Journal of Ethnopharmacology 190 (2016) 272–287

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Systematic understanding the mechanisms of vitiligo pathogenesis and its treatment by Qubaibabuqi formula

Tianli Pei [a,b], Chunli Zheng [a], Chao Huang [a], Xuetong Chen [a], Zihu Guo [a], Yingxue Fu [a], Jianling Liu [b], Yonghua Wang [a,*]

[a] Center of Bioinformatics, College of Life Science, Northwest A & F University, Yangling, Shaanxi 712100, China
[b] Key Laboratory of Resource Biology and Biotechnology in Western China, Northwest University, Ministry of Education, China

ARTICLE INFO

Article history:
Received 22 August 2015
Received in revised form
16 May 2016
Accepted 1 June 2016
Available online 2 June 2016

Keywords:
Systems pharmacology
Vitiligo
Molecular pathogenesis
Qubaibabuqi formula
Herbal medicines

Chemical compounds studied in this article:
Butin (PubChem CID: 92775)
Butein (PubChem CID: 5281222)
Galangin (PubChem CID: 5281616)
Psoralen (PubChem CID: 6199)
Kaempferol (PubChem CID: 5282102)
Cholesterol (PubChem CID: 5997)
Isopsoralen (PubChem CID: 10658)

ABSTRACT

Ethnopharmacological relevance: Vitiligo is a depigmentation disorder, which results in substantial cosmetic disfigurement and poses a detriment to patients' physical as well as mental. Now the molecular pathogenesis of vitiligo still remains unclear, which leads to a daunting challenge for vitiligo therapy in modern medicine. Herbal medicines, characterized by multi-compound and multi-target, have long been shown effective in treating vitiligo, but their molecular mechanisms of action also remain ambiguous.
Materials and methods: Here we proposed a systems pharmacology approach using a clinically effective herb formula as a tool to detect the molecular pathogenesis of vitiligo. This study provided an integrative analysis of active chemicals, drug targets and interacting pathways of the Uygur medicine Qubaibabuqi formula for curing Vitiligo.
Results: The results show that 56 active ingredients of Qubaibabuqi interacting with 83 therapeutic proteins were identified. And Qubaibabuqi probably participate in immunomodulation, neuromodulation and keratinocytes apoptosis inhibition in treatment of vitiligo by a synergistic/cooperative way.
Conclusions: The drug-target network-based analysis and pathway-based analysis can provide a new approach for understanding the pathogenesis of vitiligo and uncovering the molecular mechanisms of Qubaibabuqi, which will also facilitate the application of traditional Chinese herbs in modern medicine.

1. Introduction

Vitiligo is a depigmentation disorder of human skin characterized by loss of cutaneous melanocytes with a complex pathogenesis (Spritz, 2006). The skin lesions are cosmetically disfiguring and are often associated with profound emotional trauma (Grimes et al., 2013), which affect 1–4% of the world population (Szczurko and Boon, 2008). Several reports have proposed a series of theories about the etiology of vitiligo, including genetic susceptibility, autoimmunity, neural dysfunctional, impaired melanocyte migration and/or proliferation, keratinocytes apoptosis and oxidative stress (Gauthier et al., 2003; Namazi, 2007). The corresponding therapies include topical therapies (Hossani-Madani and Halder, 2010), the suppression of the immune response and the regulation of the proliferation of melanocytes (Grimes, 1993). However, the success rate of these therapies is limited by failure to follow the primary pathogenesis of vitiligo. For example, one of the most common treatments for vitiligo is the application of psoralens followed by exposure to ultra-violet light (sun light), but only 61% of patients achieve more than 25% repigmentation (Grimes, 1993). More seriously, such type of drugs can trigger some side effects such as cutaneous atrophy, perioral dermatitis, phototoxic reactions, as well as long-term carcinogenic risk (Morison et al., 1998; Travis and Silverberg, 2004).

Traditional Chinese Medicine (TCM) has been used to treat various human diseases for over 4000 years (Tang et al., 2009). A number of Chinese herbal compounds have been successfully applied to treat vitiligo, such as Uygur medicine Qubaibabuqi formula in Xinjiang province (China) (Commission, 1999; Liu et al., 2011). Qubaibabuqi is composed of five herbs, *Vernonia anthelmintica* (Linn.) Willd. (Compositae), *Psoralea corylifolia* Linn. (Fabaceae), *Alpinia officinarum* Hance. (Zingiberaceae), *Operculina turpethum* (Linn.) Silva. Manso. (Convolvulaceae), *Plumbago zeylanica* Linn. (Plumbaginaceae). Clinical studies show that Qubaibabuqi can treat vitiligo through promoting melanocyte proliferation and improving tyrosinase activity *in vitro* (Huo et al.,

* Corresponding author.
E-mail address: yh_wang@nwsuaf.edu.cn (Y. Wang).

http://dx.doi.org/10.1016/j.jep.2016.06.001
0378-8741/

2012). And Qubaibabuqi can also increase epidermal melanocytes and production of black granules and enhance the expression of tyrosine *in vivo* (Peng et al., 2011). Despite the therapeutic effect of Qubaibabuqi is attractive, the molecular mechanism of action has not been completely understood. Although some traditional approaches including chemical analysis (Mok and Chau, 2006), bioactivity test (Borisy et al., 2003) have been performed to study Qubaibabuqi, its pharmacological mechanism has not been fully elucidated.

Recently, systems pharmacology, as an emerging field that integrates systems biology and pharmacology, provides a new approach to explore TCM across multiple scales of complexity ranging from molecular and cellular levels to tissue and organism levels (Berger and Iyengar, 2009). Systems pharmacology has made a significant contribution to investigate the molecular mechanisms of TCM through pharmacokinetic evaluation (absorption, distribution, metabolism, excretion (ADME) properties of herbs), target prediction and network/pathway analysis (Huang et al., 2013). Successful sample applications of systems pharmacology in disclosing the underlying actions mechanisms of TCM include the treatment of stroke, depression and cardiovascular diseases (Huang et al., 2013; Zhang et al., 2014; Zheng et al., 2014).

Here, based on the systems pharmacology framework, we exploited the clinically effective formula Qubaibabuqi as a tool to detect the molecular pathogenesis of vitiligo and identify the action mechanism of Qubaibabuqi. Firstly, the active ingredients of Qubaibabuqi were obtained via oral bioavailability, drug-likeness and Caco-2 permeability evaluation at a molecular level. Then, by utilizing the active ingredients as baits, we predicted the potential targets and further constructed the drug-target interactions at

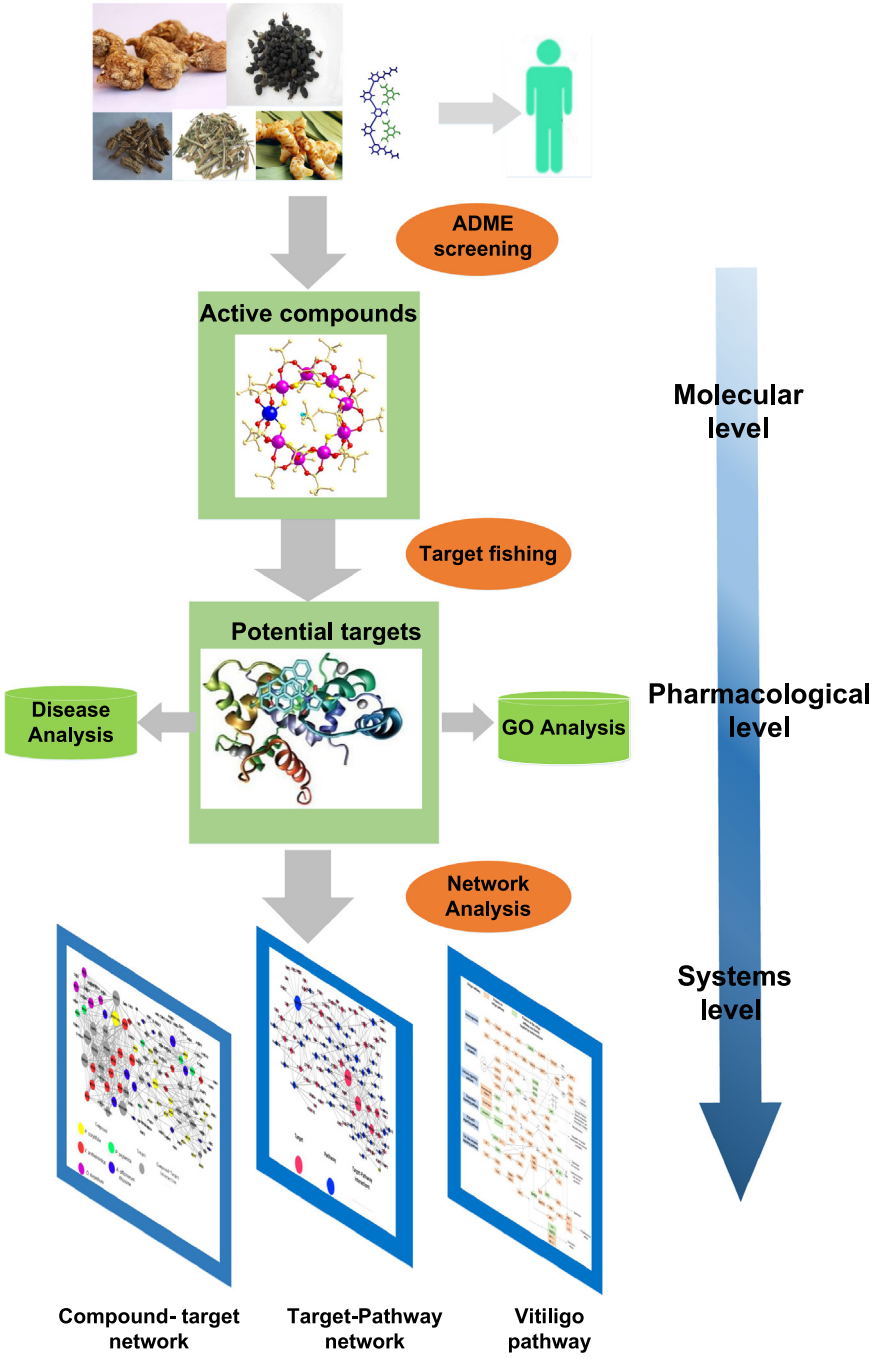


Fig. 1. Systems pharmacology approach workflow.

pharmacological level. Subsequently, we utilized the targets as baits to fish corresponding pathways from KEGG database (Kyoto Encyclopedia of Genes and Genomes, http://www.genome.jp/kegg/). And target-pathway network were constructed for network analysis. Finally, an integrated "vitiligo pathway" was constructed to dissect the molecular pathogenesis of vitiligo at a systems level. The results not only significantly improve our understanding of vitiligo pathogenesis, but also dissect the molecular mechanism of action of Qubaibabuqi, which promote the development of TCM in the treatment of complex diseases.

2. 2. Materials and methods

The protocol of the integrated systems pharmacology approach includes five main steps as follows (Fig. 1):

(1) Molecular database building. The chemicals of all five herbs in Qubaibabuqi were collected from our previously developed Traditional Chinese Medicine Systems Pharmacology Database (TCMSP, http://lsp.nwsuaf.edu.cn/tcmsp.php (Ru et al., 2014)).
(2) Pharmacokinetic evaluation. Oral bioavailability screening, drug likeness evaluation and Caco-2 permeability filtering were applied to screen out the active compounds of Qubaibabuqi.
(3) Drug targeting. Our previously developed *in silico* model weighted ensemble similarity (WES) was employed to predict the direct targets of the obtained active compounds. GO analysis and disease analysis were performed to identify the targets of active compounds.
(4) Network construction and analysis. Drug-target network and target-pathway network were constructed to interpret the therapeutic mechanisms of Qubaibabuqi for vitiligo and improve our understanding of vitiligo pathogenesis.
(5) The integrated "vitiligo pathway" was constructed to better elaborate vitiligo pathogenesis and the holistic mechanisms of Qubaibabuqi.

2.1. Active compounds screening

To gain the potential active compounds from Qubaibauqi, we applied an intergrated model including PreOB (predicts oral bioavailability), PreDL (predicts drug-likeness) and PreCaco-2 (predicts Caco-2 permeability) in this work.

2.1.1. PreOB

Oral bioavailability (OB) is one of the most vital pharmacokinetic properties of orally administered drugs because it plays an important role for the efficiency of the drug delivery to the systemic circulation. In this work, OB value was calculated by an in-house model OBioavail1.1 (Ru et al., 2014). And the threshold of OB value was set to 33% by taking into account of the following controls: 1) extracting information from the studied herbs should be as much as possible with the least number of molecules; 2) reasonably explaining the obtaining model by the reported pharmacological data. Therefore, the compounds with OB $\geq$33% were screened out for further analysis (Wang et al., 2013).

2.1.2. PreDL

To screen out the drug-like molecules from Qubaibabuqi, in this work, based on the molecular descriptors and tanimoto coefficient, a self-constructed model PreDL was performed to calculate the drug-likeness index of these compounds. The drug-likeness evaluation approach is shown below:

$$T(A, B)=\frac{A\cdot B}{\|A\|^2+\|B\|^2-A\cdot B} \quad (1)$$

where A is the molecular descriptors of herbal ingredients, and B shows the average molecular properties of all molecules in DrugBank database (http://www.drugbank.ca/). In this work, the compounds with DL $\geq$0.18 were selected as candidate compounds for further research.

2.1.3. PreCaco-2

For orally administered drugs, one of the greatest problems is its movement across the intestinal epithelial barrier, which determines the rate and extent of human absorption and ultimately affects its bioavailability. Thus, in this work a Caco-2 permeability prediction model preCaco2 (Li et al., 2007) was applied to predict the drug absorption. And the threshold of Caco-2 permeability was set to 0.4 in this study. Finally, compounds with OB $\geq$33%, DL $\geq$0.18 and Caco2 $\geq$0.4 were regarded as active compounds for further analysis.

2.1.4. Hepatotoxicity assessment of active compounds

Hepatotoxicity is of great concern for novel pharmaceutical drugs and patient safety. Thus, the identification of adverse hepatic effects is of great importance. *In-silico* model is a desirable tool for hepatotoxicity over the experimental methods in terms of resource- and time-saving (Cumming et al., 2013). Recently, several quantitative structure–activity relationship (QSAR)-based models for human hepatotoxicity have been reported (Greene et al., 2010; Huang et al., 2015; Matthews et al., 2009). In this work, a developed QSAR model was introduced to predict the hepatotoxicity of the active compounds of Qubaibabuqi (Mulliner et al., 2016).

2.2. Drug targeting

In order to clarify the pathogenesis of vitiligo and elucidate the mechanisms of action of drugs, building the compound-target interaction profiles is essential (Rix and Superti-Furga, 2009). In this work, we performed the WES algorithm to predict the direct targets of the active ingredients based on a large-scale of drug-target relationships (Zheng et al., 2015). WES model performs well in predicting the binding (sensitivity 85%, SEN) and the nonbinding (specificity 71%, SPE) patterns, with the accuracy of 78%, the precision (PRE 74%) and the area under the receiver operating curves (AUC) of 0.85, respectively. In this work, according to the possibilities of the compound-target interactions in the WES model, the targets with likelihood score $\geq$7 were selected as candidate targets for further analysis. In addition, the obtained targets were further mapped to Uniprot (http://www.uniprot.org/) for normalization.

2.3. Network construction

For the sake of clarifying vitiligo pathogenesis and interpreting the mechanisms of Qubaibabuqi for vitiligo at a network level, in this study, we established two kinds of visualized networks: 1) Compound-target network (C-T network). All active compounds in Qubaibabuqi and their potential targets were utilized to generate a bipartite graph of drug-target interactions in which a compound and a target are linked with each other if the drug target the protein. 2) Target-pathway network (T-P network). We utilized the targets as baits to fish corresponding pathways from KEGG database (Kyoto Encyclopedia of Genes and Genomes, http://www.genome.jp/kegg/).

The bipartite graphs were constructed by Cytoscape version 2.8.3, which is a published source for biological network

visualization and data integration (Smoot et al., 2011). In the network, the compounds, targets and pathways are represented by nodes, and the interaction between two nodes is represented by an edge. In addition, the importance of each node in the networks was evaluated by one crucial topological parameters namely degree (Azuaje et al., 2011). And degree was analyzed by plugin NetworkAalyzer of Cytoscape. The degree of a node is the number of edges associated with the node.

2.4. Vitiligo pathway analysis

Based on the current knowledge of vitiligo pathology, we constructed an integrated "vitiligo pathway". First, according to the pathological and clinical data, the pathways in the T-P network which were not directly and closely related to vitiligo were detached. Then, we manually assembled a relatively complete vitiligo pathway. In addition, we made a nearness analysis about the correlativity between herbal medicine targets p and the "vitiligo pathway" related proteins p' based on protein-protein interaction (PPI) network by the expression (Huang et al., 2013).

$$\varphi_{pp'} = \frac{1}{nm}\sum_{i=1}^{n}\sum_{j=1}^{m} e^{-D^2} p_i p_j' \quad (2)$$

where p_i represents the herbal medicines target, p_j' is the 'vitiligo Pathway' related protein, and Dp_ip_j' is the shortest distance between p_i and p_j' on the PPI network. In addition, n and m represent the number of herbal medicine target p and vitiligo pathway related protein p' respectively. If two proteins are unconnected on the PPI network, the Dp_ip_j' is defined as ∞.

3. Results and discussion

3.1. Active compounds screening

A total of 308 compounds (as displayed in Table S1) were collected from the five herbs of Qubaibabuqi. In order to screen out the active compounds, it is significant to evaluate the ingredients' ADME properties including oral bioavailability, drug-likeness and Caco-2 permeability. As a result, 51 potential active molecules with OB $\geq$ 33%, DL $\geq$ 0.18 and Caco-2 $\geq$ 0.4, accounting for 16.5% of all 308 ingredients of Qubaibabuqi, were obtained. So as to obtain a more accurate result, some certain rejected ingredients, which have relatively poor pharmacokinetic properties, but are the most abundant and active ingredients of certain herbs, were also selected as the active components for further research. For instance, kaempferol with relatively poor Caco-2 (0.26) was retained for further analysis since it is the major constituent of *A. officinarum* (García-Mediavilla et al., 2007). However Operculinosides A, operculinosides B, operculinosides C and operculinosides D have poor ADME properties, they are all isolated from the root of *O. turpethum*, and have been confirmed to exhibit potent hepatoprotective activity, which probably play significant role in curing vitiligo (Ding et al., 2011). Thus, the four compounds were also chosen for further analysis. Finally, a total of 56 active ingredients were obtained in this study (as shown in Table 1).

The five herbs of Qubaibabuqi, Vernonia anthelmintica (Linn.) Willd. (*V. anthelmintica*), Psoralea corylifolia Linn. (*P. corylifolia*), Alpinia officinarum Hance. (*A. officinarum*), Operculina turpethum (Linn.) Silva. Manso. (*O. turpethum*) and Plumbago zeylanica Linn. (*P. zeylanica*) are traditionally used by Uygur to cure vitiligo (Commission, 1999, 2005; Kotiyal and Sharma, 1992; Kohli et al., 2010). For 56 ingredients, most of them have been validated to display vital biological activities including anti-inflammatory, immune-regulatory activities and modulating the activity of tyrosinase. For instance, butin (M242, OB=75.3%, DL=0.21, Caco-2=0.41) and butein (M243, OB=97.9%, DL =0.18, Caco-2=0.4) with favorable pharmacokinetic profiles come from *V. anthelmintica*, and they have a range of pharmacological properties including antioxidant, anti-inflammatory activities (Zhang et al., 2014; Semwal et al., 2015). The flavonoid galangin (OB=45.5%, DL=0.21, Caco-2=0.54) from *A. officinarum*, which exhibits antioxidant, antimicrobial activities (Pepeljnjak and Kosalec, 2004; Russo et al., 2002). And galangin is able to cure vitiligo by promoting synthesis of tyrosinase (Huo et al., 2014). Besides, it is worth noting that psoralen (M103) is a common ingredient of *P. zeylanica* and *P. corylifolia*, indicating that the active compound may show synergetic pharmacological effects on vitiligo. These candidate compounds could be the key elements for curing vitiligo.

3.1.1. Hepatotoxicity assessment of active compounds

The hepatotoxicity of the 56 active compounds in Qubaibabuqi were successfully predicted by the developed QSAR model. The hepatotoxicity-positive and hepatotoxicity-negative predictive values were 12 (21.4%) and 44 (78.6%), respectively (as shown in Table 1). Positive value represents hepatotoxic compounds and negative value represents non- hepatotoxic compounds. For compounds where the hepatotoxicity is clearly dose-dependent, it may be possible to define a safe dose. For example, however plumbagin from *P. zeylanica* can cause hepatotoxic effects by unbalancing of the redox defense system, the low concentrations of plumbagin (1 mg/kg/day) does not cause liver injury (Sukkasem et al., 2016). Moreover, literature review indicate that some active compounds of Qubaibabuqi such as kaempferol (Shih et al., 2013) and lupeol acetate (Kumar et al., 2009), exhibit a protective influence on the liver against toxicity induced by hepatotoxicity-positive compounds. While with different clinical manifestations of the known hepatotoxic ingredients in herbal medicines, most chemicals were correctly predicted by the developed model. For example, psoralen and its related chemicals seem to be causes of *P. corylifolia* related liver injury from case reports (Cheung et al., 2009). In our study, psoralen and isopsoralen were also predicted hepatotoxic potential.

3.2. Drug targeting and analysis

We obtained 140 candidate targets for the 56 compounds with 610 connections between them (Table S2). The results show that the most compounds act on more than one target, demonstrating various pharmacological effects of the bioactive molecules. For instance, kaempferol (M133) from *A. officinarum* can interact with 16 targets, and stigmasterol (M247) from *V. anthelmintica* and *P. corylifolia* target on 14 different proteins.

As we know, there are three major hypotheses for the pathogenesis of vitiligo that are not exclusive of each other: autoimmune, melanocyte damage and neural dysfunctional (Passeron and Ortonne, 2005). In addition, accumulating studies indicate that some of these genetic factors in the pathogenesis of vitiligo may be shared with a number of other autoimmune diseases, including thyroid disease, pernicious anaemia, Addison's disease, systemic lupus erythematosus, and inflammatory bowel disease (Alkhateeb et al., 2003; Amerio et al., 2006; Spritz, 2007). Hence, targets which are involved in the pathologies of vitiligo and these autoimmune diseases are potentially the therapeutic targets for vitiligo. Based on above strategies, 140 candidate targets were further mapped to PharmGkb, TTD and CTD database to gain their related diseases, so as to delete noise and errors (Table S3). As displayed in Table 2, we finally retrieved 83 potential targets.

In order to validate whether the 83 selected targets indeed match for vitiligo, we performed a Gene Ontology (GO) analysis for their biological process (Ashburner et al., 2000). The 83 potential

Table 1
Chemical information of 56 active compounds and their network parameters.

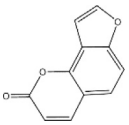
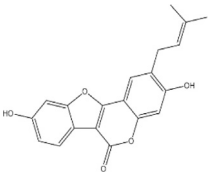
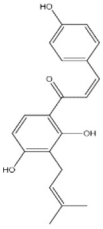
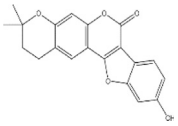
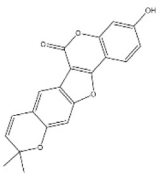
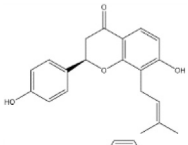
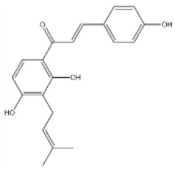
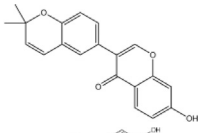
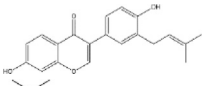
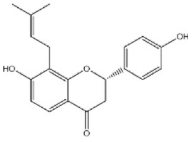
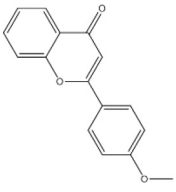
ID	Compounds	Structure	OB	DL	Caco2	Degree	Hepato-toxicity	Herb
M001	Isopsoralen		33.8	0.2	1.04	9	Positive	*P. corylifolia*
M002	Psoralidin		54.6	0.58	0.71	2	Positive	*P. corylifolia*
M003	Corylifolinin		40.9	0.27	0.5	3	Positive	*P. corylifolia*
M005	Isopso-ralidin		49.1	0.77	0.85	6	Positive	*P. corylifolia*
M010	Sophoracoumestan A		61.1	0.78	0.77	7	Negative	*P. corylifolia*
M015	Islbavachin		55.6	0.32	0.7	3	Negative	*P. corylifolia*
M017	Isobavachalcone		43.9	0.27	0.71	2	Negative	*P. corylifolia*
M024	Corylin		61.1	0.45	0.84	10	Negative	*P. corylifolia*
M025	Neobavaisoflavone		55.2	0.34	0.8	6	Negative	*P. corylifolia*
M034	Isobavachin		38.1	0.32	0.72	3	Positive	*P. corylifolia*
M039	4-methoxy flavone		37.7	0.18	1.09	14	Positive	*P. corylifolia*

Table 1 (*continued*)

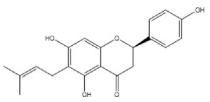
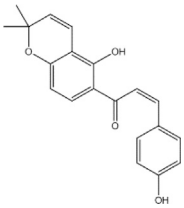
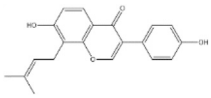
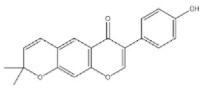
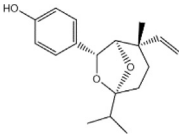
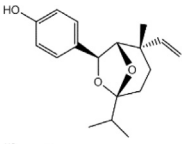
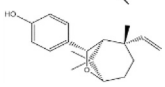
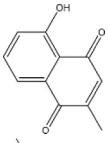
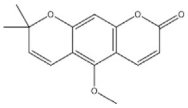
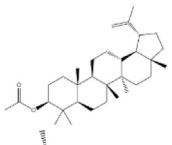
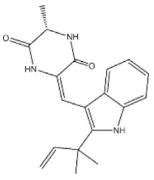
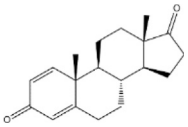
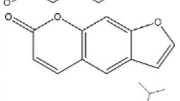
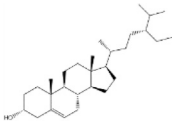
ID	Compounds	Structure	OB	DL	Caco2	Degree	Hepato-toxicity	Herb
M040	6-prenylnaringenin		49.1	0.37	0.49	2	Positive	*P. corylifolia*
M042	Isobavachromene		73.1	0.34	0.59	3	Negative	*P. corylifolia*
M046	8-prenyldaidzein		52.1	0.33	0.79	4	Negative	*P. corylifolia*
M047	Erythrinin A		47.3	0.46	0.86	8	Negative	*P. corylifolia*
M059	Psoracorylifols B		37.5	0.2	1.07	6	Negative	*P. corylifolia*
M060	Psoracorylifols C		41.8	0.2	1.05	5	Negative	*P. corylifolia*
M061	Psoracorylifols D		76.8	0.19	1.24	9	Negative	*P. corylifolia*
M063	Plumbagin		29.3	0.08	0.55	11	Positive	*P. zeylanica*
M081	Xanthoxyletin		73.1	0.21	1	8	Negative	*P. zeylanica*
M088	Lupeol acetate		42.3	0.76	1.47	9	Negative	*P. zeylanica*
M094	Neoechinulin A		62.3	0.31	0.68	3	Negative	*P. zeylanica*
M099	Androsta-1,4-diene-3,17-dione		46.56	0.35	0.76	12	Positive	*P. zeylanica*
M103	Psoralen		39.6	0.2	1.07	10	Positive	*P. zeylanica* *P. corylifolia*
M132	Sitosterol		36.9	0.75	1.32	14	Negative	*A. officinarum*

Table 1 (*continued*)

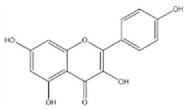
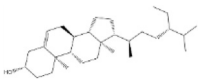
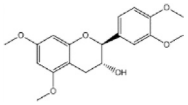
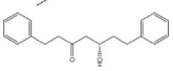
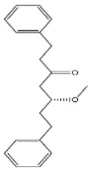
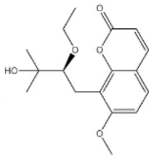
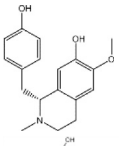
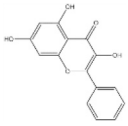
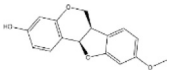
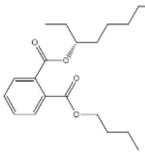
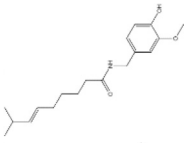
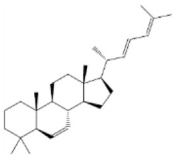
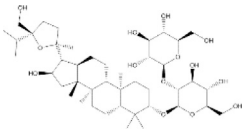
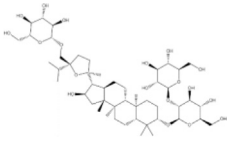
ID	Compounds	Structure	OB	DL	Caco2	Degree	Hepato-toxicity	Herb
M133	Kaempferol		42.1	0.24	0.26	16	Negative	*A. officinarum*
M157	Poriferast-5-en-3beta-ol		36.9	0.75	1.44	14	Negative	*A. officinarum*
M188	(2S, 3R)-2-(3,4-dimethoxyphenyl)-5, 7-dimethoxychroman-3-ol		51.9	0.37	0.65	3	Negative	*A. officinarum*
M189	1,7-diphenyl-5-hydroxy-3-heptanone		61.9	0.18	0.97	4	Negative	*A. officinarum*
M198	5-methoxy-1,7-diphenyl-3-heptanone		68.3	0.2	1.3	4	Negative	*A. officinarum*
M200	7-Methoxy-8-(2′-ethoxy-3′-hydroxy-3′-methybutyl)coumarin		72.8	0.21	0.6	3	Negative	*A. officinarum*
M206	(R)-N-Methylcoclaurine		84.9	0.26	0.92	6	Negative	*A. officinarum*
M207	Galangin		45.5	0.21	0.54	13	Negative	*A. officinarum*
M209	Medicarpin		49.3	0.34	1.01	2	Negative	*A. officinarum*
M219	Butyl-2-ethylhexyl phthalate		44.5	0.22	1.16	4	Positive	*A. officinarum*
M222	Capsaicin		48.6	0.2	0.95	13	Negative	*A. officinarum*
M223	Cholesta-6,22,24-trien, 4,4-dimethyl		45.1	0.71	1.89	12	Negative	*A. officinarum*
M230	Operculinosides A		33.8	0.14	−1.93	14	Negative	*O. turpethum*
M231	Operculinosides B		11.1	0.05	−2.93	12	Negative	*O. turpethum*

Table 1 (*continued*)

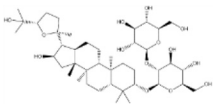
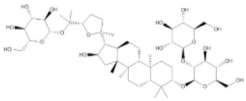
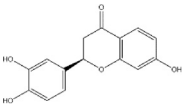
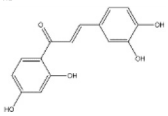
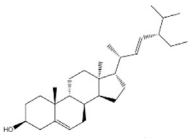
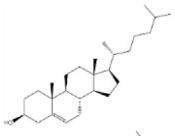
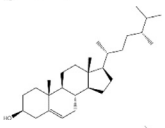
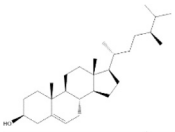
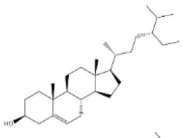
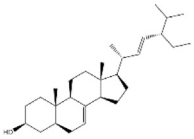
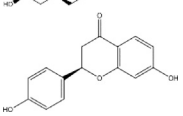
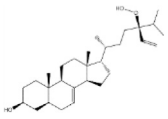
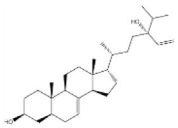
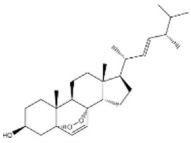
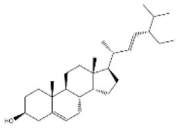
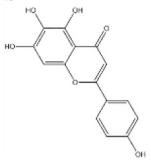
ID	Compounds	Structure	OB	DL	Caco2	Degree	Hepato-toxicity	Herb
M232	Operculinosides C		31.5	0.15	−2.08	13	Negative	*O. turpethum*
M233	Operculinosides D		11.2	0.05	−3.13	14	Negative	*O. turpethum*
M242	Butin		75.3	0.21	0.41	7	Negative	*V. anthelmintica*
M243	Butein		97.9	0.18	0.4	9	Negative	*V. anthelmintica*
M246	Vernolic acid		37.6	0.19	0.71	7	Negative	*V. anthelmintica*
M247	Stigmasterol		43.8	0.76	1.26	14	Negative	*V. anthelmintica* *P. corylifolia*
M253	Cholesterol		37.9	0.68	1.33	16	Negative	*V. anthelmintica*
M254	Campesterol		37.6	0.71	1.35	14	Negative	*V. anthelmintica*
M255	22-dihy-drobrassicasterol		37.6	0.71	1.36	14	Negative	*V. anthelmintica*
M256	Sitosterol		36.9	0.75	1.34	15	Negative	*V. nthelmintica* A. officinarum *O. turpethum* *P. zeylanica*
M257	Spinasterol		43	0.76	1.37	14	Negative	*V. anthelmintica*
M258	Liquiritigenin		72.6	0.18	0.51	5	Negative	*V. anthelmintica*
M280	24ζ-hydroperoxy-24-vinyllathosterol		38.2	0.82	0.75	13	Positive	*V. anthelmintica*

Table 1 (*continued*)

ID	Compounds	Structure	OB	DL	Caco2	Degree	Hepato-toxicity	Herb
M281	24Rζ-hydroxyl-7,28-dien-ergota-3β-sitosterol		39.5	0.79	0.95	13	Negative	*V. anthelmintica*
M282	5α,8α-epidioty-6,22-dien-ergota-3β-ol		44.4	0.82	0.91	13	Negative	*V. anthelmintica*
M284	Stigmast-5,22-dien-3β-ol		43.8	0.76	1.43	14	Negative	*V. anthelmintica*
M307	Isocarthamidin		39	0.24	0.41	9	Negative	*V. anthelmintica*

targets were mapped to DAVID (The Database for Annotation, Visualization and Integrated Discovery, http://david.abcc.ncifcrf.gov) bioinformatics resources to systematically analyzing their biological process (Huang et al., 2008). Fig. 2 lists the top 20 significantly enriched GO terms, the results show that the majority of these targets are strongly associated with various biological processes, including regulation of neurological system process, inflammatory response, and regulation of apoptosis. These biological processes are all associated with the pathogenesis of vitiligo (Gauthier et al., 2003; Namazi, 2007).

3.3. Network construction and analysis

3.3.1. Compound-target network and analysis

As shown in Fig. 3, a graph of C-T interaction was built based on 139 nodes (56 potential compounds and 83 potential targets) and 493 edges. C-T network analysis display that the average degree number of targets per compound is 7.086, elucidating the multi-target properties of Qubaibabuqi. Cholesterol (M253, degree = 16) from *V. anthelmintica* and kaempferol (M133, degree = 16) from *A. officinarum* exhibited the highest degree number of interactions with various protein targets. We speculate that the top two ingredients might be the crucial elements in the treatment of vitiligo. Previous findings suggest that cholesterol can increase melanogenesis in epidermal melanocytes and melanoma cells (Schallreuter et al., 2009), and kaempferol is effective in reducing the harmful immune responses such as chronic inflammation and autoimmunity (Lin et al., 2011). Kaempferol also can regulate adaptive humoral immunity by managing primary B cells and Tfh cells in vivo (Nagashima et al., 2011). Thus, the two key active ingredients of Qubaibabuqi work mainly by modulating melanogenesis and innate immune system.

Meanwhile, many potential targets are also linked with multiple compounds of different herbs, which might exhibit synergism effects or additive effects of Qubaibabuqi in the treatment of vitiligo. For instance, ADCY1 (Brain adenylate cyclase 1) is targeted by 22 active compounds from five herbs, which might provide additive effects to increase melanin synthesis via regulating the expression of MITF (Microphthalmia associated transcription factor). (Rodríguez and Setaluri, 2014). SCD (Acyl-CoA desaturase) and CCL5 (C-C motif chemokine 5) interacting with various ingredients of Qubaibabuqi formula play a crucial role in modulating immune system. BCHE (Cholinesterase) and PPP3CA4 (Serine/threonine protein phosphatase 2B catalytic subunit, alpha isoform) are involved in the regulation of neurological system process. All of these suggest that Qubaibabuqi probably treat vitiligo by increasing melanin synthesis, modulating immune system and nervous system.

3.3.2. Target-pathway network and analysis

The result displays that 75 targets are further mapped to 181 pathways (Table S4), which show an average degree of 6.85 per target and 2.8 per pathway. While, 8 of 83 targets have not been mapped into pathways. In the T-P network, we discover that several target proteins (31/75) are mapped to multiple pathways (≥ 5), demonstrating that these targets may intercede the interactions and cross-talk between different pathways. Meanwhile, numerous pathways (76/181), also regulated by multiple target proteins (≥ 3), might be the key factors for vitiligo.

As shown in Fig. 4, those pathways tightly interact with targets such as Calcium signaling pathway (hsa04020, degree = 10), Steroid hormone biosynthesis pathway (hsa00140, degree = 9), Serotonergic synapse pathway (hsa04726, degree = 9), should be the crucial pathways. For instance, steroid hormone biosynthesis pathway has anti-inflammatory and immunomodulatory effects by adjusting the level of steroids (Ongenae et al., 2003). Some targets involved in the function of neurosecretion and neuronal excitability and regulation of synaptic transmission locate in Serotonergic synapse pathway and Calcium signaling pathway. In addition, some pathways like melanogenesis pathway (hsa04916, degree = 3), MAPK signaling pathway (hsa04010, degree = 5), PI3K-AKT signaling pathway (hsa04151, degree = 5), have been testified as accurate target pathways for the treatment of vitiligo (Becatti et al., 2010; Lee et al., 2007). Melanogenesis pathway controlled melanin synthesis by regulating activity of tyrosinase. MAPK and NF-kB pathway regulate the expression of many genes, including those involved in responses ranging from inflammation and immunity, to cell growth and proliferation. These illustrate that

Table 2
The information of vitiligo-related targets of herbs.

ID	UniProt	Protein names	Gene names	Organism
T01	P47989	Xanthine dehydrogenase	XDH	*Homo sapiens*
T02	P30291	Serine/threonine-protein kinase	WEE1	*Homo sapiens*
T03	P11473	Vitamin D3 receptor	VDR	*Homo sapiens*
T04	P16662	UDP-glucuronosyltransferase 2B7	UGT2B7	*Homo sapiens*
T05	Q3KRE8	Tubulin beta chain	TUBB2B	*Homo sapiens*
T06	Q9UBN4	Short transient receptor potential channel 4	Trpc4	*Homo sapiens*
T07	P08842	Steryl-sulfatase precursor	STS	*Homo sapiens*
T08	P18405	Steroid 5-alpha-reductase 1	SRD5A1	*Homo sapiens*
T09	Q9NYA1	Sphingosine kinase 1	SPHK1	*Homo sapiens*
T10	Q05940	Synaptic vesicular amine transporter	SLC18A2	*Homo sapiens*
T11	P49281	Natural resistance-associated macrophage protein 2	SLC11A2	*Homo sapiens*
T12	O00767	Acyl-CoA desaturase	SCD	*Homo sapiens*
T13	P17707	S-adenosylmethionine decarboxylase proenzyme	AMD1	*Homo sapiens*
T14	P23219	Prostaglandin G/H synthase 1	PTGS1	*Homo sapiens*
T15	Q9Y263	Phospholipase A-2-activating protein	PLAA	*Homo sapiens*
T16	O15212	Prefoldin subunit 6	PFDN6	*Homo sapiens*
T17	Q9 μGN5	Poly (ADP-ribose) Polymerase-2	Parp2	*Homo sapiens*
T18	P11926	Ornithine decarboxylase	ODC1	*Homo sapiens*
T19	P04150	Glucocorticoid receptor	NR3C1	*Homo sapiens*
T20	P51843	nuclear receptor subfamily 0 group B member 1	NR0B1	*Homo sapiens*
T21	P29475	Nitric Oxide Synthase, brain	NOS1	*Homo sapiens*
T22	Q96P20	NACHT, LRR and PYD domains-containing protein 3	NLRP3	*Homo sapiens*
T23	P19838	Nuclear factor NF-kappa-B p105 subunit	NFKB1	*Homo sapiens*
T24	O14561	Acyl carrier protein, mitochondrial	NDUFAB1	*Homo sapiens*
T25	P10636	Microtubule-associated protein tau	MAPT	*Homo sapiens*
T26	P45985	Dual specificity mitogen-activated protein kinase kinase 4	MAP2K4	*Homo sapiens*
T27	P27338	Amine oxidase [flavin-containing] B	MAOB	*Homo sapiens*
T28	Q12791	Large conductance calcium-activated potassium channel subfamily M alpha member 1 isoform b	KCNMA1	*Homo sapiens*
T29	P10145	interleukin 8 precursor	IL8	*Homo sapiens*
T30	P28335	5-hydroxytryptamine receptor 2C	Htr2c	*Homo sapiens*
T31	P28223	5-hydroxytryptamine receptor 2A	HTR2A	*Homo sapiens*
T32	P80365	11-beta-Hydroxysteroid Dehydrogenase 2	HSD11B2	*Homo sapiens*
T33	P15428	15-hydroxyprostaglandin dehydrogenase [NAD+]	HPGD	*Homo sapiens*
T34	Q9Y2T3	Guanine deaminase	GDA	*Homo sapiens*
T35	P10253	Lysosomal alpha-glucosidase	GAA	*Homo sapiens*
T36	P11413	glucose-6-phosphate 1-dehydrogenase isoform b	G6PD	*Homo sapiens*
T37	P23945	Follicle stimulating hormone receptor	FSHR	*Homo sapiens*
T38	P11308	Transcriptional regulator	ERG	*Homo sapiens*
T39	P55245	Epidermal growth factor receptor	EGFR	*Homo sapiens*
T40	Q9NRD8	Thyroid oxidase 2	DUOX2	*Homo sapiens*
T41	P14416	Dopamine D2 receptor	DRD2	*Homo sapiens*
T42	Q9UBM7	7-dehydrocholesterol reductase	DHCR7	*Homo sapiens*
T43	P10635	Cytochrome P450 2D6	CYP2D6	*Homo sapiens*
T44	P11509	Cytochrome P450 2A6	CYP2A6	*Homo sapiens*
T45	O15528	25-hydroxyvitamin D-1 alpha hydroxylase, mitochondrial	CYP27B1	*Homo sapiens*
T46	Q16678	Cytochrome P450 1B1	CYP1B1	*Homo sapiens*
T47	P05177	Cytochrome P450 1A2	CYP1A2	*Homo sapiens*
T48	P04798	Cytochrome P450 1A1	CYP1A1	*Homo sapiens*
T49	P11511	Aromatase	CYP19A1	*Homo sapiens*
T50	P05093	Steroid 17-alpha-Monooxygenase (CYP17)	CYP17A1	*Homo sapiens*
T51	P34972	Cannabinoid receptor 2	CNR2	*Homo sapiens*
T52	P09483	Neuronal acetylcholine receptor subunit alpha-4	CHRNA4	*Homo sapiens*
T53	P08172	Muscarinic acetylcholine receptor M2	CHRM2	*Homo sapiens*
T54	O00748	Carboxylesterase 2 (intestine, liver)	CES2	*Homo sapiens*
T55	P51684	C-C chemokine receptor type 6	CCR6	*Homo sapiens*
T56	P16152	Carbonyl reductase [NADPH] 1	CBR1	*Homo sapiens*
T57	Q16790	Carbonic anhydrase 9	CA9	*Homo sapiens*
T58	O43570	Carbonic anhydrase 12	CA12	*Homo sapiens*
T59	P06276	Cholinesterase	BCHE	*Homo sapiens*
T60	O14983	Sarcoplasmic/endoplasmic reticulum calcium ATPase 1	ATP2A1	*Homo sapiens*
T61	P05023	Sodium/potassium-transporting ATPase subunit alpha-1	ATP1A1	*Homo sapiens*
T62	Q9UHC3	Amiloride-sensitive cation channel 3	ASIC3	*Homo sapiens*
T63	P15207	Androgen Receptor	AR	*Homo sapiens*
T64	P05067	Amyloid beta A4 protein	APP	*Homo sapiens*
T65	Q16853	Membrane primary amine oxidase	AOC3	*Homo sapiens*
T66	P09917	Arachidonate 5-lipoxygenase	ALOX5	*Homo sapiens*
T67	P05091	Aldehyde dehydrogenase	ALDH2	*Homo sapiens*
T68	P35869	Aryl hydrocarbon receptor	AHR	*Homo sapiens*
T69	O95622	Adenylate cyclase type V	ADCY5	*Homo sapiens*
T70	Q08828	Brain adenylate cyclase 1	ADCY1	*Homo sapiens*
T71	P08183	P-glycoprotein 1	ABCB1	*Homo sapiens*
T72	Q08209	Serine/threonine protein phosphatase 2B catalytic subunit, alpha isoform	PPP3CA	*Homo sapiens*
T73	P30989	neurotensin receptor type 1	NTSR1	*Homo sapiens*
T74	O60502	Protein O-GlcNAcase	MGEA5	*Homo sapiens*
T75	Q9Y251	Heparanase	HPSE	*Homo sapiens*

Table 2 (*continued*)

ID	UniProt	Protein names	Gene names	Organism
T76	P49841	Glycogen synthase kinase-3 beta	GSK3B	*Homo sapiens*
T77	Q04760	Glyoxalase I	GLO1	*Homo sapiens*
T78	Q13451	Peptidyl-prolyl cis-trans isomerase	FKBP5	*Homo sapiens*
T79	P15169	Carboxypeptidase N, catalytic subunit	CPN1	*Homo sapiens*
T80	Q00534	Cyclin-Dependent Kinase 6	CDK6	*Homo sapiens*
T81	P13501	C-C motif chemokine 5	CCL5	*Homo sapiens*
T82	Q96CA5	Baculoviral IAP repeat-containing protein 7	BIRC7	*Homo sapiens*
T83	P33527	Multidrug resistance-associated protein 1	ABCC1	*Homo sapiens*

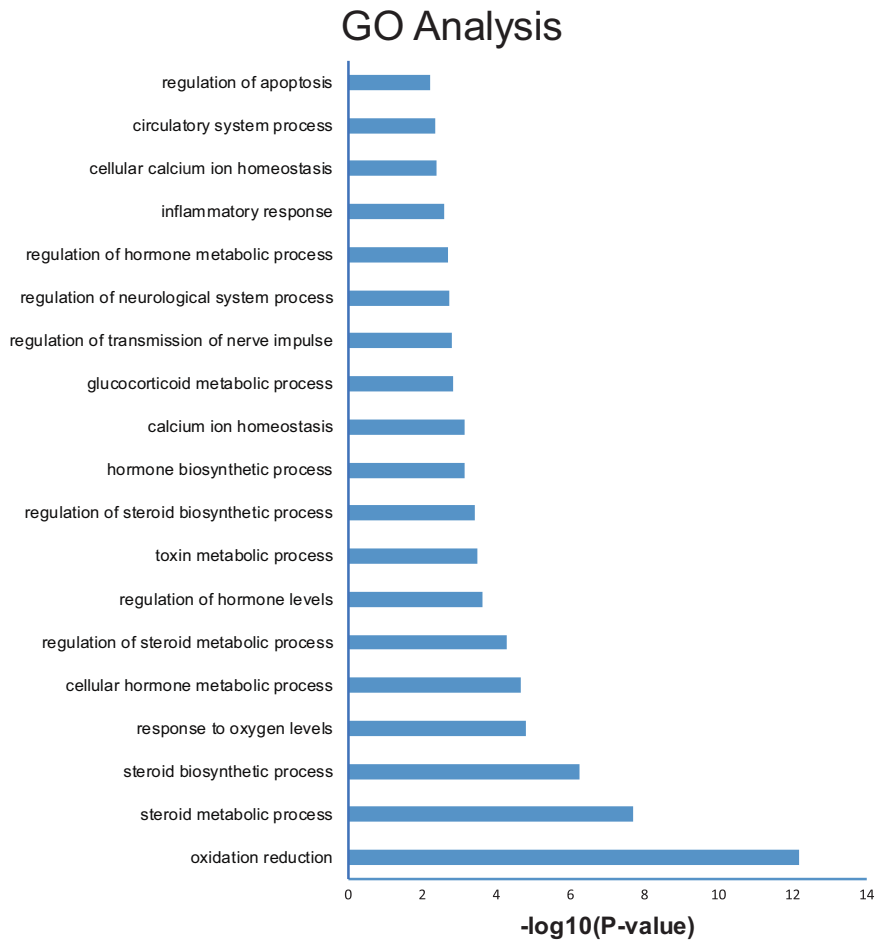


Fig. 2. Gene Ontology (GO) analysis of therapy target genes. The y-axis shows significantly enriched 'Biological Process' categories in GO of the target genes, and the x-axis shows the enrichment scores of these terms (P-value < 0.05).

Qubaibabuqi integrates multiple signaling pathways to regulate immune system, nervous system and melanin synthesis.

3.4. Vitiligo pathway

An integrated "vitiligo pathway" was constructed by integrating the key pathways that obtained through the T-P network analysis, including melanogenesis pathway (hsa04916), Serotonergic synapse pathway (hsa04726), Calcium signaling pathway (hsa04020), Chemokine signaling pathway (hsa04062), PI3K-AKT signaling pathway (hsa04151), Toll-like receptor signaling pathway (hsa04620). The target proteins of the integrated "vitiligo pathway" exhibit markedly close functional relationship with the vitiligo related proteins (ultimate nearness = 0.0063, nearness = 0.0152, $p < 0.01$). As can be seen from Fig. 5, the vitiligo pathway can be separated into three representative therapeutic modules (Immunoregulation module, Melanogenesis module and Neurological module).

3.4.1. Immunoregulation module

Humoral and cell-mediated immune mechanisms are likely to be involved in melanocyte destruction of vitiligo (Laddha et al., 2013). As shown in Fig. 5, toll-like receptor signaling pathway and chemokine signaling pathway involve in modulation of immune and proinflammatory responses. For instance, The chemokine IL-8 (cytokine interleukin-8) in toll-like receptor pathway plays an vital role in immune responses by induction of release of lysosomal enzymes and CD4+ and CD8+ human peripheral blood T lymphocytes (Mukaida et al., 1998). Neoechinulin A (M094) from *P. zeylanica* might play a vital role in vitiligo treatment by controling IL-8. In addition, chemokine signaling pathway can lead to Nitric Oxide (NO) induction, and NO is gaining recognition as an important biological mediator in inflammation and immunity. All these indicate that Qubaibabuqi may cure vitiligo by regulating

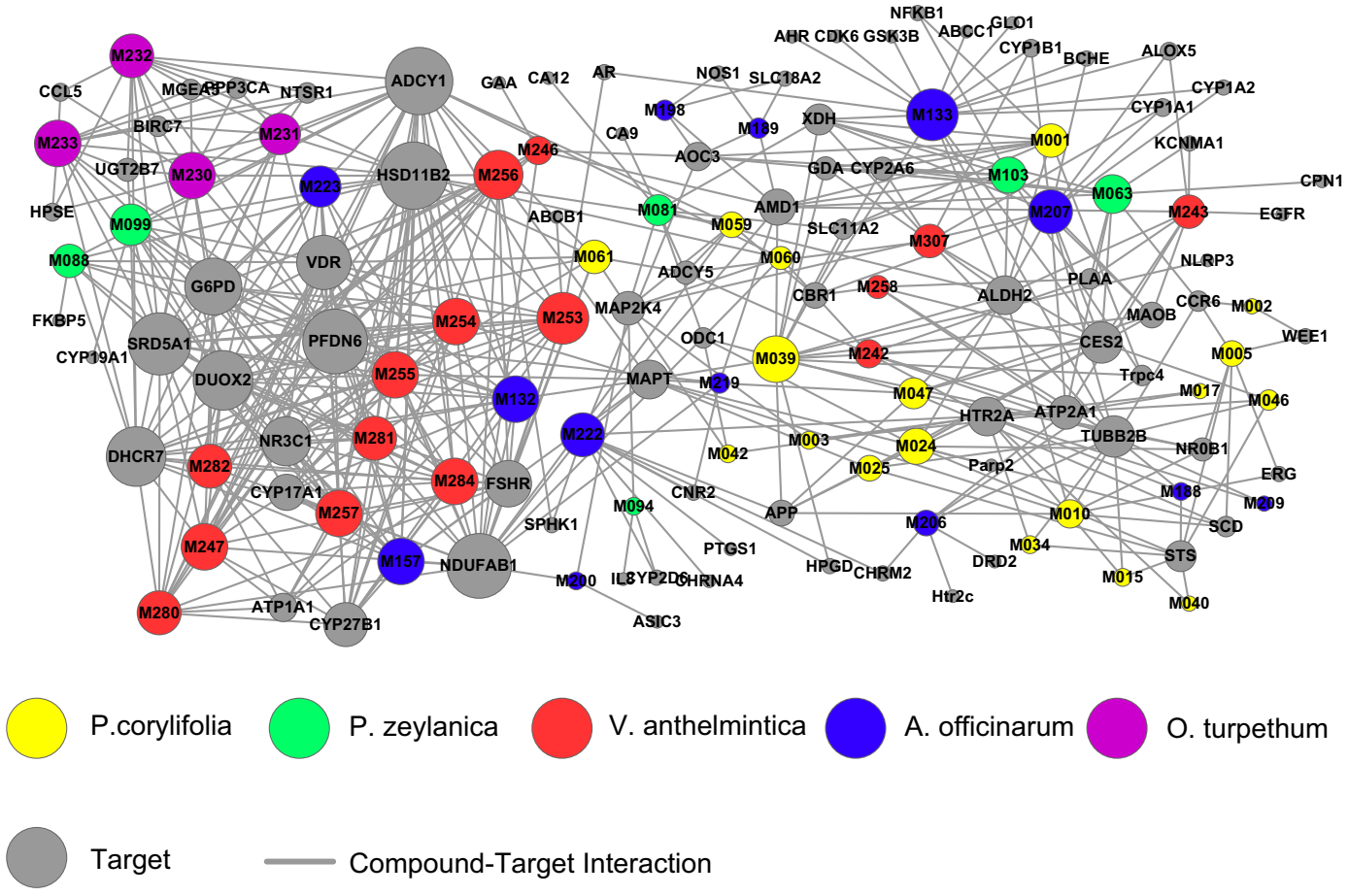


Fig. 3. C-T network. A compound node and a protein node are linked if the protein is targeted by the corresponding compound. Node size is proportional to its degree.

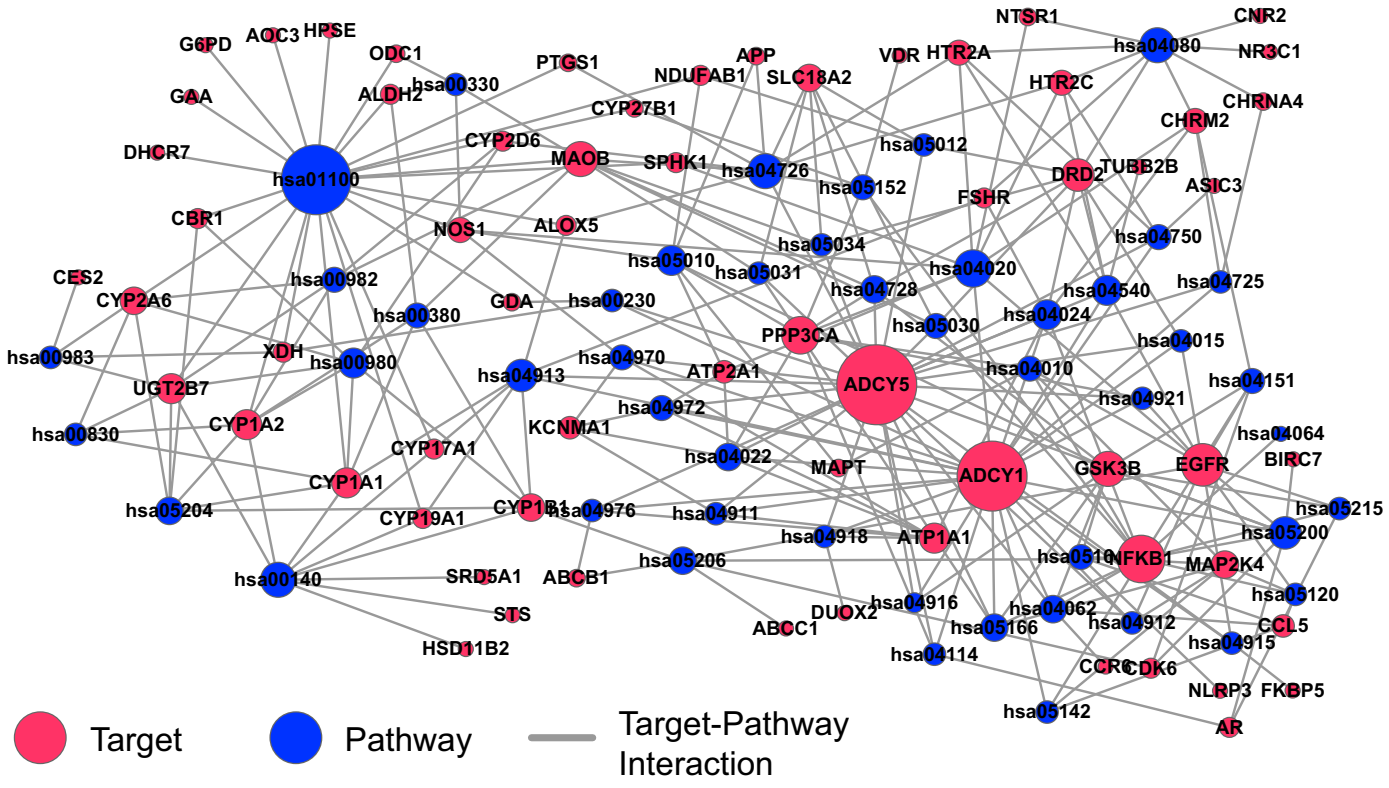


Fig. 4. T-P network. The T-P network is built by a target and a pathway if the pathway is lighted at the target. Node size is proportional to its degree. The information of pathways is obtained by mapping the target proteins to the KEGG pathway database.

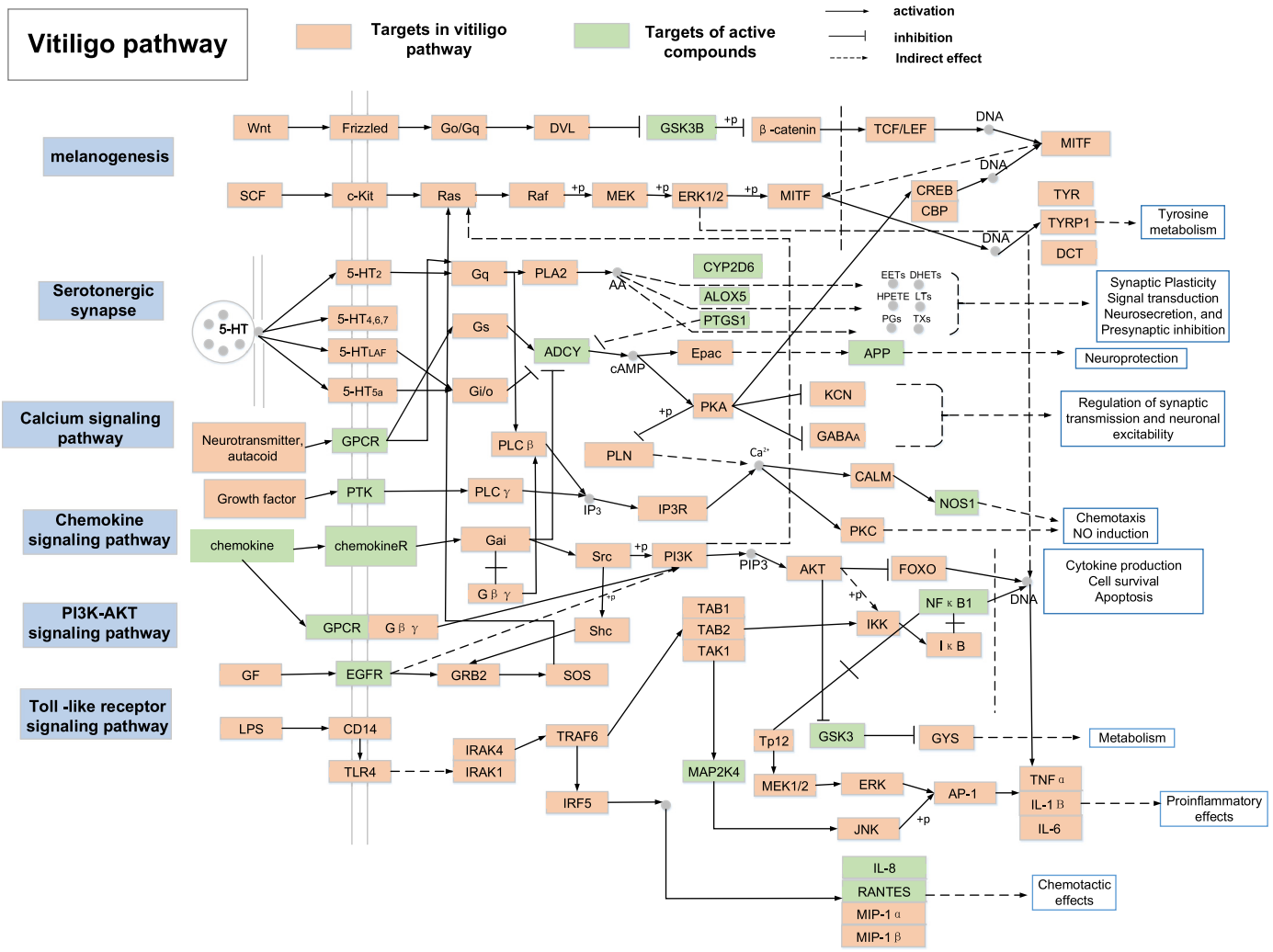


Fig. 5. Distribution of target proteins of Qubaibabuqi on the compressed 'vitiligo pathway'. Six pathways (lightsky-blue) form the compressed vitiligo pathway. Arrows represent activation effect, T-arrows represent inhibition effect and segments show activation effect or inhibition effect. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

immune system.

3.4.2. *Melanogenesis module*

It's well known that vitiligo is characterized by the destruction of melanocytes. As displayed in Fig. 5, the melanogenesis signaling pathway plays an important role for promoting melanin biosynthesis in the human epidermis. For example, GSK3β (phosphorylation of glycogen synthase kinase 3β) in melanogenesis signaling pathway is modulated by kaempferol (M133) (Zhou et al., 2015a). Inhibition of GSk3β can lead to β-catenin accumulation. The accumulated β-catenin can increase the expression of MITF by forming a complex with the lymphoid-enhancing factor/T-cell factor (LEF/TCF) transcription factor. And MITF can promote melanin synthesis by up-regulating the expression of tyrosinase, tyrosinase-related protein-1 (TRP-1), and dopachrome tautomerase (Dct) (Su et al., 2013).

The PI3K-AKT signaling pathway also takes part in regulation of melanin synthesis by inhibiting keratinocytes (KCs) apoptosis. KCs contribute to melanocyte homeostasis. The results of a comparison between depigmented and normally pigmented epidermis in patients with vitiligo showed that the KCs in the depigmented epidermis were more vulnerable to apoptosis (Lee, 2012). As shown in Fig. 5, some targets in the PI3K-AKT signaling pathway engage in equaling the levels between cellular survival and apoptosis. To take NF-κB1 for example, NF-κB1 can be regulated by isopsoralen (M001) and psoralen (M103). And previous study shows that NF-κB1 can reduce apoptosis of vitiliginous keratinocytes (Lee, 2012). Thus, Qubaibabuqi might promote the survival of vitiliginous KCs, which contributes to proliferation and differentiation of melanocytes. And these show that Qubaibabuqi may cure vitiligo by promoting melanin biosynthesis.

3.4.3. *Neurological module*

Neural factors may play an important role in the pathogenesis of vitiligo (Cucchi et al., 2000). The skin is highly innervated by a plenitude of nerve fiber subpopulations. Melanocyte itself also intimately contacts with nerve fibers to form 'synaptic-like structure' and its functions may be directly regulated by the mediators contained in terminals of intra-epidermal nerve fibers (Zhou et al., 2015b). As can be seen from the Fig. 5, some targets on Serotonergic synapse pathway and Calcium signaling pathway involves in the function of neurosecretion and neuronal excitability and the regulation of synaptic transmission. For example, G-protein Coupled Receptors (GPCRs) are key transmembrane recognition factors for regulatory signals such as neurotransmitters. And then, in the central nervous system, various GPCRs modulate sympathetic activity (Vizi, 2000). Our results show that GPCRs can be regulated by capsaicin (M222) from *A. officinarum*. Thus, all

above suggest that Qubaibabuqi may treat vitiligo by regulating the nervous system.

4. Conclusions

In this work, we utilized a systems pharmacology approach by integrating the ADME screening, targets prediction, network analysis and pathway analysis to dissect the underlying mechanisms of action of Qubaibabuqi and detect the pathogenesis of vitiligo. The main findings are as follows:

(1) 56 bioactive compounds and 83 target proteins were obtained in this study, demonstrating a multi-drug-multi-target paradigm of Qubaibabuqi. These compounds and targets might serve to guide our further study of this botanical drug.
(2) Target and C-T network analysis together display that some vital compounds of Qubaibabuqi such as butin, psoralen, kaempferol and cholesterol may play an important role in the treatment of vitiligo, and Qubaibabuqi positively aiming for some targets like ADCY1, SCD and BCHE exhibits the therapeutic effects against vitiligo by strengthening immunological response, promoting melanogenesis and balancing the nervous system.
(3) The T-P network and integrated vitiligo pathway display that Qubaibabuqi might together act on various pathways involved in the pathogenesis of vitiligo, which further demonstrate the three pathogenesis of vitiligo: autoimmune, melanocyte damage and neural dysfunctional.
(4) This work offers a new approach for understanding the mechanisms of pathogenesis of vitiligo and the action mechanism of Qubaibabuqi on vitiligo from molecular level to pathway level. The results will facilitate the widespread application of traditional medicines in modern medicine.

Author contributions and email address

Yonghua Wang (E-mail: yh_wang@nwsuaf.edu.cn) formulated the idea of the paper and supervised the research. Tianli Pei (E-mail: peitianli1990@163.com) and Chunli Zheng (E-mail: chunlizheng@nwsuaf.edu.cn) performed the research and wrote the paper., Chao Huang (E-mail: huangchao@nwsuaf.edu.cn), Xuetong Chen (E-mail: xuetongchen@hotmail.com), Yingxue Fu (E-mail: yingxue_fu@nwsuaf.edu.cn), Zihu Guo (E-mail: guozihu2010@yahoo.com) and Jianling Liu (E-mail: liujl@nwu.edu.cn) prepared tables and figures. All authors reviewed the manuscript.

Acknowledgements

This work was supported by Grants from Northwest A & F University, National Natural Science Foundation of China (31170796 and 81373892). It also was supported in part by Grants from, National Natural Science Foundation of China (31540008).

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jep.2016.06.001.

References

Alkhateeb, A., Fain, P.R., Thody, A., Bennett, D.C., Spritz, R.A., 2003. Epidemiology of vitiligo and associated autoimmune diseases in Caucasian probands and their families. Pigm. Cell Res. 16 (3), 208–214.
Amerio, P., Tracanna, M., De Remigis, P., Betterle, C., Vianale, L., Marra, M., Di Rollo, D., Capizzi, R., Feliciani, C., Tulli, A., 2006. Vitiligo associated with other autoimmune diseases: polyglandular autoimmune syndrome types 3B+ C and 4. Clin. Exp. Dermatol. 31 (5), 746–749.
Ashburner, M., Ball, C.A., Blake, J.A., Botstein, D., Butler, H., Cherry, J.M., Davis, A.P., Dolinski, K., Dwight, S.S., Eppig, J.T., 2000. Gene Ontology: tool for the unification of biology. Nat. Genet. 25 (1), 25–29.
Azuaje, F.J., Zhang, L., Devaux, Y., Wagner, D.R., 2011. Drug-target network in myocardial infarction reveals multiple side effects of unrelated drugs. Sci. Rep. 1.
Becatti, M., Prignano, F., Fiorillo, C., Pescitelli, L., Nassi, P., Lotti, T., Taddei, N., 2010. The involvement of Smac/DIABLO, p53, NF-kB, and MAPK pathways in apoptosis of keratinocytes from perilesional vitiligo skin: protective effects of curcumin and capsaicin. Antioxid. Redox Signal. 13 (9), 1309–1321.
Berger, S.I., Iyengar, R., 2009. Network analyses in systems pharmacology. Bioinformatics 25 (19), 2466–2472.
Borisy, A.A., Elliott, P.J., Hurst, N.W., Lee, M.S., Lehár, J., Price, E.R., Serbedzija, G., Zimmermann, G.R., Foley, M.A., Stockwell, B.R., 2003. Systematic discovery of multicomponent therapeutics. P. Natl. Acad. Sci. USA 100 (13), 7977–7982.
Cheung, W.I., Tse, M.L., Ngan, T., Lin, J., Lee, W.K., Poon, W.T., Mak, T.W., Leung, V.K. S., Chau, T.N., 2009. Liver injury associated with the use of Fructus Psoraleae (Bol-gol-zhee or Bu-gu-zhi) and its related proprietary medicine. Clin. Toxicol. 47 (7), 683–685.
Commission, C.P., 1999. Drug Standard of Ministry of Public Health of the People's Republic ofChina (Uygur Medicine Volumes). Xinjiang Health Science and Technology Publishing House, Urumqi, 169.
Commission, C.P., 2005. Pharmacopoeia of the People's Republic of China, Uyghur Medicine Volume. Chinese Medical Science and Technology Press, Beijing, China, p. 150.
Cucchi, M.L., Frattini, P., Santagostino, G., Orecchia, G., 2000. Higher plasma catecholamine and metabolite levels in the early phase of nonsegmental vitiligo. Pigm. Cell Res. 13 (1), 28–32.
Cumming, J.G., Davis, A.M., Muresan, S., Haeberlein, M., Chen, H., 2013. Chemical predictive modelling to improve compound quality. Nat. Rev. Drug. Discov. 12 (12), 948–962.
Ding, W., Zeng, F., Xu, L., Chen, Y., Wang, Y., Wei, X., 2011. Bioactive dammarane-type saponins from *Operculina turpethum*. J. Nat. Prod. 74 (9), 1868–1874.
García-Mediavilla, V., Crespo, I., Collado, P.S., Esteller, A., Sánchez-Campos, S., Tuñón, M.J., González-Gallego, J., 2007. The anti-inflammatory flavones quercetin and kaempferol cause inhibition of inducible nitric oxide synthase, cyclooxygenase-2 and reactive C-protein, and down-regulation of the nuclear factor kappaB pathway in Chang Liver cells. Eur. J. Pharmacol. 557 (2), 221–229.
Gauthier, Y., Andre, M.C., Taïeb, A., 2003. A critical appraisal of vitiligo etiologic theories. Is melanocyte loss a melanocytorrhagy? Pigm. Cell Res. 16 (4), 322–332.
Greene, N., Fisk, L., Naven, R.T., Note, R.R., Patel, M.L., Pelletier, D.J., 2010. Developing structure – activity relationships for the prediction of hepatotoxicity. Chem. Res. Toxicol. 23 (7), 1215–1222.
Grimes, P., 1993. Vitiligo. An overview of therapeutic approaches. Dermatol. Clin. 11 (2), 325–338.
Grimes, P.E., Hamzavi, I., Lebwohl, M., Ortonne, J.P., Lim, H.W., 2013. The efficacy of afamelanotide and narrowband UV-B phototherapy for repigmentation of vitiligo. JAMA Dermatol. 149 (1), 68–73.
Hossani-Madani, A., Halder, R., 2010. Topical treatment and combination approaches for vitiligo: new insights, new developments. G. Ital. Dermatol. Venereol. 145 (1), 57–78.
Huang, C., Zheng, C., Li, Y., Wang, Y., Lu, A., Yang, L., 2013. Systems pharmacology in drug discovery and therapeutic insight for herbal medicines. Brief. Bioinform., bbt035
Huang, D.W., Sherman, B.T., Lempicki, R.A., 2008. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat. Protoc. 4 (1), 44–57.
Huang, S.-H., Tung, C.-W., Fülöp, F., Li, J.-H., 2015. Developing a QSAR model for hepatotoxicity screening of the active compounds in traditional Chinese medicines. Food Chem. Toxicol. 78, 71–77.
Huo, S.-x., Kang, Y.-T., Peng, X.-m., Gao, L., Tang, X.-q., Peng, Y., Yan, M., 2012. Effects of Qubaibabuqi Capsule containing serum on proliferation and migration of Human Melanoma A375 Cells. Chin. J. Pharmacol. Toxicol. 1, 016.
Huo, S.X., Liu, X.M., Ge, C.H., Gao, L., Peng, X.M., Zhao, P.P., Yan, M., 2014. The effects of galangin on a mouse model of vitiligo induced by hydroquinone. Phytother. Res. 28 (10), 1533–1538.
Kohli, K., Nipanikar, S., Kadbhane, K., 2010. A comprehensive review on trivrit (operculina Turpethum Syn. Ipomoea Turpethum). Int. J. Pharm. Biol. Sci. 1 (4), 452.
Kotiyal, J., Sharma, D., 1992. Phytochemical studies of Psoralea species-A review. Bull. Medico-Ethnobot. Res. 13, 209–223.
Kumar, R., Kumar, S., Patra, A., Jayalakshmi, S., 2009. Hepatoprotective activity of aerial parts of Plumbago zeylanica linn against carbon tetrachloride-induced hepatotoxicity in rats. Int. J. Pharm. Pharm. Sci. 1, 171–175.
Laddha, N.C., Dwivedi, M., Mansuri, M.S., Gani, A.R., Ansarullah, M., Ramachandran, A., Dalai, S., Begum, R., 2013. Vitiligo: interplay between oxidative stress and immune system. Exp. Dermatol. 22 (4), 245–250.
Lee, J., Jung, K., Kim, Y.S., Park, D., 2007. Diosgenin inhibits melanogenesis through the activation of phosphatidylinositol-3-kinase pathway (PI3K) signaling. Life Sci. 81 (3), 249–254.
Lee, A.Y., 2012. Role of keratinocytes in the development of vitiligo. Ann. Dermatol. 24 (2), 115–125.
Li, L., Li, Y., Wang, Y., Zhang, S., Yang, L., 2007. Prediction of human intestinal absorption based on molecular indices. Int. J. Mol. Sci. 23, 286–291.

Lin, M.-K., Yu, Y.-L., Chen, K.-C., Chang, W.-T., Lee, M.-S., Yang, M.-J., Cheng, H.-C., Liu, C.-H., Chen, D.-C., Chu, C.-L., 2011. Kaempferol from Semen cuscutae attenuates the immune function of dendritic cells. Immunobiology 216 (10), 1103–1109.
Liu, Y.-t., Zeng, W.-h., Wang, J.-w., Zhang, H.-b., 2011. Clinical efficacy of the Qubai Babuqi Tablets in the treatment of vitiligo. Chin. J. Aesthetic. Med. 9, 049.
Matthews, E.J., Ursem, C.J., Kruhlak, N.L., Benz, R.D., Sabaté, D.A., Yang, C., Klopman, G., Contrera, J.F., 2009. Identification of structure-activity relationships for adverse effects of pharmaceuticals in humans: Part B. Use of (Q) SAR systems for early detection of drug-induced hepatobiliary and urinary tract toxicities. Regul. Toxicol. Pharm. 54 (1), 23–42.
Mok, D.K., Chau, F.-T., 2006. Chemical information of Chinese medicines: a challenge to chemist. Chemom. Intell. Lab. Syst. 82 (1), 210–217.
Morison, W.L., Baughman, R.D., Day, R.M., Forbes, P.D., Hoenigsmann, H., Krueger, G. G., Lebwohl, M., Lew, R., Naldi, L., Parrish, J.A., 1998. Consensus workshop on the toxic effects of long-term PUVA therapy. Arch. Dermatol. 134 (5), 595–598.
Mukaida, N., Harada, A., Matsushima, K., 1998. Interleukin-8 (IL-8) and monocyte chemotactic and activating factor (MCAF/MCP-1), chemokines essentially involved in inflammatory and immune reactions. Cytokine Growth Factor Rev. 9 (1), 9–23.
Mulliner, D., Schmidt, F., Stolte, M., Spirkl, H.-P., Czich, A., Amberg, A., 2016. Computational models for human and animal hepatotoxicity with a global application scope. Chem. Res. Toxicol.
Nagashima, T., Ichimiya, S., Kikuchi, T., Saito, Y., Matsumiya, H., Ara, S., Koshiba, S., Zhang, J., Hatate, C., Tonooka, A., 2011. Arachidonate 5-lipoxygenase establishes adaptive humoral immunity by controlling primary B cells and their cognate T-cell help. Am. J. Pathol. 178 (1), 222–232.
Namazi, M., 2007. Neurogenic dysregulation, oxidative stress, autoimmunity, and melanocytorrhagy in vitiligo: can they be interconnected? Pigm. Cell Res. 20 (5), 360–363.
Ongenae, K., Van Geel, N., Naeyaert, J.M., 2003. Evidence for an autoimmune pathogenesis of vitiligo. Pigm. Cell Res. 16 (2), 90–100.
Passeron, T., Ortonne, J.-P., 2005. Physiopathology and genetics of vitiligo. J. Autoimmun. 25, 63–68.
Peng, Y., Huo, S.-x., Kang, Y.-t., Yan, M., 2011. Curative effect of victoria medicine qubaibabuqi capsule on experimental vitiligo in guinea pigs. Herald. Med. 7, 009.
Pepeljnjak, S., Kosalec, I., 2004. Galangin expresses bactericidal activity against multiple-resistant bacteria: MRSA, Enterococcus spp. and Pseudomonas aeruginosa. FEMS Microbiol. Lett. 240 (1), 111–116.
Rix, U., Superti-Furga, G., 2009. Target profiling of small molecules by chemical proteomics. Nat. Chem. Biol. 5 (9), 616–624.
Rodríguez, C.I., Setaluri, V., 2014. Cyclic AMP (cAMP) signaling in melanocytes and melanoma. Arch. Biochem. Biophys. 563, 22–27.
Ru, J., Li, P., Wang, J., Zhou, W., Li, B., Huang, C., Li, P., Guo, Z., Tao, W., Yang, Y., 2014. TCMSP: a database of systems pharmacology for drug discovery from herbal medicines. J. Cheminform. 6 (1), 13.
Russo, A., Longo, R., Vanella, A., 2002. Antioxidant activity of propolis: role of caffeic acid phenethyl ester and galangin. Fitoterapia 73, S21–S29.
Semwal, R.B., Semwal, D.K., Combrinck, S., Viljoen, A., 2015. Butein: From ancient traditional remedy to modern nutraceutical. Phytochem. Lett. 11, 188–201.
Schallreuter, K.U., Hasse, S., Rokos, H., Chavan, B., Shalbaf, M., Spencer, J.D., Wood, J. M., 2009. Cholesterol regulates melanogenesis in human epidermal melanocytes and melanoma cells. Exp. Dermatol. 18 (8), 680–688.
Shih, T.-Y., Young, T.-H., Lee, H.-S., Hsieh, C.-B., Hu, O.Y.-P., 2013. Protective effects of kaempferol on isoniazid-and rifampicin-induced hepatotoxicity. AAPS J. 15 (3), 753–762.
Smoot, M.E., Ono, K., Ruscheinski, J., Wang, P.-L., Ideker, T., 2011. Cytoscape 2.8: new features for data integration and network visualization. Bioinformatics 27 (3), 431–432.
Spritz, R.A., 2006. The genetics of generalized vitiligo. Exp. Dermatol. 15 (10), 850–851.
Spritz, R.A., 2007. The genetics of generalized vitiligo and associated autoimmune diseases. Pigm. Cell Res. 20 (4), 271–278.
Su, T.-R., Lin, J.-J., Tsai, C.-C., Huang, T.-K., Yang, Z.-Y., Wu, M.-O., Zheng, Y.-Q., Su, C.-C., Wu, Y.-J., 2013. Inhibition of melanogenesis by gallic acid: possible involvement of the PI3K/Akt, MEK/ERK and Wnt/β-catenin signaling pathways in B16F10 cells. Int. J. Mol. Sci. 14 (10), 20443–20458.
Sukkasem, N., Chatuphonprasert, W., Tatiya-aphiradee, N., Jarukamjorn, K., 2016. Imbalance of the antioxidative System by plumbagin and Plumbago indica L. Extract Induces Hepatotoxicity in Mice. J. Intercult. Ethnopharmacol. 5 (2), 137.
Szczurko, O., Boon, H.S., 2008. A systematic review of natural health product treatment for vitiligo. BMC Dermatol. 8 (1), 2.
Tang, F., Zhang, Q., Nie, Z., Yao, S., Chen, B., 2009. Sample preparation for analyzing traditional Chinese medicines. TrAC Trends Anal. Chem. 28 (11), 1253–1262.
Travis, L.B., Silverberg, N.B., 2004. Calcipotriene and corticosteroid combination therapy for vitiligo. Pediatr. Dermatol. 21 (4), 495–498.
Vizi, E.S., 2000. Role of high-affinity receptors and membrane transporters in nonsynaptic communication and drug action in the central nervous system. Pharmacol. Rev. 52 (1), 63–90.
Wang, X., Xu, X., Li, Y., Li, X., Tao, W., Li, B., Wang, Y., Yang, L., 2013. Systems pharmacology uncovers Janus functions of botanical drugs: activation of host defense system and inhibition of influenza virus replication. Integr. Biol. 5 (2), 351–371.
Zhang, J., Li, Y., Chen, X., Pan, Y., Zhang, S., Wang, Y., 2014. Systems pharmacology dissection of multi-scale mechanisms of action for herbal medicines in stroke treatment and prevention. PLoS One 9, 8.
Zheng, C., Guo, Z., Huang, C., Wu, Z., Li, Y., Chen, X., Fu, Y., Ru, J., Shar, P.A., Wang, Y., 2015. Large-scale direct targeting for drug repositioning and discovery. Sci. Rep. 5.
Zheng, C., Wang, J., Liu, J., Pei, M., Huang, C., Wang, Y., 2014. System-level multi-target drug discovery from natural products with applications to cardiovascular diseases. Mol. Divers. 18 (3), 621–635.
Zhou, M., Ren, H., Han, J., Wang, W., Zheng, Q., Wang, D., 2015a. Protective effects of kaempferol against myocardial ischemia/reperfusion injury in isolated rat heart via antioxidant activity and inhibition of glycogen synthase kinase-3. Oxid. Med. Cell. Longev. 2015.
Zhou, J., Feng, J.-Y., Wang, Q., Shang, J., 2015b. Calcitonin gene-related peptide cooperates with substance P to inhibit melanogenesis and induces apoptosis of B16F10 cells. Cytokine 74 (1), 137–144.