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Systems pharmacology-based approach for dissecting the addition and subtraction theory of traditional Chinese medicine: An example using Xiao-Chaihu-Decoction and Da-Chaihu-Decoction

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ABSTRACT

Background: Addition and subtraction theory (AST), a basic theory of herb combination in traditional Chinese medicine (TCM), is often used to add or subtract the "fundamental formulae" to generate more targeted prescriptions. This theory plays a core role in individualized medicine and compound compatibility of TCM. However, the mechanisms underlying AST have largely remained elusive.
Methods: An integrated platform of systems pharmacology was proposed for revealing how the oral administration, drug half-life, and target interactions affect the pharmacological functions of herbal medicines. This platform was further applied on two classical prescriptions, i.e., Xiao Chaihu decoction (XCHD) and Da Chaihu decoction (DCHD) to dissect the addition and subtraction theory (AST).
Results: We uncovered the candidate compounds, key molecular targets and interaction network involved in XCHD and DCHD, and summarized its pharmacological characters and therapeutic indications. The results show that the "fundamental formula" is responsible for the major therapeutic effects, whereas the "additive herbs" synergistically enhance the treatment outcomes by targeting the same or complementary proteins between the foundational and additive herbs.
Conclusion: This work has established a novel method to comprehensively understand the mechanism of AST, which would be beneficial for the TCM recipe optimization as well as the production of new herbal formula with desirable therapeutic effects.

1. Introduction

Traditional Chinese medicine (TCM) is a comprehensive medicinal system that has been used for maintaining health for thousands of years. Several basic theories, with high applicability, have been established in TCM, which include Sovereign-Minister-Assistant-Envoy (Jun-Chen-Zuo-Shi in Chinese), pharmacological synergy, syndrome differentiation, as well as addition and subtraction theory (AST) [1]. By using the emerging new methods, systems biology and/or network pharmacology, the mystery of some TCM theories have been investigated, such as Jun-Chen-Zuo-Shi [2–5], pharmacological synergy [6–8], syndrome differentiation [9]. However, little is focused on dissecting the underlying mechanisms of the herbal addition and subtraction theory.

The addition and subtraction theory, briefly, adding or removing one or more herbal medicines or dosage from an original "foundational formula", and thus modifying it into another new formula. This fundamental theory plays a core role in individualized medicine, which examines the relationship between personality traits and susceptibility to pathology or drug response [10]. Here, we select two typical formulae, i.e., Xiao Chaihu Decoction (XCHD, Minor *Radix bupleuri* Decoction) and Da Chaihu Decoction (DCHD, Major *Radix bupleuri* Decoction) to illuminate the potential mechanisms of AST. The XCHD is composed of seven herbs including *Radix bupleuri* (RB., Chaihu), *Radix scutellariae* (RS., Huangqin), *Rhizoma pinelliae* (RP., Banxia), *Rhizoma zingiberis recens* (RZR., Shengjiang), *Fructus jujubae* (FJ., Dazao), *Licorice* (L., Gancao) and *Panax ginseng* (PG., Renshen). While, the DCHD is derived from XCHD, which subtracts *P. ginseng* and *Licorice*, simultaneously, adds *Fructus aurantii immaturus* (FAI., Zhishi) and *Paeonia lactiflora* (PL., Shaoyao). Specifically, the five common herbs of the two prescriptions are termed as "foundational formula" or "foundational herbs" (RB., RS., RP., RZR., FJ.), with the

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other two herbs as "additive herbs" (L., and PG. in XCHD; FAI., and PL. in DCHD).

In clinic, XCHD is responsible for "shao-yang zheng", yet, DCHD is adopted for syndrome accompanied with "shao-yang and yang-ming zheng". XCHD is effective on reliving fever, treating respiratory diseases with mild syndromes, while DCHD is mainly applied to treat acute or chronic digestive diseases. Although the two prescriptions have gained great success in clinical applications for thousands of years, the underlying molecular mechanisms involved in the two formulae and their combination rule are still mysterious.

Recently, systems pharmacology, as a new emerging filed featured with multi-disciplines and mutli-techniques, has made a significant contribution to TCM pharmacological researches [11–13]. This approach combines pharmacokinetics evaluation and drug–target network analysis to obtain a global understanding of the multiple mechanisms of drug actions [12]. Because of its non-reductive (allowing researchers to analyze multiple components at once rather than studying a single compound in isolation) and holistic characteristics, systems pharmacology is particularly applicable to meet the demand of TCM research [4,12,14].

To get a deep understanding of AST, firstly, all the ingredients from herbs were explored to build a compound database, subsequently, the oral bioavailability and drug half-life were calculated to screen out the potential active compounds, finally, the potential targets were predicted. The acquired pharmacological data were further integrated into drug–target and target–disease network, highlighting the mechanisms involved in the herb functions and the addition and subtraction theory. The systems pharmacology approach framework for the present work is shown in Fig. 1.

2. Materials and methods

2.1. Dataset construction

All ingredients of the two formulae (9 herbs) were manually collected and have been uploaded to the database TCMSP: Traditional Chinese Medicines for Systems Pharmacology Database and Analysis Platform (http://sm.nwsuaf.edu.cn/lsp/tcmsp.php) [15]. TCMSP has integrated herb information, ingredient pharmacokinetic parameters, targets, and diseases. Taken together, 1341 molecules were obtained, of which 288 in Chaihu, 190 in Renshen, 126 in Dazao, 154 in Shaoyao, 280 in Gancao, 116 in Banxia, 58 in Huangqin, 64 in Shengjiang and 65 in Zhishi.

2.2. Oral bioavailability screening

For orally administered drugs, oral bioavailability (OB) is undoubtedly one of the most important pharmacokinetic parameters because it indicates the capability of a given compound delivering to the systemic circulation. Due to the difficulties in obtaining OB values by experimental methods, especially for the herbal active components, in silico OB prediction is undoubtedly the best choice to exclude the compounds that are not likely to be drugs. In this work, the OB values were calculated by a robust in-house tool OBioavail1.1 [4]. Molecules with OB $\geq$ 30% were retained as candidate bioactive compounds for further analysis. Besides, glycosides in medicinal herbs are usually metabolized to liberate aglycone by intestinal bacteria [16], thus the metabolites were also taken into account. The threshold used here is for (1) extracting information from the herbs as much as possible with the least number of components; (2) reasonably explaining the obtaining model by the reported pharmacological data.

2.3. Drug half-life prediction

Drug half-life ($t_{1/2}$), is beyond doubt one of the most important properties as it evaluates the timescale over which the compound may elicit therapeutic [17]. Fortunately, we have recently developed an in silico PreDHL model to calculate the drug half-life by a stepwise forward selection method [18]. This model is supported by 169 drugs with known half-life values, which were deposited in the DrugBank database [19]. The optimal model is as following:

$$\begin{aligned} Y(t_{1/2}) = {} & 13.310(\pm 2.202) + [13.376(\pm 2.273) \times n\mathrm{ArCO}] \\ & + [7.092(\pm 0.937) \times \mathrm{H7m}] + [0.053(\pm 0.007) \\ & \times D/\mathrm{Dr09}] + [19.377(\pm 4.052) \\ & \times N - 070] - [7.598(\pm 2.283) \times C - 033] \\ & - [347.423(\pm 104.591) \times \mathrm{JGI6}] + [32.752(\pm 8.522) \\ & \times n\mathrm{RC}{=}N] - [0.100(\pm 0.030) \times \mathrm{Mor02e}] \end{aligned} \quad (1)$$

$$R^2 = 0.65, \quad Q^2 = 0.62, \quad F = 27.272, \quad \mathrm{SEE} = 8.127,$$
$$N_{\mathrm{training}} = 126, \quad N_{\mathrm{test}} = 43$$

where R^2 is the basis of conventional correlation coefficient, Q^2 is the external-validated correlation coefficient, F is the mean square ratio, SEE is the standard error for estimating the training set, N_{training} and N_{test} are the number of compounds in the training set and test set, respectively. The parameters are the number of carboxylic acids (aromatic, nArCO), H autocorrelation of lag 7/weighted by atomic masses (H7m), distance/detour ring index of order 9 (D/Dr09), Ar–NH–Al (N-070), R-CH..X (C-033), mean topological charge index of order 6 (JGI6), number of imines (aliphatic, nRC=N), and 3D-MoRSE-signal 02/weighted by atomic Sanderson electronegativities (Mor02e) which are also included in the optimal model.

2.4. Chemical space comparisons

In order to investigate differences in molecular properties and structural features among candidate compounds, complementary compounds (total chemicals in all herbs excluding candidate compounds), the compounds in OBioavail1.1 model, and drugs in DrugBank, six representative drug-related physicochemical properties including molecular weight (MW), octanol–water partition coefficient (AlogP), hydrogen bond donors/acceptors (nHDon and nHAcc), number of rotatable bonds (RBN), and topological polar surface area (TPSA) were calculated. These parameters describe the properties of druglikeness, and are crucial for orally bioavailable chemicals and can reflect the basic characteristics of a molecule. Meanwhile, the distribution histogram of the physicochemical properties was carried out considering all six of the above mentioned descriptors, and Wilcoxon rank sum test was employed to analyze the differences of variables in the property spaces.

2.5. Drug targeting analysis

The potential target prediction for the candidate compounds were performed based on the systematic drug targeting tool (SysDT) developed in our previous work [13], which efficiently integrated the chemical, genomic and pharmacological information for drug targeting on a large scale by both random forest (RF) and support vector machine (SVM) methods. This model shows an impressive performance of prediction for drug–target interaction, with a concordance of 86%, a sensitivity of 80% and a specificity of 93%. Here, the targets with predicted probability larger than 0.7 for RF and 0.8 for SVM were considered as potential targets of the query compounds.

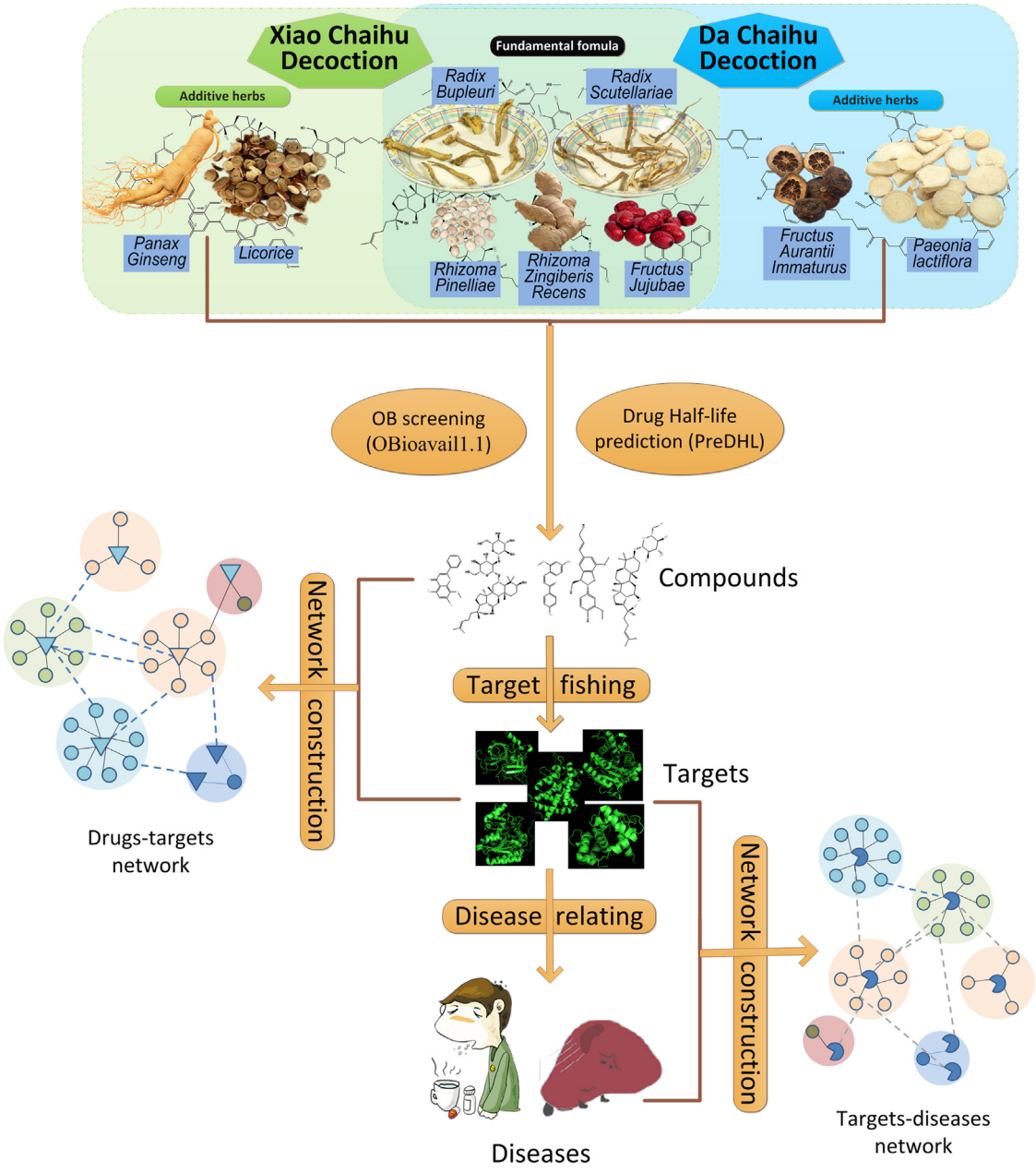

Fig. 1. Systems pharmacology approach framework.

2.6. *Network construction*

To systematically understand the therapeutic and synergic mechanisms of the multi-component herbal medicines, the predicted active ingredients and corresponding targets were analyzed by two kinds of visualized networks: (1) drug–target network, in which all active ingredients are linked to their targets; (2) target–disease network, where the potential targets are linked to related diseases. The targets–diseases were retrieved from TTD (Therapeutic Targets Database) and PharmGKB. In these networks, compounds, targets and diseases are represented as nodes, while the edges indicate interaction or relatedness.

3. Results and discussion

Syndrome differentiation/pattern classification (Zheng) is basic in TCM theory. Shao-yang patterns (also known as Midstage pattern) are externally contracted febrile disease patterns that fit neither the exterior patterns nor the interior patterns categories. They are characterized by alternating fever and chills, bitter fullness in the chest and rib-side, vexation, no desire for food and drink, bitter taste in the mouth, dry pharynx, dizzy vision, and string-like pulse [20]. While, the combined shao-yang and yang-ming pattern is characterized by shao-yang signs such as alternating fever and chills, and bitter fullness in the chest and rib-side, as well as yang-ming signs such as fullness and pain in the abdomen, constipation, and yellow tongue fur [20].

According to the syndrome differentiation, the TCM practitioners add or subtract medicinal herbs from a “foundational formula” to produce a new formula with new therapeutic effects. For instance, XCHD and DCHD encompass seven herbs but five belong to both prescripts. Despite the high acceptance of AST in practice for building herbal medicinal prescriptions, so far, the underlying mechanism remains unclear due to the fact that one prescription is not a simple quantitative addition of different

herbs [21]. Thus in this work, we applied a systems pharmacology approach which combined poly-pharmacology and network biology techniques to uncover the mechanism of AST from a molecular/systematic level.

3.1. Candidate compounds screening

It is generally known that pharmacokinetic properties directly affect the effectiveness of drugs. Drug oral bioavailability screening is crucial to detect the potential active compounds, leading to obtainment of 634 potential ingredients with OB $\geq$ 30% from the 9 herbs. These ingredients with good OB in this work could offer a significant research clue for probing the potential bioactive substances in the future. In order to obtain a more accurate result, the compounds with reported bioactivities were selected to deep analysis. Besides, four compounds beyond the OB screening but abundant in each herb were also included. Taken together, 63 key compounds were obtained and further divided into fast-elimination, mid-elimination and slow-elimination groups when their half-life less than 4 h, between 4 and 8 h and more than 8 h respectively (Table 1).

For all these 63 ingredients, all of them have been proved to exhibit significant bioactivity including anti-inflammatory, antioxidant and anti-cytotoxicity activities. For instance, the ingredients petunidin (RB.4) and quercetin (RB.1), obtaining from *R. bupleuri*, showed the OB of 30.0% and 46.4% respectively. In line with previous study, these compounds present anti-inflammatory, anti-proliferative, antioxidant and hepatoprotective activity, and thus exhibit the anti-cancer activity in colon cancer [22,23]. The flavones baicalein (RS.3, OB=33.5%) and wogonin (RS.1, OB=30.7%) from *R. scutellariae*, exhibiting anti-inflammatory activity and novel antiviral activity against dengue virus, meanwhile, they are benefit for colitis and some other complex diseases [24,25]. Remarkably, although 6-gingerol (RZR.6) and 6-shogaol (RZR.7) in *R. zingiberis recens* with low OB (19.9% and 8.7% respectively), both of them are the most abundant constituents and found to inhibit the growth of human cancer cells, as well as induce apoptosis through modulation of mitochondrial functions [26]. Therefore, these two compounds are also involved as the candidate bioactive ingredients for further target prediction and network analysis.

Besides, accurate prediction of drug half-life is a key issue for evaluating the fate of drugs. As shown in Table 1, 19% (12 of 63) ingredients with half-life under 4 h were in the fast-elimination group, 13% (8 of 63) chemicals under the half-life between 4 and 8 h were in the mid-elimination group. Especially, 43 of 63 (68%) compounds follow the half-life ranged from 9 to 24 h. Indeed, the drug compounds with longer drug half-life seem to be more efficient than that with shorter ones [27].

3.2. Differences in chemical space

Generally, systematic investigations of chemical space are used of assessing the scaffold diversity of compound data sets. Ingredients come from XCHD and DCHD, OBioavail1.1 model, and DrugBank are compared based on six important drug-associated descriptors including the MW, nHDon, nHAcc, RBN, TPSA and ALogP (Fig. 2 and Table 2). In the result, it is seen that the MW of candidate compounds are similar (p=0.997) to complementary compounds, while significantly lower than that of model and DrugBank compounds. There is no difference between candidate and model compounds for the median lipophilicity AlogP value (P=0.685), but significant difference for complementary and DrugBank compounds (P=2E-4, P=0.047, respectively). The median value of RBN for the candidate compounds are significantly different from that of complementary, model and DrugBank compounds (P=0.009, P=1E-4, P=1E-4, respectively). For nHDon, nHAcc and TPSA, it is seen that the candidate compounds are similar to that of model and DrugBank compounds, and are significant different from complementary compounds.

In summary, the distribution profiles of these basic, physicochemical properties for candidate compounds are obviously different from those of the complementary compounds, suggesting highly representativeness of candidate compounds. Notably, it is clearly that the candidate compounds are more similar to those of OBioavail1.1 model and DrugBank compounds, suggesting highly drug likeness property of candidate compounds.

3.3. Target identification

Generally, TCM prescription contains numerous pharmacological compounds, which offers bright prospects for controlling and preventing of complex diseases in a synergistic manner. To understand the underlying mechanism of such synergistic effect, it is important to reveal the therapeutic targets of drugs. By Applying SysDT approach, a total of 65 pharmacological targets (Table S1) have been identified for the bioactive ingredients, in which two or more active ingredients target on multiple proteins simultaneously. For example, molecule quercetin (RB.1, from *R. bupleuri*) can attenuate the function of androgen receptor (AR), which is potential to become a chemopreventive and/or chemotherapeutic agent for prostate cancer [27]. Besides, Baicalein (RS.3) and its metabolite baicalin (RS.8/RP.4), both show interactions on AR in our prediction. Interestingly, experimental evidence shows that orally administered baicalein exhibits dose-dependent growth inhibitory effects on human prostate cancer cells [28].

3.4. Network pharmacology analysis

TCM prescriptions are considered as multicomponent therapeutics, that is, two or more herbs/ingredients simultaneously interact with multiple targets. Here, we mapped the ingredients, targets and diseases relevant to XCHD and DCHD onto the drug–target and target–disease networks, respectively.

3.4.1. Drug–target network

All of the 63 ingredients, 34 (53%) are tightly linked with more than 10 targets, and 23 chemicals are connected to more than 14 targets. The compound FJ.1 (protopine, an ingredient in *Fructus Jujubae*) exhibits the highest number of interactions with 26 protein targets, following are RZR.4 (Linalool, an ingredient in *R. zingiberis recens*, 20 targets), RB.1 and RP.2 (quercetin and ephedrine from *R. bupleuri* and *R. pinelliae*, respectively) with 19 protein targets. Simultaneously, we have also found that many potential targets interact with multiple ingredients of different herbs. The Prostaglandin G/H synthase 1(PGHS-1), androgen receptor and beta-2 adrenergic receptor, are the examples of highly connected targets which have been targeted by all the herbs. Indeed, higher expression levels of PGHS-1 were detected in various cancers including lung and colon carcinomas [29,30]. While, the chemical quercetin (RB.1) can down-regulate PGHS-1 and androgen receptor levels [31,32], as well as acts as a natural inhibitor of 5-hydroxytryptamine type II receptor [33]. By analyzing the drug–target network, we conclude that the two formulae exhibit different therapeutic effects via targeting the same or different protein.

3.4.1.1. Overlapped and respective targets for each formula. In order to profoundly understand the similarities and differences between DCHD and XCHD at a molecular level, we further analyzed the overlapped targets and respective targets for these two formulae.

Table 1
Information for candidate active compounds.

Drug ID	Molecule name	CAS number	OB (%)	Half-life ($t_{1/2}$)/h	Elimination rate
FAI.1	Sinensetin	2306-27-6	50.6	15.5	c
FAI.2	Eriodictyol	552-58-9	41.4	15.8	c
FAI.3	Synephrine	532-80-9	75.3	4.0	b
FAI.4	Hesperidin	520-26-3	47.7	16.5	c
FAI.5	Didymin	14259-47-3	38.6	16.6	c
FAI.6	N-methyltyramine	370-98-9	75.5	2.1	a
FAI.7	Dehydrodiconiferyl alcohol	4263-87-0	50.8	7.5	b
FAI.8	Poncimarin	55916-48-8	63.6	2.3	a
FJ.1	Protopine	130-86-9	59.3	23.5	c
FJ.2	Zizybeoside I	10-44-6	13.9	2.3	a
FJ.3	Stepharine	2810-21-1	31.5	10.2	c
FJ.4	Zizybeoside II	81417-79-0	22.8	1.6	a
FJ.5	Mauritine D	–	89.1	4.9	b
L.1	Isoliquiritigenin	237–316-5	85.3	17.6	c
L.2	Echinatin	34221-41-5	66.6	15.8	c
L.3	Licochalcone B	58749-23-8	76.8	16.5	c
L.4	Licoricone	51847-92-8	63.6	15.6	c
L.5	Liquiritin	551-15-5	65.7	16.2	c
L.6	Glabridin	59870-68-7	53.2	1.2	a
L.7	Glabrene	60008-03-9	46.3	3.1	a
L.8	Liquiritigenin	41680-09-5	71.1	18.2	c
PG.1	Protopine	130-86-9	59.3	23.5	c
PG.2	panaxynol	21852-80-2	42.4	6.9	b
PG.3	Frutinone A	38210-27-4	65.9	19.1	c
PG.4	Ginsenoside-Rf	52286-58-5	17.3	12.9	c
PG.5	Ginsenoside-Rg3	14197-60-5	17.7	13.5	c
PG.6	Ginsenoside-Rh2	78214-33-2	36.3	11.1	c
PG.7	Alloaromadendrene	25246-27-9	53.5	12.6	c
PL.1	Benzoic acid	74239-84-2	31.5	12.1	c
PL.2	Cianidanol	490-46-0	49.7	0.4	a
PL.3	Gallic acid	5995-86-8	31.7	11.8	c
PL.4	Paeoniflorin	23180-57-6	53.9	13.6	c
PL.5	Albiflorin	39011-90-0	30.2	7.5	b
RB.1	Quercetin	482-36-0	46.4	14.4	c
RB.10	Saikosaponin D	20874-52-6	34.4	15.2	c
RB.2	Carvone	99-49-0	49.5	11.8	c
RB.3	Kaempferol	520-18-3	41.9	14.7	c
RB.4	Petunidin	13270-60-5	30.0	1.2	a
RB.5	Vanillin	8014-42-4	52.0	11.8	c
RB.6	Alloaromadendrene	25246-27-9	54.0	12.1	c
RB.7	Iso-liquiritigenin	237–316-5	85.3	17.6	c
RB.8	Cubebin	18423-69-3	57.1	16.4	c
RB.9	Saikosaponin A	20736-09-8	32.4	15.3	c
RP.1	Trigonelline	535-83-1	60.1	11.7	c
RP.2	Ephedrine	299-42-3	43.3	2.6	a
RP.3	Homogentisic acid	451-13-8	92.4	4.8	b
RP.4	Baicalin	21967-41-9	40.1	17.1	c
RS.1	Wogonin	632-85-9	30.7	17.7	c
RS.2	Wogonoside	51059-44-0	45.1	17.9	c
RS.3	Baicalein	491-67-8	33.5	16.2	c
RS.4	Eriodictyol	552-58-9	41.4	15.8	c
RS.5	Neobaicalein	55084-08-7	69.5	16.1	c
RS.6	Oroxylin A	480-11-5	41.4	17.1	c
RS.7	Skullcapflavone I	41060-16-6	76.3	16.8	c
RS.8	Baicalin	21967-41-9	40.1	17.1	c
RZR.1	Nerolidol	7212-44-4	40.4	4.7	b
RZR.2	Isoeugenol	97-54-1	70.1	0.6	a
RZR.3	Vanillin	8014-42-4	52.0	11.8	c
RZR.4	Linalool	78-70-6	38.3	6.3	b
RZR.5	Thymol	89-83-8	41.5	11.3	c
RZR.6	6-Gingerol	1391-73-7	35.6	2.9	a
RZR.7	6-Shogaol	555-66-8	31.0	3.8	a
RZR.8	Alloaromadendrene	25246-27-9	53.5	12.6	c

***Abbreviations*:** *Radix bupleuri* (RB.); *Radix scutellariae* (RS.); *Rhizoma pinelliae* (RP.); *Rhizoma zingiberis recens* (RZR.); *Fructus jujubae* (FJ.); *Licorice* (L.); *Panax ginseng* (PG.); *Fructus aurantii immaturus* (FAI.); *Paeonia lactiflora* (PL.)

[a] Fast-elimination.
[b] Mid-elimination.
[c] Slow-elimination.

Here we noted that there are 22 (33%) overlapped targets (Fig. 3, black) which are simultaneously targeted by compounds from all 9 herbs. Indeed, further observation of the drug–target network shows that the above mentioned targets display high degrees (>8). Take the example of Prostaglandin G/H synthase 2, the highest connected target, is linked to 78% (49 of 63) of the compounds. Followed by Dipeptidyl peptidase IV (degree=39), Carbonic anhydrase II (degree=34), Prostaglandin G/H synthase 1

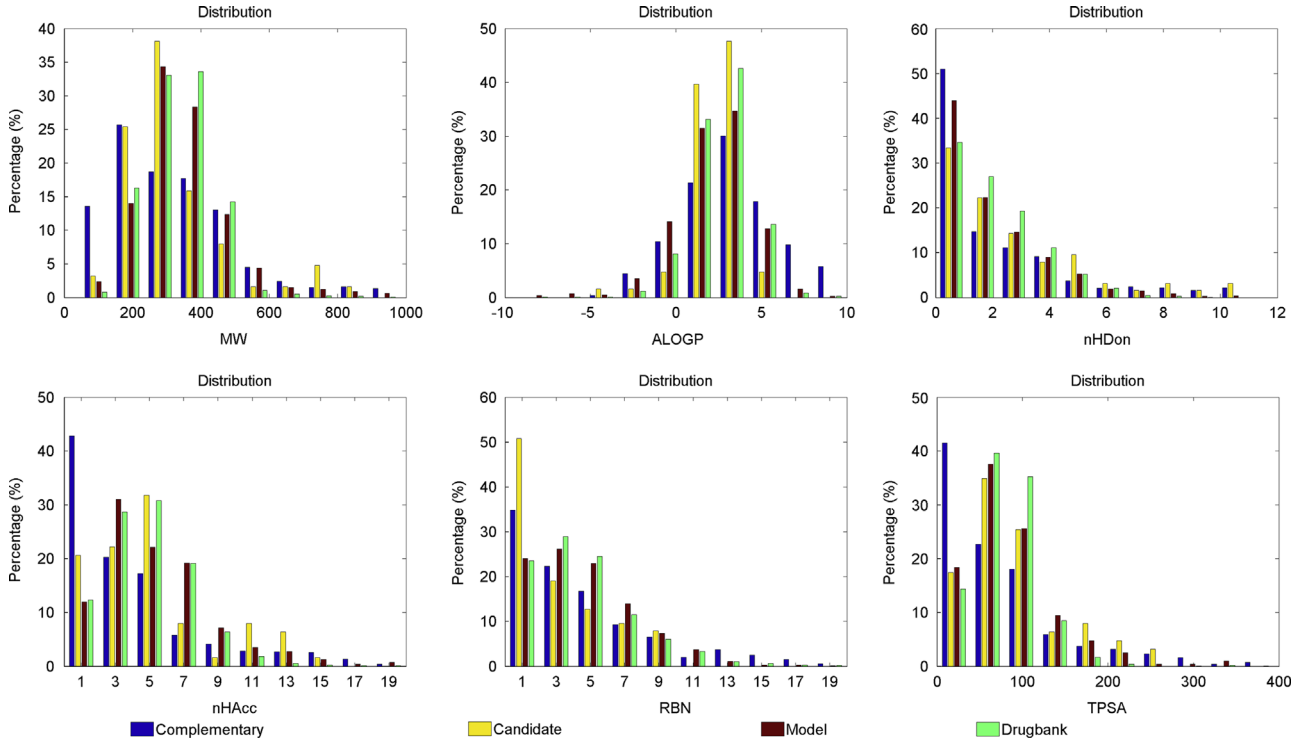

Fig. 2. The profile distributions of six important molecular properties for candidate compounds, complementary compounds (total chemicals in all herbs excluding candidate compounds), the compounds in OBioavail1.1 model, and drugs in DrugBank.

Table 2
Comparison of molecular properties.

Index	Median	Median (*p*-value)		
	Candidate compounds	Complementary compounds	Model compounds	DrugBank compounds
MW	286.3	290.29 (P=0.997)	325.79 (P=0.013)*	327.36 (P=0.012)*
ALogP	2.334	3.115 (P=2E−4)***	2.268 (P=0.685)	2.65 (P=0.047)*
RBN	2	4 (P=0.009)**	4 (P=1E−4)***	4 (P=1E−4)***
*n*HDon	2	2 (P=0.041)*	2 (P=0.096)	2 (P=0.285)
*n*HAcc	5	4 (P=0.002)**	5 (P=0.428)	5 (P=0.775)
TPSA	77.76	57.53 (P=0.007)**	72.83 (P=0.568)	75.43 (P=0.560)

* $P<0.05$.
** $P<0.01$.
*** $P<0.001$.

(degree=34), and Cell division protein kinase 2 (degree=32). The Prostaglandin G/H synthase 1,2 (also known as COX-1,2), which are over expressed in colorectal tumor specimens as compared with normal mucosa. Therefore, the selective inhibition of the COX-1,2 is thought as an effective measure on preventing and treating colorectal cancer [34,35]. In addition, previous study shows that the inhibition of cell division protein kinase 2 (CDK2) has been proposed as a potential therapeutic approach in endocrine-resistant disease [36]. All the above evidences support that both of the Da Chaihu Decoction and Xiao Chaihu Decoction might share the similar action mechanisms through targeting the overlapped proteins.

Interestingly, several targets have been identified for the additive herbs of each formula. We summarized as follows: HTR3A, IDO, IL1B, OPRD1, OPRM1, PDE3A, PDE4 and SLC5A2 (Fig. 3, red) are for *P. ginseng* and *Licorice*; while, there are 14 proteins including ADRA1A, ADRA2A, ADRA2B, ADRA2C, ADRB1, LOX15, ALOX5, CCR4, eNOS, HMOX1, LGALS3, MGAM, PYGM and TNF (Fig. 3, yellow) are for *Paeonia lactiflora* and *Fructus aurantii immaturus*. Actually, a previous study has revealed that eNOS acts as a potential target against vascular senescence, dysfunction and atherosclerosis. Meanwhile, Da Chaihu Decoction has also been reported to have inhibitory effects on the progression of atherosclerotic lesions [37]. This target preference by Xiao Chaihu Decoction and Da Chaihu Decoction illustrates the essence of the differences between the two decoctions. Compounds from the additive herbs reinforce/strengthen preferentially on disease associated targets, thus achieving respective therapeutic efficiency for the two decoctions [38]. Therefore, changes in prescription (like in XCHD and DCHD), i.e. adding or subtracting herbs based on the patient's symptoms and dysfunction proteomes, can achieve preference and/or collaboration among herbs, thereby reaching the therapeutic goals.

3.4.2. Target–disease association analysis

All of 65 drug targets were projected into TTD to obtain their corresponding diseases, finally, 93 diseases which belong to 20 categories derived from the MeSH 'Diseases [C]' branch, were collected. The target–disease interactions were visualized in the Target-disease Network (Fig. 4).

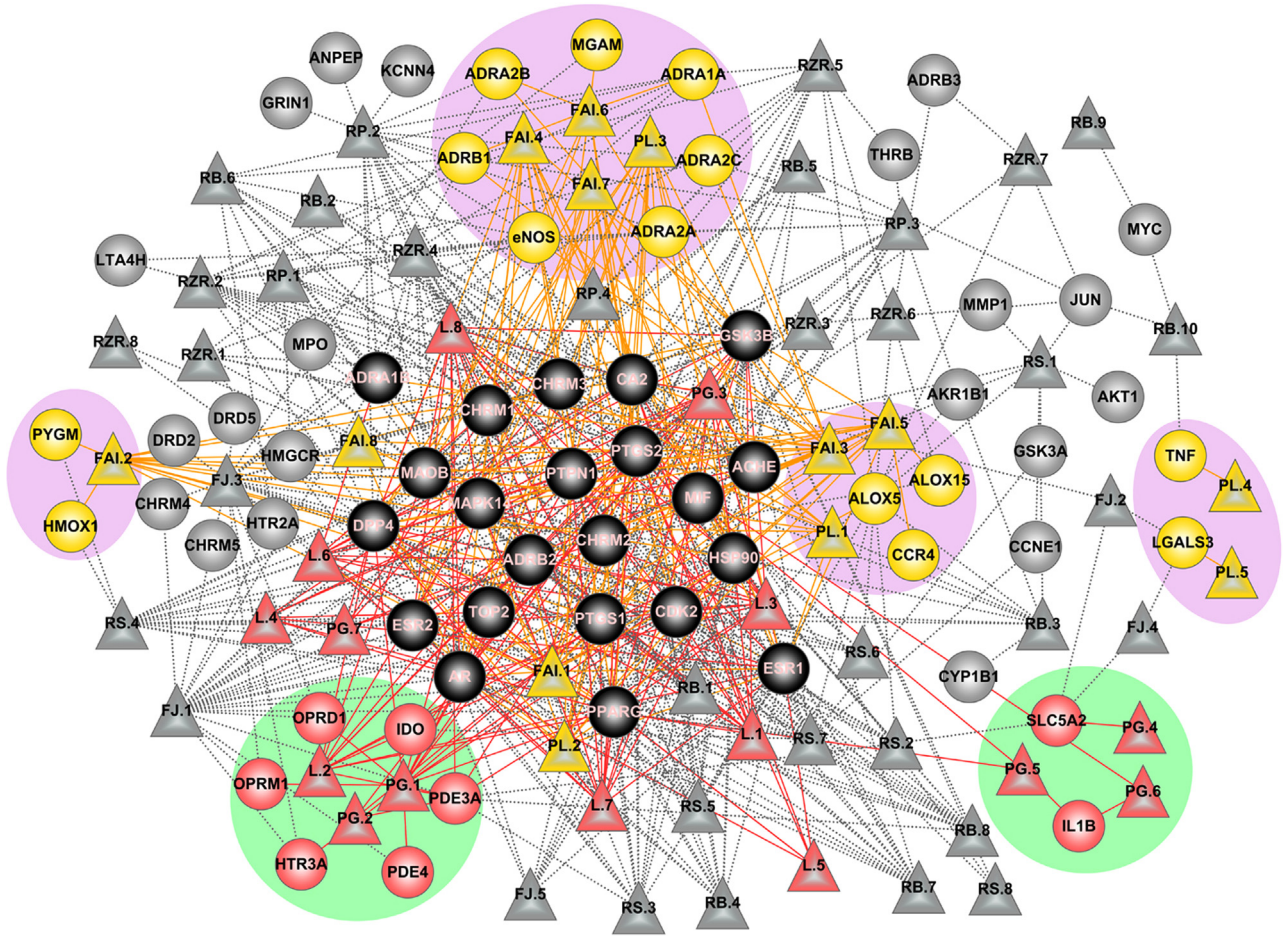

Fig. 3. Drug–target network. Drug–target interactions are depicted as connecting lines between drugs (compounds, triangles) and targets (circles). The black nodes (circles) represent targets that targeting by all the nine herbs. Drugs belonging to the L. & PG. are indicated in red (highlighted in green background), and their corresponding strengthened targets and the linked lines are also indicated in the same color. Drugs belonging to PL. & FAI. are indicated in yellow (highlighted in purple background), and their linked targets and the corresponding lines are also in yellow. Drugs in the fundamental herbs and corresponding targets are colored in gray, and linked by gray dashed lines. (*Radix bupleuri* (RB.); *Radix scutellariae* (RS.); *Rhizoma pinelliae* (RP.); *Rhizoma zingiberis recens* (RZR.); *Fructus jujubae* (FJ.); *Licorice* (L.); *Panax ginseng* (PG.); *Fructus aurantii immaturus* (FAI.); *Paeonia lactiflora* (PL.)). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

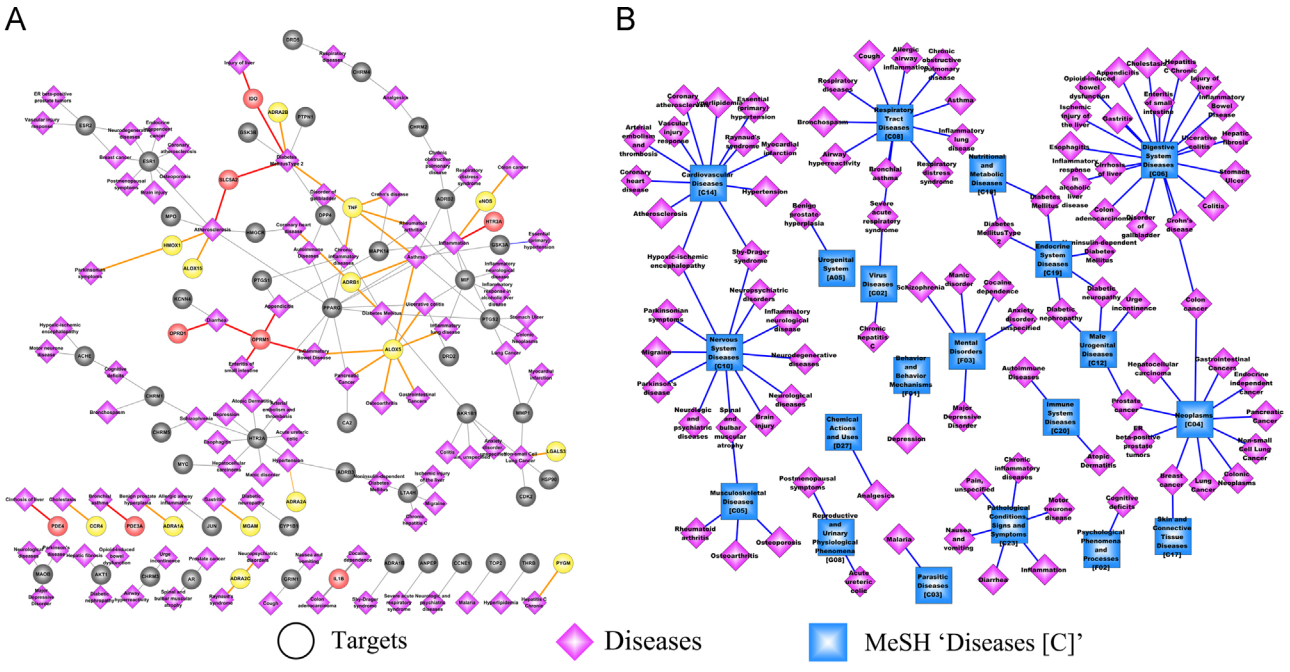

Fig. 4. Target–disease associations. (A) Potential targets (circles) are connected with those related diseases (diamonds, purple), of which circles in different colors are the same meaning to Fig. 3. (B) Diseases (purple diamonds) and MeSH 'Diseases [C]'(blue squares) are linked. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

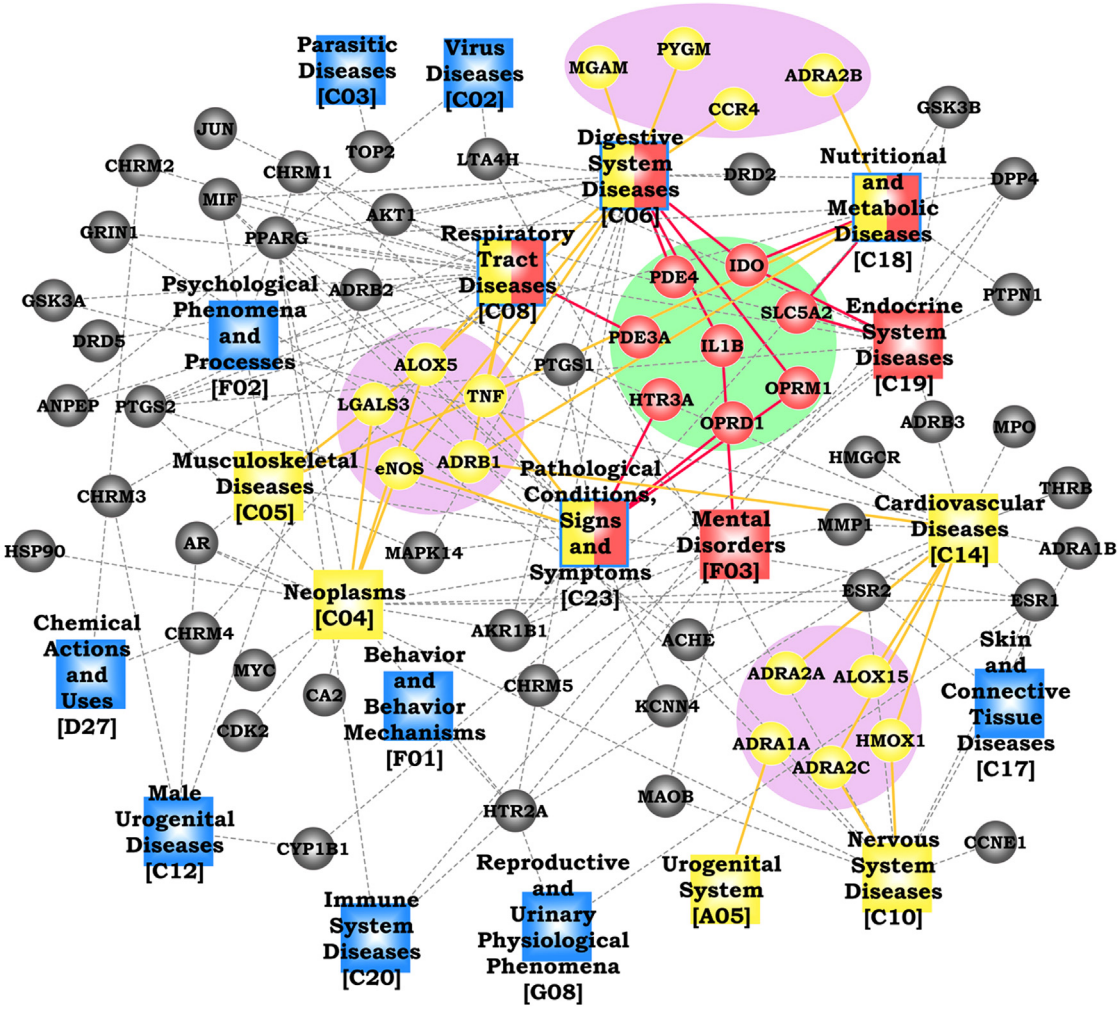

Fig. 5. Target–disease network. Network representation of the target–disease (MeSH 'Diseases [C]') interactions with targets (circles) and diseases (squares). Where red and yellow circles (highlighted in green and purple background) are proteins strengthened by XCHD additive herbs and DCHD additive herbs, respectively. And gray circles are proteins targeted by the fundamental herbs. The squares display in red means the diseases targeted by XCHD additive herbs and/or fundamental herbs. The squares display in yellow means the diseases targeted by DCHD additive herbs and/or fundamental herbs. The shared diseases display a split color code. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

As shown in Fig. 4A, the T–D network is comprised of 158 nodes including 65 targets and 93 diseases, the circles and diamonds represent proteins and diseases, respectively. Both targets and diseases exhibit mutual connectedness, which verify the comprehensive therapeutic effects of TCM formulae. For instance, the peroxisome proliferator activated receptor gamma (PPARγ), connected with 10 different diseases including asthma, pancreatic cancer, ulcerative colitis, inflammatory bowel disease, and atherosclerosis, etc. Clinical evidence shows that PPARγ can improve disease manifestations including digestive system diseases, endocrine system diseases, as well as immune system diseases [39,40]. Macrophage migration inhibitory factor (MIF, degree=6), an inflammatory factor that regulates the function of macrophages, and simultaneously acts on inflammatory lung disease, etc. [41]. Indeed, XCHD and DCHD have several experimentally proved pharmacological activities, including prevention of experimental hepatotoxicity, immunomodulatory effect, antineoplastic activity and promotion of liver regeneration [42,43].

On the other hand, we found that a disease usually associated with multiple protein targets (Fig. 4A), such as ulcerative colitis which is connected to ALOX5, DRD2, MIF, PPARG, the disease asthma is connected to ADRB1, ADRB2, ALOX5, GSK3A, etc. These results suggest that most complex chronic diseases are not caused by changes in a single biomacromolecule interaction, but by an unbalanced regulating pathway resulting from the dysfunctions of multiple components or gene products. The target–disease association offers a clear light upon the relationships of the target proteins and their corresponding diseases.

Fig. 4B gives a global insight into disease categories, which belong to the sub-branches of digestive system diseases, respiratory tract diseases, endocrine system diseases, etc. This is consistent with that both Da chaihu Decoction and Xiao Chaihu Decoction are used for the treatment of ulcerative colitis, inflammatory bowel disease, and gastritis. The results also show that the digestive system diseases are the most connected branches, which contain hepatitis C chronic, disorder of gallbladder, esophagitis, gastritis, and other 16 diseases (more details were shown in Table S1).

3.4.2.1. The preference of the two formulae on diseases. As shown in Fig. 5, some gene or gene products are related with multiple diseases, such as ADRB1 (beta-1 adrenergic receptor), which connects to respiratory tract diseases, cardiovascular diseases, endocrine system diseases and nutritional and metabolic diseases [44]. ESR1 (estrogen receptor) also links with many diseases including cardiovascular diseases, neoplasms, nervous

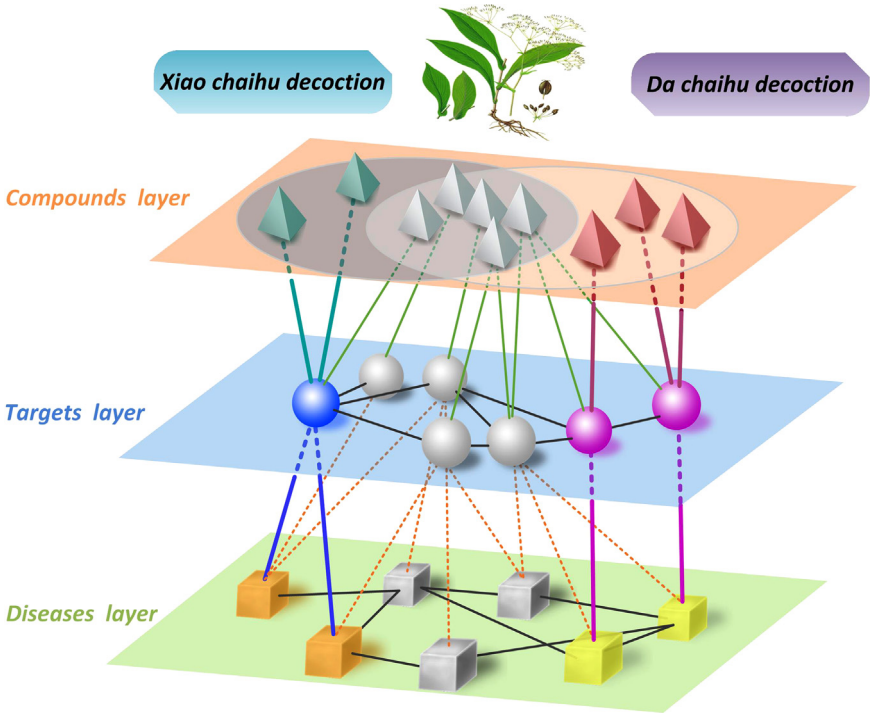

Fig. 6. The model of mechanisms for addition and subtraction theory of TCM.

system diseases [45]. Remarkably, many diseases are related with more than one gene products, such as asthma which links to ADRB1, ADRB2, ALOX5, PPARG, PTGS2 and TNF. These mutual connections bring enormous challenges on treating complex diseases by monotherapies. Therefore, understanding the mechanisms of TCM on complex diseases in the context of network could inspire advancement of multiple-component therapies.

Further, we have analyzed the formulae preference on diseases. Interestingly, we found that the targets shared by the fundamental herbs play a basic role in the treatment of diseases, which focus on digestive system diseases, respiratory tract diseases, endocrine system diseases, nervous system diseases, neoplasms, and cardiovascular diseases (Fig. 5). This feature indicates that the fundamental formula may account for the basic therapeutic effects, via modulating the major symptoms of body. For instance, the pro-inflammatory mediator macrophage migration inhibitory factor (MIF), acts as the common target of the foundational herbs, and serves as a key regulator in ulcerative colitis, inflammatory neurological disease, and inflammatory lung disease [46,47]. The target beta-2 adrenergic receptor (ADRB2), its genetic polymorphism makes a significant influence on asthma [48], and stimulating beta-2 adrenergic receptors by beta-2-agonists can relax airway smooth muscle and then regulate chronic obstructive pulmonary disease and respiratory distress syndrome [49]. Estrogen receptor (ESR1) and estrogen receptor beta (ESR2), both were well-illustrated to improve vascular function and reduce atherosclerosis, and studies in monkeys indicated that estrogen replacement therapy results in a large reduction in atherosclerosis [45]. These results support that the fundamental herbs of both formulae mainly target aberrant proteins which may give rise to body disorders, thereby regaining the body balance for heath recovery.

As representative formula for shao-yang zheng, XCHD significantly treats disease accompanying symptoms of alternating fever and chills, no desire for food and drink, and dry pharynx. Notably, the proteins targeted by additive herbs (Ren Shen/Panax ginseng and Gan Cao/Licorice) in XCHD mostly strengthen on digestive system diseases and endocrine system diseases (Fig. 5, red). For example, Interleukin-1 beta (IL1B), a target reinforced by licorice in XCHD, experimental data revealed that IL-1 beta was significantly overexpressed in esophageal squamous cells compared to nonmalignant tissues [50], as well as combinational effects with IL-1 alpha and TNF-alpha on colon adenocarcinoma (digestive system diseases, shown in Fig. 4B) [51]. Another target sodium/glucose cotransporter 2 (SGLT2) strengthened by XCHD, is almost exclusively expressed in the proximal renal tubules, and serves as a new treatment target for diabetes mellitus type-2 (endocrine system diseases, shown in Fig. 4B) [52]. The results show that the fundamental and additive herbs in XCHD could significantly enhance the therapeutic efficiency on asthma, respiratory distress syndrome, bronchospasm, and airway hyperreactivity, which fall into the respiratory tract diseases accompanied with the shao-yang symptoms.

In comparison, DCHD mostly treats diseases with “shao-yang and yang-ming zheng” including symptoms of fullness, pain in the abdomen, and constipation. Remarkably, the proteins that reinforced by Shao Yao (*P. lactiflora*) and Zhi Shi (*F. aurantii immaturus*) in Da chaihu decoction, are not only involved in digestive system diseases, but also in cardiovascular diseases, respiratory tract diseases and neoplasms (Fig. 4 yellow). For example, the ALOX5 and TNF could strengthen asthma, and ADRA2A and HMOX1 are related with hypertension and atherosclerosis, respectively [53]. HO-1 has been recognized to have major immunomodulatory and anti-inflammatory properties [54]. Clearly, ulcerative colitis, cholestasis, colon cancer, and gastritis, belonging to digestive system diseases, are closely related with shao-yang and yang-ming zheng of fullness, pain in the abdomen and constipation. To sum up, we conclude that the overall therapeutic difference in DCHD and XCHD, mainly due to their respective additive herbs exerting different pharmacological activities (Fig. 6).

Taken together, the above results provide new insights into TCM therapeutic efficiency, that is, addition or subtraction herbs in prescription could result from two strategies: (1) the foundational formula takes charge of the basic therapeutic effects, through intensively targeting the dysfunction of whole body; (2) compounds in

additive herbs strengthen or assist functions to the foundational formula (Fig. 6).

4. Conclusion

Traditional Chinese medicine (TCM), a key branch of natural medicine, plays an important role in the treatment of diseases because of their reliable clinical performance. Although TCM has accumulated a mass of clinical experience and given lots of successful applications, its mysteries remain uncovered due to the difficulties in identifying bioactive substances, as well as in identifying their therapeutic targets. Furthermore, it is still insufficient in systematically and holistically to elucidate the underlying mechanism of the basic theories in TCM on molecular level, such as AST.

In this work, a new integrated platform of system pharmacology was proposed, which evaluates the oral bioavailability, drug half-life, target proteins as well as the combined interactions of the herbal medicines, for dissecting the addition and subtraction theory. Profound investigation was applied on two classical prescriptions from AST, Xiao Chaihu decoction and Da Chaihu decoction. The main findings are as follows:

(i) A total of 63 bioactive ingredients with 65 potential targets in the two prescriptions were obtained, which explains why they have anti-inflammatory, antioxidant and anti-cytotoxicity activities.

(ii) The drug–target and target–disease network analyses show the mechanisms of AST are mainly involved in two aspects: (1) the “fundamental formula” is mainly responsible for basic therapeutic effects, via targeting the major dysfunction of the whole body; (2) the “additive herbs” exhibit reinforced or assistant function to the foundational formula and removing major diseases-associated syndromes. Therefore, the foundational and additive herbs achieve a complementary synergistic effect through the different mode of actions on various diseases. The systems pharmacology approach developed in our study provides new insights into understanding the mechanism of AST, as well as offers new clues in personalizing therapies and TCM recipe optimization.

Conflict of interest statement

The authors declared that they have no competing interests.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.compbiomed.2014.05.007.

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