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Systems pharmacology in drug discovery and therapeutic insight for herbal medicines

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Abstract

Systems pharmacology is an emerging field that integrates systems biology and pharmacology to advance the process of drug discovery, development and the understanding of therapeutic mechanisms. The aim of the present work is to highlight the role that the systems pharmacology plays across the traditional herbal medicines discipline, which is exemplified by a case study of botanical drugs applied in the treatment of depression. First, based on critically examined pharmacology and clinical knowledge, we propose a large-scale statistical analysis to evaluate the efficiency of herbs used in traditional medicines. Second, we focus on the exploration of the active ingredients and targets by carrying out complex structure-, omics- and network-based systematic investigations. Third, specific informatics methods are developed to infer drug–disease connections, with purpose to understand how drugs work on the specific targets and pathways. Finally, we propose a new systems pharmacology method, which is further applied to an integrated platform (Herbal medicine Systems Pharmacology) of blended herbal medicine and omics data sets, allowing for the systematization of current and traditional knowledge of herbal medicines and, importantly, for the application of this emerging body of knowledge to the development of new drugs for complex human diseases.

Keywords: *systems pharmacology; herbal medicines; ADME; network pharmacology; depression*

INTRODUCTION

Traditional herbal medicines are plant-derived natural products, which play an important role in health maintenance for the people of Asia, and are becoming more frequently used in the western countries [1]. As a gorgeous cradle of new active compounds in drug discovery, herbal medicines, an imperative group of natural products remedies delegating more multiplicity in structure, bioactivity and less toxicity [2], have attracted extensive attention worldwide [3]. Nowadays, there is a growing recognition in the west that single drug remedies are not enough to treat disease, and the concept of 'one disease—one target—one-size-fits-all' is shifting toward more comprehensive therapeutic strategies. Therefore, herbal medicines, featured as abundant bioactive ingredients and multiple targets, are considered more effective, particularly for the complex chronic

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diseases such as schizophrenia, bipolar disorder, diabetes, cardiovascular diseases, depression and so forth [4].

In modern medicine, chronic diseases are often treated with prolonged administration of chemical drugs, which might result in long-term toxicity or even resistance. Thus, the combinations of drugs is thought to be the most effective way of countering biological buffering, which allows for the reduced dosing of each agent while increased therapeutic selectivity as well [5]. Interestingly, herbal medicines might overcome the shortage of either the long-period toxicity or resistance in two ways: (i) being natural and therefore 'healthier' than the synthetic chemicals and (ii) containing combinations of bioactive compounds and thus providing synergistic effects. One famous example is herbal medicine St. John's wort, which has been widely used to treat mild-to-moderate depression in Europe and United States. Despite the attractiveness of herbal medicines, the clinical evidence that props up the use of most them is still limited, awaiting discovery of methods sufficient to increase the understanding of herbs.

Herbal concoctions are a complex system, which contains many active compounds that may also hit multiple biological targets involved in various pathogenesis. However, in most cases, we do not know what specific ingredients in a particular herb work to treat a disease, and the factors determining how effective the herb will be are still unclear. Thus, a question arises that is it possible to develop a method that could measure the whole body's response to a mixture of herbs? In addition, it is more difficult to translate ancient interpretations of diseases into those used in modern medicine, i.e. translate into modern biochemical and biological meanings to reduce the irreconcilable differences between traditional medicine and western science [6].

In this work, we have extensively reviewed current available *in silico* methods that are particularly associated with the discovery and development of herbal medicines. Based on the survey of systems pharmacology, bioinformatics and computational chemistry methods and models, from a systems perspective, we deciphered the molecular logic underlying the combinatorial/synergistic effects using multi-component herbs. Particularly, as an example, we depict the systems-level treating effects of antidepression herbal medicines by using the drug-target-disease mapping and the pharmacokinetic screening techniques. To the best of our knowledge, this is the first description of a comprehensive dissection of herb–disease connections using systems pharmacology methods. We believe that this strategy of gaining a functional/systems understanding of an herb medicine may serve as a model for further mode-of-action studies and novel drug development.

WHY ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION EVALUATION FOR HERBAL MEDICINES?

Absorption, distribution, metabolism and excretion (ADME) evaluations of drugs are critical procedures in drug discovery and development [7]. In the late 1990s, unfavorable pharmacokinetic properties were the primary causes of costly late-stage failures in drug development [8]. Consequently, it has become extensively appreciated that further efforts should be put into the area with the least delay possible [9].

To be specific, herbal medicine is a multifaceted system consisting of manifold components. However, only a few of them exhibit favorable ADME properties [10] with potential of a biological effect (Figure 1). The traditional process of drug development for herbs follows a separation, purification and structure elucidation way to identify discrete valid entities [11]. Although the past two decades have witnessed increasing application of various ADME studies in drug discovery [12], only ~30% of the most commonly used herbal medicines in the United States performed *in vitro* ADME evaluations [3]. Clearly, the large numbers of components in herbs make the screening and analysis of their bioactive components extremely challenging. Therefore, in the following part, we concentrate on the introduction of *in silico* ADME methods, featured as cost-effective and time-saving strategy, to herbal medicine studies for facilitation of the plant-origin drug discovery (Figure 1).

HOW TO PREDICT ADME PROPERTIES?

Approaches to depicting ADME properties count on experimental or *in silico* tools, used alone or in amalgamation [13]. Nevertheless, experimental tools including physicochemical methods and biological assays have two major drawbacks of higher

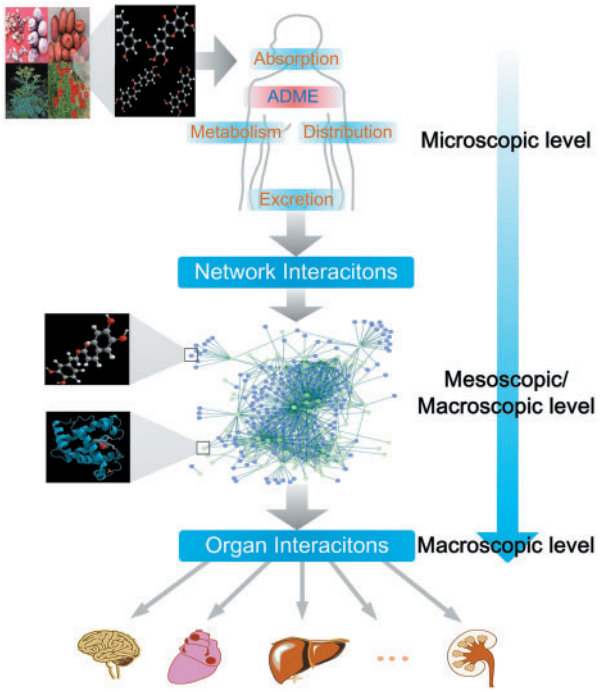


Figure 1: The process of herbal medicines interacts with body in molecular/network/organ levels. The global systems analysis frames a hierarchy of functional domains of macroscopic level, mesoscopic/macroscopic level and microscopic level. Molecules in herbs derived from ADME screening are interacted with proteins. These loci of interaction, termed connectivity nodes, interact with other nodes across the entire network. The molecule-induced stimulus perturbs organs, with the final result being detectable or measurable therapeutic effects.

throughput and shorter time for data turnaround [14–17]. Owing to this reason, theoretical approaches appear to be a good alternative to the prediction of ADME properties. With the use of *in silico* models, the global number of compounds to be synthesized and experimentally tested is boiled down to better suit to the capacity of subsequent analysis [17] and to advance the veracity and efficiency of the studies.

Absorption

Following oral administration, drugs are normally assimilated by passive diffusion, carrier-mediated uptake or active transportation through the lining of the stomach or intestinal epithelial cell before reaching the general circulation [13, 18, 19]. The currently obtainable computational models about absorption can be mainly classified into two categories—empirical and mechanistic [20]. Empirical models about logS, logP, $Caco_2$ passive permeability use statistical tools to explore the, either linear [13, 21] or nonlinear [22, 23], relationships between certain structural descriptors and the observed absorption properties [24]. Contrariwise, mechanistic models use the quantum/molecular mechanics methods to estimate the atomic interactions between micro-molecules and macromolecules and thus are more predictive due to a more expanded chemistry space, which yet has not been applied to the absorption prediction. In the future, the prime methodology, we assume, will be the one that uses both experimental and *in silico* methods in a complementary way to model the drug absorption process.

Distribution

Tissue distribution is a significant determinant of the pharmacokinetic profile of drugs [9], which principally comes down to blood–brain barrier (BBB), transporters and plasma protein binding. For an outline of the utmost prominent work about BBB penetration, the currently proposed models diverge considerably in terms of the methodological approaches ranging from the artless regression equations unfolding logBBB and the transporter properties [25] as a linear combination of selected physicochemical properties, to intricate models exploiting sophisticated and stylish statistical techniques and large pools of theoretical descriptors [26–29].

However, most of the models have several defects for the merely use of logBBB value, which is frequently fitted 'as is' disregarding the relationship with plasma protein binding as a sole factor [29], thus overlooking other determinants of the permeability process. Besides, the character of active transporters such as P-glycoprotein (P-gp) is underestimated and the intricate nature of BBB is deserted, which cooperatively give rise to misleading conclusions [30]. To disentangle the P-gp modulating activity of drugs, diverse theoretical methods by modeling of P-gp substrates, nonsubstrates and inhibitors have been created. These methods include logP, molecular weight, amphiphilicity and so forth, which were testified to dedicate smartly toward the interactions with P-gp [31–34].

Metabolism

Of various ADME endpoints, metabolism might be the most challenging one to evaluate and predict, as it is a complex biological process that encompasses a number of—often competing—mechanisms and

enzymatic systems [9]. Approaches to metabolism are conceivable to note an evolution from rule-based methods to recent structure-based models [35–38]. Primarily, rule-based approaches use data mining techniques to abstract generalized rules to determine the part of a molecule that undergoes metabolic alteration from a large database [39] but usually ending up with tiny veracity. To enhance the accuracy veracity of prediction, the ligand-based approaches subsequently emerged on the prediction stage [40, 41]. As the dimensions and composition of the data set are unset, the performances of these models are generally prejudiced. Ultimately, the protein-based methods using the 3D-structure information, which could be applied in molecular docking approaches, were further developed [42]. This is particularly for herbal chemicals whose structure-activity relationship studies have been successfully used to explore the interactions of naturally occurring compounds with cytochrome P450, such as flavonoids, piperine [43] and so forth.

Excretion

Drug excretion refers to its irreversible removal process from the body in a chemically altered or, sometimes, unbroken form, which normally occurs via three chief routes: in bile via the liver, in urine via the kidneys or in exhaled air via the lungs [44]. As these procedures are determined by a great many physicochemical and physiological factors such as the blood flow, protein binding and lipophilicity [9], the development of an integrated model for modeling excretion is actually challenging or even impossible. Therefore, this part is omitted in this review, as the technique is still in its initial stage.

As aforementioned, though having been integrated into modern drug development, generally speaking *in silico* ADME studies have not yet been put into herbal remedy discovery [3, 45]. Recently, we have developed a set of new ADME strategies for visualizing active ingredients and exploring the mechanisms of action of herbs [11, 46–48]. In the 'Case study' section, we will systematically introduce the procedures to carry out these computational ADME techniques on medicinal herb studies.

HOW TO PREDICT DRUG TARGETS?

With the explosion of biomedical data and information generated from a variety of innovative technologies, we are embracing an exciting omics drug discovery era. Clearly, in a systems level to search potential compound and target interactions, the 'dry' experiment (computational method) should be the first choice, owing to the shortages of the 'wet' experiment as time-consuming, expensive and also being limited in small scale [49].

Text mining

Text mining can be defined as 'the computational unearthing of newfangled, formerly unknown information, by automatically mining information from various written resources' [50]. This technique has grown into one of the most important stage of imminent drug discovery pipelines, which might be beneficial to select appropriate targets and better fathom the cellular mechanisms or phenotypes of human diseases. It has also been applied for identifying disease-associated entities (genes/proteins), disease-related networks [51, 52] and even the interactions of herbal active ingredients and the targets [53]. It goes without saying that text mining makes great contribution to spring biological entities and dig from an astronomically large number of exploration articles. However, owing to the term variation and term ambiguity of biomedical entities, the full text of article is often restricted to be access to limited information.

Chemogenomic method

Chemogenomic method has arisen as a newfangled discipline in target prediction, which drew a bead on exploiting the much larger chemical space [54]. Chemogenomic approach consists of the ligand-based, target-based and target-ligand methods, which have been blossomed out into revealing the novel relationship between compounds and targets [55–57]. The ligand-based chemometric approach is based on the motivating hypothesis that two similar molecules on the cards have analogous characters and will combine to the same group of proteins, such as the Similarity Ensemble Approach [58]; another representative example is the pharmacophore method [54]. Based on chemical feature matching and shape complementarity in binding site, target-based method adopts two strategies: docking and reverse (or inverse) docking [59–61]. Before these two approaches, the target-ligand approach is an intricacy forecast system, which incorporates the ligand chemical space, target space and the presently known drug-target connections information. It is capable

of predicting ligands or targets for a specified target or ligand without prior attempting to define a series of specific similar receptors or ligands [56]. Remarkably, the optimal models have shown impressive performance in prediction of drug-target associations for herbal medicines [11, 46, 62].

Database searching

In the wake of information explosion by multifarious groundbreaking technologies and the spring up of target data, we are fortunately on the brink of a stimulating era that plentiful of databases warehousing a variety of data are updating as time goes on. Graceful examples are Therapeutic Targets Database (TTD) [63] and DrugBank [64]. Such information has resulted in the integration of further resources and computational methods, such as herbal ingredient targets database [53], TcmSP^{TM} [65] and TCM Database@Taiwan [66], which have served as valuable platforms for analysis of targets and drug actions.

Owing to the inherent limitations and challenges of various approaches, we suggest that a combination of different approaches should be adopted to circumvent the drawbacks of a single method. In the 'Case study' section, we will give an example to illustrate how to combine all these different methods together to fulfill the task of a systematic identification of active ingredients, as well as the elucidation of action mechanisms for herbal medicines.

HOW INTERGRATE NETWORK ANALYSIS INTO PHARMACOLOGY?

Network pharmacology, the new paradigm in drug study, covers systems biology and pharmacology, which not only attempts to comprehend the role of networks for drug action in biological systems but also exploits the information to notify and guide drug development along with endeavors to tackle the two major sources of attrition in drug development—efficacy and toxicity [67, 68]. Using various scales network-based approaches to visualize and analyze dissimilar types of biologically pertinent interaction data has converted progressively more prevalent in recent years [69] and permits us to unequivocally trail drug actions from molecule-level interactions to organismal physiology [70]. Normally, the network-based approaches can be divided into two expansive spaces: static network and dynamic network.

Static network

In general, static network is superlative conceptualized, as the computerized reconstruction of molecular anatomy tells us the mutual effect of molecules, which gravitates toward wider and coarser in latitude. With regard to this network, bulky interaction data sets with thousands of nodes and edges can be visualized interactively rather than statically. Several methodologies are possible to assess the topographical properties based on key concepts variants of centrality and eigenvector centrality to describe and quantify the complex static network.

Variants of centrality in a network include degree, closeness and betweenness. Each has made a vital contribution in its own way: degree conveys us how much access a particular node has to the other nodes; closeness could appraisal the time required for information to propagate to a given node in a network by calculating the length of the path between them [71]; betweenness corresponds to the total number of nonredundant shortest paths going through a certain node or edge [72] and, therefore, indicates the reliance of a network on a specific node for sustaining connectedness. However, these topological features of the centrality variants do not take the importance of other nodes or the significance of all paths resemble eigenvector centrality into account, which integrates not only the number of a node's links and the strength of those ties but also the centrality of other nodes [73].

It has been a long-standing goal in systems pharmacology to find relations between the topological properties and functional features of herb-disease connections through static networks study [11, 73]. Although being not entirely comprehended, the proposed action mechanisms are suited for elucidating the disease therapies and guiding drug usage including especially the curative effects of a combination therapy.

Dynamic network

Dynamic network is a renovation of molecular physiology, a description of how the state of a system progresses as time goes on with emphasis on the particulars of a single subsystem [74, 75]. Compared with static network, which is less challenging from an experimental perspective, dynamic network obliges temporally, sometimes spatially resolved data or even more data. The description for dynamic network habitually entails of equation that labels the time dependence of every last of the

state variables of the system. Ordinary differential equation [76], partial differential equation [77] and stochastic simulation [78] are generally applied in dynamic network modeling and analysis. To date, a great deal of leverage can be gained in helping understanding the interlocking network behaviors, including sensitivity analysis, an established practice lying in evaluating a subset of parameters to simplify and better the redundancy parameters [79]; control analysis, a mechanism that systematically controls the state of the molecules (drugs, proteins, etc.) in a network [75]; bifurcation analysis, a process to trace time-varying change (s) for the state of a system in a multidimensional space where each dimension signifies a particular concentration of the biochemical factors involved [80].

Exploiting dynamic network to integrate diverse data sets has been deemed as a feasible track toward illuminating the origins of specific systemic diseases [81] like stroke and total cardiovascular disease [82]. Uncovering the origins of diseases not only can help us understand the diseases in genetic, pathway, protein and organ levels but also may provide better therapy of them. In parallel, past records have demonstrated that herbal medicines are ideal alternative medicines to western drugs for the cure of systemic diseases including in especial the chronic diseases [83]. Therefore, bridging network pharmacology and herbal medicines will faultlessly clarify the therapeutic mechanisms for herbal medicines, which, in turn, could aid in drug discovery. In the 'Case study' section, we will dissect the role that the networks techniques play across the traditional herbal medicines discipline, as exemplified by the anti-depression herbal medicines.

SYSTEMS PHARMACOLOGY PLATFORM FOR HERBAL MEDICINES

Based on the previous series of research, we propose a novel integrated Herbal medicine systems pharmacology (HmSP) platform for the purpose of investigating how herbs interact with the human body from a molecular level (gen, protein) to the organism level (Figure 2). The detailed process is as following: (i) performing a large-scale data mining and statistical analysis for effective herbs relevant to the disease of interest; (ii) chemical database building for the herbs; (iii) *in silico* ADME predicting to obtain potential active compounds; (iv) target fishing by a combinatorial approach integrating text mining, chemometric and chemogenomic methods; (v) generation of drug-target, drug-pathway and drug-disease networks; (vi) data processing, visualizing and association study of herbs–diseases–organisms. The key techniques in the HmSP platform have been successfully applied in our previous work to explore the mechanisms of action of herbal medicines in the treatment of cardiovascular diseases and virus diseases [46, 48, 62]. A complete application of this platform is provided in the following section, exemplified by the depression disease and its treatment by herbs.

CASE STUDY

Depression is a kind of bad mood that belongs to obsessive neurosis and is usually caused by many factors including the genetic, physical/chemical and psychological ones. This disease has become a great concern during more recent years, as 15% of the people in developed countries suffer from severe depression [84], of which ~15% of may even commit suicide [85]. Recently, depression is commonly treated with selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine, Citalopram and so forth. However, single-agent applications could not surmount the inherent characteristics of the disease systems, such as redundancy and multi-functionality, bringing about the trend of system-level intervention like using drug combinations [86]. Actually, depression has been clinically treated with specific herbs or herbal combinations for many years. In the following sections, we fully illustrate why and how the proposed HmSP helps us to dissect the mechanisms of those anti-depression herbal medicines.

Anti-depression herbs determining and compound database building

To obtain anti-depression herbs, PubMed and the clinical trial database (www.Clinicaltrials.gov) were investigated by a large-scale text mining with the keywords 'herbal medicine' and 'depression'. As a result, 105 reported anti-depressive herbs were collected. To lessen possible bias and further evaluate the relationships between the herbs and depression, a parameter, i.e. the ratio of the number of anti-depressive-herb-related articles/the number of herb-related articles is calculated. The hypergeometric distribution was applied to obtain the chance

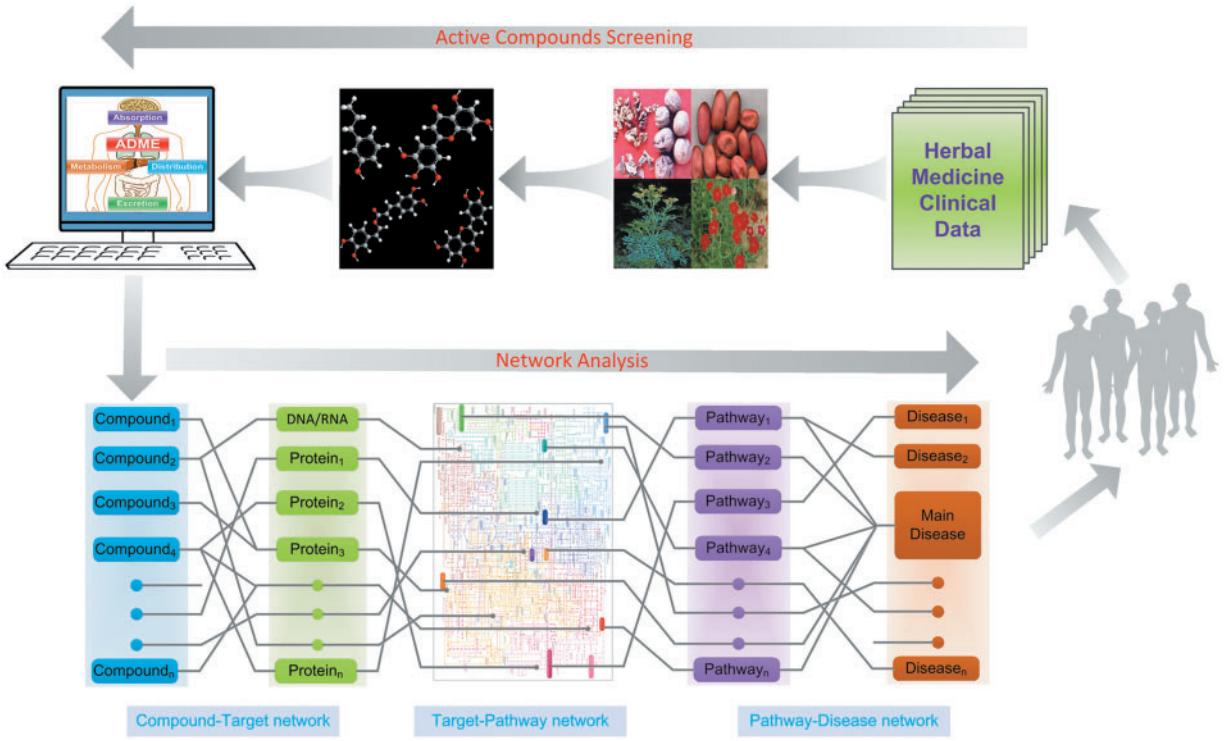


Figure 2: Workflow for systems pharmacology-based herbal pharmacology study. In the active compounds recursively screening process, chemicals for herbs relevant to certain diseases obtained by a large-scale data mining and statistical analysis were evaluated by *in silico* ADME screening to obtain potential active compounds. In the network analysis process, three levels network drug–target, drug–pathway and pathway–disease are generally generated to realize data processing and visualizing and announce associations of herbs–diseases–organisms. The biochemical pathway map is taken from http://www.genome.jp/kegg.

improbable of co-occurrences of each herb and depression to a certain level in at least k articles:

$$p = 1 - \sum_{i=1}^{k-1} f(i) = 1 - \sum_{i=0}^{k-1} \frac{\binom{K}{i}\binom{N-K}{n-i}}{\binom{N}{n}} \quad (1)$$

where N is the total number of articles in PubMed (22 188 039 articles, as given by GoPubMed, access time: October 9, 2012), K is the amount of literatures associated with depression (285 790 articles, as given by GoPubMed), n is the quantity about one single herb, k is the number of papers about the effects of corresponding herbs on depression. GoPubMed was used to get the value of N, K, n and k. P-value indicates the consequence of relevance between each herb and depression (significant when $P < 0.01$) [87].

The results show that 16 herbs were significantly correlated with the depression disease, among which *Cannabis sativa* and *Ginkgo biloba* are found to be the top well studied herbs ones (Table 1). *Hypericum perforatum* obtains the highest ratio (32.13%; $P \ll 0.01$), supporting the fact that *H. perforatum* is the sole herbal alternative to classic synthetic antidepressants in the treatment of mild to moderate depression [88]. And following are *Semen nelumbinis*, *Acorus tatarinowii*, *Albizia julibrissin*, *Radix Bupleuri*, *Passiflora perpera*, *Rhodiola rosea*, *C. sativa*, *Piper methysticum*, *Valeriana officinalis*, *Magnolia Officinalis* and so forth (Table 1).

Further, 1815 chemical components of these herbs were extracted from our database TcmSP™. As a chemically oriented herbal encyclopedia, TcmSP™ is a unique systems pharmacology platform of Chinese herbal medicines that includes >500 medicinal herbs and >30 000 chemical components and their potential targets.

ADME screening

Four most ADME-relevant parameters, i.e. the human oral bioavailability (OB), 'drug-likeness' (DL), the BBB and $Caco_2$ permeability were obtained for each compound of these herbs based on our previous work [10, 89–91], respectively. In our previous studies, the optimal OB predicting model was supported by a data set of 805 structurally diverse

drug (western drugs) with determination coefficient (R^2) of 0.80 and standard error of estimate (SEE) of 0.31 for test sets; the optimized BBB model is a qualitative model containing 190 related but chemically diverse compounds, which are either penetrating or non-penetrating cross the BBB; the $Caco_2$ permeability model was construed by 100 drug molecules, which showed satisfactory statistical results ($R^2 > 0.8$). To achieve more promising drugs, the filtering criteria were defined as follows: DL ≥ 0.18; BBB ≥ 0; OB $\geq 30\%$ or $Caco_2 \geq -0.4$ (with all corresponding data having been uploaded to TcmSPTM).

Table 1: Correlations between herbs with anti-depression

Herb name	Volume of articles	
	Total	Relevant to depression disease (Rate; *p*-value)
Hypericum perforatum (H. perforatum)	1718	552 (32.13%; $p \ll 0.01$)
Semen nelumbinis (S. nelumbinis)	23	5 (21.74%; $p \ll 0.01$)
Acorus tatarinowii (A. tatarinowii)	33	5 (15.155%; $p \ll 0.01$)
Radix bupleuri (R. bupleuri)	154	21 (13.64%; $p \ll 0.01$)
Albizia julibrissin (A. julibrissin)	43	5 (11.63%; $p \ll 0.01$)
Passiflora perpera (P. perpera)	109	12 (11.01%; $p \ll 0.01$)
Rhodiola rosea (R. rosea)	399	26 (6.52%; $p \ll 0.01$)
Cannabis sativa (C. sativa)	11506	740 (6.43%; $p \ll 0.01$)
Piper methysticum (P. methysticum)	662	42 (6.34%; $p \ll 0.01$)
Valeriana officinalis (V. officinalis)	733	39 (5.32%; $p \ll 0.01$)
Magnolia officinalis (M. officinalis)	267	12 (4.49%; $p \ll 0.01$)
Perilla frutescens (P. frutescens)	207	10 (4.49%; $p \ll 0.01$)
Paeonia lactiflora (P. lactiflora)	310	12 (3.87%; $p \ll 0.01$)
Lavandula pedunculata (L. pedunculata)	1315	42 (3.19%; $p \ll 0.01$)
Crocus sativus (C. sativus)	658	21 (3.19%; $p \ll 0.01$)
Ginkgo biloba (G. biloba)	3034	95 (3.13%; $p \ll 0.01$)

As shown in Figure 3, ~50% molecules (907/1815) are orally bioavailable, whereas just 25% (454/1815) provide drug-like characters. What's stirring is that larger than 80% compounds (1452/1815) can easily overcome the BBB and be readily absorbed by $Caco_2$ cell monolayers. Here, among the 273 compounds after ADME screening, 47 representative compounds including ADME favorable/literature-reported active agents were singled out and displayed in Table 2 with their structures and ADME parameters listed. As an illustration, three representative herbs were specified in detail to interpret this screening principle.

Hypericum perforatum

The predicted active ingredients in *H. perforatum*, which have favorable ADME features are hyperforin, kaempferol and rutin. Surprisingly, compounds hyperforin and kaempferol have been experimentally demonstrated to have noteworthy antidepressant activity [10, 92–94]. Besides, despite of showing substandard OB and DL properties, hypericin has also desirable neuro-activation property [95]. Analogously, rutin and amentoflavone are also not highly orally bioavailable but showing therapeutic effects [88, 96, 97] owing to the synergistic antidepressant effects [98].

Semen nelumbinis

As shown in Table 2, the leading ADME-favorable compounds in *S. nelumbinis* are prevailingly grouped

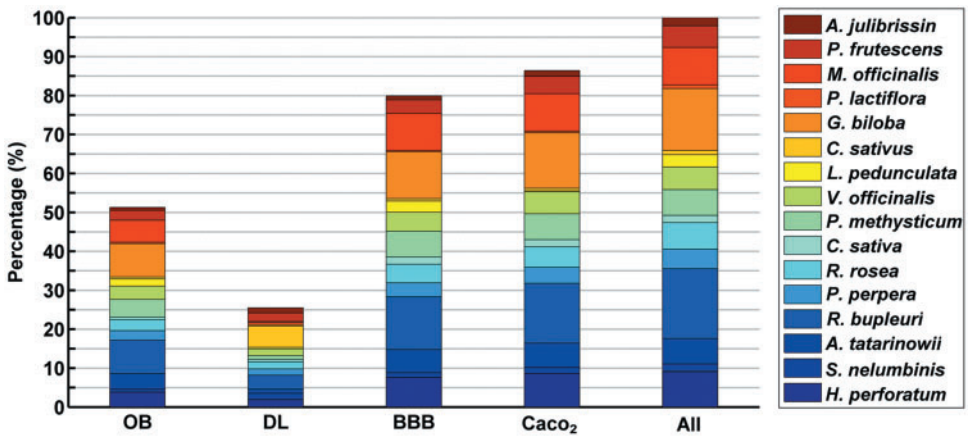
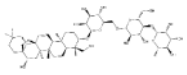
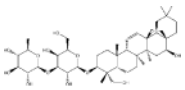
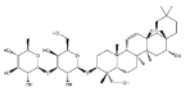
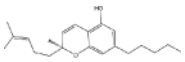
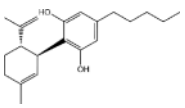
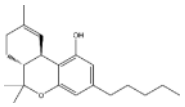
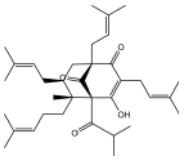
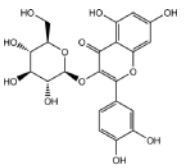
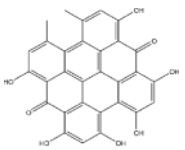
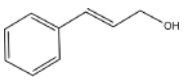
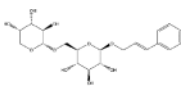
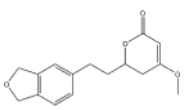
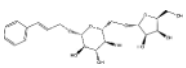


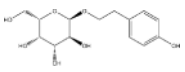
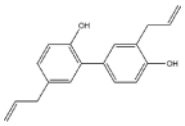
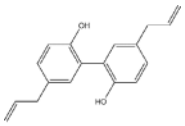
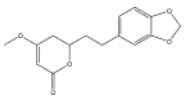
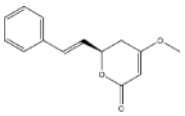
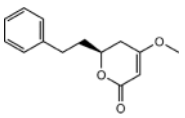
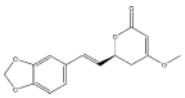
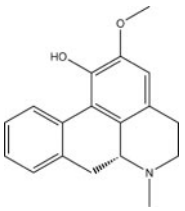
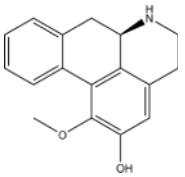
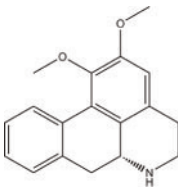
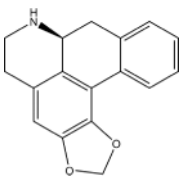
Figure 3: ADME screening. The meanings of the 16 colors are shown in the right of the figure. Characters in the abscissa are as follows: OB- oral bioavailability value (≥30%); DL- Drug-likeness (≥0.18); BBB- the BBB (≥0); $Caco_2$ (≥−0.4); All- the number of compositions of the 16 herbs. Ordinate reveals the percentage of compounds satisfy the qualifications above separately.

Table 2: Representative active compounds BBB score ≥0 or $Caco_2$ score ≥−0.4 represent that molecules can cross the BBB and be absorbed by $Caco_2$ cell line

Number	Compounds	Structure	OB	DL	BBB	$Caco_2$	Herb
M019	Saikosaponin c		54.22	0.63	0.01	0.29	*R. bupleuri*
M020	Saikosaponin a		25.06	0.63	0.01	0.04	*R. bupleuri*
M021	Saikosaponin d		16.78	0.63	0.01	0.17	*R. bupleuri*
M025	Cannabichromene		52.07	0.24	0.06	1.15	*C. sativa*
M027	Cannabidiol		3.97	0.21	0.06	1.38	*C. sativa*
M029	Tetrahydrocannabinol		13.39	0.32	0.05	1.45	*C. sativa*
M037	Hyperforin		44.03	0.60	0.03	0.77	*H. perforatum*
M038	Isoquercitrin		35.78	0.77	0.0 1	−1.47	*H. perforatum*
M039	Hypericin		14.52	0.08	0.00	0.28	*H. perforatum*
M042	Cinnamyl alcohol		44.42	0.02	0.12	−1.65	*R. rosea*
M046	Rosavin		48.85	0.58	0.12	0.86	*R. rosea*
M047	Rosin		48.85	0.58	0.12	0.86	*R. rosea*
M048	Rosarin		51.95	0.57	0.12	1.12	*R. rosea*

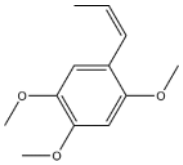
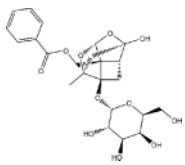
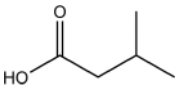
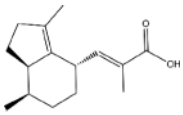
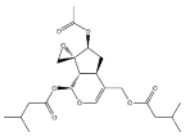
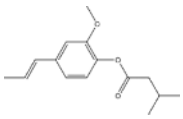
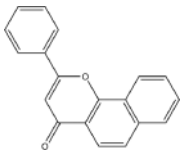
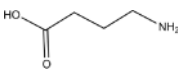
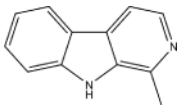
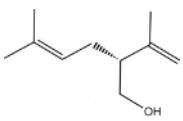
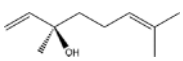
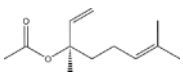
(continued)

Table 2: Continued

Number	Compounds	Structure	OB	DL	BBB	$Caco_2$	Herb
M049	Salidroside		26.83	0.20	0.05	0.66	*R. rosea*
M055	Honokiol		37.34	0.15	0.08	1.45	*M. officinalis*
M056	Magnolol		44.72	0.15	0.08	1.37	*M. officinalis*
M059	Dihydromethysticin		65.23	0.20	0.02	0.03	*P. methysticum*
M074	(+)-kawain		18.39	0.10	0.01	−0.49	*P. methysticum*
M075	Dihydrokavain		69.61	0.10	0.04	−1.71	*P. methysticum*
M078	Methysticin		10.51	0.21	0.06	0.81	*P. methysticum*
M080	Lirinidine		19.80	0.36	0.11	0.40	*S. nelumbinis*
M081	Asimilobine		11.41	0.33	0.05	−0.96	*S. nelumbinis*
M082	Nornuciferine		46.72	0.36	0.09	1.23	*S. nelumbinis*
M083	Anonaine		24.50	0.47	0.11	1.04	*S. nelumbinis*

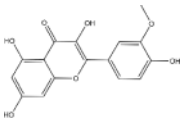
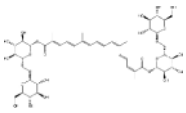
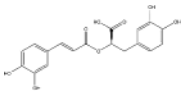
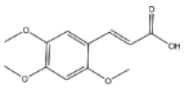
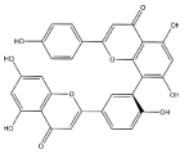
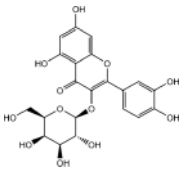
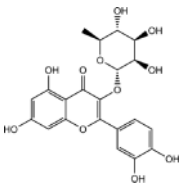
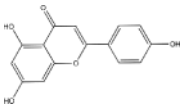
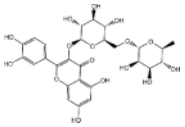
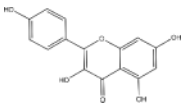
(continued)

Table 2: Continued

Number	Compounds	Structure	OB	DL	BBB	$Caco_2$	Herb
MI04	Betaasarone		16.79	0.06	0.06	1.45	*A. tatarinowii*
MI07	Paeoniflorin		10.99	0.79	−0.12	−1.74	*P. lactiflora*
MI08	Isovaleric acid		36.76	0.01	0.07	0.98	*V. officinalis*
MI09	Valerenic acid		43.64	0.10	0.10	1.51	*V. officinalis*
MI10	Didrovaltrate		100.00	0.50	0.04	1.12	*V. officinalis*
MI22	Isoeugenyl-isovalerate		54.00	0.09	0.05	1.17	*V. officinalis*
MI28	Benzoflavone		60.31	0.32	0.09	0.64	*P. perpera*
MI29	Gaba		91.95	0.01	0.04	−0.24	*P. perpera*
MI37	Harmane		38.58	0.10	0.11	1.52	*P. perpera*
MI42	Lavandulol		50.40	0.02	0.07	1.24	*L. pedunculata*
MI43	Linalool		43.59	0.02	0.06	1.29	*L. pedunculata*
MI44	Linalyl acetate		24.42	0.04	0.07	1.43	*L. pedunculata*

(continued)

Table 2: Continued

Number	Compounds	Structure	OB	DL	BBB	$Caco_2$	Herb
MI67	Isorhamnetin		11.62	0.31	−0.03	0.28	*G. biloba*
MI74	Crocetin		36.95	0.26	0.00	0.50	*C. sativus*
MI77	Crocin		33.44	0.26	0.00	0.59	*C. sativus*
MI78	Rosmarinic acid		48.60	0.46	−0.12	0.82	*P. frutescens*
MI79	2,4,5-trimethoxycinnamic acid		15.17	0.09	0.01	0.82	*P. frutescens*
M201	Amentoflavone		2.79	0.65	0.01	−0.25	*H. perforatum; G. biloba*
M204	Hyperoside		35.50	0.28	0.00	0.028	*R. bupleuri*, *H. perforatum*, *S. nelumbinis*
M206	Quercitrin		46.90	0.74	0.00	0.04	*H. perforatum*, *A. julibrissin*, *G. biloba*
M212	Apigenin		45.09	0.21	0.02	0.41	*P. frutescens*, *P. perpera*, *G. biloba*, *V. officinalis*
M216	Rutin		47.46	0.28	0.00	0.05	*R. bupleuri*, *H. perforatum*, *R. rosea*, *S. nelumbinis*, *G. biloba*
M217	Kaempferol		67.43	0.24	0.02	0.15	*R. bupleuri*, *H. perforatum*, *A. julibrissin*, *R. rosea*, *V. officinalis*, *P. perpera*, *G. biloba*

into flavonol glycosides and various alkaloids. The former consists of isoquercitrin and hyperoside, whereas anonaine, asimilobine, lirinidine and nornuciferine belong to alkaloids. Of these components, conformably to animal models of depression-like symptom [99], the flavonol glycosides display powerful antidepressant effect. Analogously, although with relatively low OB values, these alkaloids also possess antidepressant activity appraised by neurotransmitter reuptake inhibition bioassay [100, 101]. In addition, the component comparison shows that isoquercitrin and hyperoside are the same active ingredients of *H. perforatum* and *S. nelumbinis*, explaining why the two herbs share similar pharmacological activities.

Rhodiola rosea

Based on ADME analysis, the potential active substances of *R. rosea* are phenylethanol derivatives (rhodioloside b and rhodioloside c) and phenylpropanoid glycosides such as rosin, rosavin and rosarin, which is consistent with the previous findings from the research on component analysis [102]. Chemicals rhodioloside b and rhodioloside c are active principals, whereas the rosavins (rosin, rosavin and rosarin) are low in activity, but the drug efficiency can be increased by applying individual components together [103]. These compounds have been applied in the pharmacological treatment of depression [104] through inhibiting monoamine oxidases A to regulate the degradation of biogenic amines [105].

The hit rate of ADME screening to obtain underlying active compounds still remains an ongoing focus in drug discovery efforts. However, the present work indicates that the integration of various requisite ADME screening tools in a single operating is effective to find the compounds with potential pharmacological activities. In the following part, we will minutely interpret the functions of these potential active ingredients in the context of networks by the pivot-target that bridging them.

Target fishing

The target fishing was then performed using a combinatorial approach integrating text mining, chemometric and chemogenomic methods. First, a text mining for all target proteins was carried out in herbal ingredient targets database (http://lifecenter.sgst.cn/hit/). Second, the virtual chemical fingerprint Similarity Ensemble Approach method was applied for target prediction (http://sea.bkslab.org/); third, the omics-based ligand-target chemogenomic model (LTC) developed by Yu *et al.* with a concordance of 82.83%, a sensitivity of 81.33% and a specificity of 93.62% was further introduced for validation with the results obtained earlier in the text [56]; fourth, mapping all the obtained targets to database UniProt (http://www.uniprot.org/) for normalization [106]; finally, the systematically evaluated target proteins were further subjected to PharmGkb [107], TTD and the Comparative Toxicogenomics Database [108] databases to delete noise and errors and to allow a more complete and greater accuracy view on the drug-target associations. As summarized in Table 3, 67 targets that relate to depression disease were finally obtained.

To unfold the relationships between these herbal targets and the depression or other diseases or known antidepressant targets, the radar chart analysis was performed as follows: First, the 36 known antidepressant targets was collected from database TTD; then, mapping herb and all known targets to databases PharmGKB, TTD and Comparative Toxicogenomics Database to build the connections with diseases. Finally, all the information was sent to Medical Subject Headings (http://www.nlm.nih.gov) for further identification of disease categories.

As shown in Figure 4, the western drugs share 15 common targets (marked in bold in Table 3) with the herbs, indicating the multi-target feature of herbal medicines. And the tendency of herbal target-disease relationship is strongly in line with those known antidepressant targets, which demonstrates that herbal medicine has similar therapeutic effects as compared with the western drugs. However, these depression-related targets are not only related to C10 (Nervous System Diseases) but also connected with other diseases such as C06, C08 and so forth. Therefore, it is suggested that in treatment of depression disease, attention should be paid to possible side effects caused by the herbs interacting with the overlapping targets of depression and other diseases [109–112].

Network construction and analysis

To decipher the action mechanism of herbal medicines and discover the most potential 'follow-on' drugs, with the bridge connection of targets, we generate two levels of networks: Compound-Target network (C-T network) and Target-Pathway network (T-P network).

Table 3: The information of depression-related targets of herbs

Number	UniProt	Protein name (gene names)	Organisms
P01	O75469	Nuclear receptor subfamily 1 group I member 2 (NR1I2)	*Homo sapiens*
P02	P00441	Superoxide dismutase [Cu-Zn] (SOD1)	*Homo sapiens*
P03	P01189	Pro-opiomelanocortin (POMC)	*Homo sapiens*
P04	P02768	Serum albumin (ALB)	*Homo sapiens*
P05	P04150	Glucocorticoid receptor (NR3C1)	*Homo sapiens*
P06	P05121	Plasminogen activator inhibitor 1 (SERPINE1)	*Homo sapiens*
P07	P05177	Cytochrome P450 1A2 (CYP1A2)	*Homo sapiens*
P08	P05231	Interleukin-6 (IL6)	*Homo sapiens*
P09	P06850	Corticoliberin (CRH)	*Homo sapiens*
P10	P07550	Beta-2 adrenergic receptor (ADRB2)	*Homo sapiens*
P11	P08183	Multidrug resistance protein 1 (ABCB1)	*Homo sapiens*
P12	P08219	Gamma-aminobutyric acid receptor subunit alpha-1 (GABRA1)	*Bos taurus*
P13	P08588	Beta-1 adrenergic receptor (ADRB1)	*Homo sapiens*
P14	**P08908**	**5-hydroxytryptamine receptor 1A (HTR1A)**	***Homo sapiens***
P15	P09038	Fibroblast growth factor 2 (FGF2)	*Homo sapiens*
P16	P14174	Macrophage migration inhibitory factor (MIF)	*Homo sapiens*
P17	**P14416**	**D(2) dopamine receptor (DRD2)**	***Homo sapiens***
P18	P14600	Substance-P receptor (Tacr1)	*Rattus norvegicus*
P19	P14842	5-hydroxytryptamine receptor 2A (Htr2a)	*Rattus norvegicus*
P20	**P14867**	**Gamma-aminobutyric acid receptor subunit alpha-1 (GABRA1)**	***Homo sapiens***
P21	**P14902**	**Indoleamine 2,3-dioxygenase 1 (IDO1)**	***Homo sapiens***
P22	P16220	Cyclic AMP-responsive element-binding protein 1 (CREB1)	*Homo sapiens*
P23	P18090	Beta-1 adrenergic receptor (Adrb1)	*Rattus norvegicus*
P24	P19327	5-hydroxytryptamine receptor 1A (Htr1a)	*Rattus norvegicus*
P25	P19643	Amine oxidase [flavin-containing] B (Maob)	*Rattus norvegicus*
P26	P20288	D(2) dopamine receptor (DRD2)	*Bos taurus*
P27	P20366	Protachykinin-1 (TAC1)	*Homo sapiens*
P28	P21396	Amine oxidase [flavin-containing] A (Maoa)	*Rattus norvegicus*
P29	**P21397**	**Amine oxidase [flavin-containing] A (MAOA)**	***Homo sapiens***
P30	P21398	Amine oxidase [flavin-containing] A (MAOA)	*Bos taurus*
P31	**P21918**	**D(1B) dopamine receptor (DRD5)**	***Homo sapiens***
P32	P21964	Catechol O-methyltransferase (COMT)	*Homo sapiens*
P33	P23415	Glycine receptor subunit alpha-1 (GLRA1)	*Homo sapiens*
P34	P23560	Brain-derived neurotrophic factor (BDNF)	*Homo sapiens*
P35	**P23975**	**Sodium-dependent noradrenaline transporter (SLC6A2)**	***Homo sapiens***
P36	**P25103**	**Substance-P receptor (TACR1)**	***Homo sapiens***
P37	P27169	Serum paraoxonase/arylesterase 1 (PON1)	*Homo sapiens*
P38	**P27338**	**Amine oxidase [flavin-containing] B (MAOB)**	***Homo sapiens***
P39	P28222	5-hydroxytryptamine receptor 1B (HTR1B)	*Homo sapiens*
P40	**P28223**	**5-hydroxytryptamine receptor 2A (HTR2A)**	***Homo sapiens***
P41	**P28335**	**5-hydroxytryptamine receptor 2C (HTR2C)**	***Homo sapiens***
P42	P28564	5-hydroxytryptamine receptor 1B (Htr1b)	*Rattus norvegicus*
P43	P28647	Adenosine receptor A3 (Adora3)	*Rattus norvegicus*
P44	P29274	Adenosine receptor A2a (ADORA2A)	*Homo sapiens*
P45	P29475	Nitric oxide synthase, brain (NOS1)	*Homo sapiens*
P46	P30543	Adenosine receptor A2a (Adora2a)	*Rattus norvegicus*
P47	**P31645**	**Sodium-dependent serotonin transporter (SLC6A4)**	***Homo sapiens***
P48	P31652	Sodium-dependent serotonin transporter (Slc6a4)	*Rattus norvegicus*
P49	P33765	Adenosine receptor A3 (ADORA3)	*Homo sapiens*
P50	P34972	Cannabinoid receptor 2 (CNR2)	*Homo sapiens*
P51	**P34998**	**Corticotropin-releasing factor receptor 1 (CRHR1)**	***Homo sapiens***
P52	P35354	Prostaglandin G/H synthase 2 (PTGS2)	*Homo sapiens*
P53	P35363	5-hydroxytryptamine receptor 2A (Htr2a)	*Mus musculus*
P54	**P41595**	**5-hydroxytryptamine receptor 2B (HTR2B)**	***Homo sapiens***
P55	P42261	Glutamate receptor 1 (GRIA1)	*Homo sapiens*
P56	**P48039**	**Melatonin receptor type 1A (MTNR1A)**	***Homo sapiens***
P57	P48974	Vasopressin V1b receptor (Avpr1b)	*Rattus norvegicus*
P58	P49840	Glycogen synthase kinase-3 alpha (GSK3A)	*Homo sapiens*
P59	P49841	Glycogen synthase kinase-3 beta (GSK3B)	*Homo sapiens*
P60	P54833	Beta-2 adrenergic receptor (ADRB2)	*Canis familiaris*
P61	P61169	D(2) dopamine receptor (Drd2)	*Rattus norvegicus*
P62	P62812	Gamma-aminobutyric acid receptor subunit alpha-1 (Gabra1)	*Mus musculus*
P63	P62813	Gamma-aminobutyric acid receptor subunit alpha-1 (Gabra1)	*Rattus norvegicus*
P64	P79208	Prostaglandin G/H synthase 2 (PTGS2)	*Ovis aries*
P65	Q01727	Melanocyte-stimulating hormone receptor (Mc1r)	*Mus musculus*
P66	Q01812	Glutamate receptor, ionotropic kainate 4 (Grik4)	*Rattus norvegicus*
P67	Q04760	Lactoylglutathione lyase (GLO1)	*Homo sapiens*

Note: The targets marked in bold are shared by herbs and western drugs

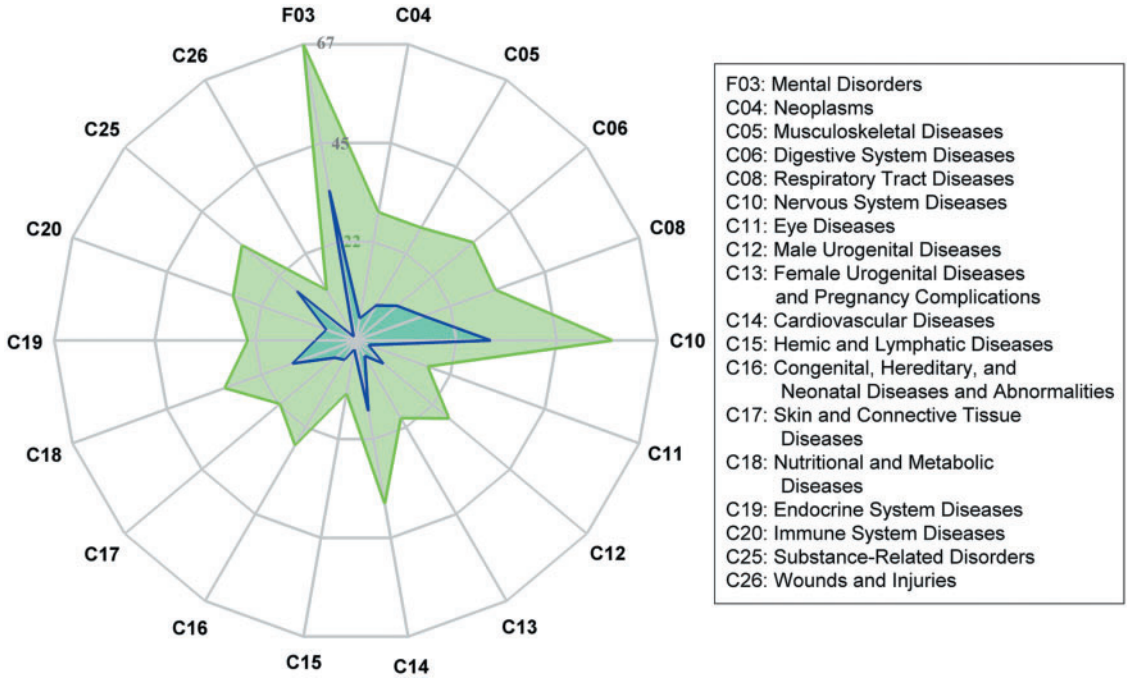


Figure 4: The equi-angular spokes radar chart. Each spoke characterize one of the diseases. The length of a spoke is distributed pro data, which is proportional to the quantity of target proteins relative to the homologous disease. A green streak for herbal medicine antidepressant targets, whereas a blue brim for western drug antidepressant targets are plotted to connect the data values for each spoke, which stretch the chart radar-like facades.

C-T network

The bipartite C-T network graph (Figure 5) was constructed for the 218 (Supplementary Table S1) of 273 compounds after ADME screening by connecting to the 67 potential targets through 1007 interactions. To visualize it, network analysis was used by evaluating the degree and betweenness of the nodes, resulting in an average degree per compound of 4.62 and 15.03 per target, respectively. Fascinatingly, a large number, ~79%, of represent active compounds are of higher (larger) than the average degrees (betweenness), which are considered to be clinically valid and labeled as 'follow-on' drugs [113].

Among the 'follow-on' drugs, M094 (4'-methyl-N-methylcoclaurine) in *S. nelumbinis* exhibits the largest number of interactions with various targets. Some compounds that are not intensively connected still have significant pharmacological activities. For example, compound M039 (betweenness = 0; degree = 1) is found to bind to protein P51 (betweenness = 0; degree = 1), a typical target for the treatment of depression. This compound is identified as remarkable antidepressant molecule in herb *H. perforatum*, which has attracted an upward devotion of pharmaceutical industry [114]. More interestingly, although the topology property of the net does not bias toward the rosavins (name: rosin, rosavin and rosarin), these rosavins all found to bind to the same Food and Drug Administration (FDA)-approved antidepressant target P54 (betweenness = 0.22; degree = 7), indicating potential synergistic mechanism in this herbal mixture for treating the disease. Finally, 34 targets from the C-T network were further demonstrated closely related to depression in the T-P network, which also contains the controversial target P52 (betweenness = 0.09; degree = 132) and so forth [115–118].

T-P network

To reflect a global view of the interactions between targets and depression therapy-associated pathways (Figure 6), the obtained 34 targets were further mapped onto 104 pathways, which show an average degree of 6.4 per target and 2.1 per pathway, respectively. The results show that most pathways are involved in a small number of targets, whereas about one-fourth of the targets locate in multiple pathways (≥8), which could be the key targets for depression treatment. To further mirror the target-pathway interactions, we applied a target-based approach to probe the pathways possibly involved in the therapeutic actions.

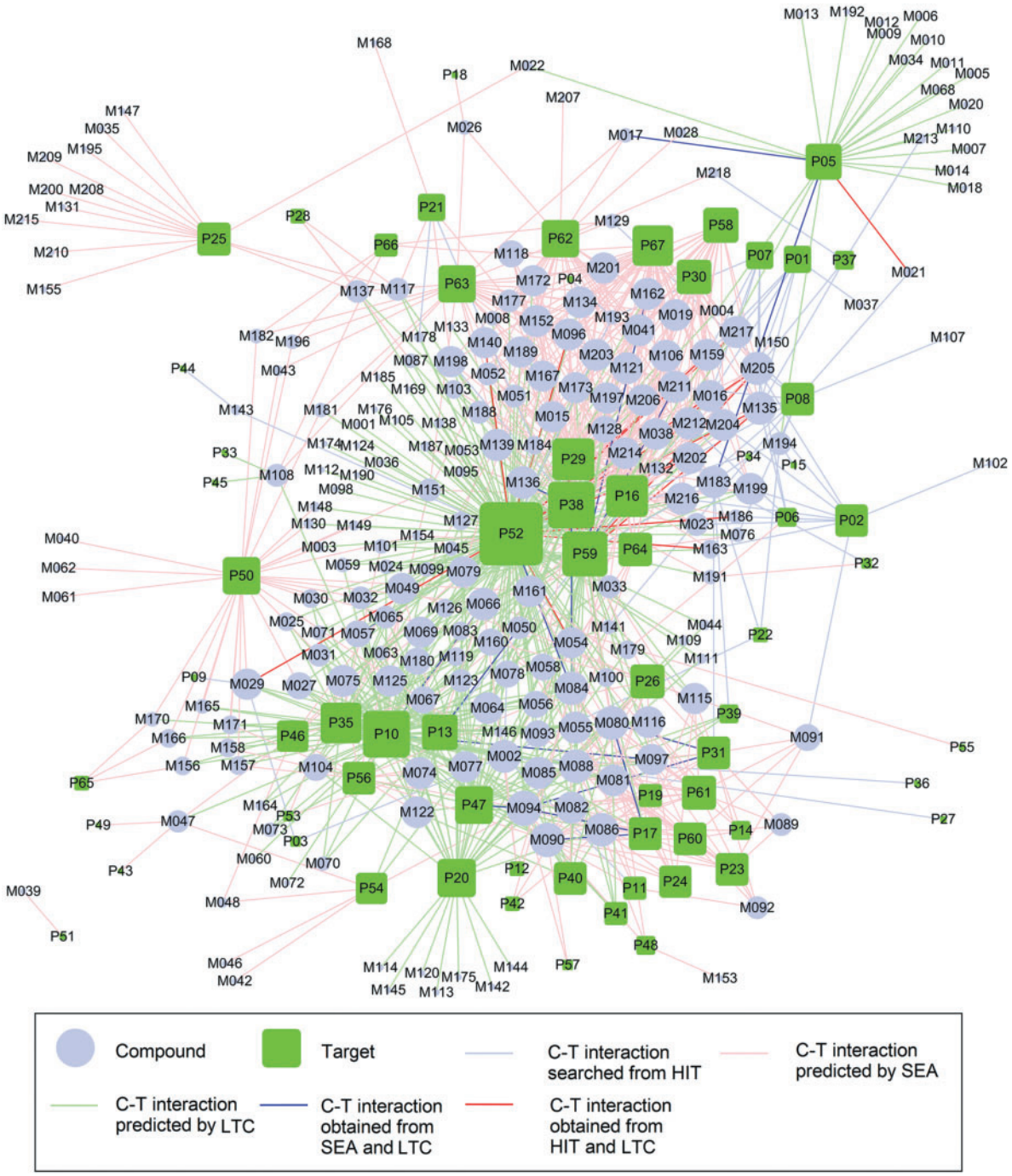


Figure 5: The C-T network. A compound node and a target protein node are linked if the protein is targeted by the corresponding compound. Node size is proportional to its degree.

Those pathways intensively connected to targets, such as neuroactive ligand-receptor interaction (degree = 15), calcium signaling pathway (degree = 9) and dopaminergic synapse (degree = 9), could be the key pathways that drugs engender their antidepressant effects. Actually, these pathways have already been testified and widely used for the depression therapies [119–121]. For instance, the pathway neuroactive ligand-receptor interaction with highest degrees could exploit the neurotransmitters glutamate, dopamine, serotonin, noradrenaline as its appetizers to adjust certain crucial pathways including of Long-term potentiation, Long-term depression and synthesis of Gap junction to cope with emotions and solace stress.

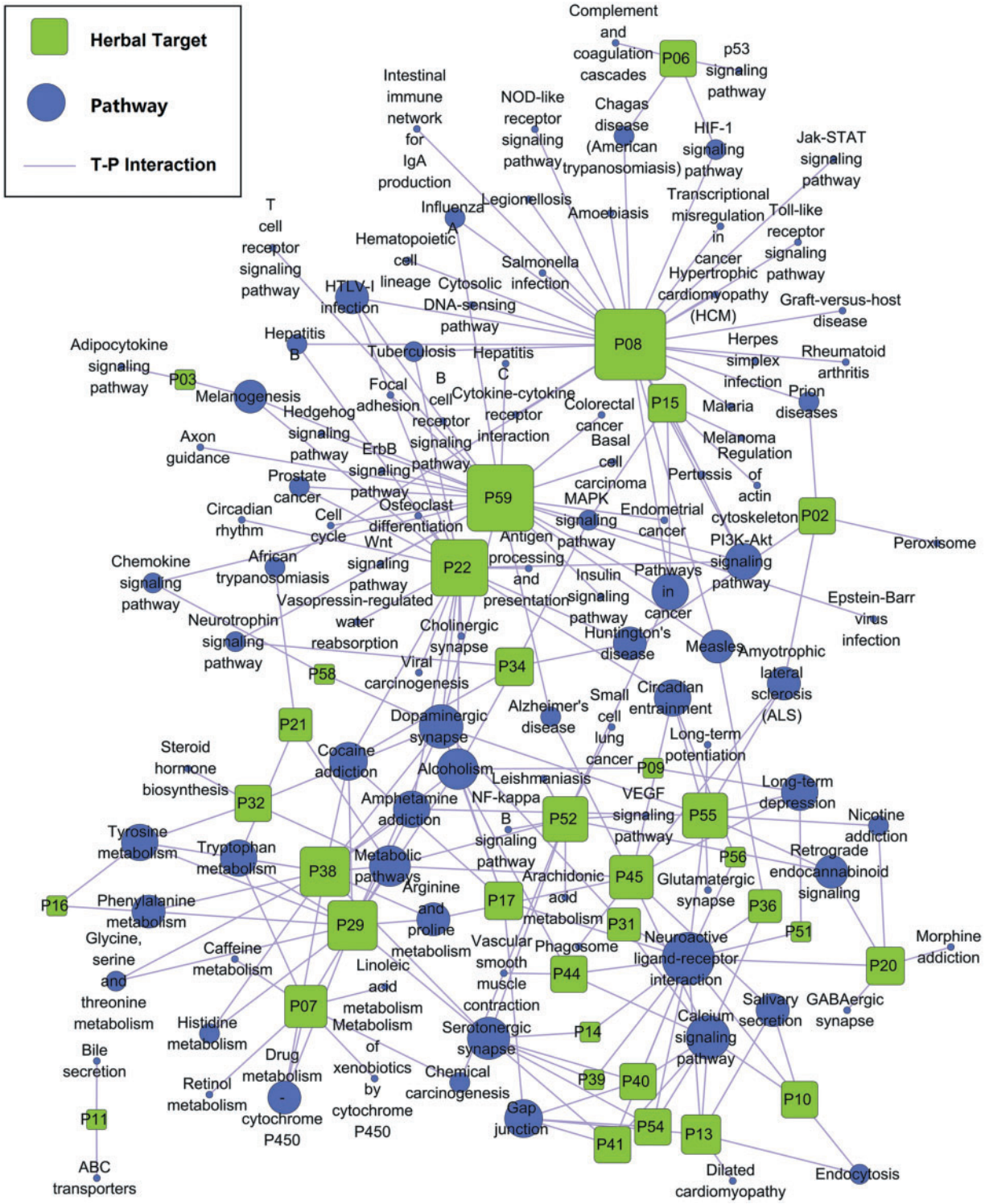


Figure 6: The T-P network. A link is placed between a target and a pathway if the pathway is lighted at the target. The area of the protein (pathway) node is proportional to the number of pathways that the target involves (the number of targets that the pathway has). The information of pathways is obtained by mapping the target proteins to the KEGG pathway database.

In addition to the highly connected pathways, some poorly onnected examples also show ideal pharmacological functions. For example, the synaptic plasticity impairment of pathway glutamatergic synapse is also therapeutically relevant to the depression [122]. This pathway is only intervened by target P55 (Glutamate receptor ionotropic, AMPA 1), existing in the neuronal membrane even before the synaptogenesis [123]. Protein P55 could be blocked by the endogenous intracellular polyamines [124], indicating that calcium-permeable receptor channels the glutamatergic synapse activity.

Depression pathway

To better recognize the integral adjustment of the antidepressant herbal medicines, an incorporated 'Depression Pathway' (Figure 7) was assembled based on the 'Basic Depression Pathway' from current knowledge of depression pathology, including pathway SSRI, selective norepinephrine (NE) reuptake inhibitor pathway, wingless-type MMTV integration site family (Wnt) signaling pathway, brain-derived neurotrophic factor (BDNF)/TrkB signaling pathway and Ca^{2+} signaling pathway [125–129]. First, the human protein–protein interaction (PPI) data from Biomolecular Interaction Network Database (BIND), Biological General Repository for Interaction Datasets (BioGRID), Database of Interacting Proteins (DIP), Human Protein Reference Database (HPRD), IntAct, Molecular INTeraction database (MINT), Mammalian Protein-Protein Interaction Database, Protein–Protein Interaction Database for PDZ-domains (PDZBase) and Reactome databases [130–138] were collected to build a comprehensive background network; then, proteins in the 'Basic Depression Pathway' were mapped to the PPIs as baits to tempt their direct partners to the extent that more herbal targets are involved in; finally, intimate proteins were gathered together on the basis of contemporary knowledge of depression pathology to clearly show the mode of action that pictures the pathway [139].

To search the relativity of the herbal targets for the 'Depression Pathway' at a higher level of organization, we delimit the nearness between herbal medicine targets p and 'Basic Depression Pathway' related proteins p' based on the PPI network by the expression:

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

where p_i represents the herbal medicine target, p_j' is the 'Basic Depression Pathway' related protein, whereas D_{pipj}' is the shortest distance between p_i and p_j' on the PPI network. n and m, respectively, represents the number of herbal medicine target p and depression pathway related protein p', which can be mapped on the PPI network (n: 44, m: 85). If two proteins are unconnected on the PPI network, the D_{pipj}' is defined as∞.

Based on the formula, the ultimate nearness between the two categories proteins is 0.0117. Statistical significance test comes before further consideration of the results with 44 randomly proteins chosen from the PPI network as a rigorous control rather than the 44 herbal targets and 85 'Depression-related proteins' fixed on the background network. The obtained nearness of each time is generally similar of the 10 000 times of randomization. For the purpose of statistical evaluation between the actual distance and those of random counterpart, the commonly used Z test is applied, and the significance is defined as the $P < 0.01$. Matching with the randomly selected 44 proteins (nearness = 0.0022), the 44 herbal targets display extremely significantly ($P \ll 0.01$) close functional linkage correlation (ultimate nearness = 0.0117) to the 85 depression pathway related proteins. Of the 67 target proteins, 43 can be plotted on the pathways, and the intracellular signaling cascades that underlie the depression and treatment response can be organized as explained in the following sections.

Direct interaction

As shown in Figure 7, 13 proteins (pink rectangles) located form upstream to downstream in sequence on the depression pathway can be targeted directly by herbal ingredients. This result potentially states that the herbs cure the depression disease through direct regulation of a set of target proteins on the pathway.

Two NE receptor proteins P13, P10 and the NE reuptake protein P35 are all located at the upstream of selective NE reuptake inhibitor pathway. The NE signaling through G-protein receptors results in the activation of Akt by disruption of PP2A. Phosphorylated Akt can phosphorylate the N-terminal serine of GSK3B, leading to the inhibition of GSK3B activity. GSK3B is a major downstream target for psychiatric illness, suggesting that the binders, such as M183 in *Perilla frutescens* bound to P32

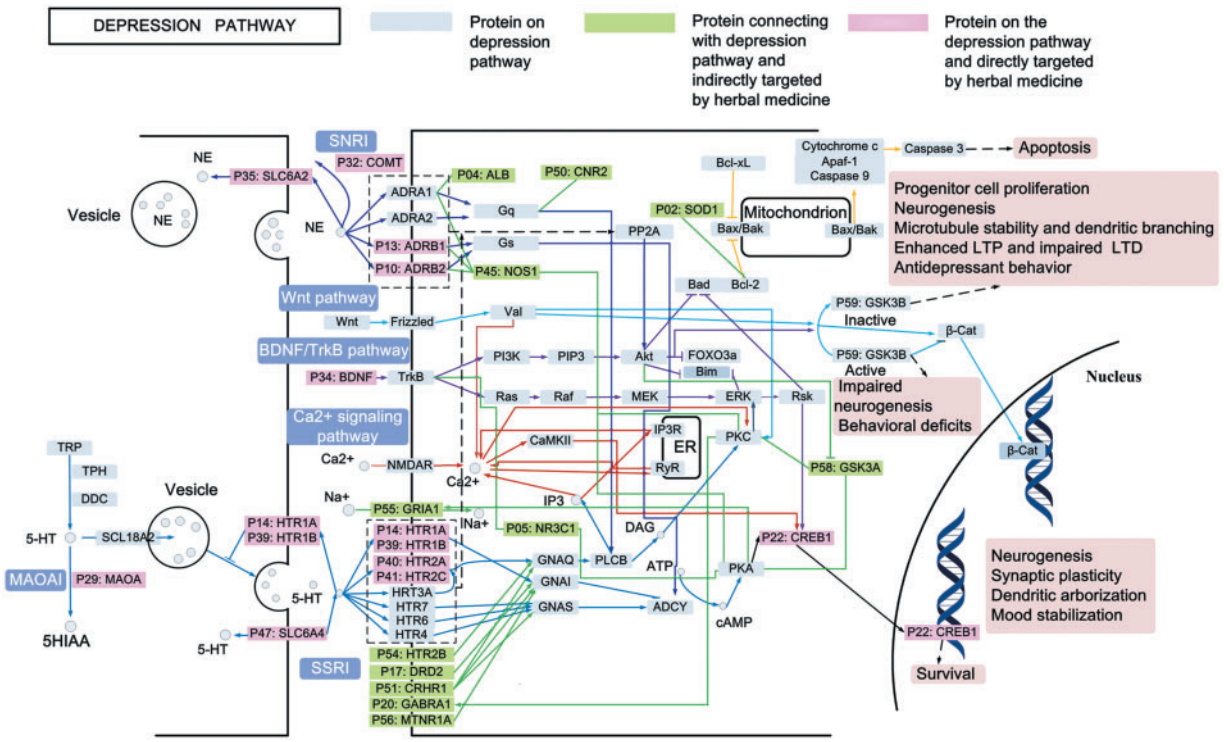


Figure 7: Distribution of target proteins of herbs on the compressed 'depression pathway'. Six pathways (lightsky-blue) form the compressed depression pathway. Pathways are marked in different colors. Arrows indicate activation, T-arrows indicated inhibition and segments indicate actions that can either be activatory or inhibitory on the specific targets. Generally, in the late phase of signaling, two waves of signaling are mediated by GSK3B and CREBI and lead to several molecular, cellular and behavioral deficits, as summarized in the boxes at the right.

and M030 in *C. sativa* to P35, might facilitate the binding of NE, thus exhibiting expected antidepressant effects.

Also, the disturbance of SSRI pathway is a serviceable treatment option for patients with depression. M002 in *R. Bupleuri* may inhibit presynaptic receptor P47, resulting in an increasing of the 5-HT concentration in the synaptic cleft, thus negatively regulating the desensitization of postsynaptic receptors P40, P41, P14 and P39 in the pathway. After the interaction with 5-HT, the main signaling linkage for the P40 and P41 receptors will activate phospholipase C, beta (PLCB) through coupling with guanine nucleotide binding protein, q polypeptide (GNAQ). The main signaling pathway for P14 and P39 receptors is via a coupling of guanine nucleotide binding protein, alpha inhibiting activity polypeptide (GNAI), leading to the decrease of cAMP formation by inhibiting the adenylate cyclase (ADCY). It, in this way, eventually actives the P22, a target of antidepressants that relates to mood stabilization located at the downstream of the pathway.

Indirect interaction

As shown in Figure 7, the major herb targets are cell membrane proteins, consist with the fact that membrane proteins account for ~70% of totally recognized drug targets [140]. It is also found that the herb targets (indicated by green rectangles), such as P45 and P51, can connect indirectly with the depression-related pathway by a bridge protein like ADRA1 or GNAI. An elegant example is the protein P45, which can be upregulated by protein kinase A (PKA) [141]. P45 exerts a negative regulation of diseases of anxiety and depression [142], inferring that its inhibition by M108 (*V. officinalis*) might promote the uptake of NE and lead an effective treatment of the diseases. In addition, the CRH signaling through P51 is also an important factor for major depression and anxiety disorders [143]. The antidepressant M039 (*H. perforatum*) may block this pathway and further disturb the GNAQ, GNAI and GNAS in the SSRI pathway to activate cAMP responsive element binding protein 1 (CREB1). All this indicates that some herbs might be potential therapeutic tools for dealing with depression through indirect actions on this pathway.

Cross-talk

The term biological cross-talk is described as one or more components of one signal transduction pathway affect(s) another. The most common form of cross-talk can be achieved between proteins of signaling cascades. A typical instance of cross-talk can be observed between the BDNF/TrkB pathway (purple) and Wnt pathway (cyan)/Ca^{2+} signaling pathway (red) in the depression disease, as in which mutual interaction are expected to occur between them owing to the common components connecting with either pathway.

For example, schematics of BDNF/TrkB pathway and Wnt pathway are coupled together to activate the intracellular signaling cascades, thus leading to the regulation of GSK3B. Inhibition of GSK3B activity may result in the stabilization of β-Cat and subsequent translocation of β-Cat to the nucleus and activation of the transcription of Wnt target genes. Thus, compounds such as M038 in *H. perforatum* exert their antidepressant effects by binding to GSK3B and finally disrupt the cross-talk between the pathways. It is also found that BDNF/TrkB pathway is involved in the activation of intracellular signaling cascades including the PI3K/Akt and MEK/ERK. Regulation of CREB by ligands like M194 (*P. frutescens*) may affect the cross-talk between the pathways, regulating the expression of genes involved in the cell proliferation, neurogenesis and mood stabilization [128].

CONCLUSION AND PERSPECTIVE

Systems pharmacology involves the application of systems biology approaches, combined with the pharmacokinetics and pharmacodynamics evaluations, to the study of drugs and their targets and effects [144–146]. Systems pharmacology analysis generally counts on a large number of variables at a genome level to construct networks for evaluating the drug action and understanding the therapeutic mechanisms. As a major tool, the network analysis based on widely existed databases permits us to form an initial understanding of the action mechanisms within the context of systems-level interactions. By linking with pathways and networks, systems pharmacology is also expected to guarantee the veracity of the predictive pharmacokinetic and pharmacodynamics models of therapeutic efficacy.

In this work, we have highlighted the principles and applications of a newly proposed HmSP in drug discovery and understanding of the therapeutic mechanisms, which is specially designed for herbal medicines. The workout offered in the case study proves the power of this methodology to obtain the potential drugs, latent targets, pathways and networks. The main findings are as follows:

(1) The proposed text mining approach is reliable to find effective herbs relevant to specific/certain disease;
(2) The DL evaluation is indispensable to screen out potential active herb ingredients with high quality and high efficiency;
(3) The strategy combining with pharmacology and network analyses is devoted to helping identify and interpret the multi-scale mechanisms of drug action, disease association and even side effects.
(4) The value of HmSP lies in its general applicability to herbal medicines for various diseases.

When faced with the challenges to rapidly develop new drugs, conventional methods usually ends up with failed results, which partly attribute to the lack of understanding of the multi-scale mechanisms that underlie the spread of effects from molecular-level interactions to organismal-level phenotypes. Although still in its infant stage, systems pharmacology has exhibited great capacity to influence the development and usage of drugs. With the evolution of systems biology and medicine, the pace of new therapeutic development will keep up with the explosion in scientific knowledge, thus facilitating the development of novel drugs.

SUPPLEMENTARY DATA

Supplementary data are available online at http://bib.oxfordjournals.org/.

Key Points

- As herbal medicines are featured as abundant bioactive ingredients and multiple targets, systems pharmacology provides the tools to understand the therapeutic mechanisms of herbal medicines intervening complex chronic diseases such as depression.
- ADME strategies that are adopted to visualize the active ingredients and explore the mechanisms of action of herbs advance the process of drug discovery.
- The strategy combining with pharmacology and network analyses is devoted to helping identify and interpret the multi-scale mechanisms of drug action, disease association and even side effects.

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