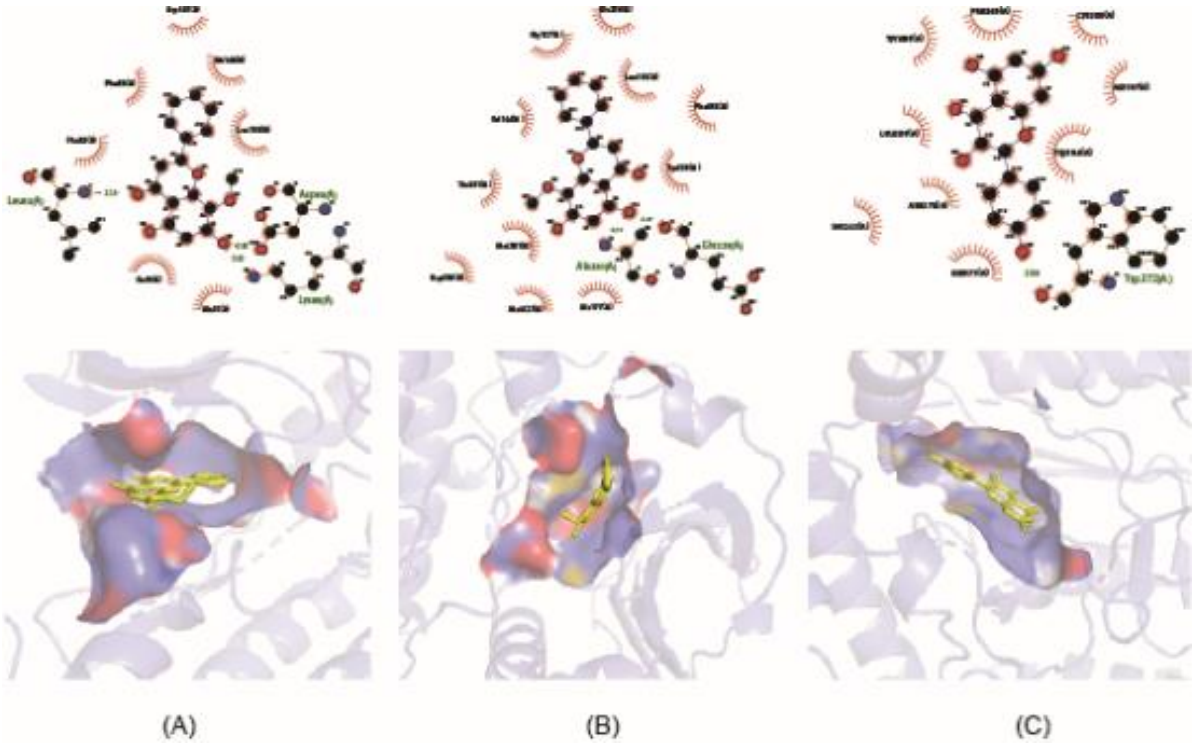

Figure 5: Molecular docking model of the top 3 hub genes and corresponding compounds. The image at the top is a 2D diagram of a protein-ligand complex containing residues that form hydrogen bonds and van der Waals forces. The lower part shows the 3D structure of the protein-ligand complex. (A) CDK2 and wogonin (affinity: -7.34); (B) AKT1 and quercetin (affinity: -7.3); (C) NOS2 and kaempferol (affinity: -7.68)

DISCUSSION

Vitiligo is a disorder of depigmentation caused by the dysfunction or destruction of melanocytes. It has been reported that BLT alleviates the symptoms of vitiligo, which may be associated with the interaction between its active compounds and related vitiligo targets [8]. The current investigation employed a network pharmacology approach to investigate the potential mechanisms underlying the anti-vitiligo effects of BLT. The active compounds and targets in BLT were retrieved from the TCMSP database. Based on the herb-compound-targets network, several hub compounds were identified, including quercetin, kaempferol, luteolin, wogonin, and beta-sitosterol. They were considered to play dominant roles in this network.

Loss of melanocytes in skin lesions is associated with the pathogenesis of vitiligo, which is possibly caused by oxidative stress. Quercetin, a widely studied flavonoid with antioxidant properties and low toxicity, is known to effectively inhibit tyrosinase activity and melanin production in melanocytes. However, there are also reports suggesting that quercetin may enhance melanogenesis by stimulating tyrosinase synthesis and activity. [9]. Guan *et al* reported that quercetin could attenuate H_2O_2-induced dilation of endoplasmic reticulum (ER), and functional tyrosinase export from the ER in melanocytes, suggesting that quercetin has certain potential in the treatment of vitiligo [10].

Kaempferol, a flavonol with versatile pharmacological properties, exhibits anti-cancer, anti-inflammatory, and anti-oxidant activities. The ability of kaempferol to stimulate melanogenesis involves upregulation of tyrosinase and microphthalmia-associated transcription factor (MITF) expression, leading to increased melanin content [11]. Inflammatory chemokines play a dominant role in vitiligo, while luteolin has been reported to restore the levels of IL-8 released from melanocytes [12].

Necrosis and apoptosis are two known mechanisms of cell death. The abnormal necrosis and apoptosis of melanocytes in vitiligo has been revealed in several studies [13]. Polymorphisms in various genes, including TYR, FAS, and FASLG, have been identified to contribute to vitiligo susceptibility by eventually controlling apoptosis [14-16]. In addition to apoptosis and necrosis, there are several other modes of melanocyte death, including pyroptosis, ferroptosis, oxeiptosis, parthanatos, and necroptosis. As demonstrated in the data from the present study, the candidate targets of BLT against vitiligo were involved in the process of apoptosis and necroptosis. As a programmed form of necrosis, necroptosis is activated by oxidative stress in melanocyte through RIPK1-RIPK3-MLKL pathway in vitiligo [17]. Taken together, these results demonstrated that the anti-vitiligo effects of BLT might be mediated by regulating melanocyte apoptosis and necroptosis.

A total of 3 hub targets, AKT1, CDK2, and NOS2 were also identified, and they might play dominant roles in the anti-vitiligo effects of BLT. The loss of protective melanin in keratinocytes (KCs) contributes to non-segmental vitiligo. Furthermore, KCs in the areas of epidermal depigmentation in vitiligo patients had ultrastructural and functional alterations of mitochondria, and presented a high rate of apoptosis. A previous study showed impaired PI3K/AKT activation in vitiliginous KCs [18]. CDK2 participates in the regulation of cell cycle, and plays an important part in a variety of biological processes. It has been proven to be involved in the pathogenesis of vitiligo by mediating a SUMOylation-induced dysregulation of cell cycle progression in KCs [19], or by mediating the effects of miRNA-21-5p in melanogenesis [20]. NOS2 catalyzes the production of nitric oxide (NO) which plays a crucial role in coordinating the skin's response to external stimuli such as heat, and ultraviolet (UV) light. It has been reported that NO stimulates MITF phosphorylation in alpaca skin melanocytes in order to facilitate melanogenesis [21]. In addition, NO also participates in the paracrine mediation of UV-induced melanogenesis [22]. The data suggested that BLT acts the 3 hub targets in order to exert its anti-vitiligo activity.

Limitations of the study

Despite the significantly major findings, this study also has limitations. Firstly, the active components and corresponding targets of BLT were identified from the public database, which may not fully represent the real situation of the anti-vitiligo effect of BLT. Such an approach may lead to both false positive and false negative results. Secondly, further experiments are needed to validate the various targets and pathways of the anti-vitiligo effects of BLT.

CONCLUSION

By means of network pharmacology, the potential targets of BLT compounds have been investigated, and the underlying mechanisms of

action of their anti-vitiligo effects have been unraveled. A total of 43 targets of BLT are candidate targets against vitiligo, and the candidate targets are significantly correlated with signal transduction, apoptosis, and necroptosis. Based on the topological analysis of the herb-compound-target-pathway network, 3 hub genes (AKT1, CDK2, and NOS2) potentially play more crucial roles in the anti-vitiligo effects of BLT. Although further validation is still required to elucidate the exact regulatory mechanism, the data generated in this study will aid relevant research in the future.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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