Network Analysis, and Experimental Validation to Uncover the Mechanism of the Four Compounds in Artemisia Annua (Qing Hao) Antimalarial

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Research

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Abstract

Background

Artemisinin is widely used to treat malaria, but the antimalarial mechanism and coordinative interactions governing the actions of artemisinin, scopoletin, arteannuin B and artemisinic acid have not been elucidated.

Methods

Based on the existence of antimalarial drugs, the antimalaria targets of artemisinin, scopoletin, arteannuin B and artemisinic acid were investigated by molecular docking using the similarity theory of chemical structure, and the antimalaria mechanism of scopoletin and its coordinative antimalaria interactions with the other three ingredients of the mixture were subsequently explored.

Results

Using the text information excavation method, the relevant proteins involved in the antimalarial effect of artemisinin were determined to be IL-6, ACHE, PC3, IPOB, CYC, TNF- α , UGT1A9, CASP3, XDH, IL-1 β , VEGF, CAT, CREB, AMPK, UGT1A6, ADR, MAPK, COX2, LB24AB and CYP450. Meanwhile, the relevant proteins involved in the antimalarial effect of scopoletin were TNF- α , PI3K, IL-8, IL-6, VEGF, IL-1 β , MAPK, CD4, SP2, CTNNB, CASP3, PRO1400, IgE, IL-4, ICAM1, p38, STAT3, TLR4 and API4. However, arteannuin B and artemisinic acid had little relevance to the abovementioned proteins. The interaction property between TNF- α and *Artemisia annua* was that the effect of the mixture of artemisinin, scopoletin, arteannuin B and artemisinic acid was greater than that of artemisinin, and the synergistic effect of the four elements was considered to be beneficial to the progress of antimalarial treatment.

Conclusion

Antimalarial targets of *Artemisia annua* ingredients were explored with data mining methods, and the antimalarial effect of scopoletin may be related to TNF. Combined application of the four elements could achieve the same antimalarial effect and reduce the clinical usage of artemisinin and scopoletin.

Background

Malaria is a major threat to human life. Together with AIDS and cancer, malaria is listed by the World Health Organization as one of the world's three major deadly diseases. Before the emergence and promotion of artemisinin, approximately 400 million people worldwide were infected with malaria, and at least 1 million people died from malaria every year. The morbidity and mortality of malaria are especially high in sub-Saharan Africa. According to WHO data for 2016, 2.7 billion dollars were spent on malaria

control and elimination worldwide by governments and international organizations [1]. Antimalarial drugs primarily include quinolines (such as chloroquine, mefloquine, and quinine) and antifolates (such as pyrimethamine and sulfadoxine). The use of these drugs has effectively controlled the global spread of malaria. However, *Plasmodium falciparum* has developed resistance to almost all antimalarial drugs [2]. China was once one of the countries most strongly affected by malaria and undertook large-scale efforts to eliminate the disease [3]. The unique chemical compositions of the traditional Chinese materia medica have significant biological activity in major diseases. Chinese medicines with clear active ingredients are rare, while *Artemisia annua* (Qing Hao) is a typical chemical composition obtained by the modern, scientifically verified traditional Chinese materia medica. The victory over the disease was finally achieved; in 2014, malaria patients were controlled to 56 individuals in China [4]. In the 1990s, artemisinin was widely used in Thailand and other countries in Southeast Asia. Next, artemisinin was widely adopted in Africa and the Americas [5]. However, neither artemisinin monotherapy nor artemisinin-based combination therapy was determined to be efficacious, and delayed parasite clearance often confused clinicians, similar to other antimalarial drugs [6–8]. *Plasmodium falciparum* has developed resistance to artemisinin in the Greater Mekong region, including Cambodia, Laos, Myanmar, Thailand and Vietnam. The World Health Organization's 2011 global plan attempted to tackle artemisinin resistance caused by the artemisinin partner drug [9]. Indeed, the primary reason for this resistance might be the artemisinin partner drug [10]. Artemisinin, if combined with another drug with low drug resistance, may not delay parasite clearance. It remains entirely possible to rely on artemisinin and its new partner drugs to end the prevalence of malaria [11]. Drugs with low drug resistance must be novel, as existing antimalarial drugs have developed strong resistance. Scopoletin, arteannuin B and artemisinic acid were considered because their chemical structures are more similar to that of artemisinin. Compounds that selectively act on two or more targets of interest would in theory perform more pharmacological actions than single-target agents [12]. 'Polypharmacology' is a new methodology that is employed in drug discovery [13]. This study uses the method of network pharmacology to perform text mining and target prediction by investigating the four components of artemisinin, artemisinin, artemisinic acid and sorghum lactone, which may have antimalarial effects in *Artemisia annua* L.

Molecular docking is an essential procedure to verify network pharmacology in structural molecular biology and computer-assisted drug design. Molecular docking could be employed to perform virtual screening on chemical compounds, rank the results, and propose structural hypotheses on how the ligands inhibit the target, which is highly valuable in lead optimization [14].

Materials And Methods

Reagents and materials

1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) was obtained from Shanghai Beinuo Biotechnology Co., Ltd. (Shanghai, China), and N-hydroxysuccinimide (NHS) was purchased from Shanghai Dibai Biotechnology Co., Ltd. Sodium acetate and dimethyl sulfoxide (DMSO) were purchased from Beijing Chemical Plant (Beijing, China). Ethanolamine hydrochloride was purchased from Shanghai

Sanying Chemical Reagent Co., Ltd. (Shanghai, China), the protein TNF- α was provided by RD Biosciences (USA), artemisinin, scopoletin, arteannuin B and artemisinic acid were provided by Chengdu Ruifensi Biological Technology Co., Ltd. (Chengdu, China), and Dulbecco's phosphate buffered saline PBS buffer (pH 4.7) was freshly prepared.

Plasma sample preparation

Approximately 40 mg of EDC and 10 mg of NHS were weighed, and 1 mL of solution was prepared with distilled water. The above solution was injected within 5 min into two channels that had been thoroughly rinsed with PBS buffer.

Fifty micrograms of TNF- α protein was dissolved in 100 μ L of PBS, and 10 μ L of the above solution was taken in three portions and diluted with sodium acetate solutions with pH values of 5.5, 6.0, and 6.5, respectively, and the final concentration was 50 μ g/mL. The flow rate was reduced to 20 μ L/min, and the left channel was rinsed for 10 min to determine the optimal pH of sodium acetate.

After determination of the optimal pH value, 1 M ethanolamine hydrochloride was injected into the two channels for 10 min to complete the sample fixation.

The four chemical compounds, scopoletin (A), artemisinin (B), artemisinic acid (C) and arteannuin B (D), were divided into 12 groups according to the combination rule of A, B, C, D, AB, AC, AD, ABC, ABD, ACD, BCD, and ABCD, and each group had 6 concentration levels. The control group was PBS buffer at pH 7.4.

Next, 19.2 mg of scutellaria lactone, 28.2 mg of artemisinin, 24.8 mg of artemisinin 2 and 23.4 mg of artemisinin were accurately weighed and dissolved in 1 mL DMSO (dimethyl sulfoxide). The DMSO solution in each group was gradient-diluted with PBS to final concentrations of 200 μ M, 66.7 μ M, 22.2 μ M, 7.41 μ M, and 2.47 μ M.

Target fishing

Known therapeutic targets for the treatment of malaria were obtained from the DrugBank database (<http://www.drugbank.ca/>, version 4.3) [15]. The prediction of drug targets based on ligand structural features primarily includes chemical similarity searches and reverse pharmacophore searches. The theoretical basis of the chemical similarity search is that small molecular compounds with similar structural or physicochemical properties can act on targets with the same or similar properties: "antimalaria" was selected as the key word, as well as the drug-target interactions whose drugs are approved by the USA Food and Drug Administration (FDA) for treating menstrual disorders. All target gene/protein identifiers (IDs) were converted into the corresponding gene symbol/UniProtKB-Swiss-Prot IDs to facilitate further data analyses. After removing redundant entries, 25 target genes corresponding to 15 known antimalarial drugs were retrieved.

Protein-protein interaction (PPI) data

PPI data were imported from the following PPI databases, including the Human Annotated and Predicted Protein Interaction Database (HAPPI, <http://bio.informatics.iupui.edu/HAPPI/>, Version 31.2) [16]. Based on the PPI network database, an interaction network of *Artemisia annua* candidate target groups and known antimalarial drug target groups was constructed, and the distribution of target nodes in metabolic pathways and the corresponding diseases was determined. As a result, a direct interaction network of key nodes was subsequently established and divided into different modules according to the functions of the nodes. According to the malaria pathway (ko05144: Malaria) in KEGG, molecules closely related to the malaria pathway were selected as candidates to be verified from the key nodes.

Network construction and topological analysis

Compound–target (C-T), target–pathway (T-P), and target–disease (T-D) networks of malaria were constructed using Cytoscape 3.2 software (<https://cytoscape.org/download.html>), a general bioinformatics software package for data integration and visualization of biological networks (Bindea *et al.*, 2009; Smoot *et al.*, 2011). An interaction network of *Artemisia annua* candidate target genes with known antimalarial drug target genes was established, consisting of 85 nodes and 298 pairs of interactions. The topological characteristic value of each node was calculated in the network, and the median of the topological characteristic value was used as the card value. A total of 32 key nodes were screened. A direct interaction network of key nodes was established and processed according to the node functions. The module was divided, the malaria pathway in Kyoto Encyclopedia of Genes and Genomes (KEGG) (ko05144: Malaria) was compared, and molecules closely related to the malaria pathway were selected as candidates to be verified from the key nodes.

Molecular docking

The molecular structures of CDK4, NFKB1, PIK3CG, MAPK1, TNF and ITGB2 protein targets (protein species is human) were searched in the database UniProt (<http://www.uniprot.org/>). The structures of scopolamine and artemisinin were downloaded from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov>). Chemical composition and protein structure were dehydrated and hydrotreated, respectively. Molecular docking and figures were generated using Discovery Studio Visualizer 2.5 software.

Probe Kd determination

Approximately 40 mg of EDC and 10 mg of NHS were weighed, and 1 mL of solution was prepared with distilled water. The above solution was injected within 5 min into two channels thoroughly rinsed with PBS buffer. Next, 19.2 mg of scopolamine, 28.2 mg of artemisinin, 24.8 mg of artemisinin, and 23.4 mg of artemisinin were precisely weighed in 1 mL DMSO (dimethyl sulfoxide) and mixed well. The TNF- α protein was immobilized on grafted sensor chips. The compound monomers and combinations are divided into 12 groups (Table 1). Each group of samples was injected from low to high concentrations, and a control group (PBS) was set at each concentration. Regression analysis when concentration curves are separated and approaching equilibrium. The dissociation constant (Kd) and its maximum value

(Bmax) were then calculated by fitting the titration curve to the single-site saturation binding equation [$Y = B_{max} \cdot X / (K_d + X)$] using GraphPad Prism software (GraphPad software Incorporated, La Jolla, CA, USA).

Table 1
Compound monomers and combinations

Group	compounds
A	Scopoletin
B	Artemisinin
C	Artemisinic acid
D	Arteannuin B
AB	Scopoletin; Artemisinin
AC	Scopoletin; Artemisinic acid
AD	Scopoletin; Arteannuin B
ABC	Scopoletin; Artemisinin; Artemisinic acid
ABD	Scopoletin; Artemisinin; Arteannuin B
ACD	Scopoletin; Artemisinic acid; Arteannuin B
BCD	Artemisinin; Artemisinic acid; Arteannuin B
ABCD	Scopoletin; Artemisinin; Artemisinic acid; Arteannuin B

Results

Compound–target network construction

There were 162 diseases related to four chemical components observed in *Artemisia annua*. Through artificial noise reduction, the top 5 diseases, ranked by frequency of occurrence, were extracted as malaria (50), cerebral malaria (7), falciparum malaria (4), visceral leishmaniasis (2), and systemic lupus erythematosus (1) (Fig. 1).

Fifteen known antimalarial drugs were retrieved from the DrugBank database (Table 2). There were 93 targets with structures similar to scopolide and 15 known antimalarial drugs (similar score greater than 0.7). Artemisinic acid had 32 targets with structures similar to 15 known antimalarial drugs (similar score greater than 0.7).

Clustering analysis

The key node interaction network was divided into three functional modules. The first functional module primarily involved immune-related pathways, the second primarily involved multiple infectious diseases,

and the third was related to drug metabolism and tumour pathways. KEGG's malaria pathway (ko05144: Malaria) comparison is shown in Fig. 2. The four key nodes were involved in four pathways: the T cell receptor signalling pathway, the Toll-like receptor signalling pathway, the TNF signalling pathway, and natural killer cell-mediated cytotoxicity, which all have important links to the malaria pathway; therefore, the joint nodes involved in these four pathways can be regarded as candidates to be verified.

Table 2
Known antimalarial drugs

Drug ID	Name
DB00254	Doxycycline
DB00908	Quinidine
DB00358	Mefloquine
DB01611	Hydroxychloroquine
DB00608	Chloroquine
DB00613	Amodiaquine
DB01103	Quinacrine
DB01087	Primaquine
DB00468	Quinine
DB04877	Voacamine
DB00440	Trimethoprim
DB01218	Halofantrine
DB00205	Pyrimethamine
DB01131	Proguanil
DB01117	Atovaquone

Molecular docking

Scopolide had suitable docking sites for TNF, NFKB1, and PIK3CG, and the docking scores were 77.9576, 57.8491, and 50.2248, respectively, with no suitable docking site being identified for CDK4 or MAPK1. There was no suitable docking site for artemisinic acid with ITGB2 (Table 3, Fig. 3).

Table 3
Docking score of scopolide and artemisinic acid
with the targets

Compounds	Targets	Docking Score
Scopolide	CDK4	-
Scopolide	NFKB1	57.8491
Scopolide	PIK3CG	50.2248
Scopolide	MAPK1	-
Scopolide	TNF	77.9576
artemisinic acid	ITGB2	-

Experimental validation of key targets

According to the binding curve of the immobilized protein, the highest binding efficiency of sodium acetate solution at pH 5.5 is 110 μ RIU/s, but the binding is unstable. The lowest binding efficiency at pH 6.5 is 68 μ RIU/s. At pH 6.0, the binding efficiency is better, and the comparison of binding is stable at 108 μ RIU/s; therefore, the optimal approach is to use sodium acetate solution with pH 6.0 for protein fixation.

TNF had good binding with scopolide, suggesting that the antimalarial effect of scopolide may be related to TNF. The binding of TNF to artemisinin, artemisinin B, and artemisinic acid was weak, but the combination of the 4 components of artemisinin, artemisinin B, artemisinic acid, and stigmalactone had good binding to TNF, suggesting that the combined application of 4 ingredients may achieve antimalarial effects by acting on TNF (Table 4, Fig. 4).

Table 4
Equilibrium dissociation constant KD for each group

Group	KD(M)	Est. error	Bmax Signal (uRIU)
A	4.05×10^{-6}	2.00×10^{-6}	42.0
B	2.01×10^{-4}	1.45×10^{-5}	38.7
C	8.91×10^{-5}	4.07×10^{-7}	72.3
D	2.93×10^{18}	1.39×10^{-7}	2.78×10^{17}
AB	1.35×10^{-3}	8.08×10^{-6}	46.7
AC	1.25×10^{-4}	3.97×10^{-6}	33.7
AD	3.29×10^{-4}	1.72×10^{-6}	15.4
ABD	1.20×10^{-4}	1.61×10^{-6}	12.7
ABC	2.69×10^{-4}	8.38×10^{-7}	25.8
ACD	1.59×10^{20}	4.91×10^{-7}	8.36×10^{18}
BCD	2.80×10^{-5}	1.78×10^{-5}	10.1
ABCD	2.17×10^{-5}	5.04×10^{-5}	12.5

Discussion

The sesquiterpene compounds represented by artemisinin are the most heavily researched compounds in *Artemisia annua*. At present, nearly 61 sesquiterpenoids, mainly artemisinin compounds, have been identified from *Artemisia annua*, including artemisinic acid, artemisinol, artemisinin ether and artemisinin. Artemisinin is a sesquiterpene lactone containing an endoperoxide bridge structure and is the main component of a number of antimalarial treatments. Research data showed that artemisinin and artemisinin can be converted to artemisinin in the original plants [17, 18]. The biosynthetic routes of artemisinin can be summarized as 9 total synthetic routes and 5 semi-synthetic routes. The synthesis method of artemisinin has a long process, high cost, and low total output, producing a maximum yield of 10%. Artemisinic acid is one of the main components of sesquiterpenes in *Artemisia annua* plants and an important precursor of artemisinin synthesis. Tu Yu's study showed that the young plants of *Artemisia annua* contained a large amount of artemisinic acid but exhibited a shortage of artemisinin. It is speculated that such sesquiterpenoids as artemisinin are converted from artemisinic acid. Levesque F and other researchers used synthetic biology to successfully produce artemisinin using genetically engineered yeast [19]. Scopolide has strong water solubility and stability to artemisinin and has pharmacological activity that reflects the efficacy of traditional artemisinin. Previous studies have shown

that scopolide has a certain antimalarial effect, and it has certain synergistic effects with artemisinin. With the rapid development of chemical genomes and pharmacological technologies, a large number of potential targets and massive biological activity data have emerged. However, with the accumulation of complex data, simple analysis methods can no longer satisfy the analytical needs of high-throughput and large-scale data [20]. The rapid development of chemical informatics has recently met the requirements of big data processing and information extraction tasks that urgently need to be solved in chemical genomics. Chemical informatics mainly studies how to properly select compounds from the diversity of compound libraries, how to describe drug molecular characteristics, how to measure the differences between different molecules, how to identify drug-like molecules, molecular structure and biological performance relationships, and how to develop corresponding computer software and hardware. This methodology includes the research tasks and content of chemometrics and computational chemistry [21]. An important application of the chemoinformatics method in the post-genomic era is predicting the potential targets of small-molecule compounds based on existing biological and chemical information and explaining their mechanism of action to accelerate the development of drugs. The prediction of drug targets is of considerable importance to the evaluation of early drug molecules and the new use of old drugs; however, due to the limitations of throughput, accuracy and cost, it is difficult to widely apply experimental methods. As a quick and low-cost method, the development of computer-aided target prediction algorithms is receiving increasing attention. According to different research strategies, the prediction of drug targets based on chemoinformatics can be divided into three categories: prediction based on ligand characteristics, prediction based on protein structural characteristics, and prediction based on data mining methods [22, 23]. According to the prediction of targets, the potential targets of scopolide are CDK4, NFKB1, PIK3CG, MAPK1, and TNF, and the potential target of artemisinic acid is ITGB2. Cyclin-dependent kinase (CDK) is a type of serine/threonine (Thr) kinase that is an important signal transduction molecule in cells, and CDK-cyclin is formed by the cyclin complex and is involved in cell growth, proliferation, dormancy, or apoptosis. During the cell cycle, cyclins are periodically and continuously expressed and degraded and are bound to CDKs and transiently activated by them. Through the activity of CDKs, cyclins catalyse the phosphorylation of different substrates to promote and transform different phases of the cell cycle. The CDK family includes CDK1-13, cyclins are divided into cyclins A-L, and different CDKs are connected to different cyclins. CDK4/6-specific activation is closely related to the proliferation of some tumours. Rb is present in approximately 80% of human tumours, and abnormalities in the cyclin D–CDK4/6–INK4–Rb pathway are common [24]. Cyclin-dependent kinase (CDK) is a type of serine/threonine (Thr) kinase that is an important signal transduction molecule in cells, and CDK-cyclin is formed by the cyclin complex and is involved in cell growth, proliferation, dormancy, or apoptosis. It is characterized by (1) p16^{INK4a} gene deletion, point mutation, or DNA methylation, leading to the inactivation of p16^{INK4a}; (2) CDK4 gene amplification or point-mutated T cells can induce other cells to activate or interfere with lysis. CD3, CD4, and CD8 cells are involved in T cell transcription of activation signals. Toll-like receptors (TLRs) play an important role in the identification of invading pathogenic microorganisms in early congenital immunity. These evolutionarily preserved receptors are homologous to the Drosophila Toll protein family in structure, and they recognize highly conserved structural motif (motif)-pathogen-associated molecular patterns

expressed only on pathogenic microorganism molecular patterns (PAMPs). TLRs are stimulated by PAMPs to initiate a signal cascade that includes several proteins, leading to the activation of the transcription factor NF- κ B, thereby inducing the secretion of pro-inflammatory cytokines and effector cytokines directly involved in the adaptive immune response. Integrin β 2 (CD18) is an important member of the integrin family of adhesion molecules. Integrin β 2 binds to different integrin subunits to form the leukocyte adhesion receptor group. Integrin β 2 is primarily expressed in white blood cells, and its ligands are TCAM, iC3b, and fibrinogen. The cytoplasmic region of integrin β 2 is linked to a variety of cytoskeletal proteins and is involved in signal transduction. The genetic defects of integrin β 2 lead to leukocyte adhesion deficiency syndrome. Integrin β 2 mainly exists in the natural killer cell-mediated cytotoxicity pathway. In this study, the biological macromolecule interaction instrument was used to verify the binding of the target protein TNF with scopolide, which had the highest score in the docking experiment. The binding effect was good. It was confirmed that scopolide could act on TNF and participate in its corresponding functions. It was confirmed that scorolactone had an antimalarial effect. However, at the same time, the binding rates of artemisinin, artemisinin B, artemisinic acid and TNF were very low, which may be observed because the three compounds do not act on TNF. This finding also showed that the mechanism of the antimalarial effect of artemisinin may be different. The combination of the four ingredients with TNF was very high, indicating that the four ingredients have a synergistic effect. The combined use of the four ingredients may reduce the amount of artemisinin and scopolamine used while achieving the same antimalarial effect. The characteristics of multicomponent, multitarget, and synergistic effects are common among traditional Chinese medicine preparations. Many experiments have indicated that TNF has a certain killing effect on Plasmodium. TNF must be assisted by a certain factor (or factors) in the body to exert its ability to damage Plasmodium, which means TNF is not a terminal effector that directly kills Plasmodium. At present, it is speculated that the immune-protective mechanism of TNF may have the following effects: (1) Enhancing the phagocytosis function of phagocytic cells: the study found that after neutrophils were treated with different doses of TNF for 30 min and incubated with *Plasmodium falciparum*, the phagocytosis of each stage of Plasmodium was strengthened, and the extent of the increase was positively correlated with the dose of TNF within a certain range. (2) Acting via reactive oxygen mediator: when TNF and macrophages were co-incubated for 30 min, the release of reactive oxygen species (ROS) from macrophages was detected, and ROS can kill Plasmodium.

Conclusion

Based on the existing literature, data mining methods were used to identify the targets of the antimalarial active ingredients artemisinin and scopolamine in *Artemisia annua*, including 20 proteins related to artemisinin's antimalarial actions (IL-6, ACHE, PC3, IPOB, CYC, TNF- α , UGT1A9, CASP3, XDH, IL-1 β , VEGF, CAT, CREB, AMPK, UGT1A6, ADR, MAPK, COX2, LB24AB, and CYP450) and 19 proteins related to the antimalarial properties of scopolamine (TNF- α , PI3K, IL-8, IL-6, VEGF, IL-1 β , MAPK, CD4, SP2, CTNNB, CASP3, PRO1400, IgE, IL-4, ICAM1, P38, STAT3, TLR4, API4), while no protein targets of artemisinic acid and arteannuin B were identified.

The combination of the four components of artemisinin, arteannuin B, artemisinic acid, and stigmalactone exhibits good binding to TNF.

Abbreviations

NHS

N-hydroxysuccinimide; DMSO:dimethyl sulfoxide; PPI:protein-protein interaction; KEGG:Kyoto Encyclopedia of Genes and Genomes; Thr:threonine; CDK:cyclin-dependent kinase; PAMP:pathogenic microorganism molecular pattern.

Declarations

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Authors' contributions

Z-yP, W-L, H-IQ integrating pharmacokinetics study, Z-xB, X-hY, network analysis, and experimental validation to research the antimalaria mechanism of Artemisinin, Scopoletin, Arteannuin B and Artemisic acid. Z-yP and H-IQ write the manuscript.

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Availability of data and materials

All relevant data are included in this report.

Ethics approval and consent to participate

There are no ethics statement in this paper.

Consent

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

The above candidate targets significantly participate in multiple immune-related pathways, such as Fc epsilon RI signaling pathway, T cell receptor signaling pathway, and Natural killer cell mediated cytotoxicity; and multiple infectious disease-related pathways, such as Hepatitis B (hepatitis B), Leishmaniasis (leish (Mann disease), Pertussis, Amoebiasis, Measles, Toxoplasmosis and Hepatitis C.

Figure 2

Establishment of an interaction network between *Artemisia annua* candidate target genes and known antimalarial drug target genes, consisting of 85 nodes and 298 pairs of interactions.

Figure 3

Molecular Docking by Discovery Studio. A Scopolide with CDK4; B Scopolide with NFKB1; C Scopolide with PIK3CG; D Scopolide with MAPK1; E Scopolide with TNF; F Artemisinin acid with ITGB2.

Figure 4

The dissociation constant(Kd) of each group. Grouping is the same as in Table 1.