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Review

Network pharmacology-based prediction of the active ingredients and potential targets of Chinese herbal *Radix Curcumae* formula for application to cardiovascular disease

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ABSTRACT

Ethnopharmacology relevance: Cardiovascular and cerebrovascular diseases (CCVD), an abnormal function of the heart, brain or blood vessels, are the biggest cause of deaths worldwide. Traditional Chinese medicine (TCM) holds a great promise for preventing such diseases in an integrative and holistic way. However, its systems-level characterization of drug–target associations is still unknown.
Methods: Here, we have constructed a computational approach by combining chemical predictors based on chemical structure, chemogenomics data linking compounds with pharmacological information, and a system biology functional data analysis and network reconstruction method.
Results: The pharmacological system generated 58 bioactive ingredients from the Chinese herbal *Radix Curcumae* formula, and predicted 32 potential targets related to the CCVD. The results indicates that *Radix Curcumae* share the most common targets with *Fructus Gardeniae* (15), while less common targets with *Moschus* and *Borneolum* (8 and 1, respectively). Further integrated network shows that *Radix Curcumae* represents the principal component for the prevention of CCVD, and other three medicines serve as adjuvant ones to assist the effects of the principal component, which together probably display synergistic actions.
Conclusions: Our work successfully explains the mechanism of efficiency of *Radix Curcumae* formula for the prevention of CCVD, and meanwhile, predicts the potential targets of the Chinese medicines, which facilitates to elucidate the compatible mechanism of the complex prescription, i.e., "jun–chen–zuo–shi", and provides basis for an alternative approach to investigate novel TCM formula on the network pharmacology level.

Contents

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1. Introduction

Cardiovascular and cerebrovascular diseases (CCVD), caused by high blood pressure, atherosclerosis, easy blood clotting, and heart enlargement, are the leading health problem all over the world claiming more than 17.3 million lives annually (http://www.who.int/mediacentre/factsheets/fs317/en/index.html). To reduce the risk of cardiovascular disease, millions of adults are treated by drug therapies which include blood pressure-lowering medications, such as diuretics, angiotensin-converting enzyme (ACE) inhibitors or beta blockers; blood-thinning medications (to reduce platelet aggregation); cholesterol-lowering medications, and/or anti-arrhythmic medications (Lawrence et al., 1992). These drugs have yielded desired responses while have also triggered serious side effects such as hot flush, fatigue, shortness of breath, headache and dizziness from some antihypertensive drugs (Toyoshima et al., 1997) and rhabdomyolysis and hepatic diseases from hypolipidemic drugs (Sgro and Escousse, 1991).

Different from the orthodox medicine focusing on a specific pathogenic process (Jiang, 2005), traditional Chinese medicine (TCM) has been recognized as a popular complementary and alternative medicine in western countries, emphasizing on individualized diagnosis and treatment of patients; maximizing the body's inherent healing ability; and treatment of the "whole" person by addressing their physical, mental, and spiritual attributes (Jonas, 1999; Xu, 2011). Presently, millions of patients around the world consume TCM, for examples, the TCM products from China have spent US$7.6 billion for US and US$2 billion for Europe in 2010, and that figure is rising at 10% per year (Cheung, 2011). For CCVD, herbal prevention has been to prevent the congestive heart failure, systolic hypertension, angina pectoris, atherosclerosis, cerebral insufficiency, venous insufficiency, and arrhythmia (Mashour et al., 1998). Moreover, several Chinese herbs have already been transformed into commercial products for treatment of CCVD, such as Compound Danshen Dripping Pills (Chinese name FufangDanshenDiwan) (Chu et al., 2011), ShexiangBaoxin Pills (Chinese name ShexiangBaoxin Wan) (Ye et al., 2004). This high rate of use translates into large out-of-pocket expenditures on TCM.

The consistency and quality control are the key issues for the application and development of TCM, since all herbal or animal medicines are mixtures of more than one active ingredient. However, the large number of TCM formulae (nearly 100,000) and the multiple active ingredients involved in each formula make it hard to distinguish the active ingredients and to identify the potential targets of the chemicals (Wang et al., 2008). During the past decade, TCM studies have followed a strategy of isolation/purification/structure identification, and pharmacological research for determining the principal bioactive components. Up to date, more than 30,000 chemicals have been isolated in hundreds of Chinese medicines, with more than 120,000 possessing pharmacological activities (http://www.tcm120.com/1w2k/tcm_species.asp). Nevertheless, this process is much resource-intensive and time-consuming. Moreover, simple quantitative analysis of one or several active components in a herbal or an animal medicine does not endorse its quality and is difficult to identify the targets of the Chinese medicines, because multiple agents contained in each formula, at least in some formulas, could hit multiple targets and exert synergistic therapeutic efficacies (Anonymous, 1979; Anonymous, 2003). Thus, a comprehensive method which could reflect the variation of most constituents in the crude drugs, and more importantly, identify the targets of the drugs, is necessary.

Systems biology, such as network pharmacology, envisions an understanding of the function and behavior of a biological system. Its technological platforms, such as genomics, proteomics and metabolomics, provide holistic approaches to study the essence of TCM syndrome and the function of herbal compound recipe (Qiu, 2007). However, the analysis of TCM based on system biology concept is still in its infancy stage (Zhao et al., 2010), and few drug–target interaction network approaches have been specifically explored for TCM. These restrict the ability of network pharmacology approaches to probe new knowledge of bioactive compounds and potential targets for TCM from a proteome- or systems-level. In this scenario, we have developed an integrated model, which combines oral bioavailability (OB) prediction, multiple drug targets prediction and validation, network pharmacology techniques, to shed light on the mystery and effectiveness of TCM (Li et al., 2012).

In this work, we take *Radix Curcumae* formula, which consists of four Chinese medicines, i.e., *Radix Curcumae* (Yujin), *Fructus Gardeniae* (Zhizi), *Moschus* (Shexiang), and *Borneolum* (Bingpian), as an example. Actually, the relatively simple medicine has been widely applied to prevent CCVD (Xu et al., 2010). More recently, it has been demonstrated that this formula can directly act on the central nervous system through blood brain barrier (BBB) and reduce brain injury and enhance functional recovery after traumatic brain injury (TBI) and stroke in different clinical trials and animal models of injury (Xu et al., 2010). However, essential components in this formula have not been determined and the underlying mechanism of the formula remains poorly defined, thus hampering its "modernization" and "globalization". Therefore, to predict the potential targets of *Radix Curcumae* formula and uncover the mechanisms of action of the active ingredients, we have applied our previously developed model (Li et al., 2012) to this formula, which offers an opportunity for deep understanding of the efficiency of *Radix Curcumae* formula for the prevention of CCVD.

2. Materials and methods

2.1. Chemical structures construction

All chemicals from these 4 medicines of *Radix Curcumae* formula were collected from (1) literature, (2) Chinese academy of sciences Chemistry Database (http://www.organchem.csdb.cn),

and (3) Chinese Herbal Drug Database. Finally, a total of 454 chemicals include 233 in *Radix Curcumae*, 147 in *Fructus Gardeniae*, 74 in *Moschus*, and 30 in *Borneolum* were collected. And the chemical structures were obtained from the Chemical Book (http://www.chemicalbook.com), NCBI PubChem database (http://www.ncbi.nlm.nih.gov/pccompound), or drawn by using ISIS Draw 2.5 (MDL Information Systems, Inc.) and further optimized by Sybyl 6.9 (Tripos Associates, St. Louis, MO) with the same parameters as our previous work (Wang et al., 2010). Considering the oral administrated medicine, the deglycosylation of the glucosides will appear in the intestinal tract by enteric bacteria (Németh et al., 2003), thus 23 compounds with glycosyl are further deglycosylated according to the rule of glycosidase hydrolysis reaction, and the resulting 21 products without overlap are obtained, which were further optimized according to procedures described as above.

2.2. OB prediction

OB *in vivo* (%F), the fraction of the orally-administered dose that reaches systemic circulation unchanged (Xu et al., 2012), is one of the most commonly used pharmacokinetic parameters in drug screening cascades. Due to the difficulties in evaluating the OB of complex TCM by "wet" experiments, especially for a formula, we have developed a robust in-house system OBioavail 1.1 (Xu et al., 2012) integrated with the metabolism (Cytochrome P450 3A4) and transport (P-glycoprotein) information to predict the compounds' OB. The detailed approach to predict the compounds' OB has been described in our previous work (Li et al., 2012; Xu et al., 2012). With this software, the compounds with poor OB can be abandoned, thus significantly reducing the large number of the original chemicals to a smaller set for a TCM formula.

In the present work, we chose the compounds with OB $\geq$ 60% as the candidate compounds in *Radix Curcumae*, *Fructus Gardeniae* and *Borneolum* while the compounds with OB $\geq$ 30% in *Moschus*. The threshold used here is mainly because: (1) Extract information from *Radix Curcumae* formula as much as possible with the least number of components. (2) The obtained model can be reasonably explained by the reported pharmacological data.

2.3. Drug-likeness prediction

Prior to the target prediction, we removed compounds deemed to be chemically unsuitable for drug by using drug-likeness index, which can be deduced as a delicate balance among molecular properties affecting pharmaco-dynamics and pharmacokinetics of molecules which ultimately affects their absorption, distribution, metabolism, and excretion (ADME) in human body like a drug. In this study, drug-likeness index of a new compound is calculated by Tanimoto similarity defined as

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

In which A represents the new compound, and B represents the average drug-likeness index of all 6511 molecules in DrugBank database (access time: June 1st, 2011, http://www.drugbank.ca/) based on Dragon soft descriptors. According to this, we removed the molecules with drug-likeness < 0.1. The compounds which overcome both the OB and drug-likeness screening are treated as candidate compounds.

2.4. Target prediction and validation

The target prediction was performed for the candidate compounds using our previously developed model (Yu et al., 2012) which integrates the chemical, genomic and pharmacological information for drug targeting on a large scale, based on Random Forest (RF) and Support Vector Machine (SVM). In this model, the results were represented by probability of interactions between each molecule and 3999 targets from DrugBank database. Here, the proteins with which the probability of each molecule interacting predicted by both methods (RF and SVM) is greater than 0.7 are chosen as targets. And the obtained targets (candidate targets) were reserved for further validation.

To validate these targets, the crystal structures of candidate targets were retrieved from RCSB Protein Data Bank (http://www.pdb.org/), and the proteins without crystal structures were modeled using the Swiss-Model Automated Protein Modeling Server (http://swissmodel.expasy.org/) with the default parameters. Then each compound was docked to its candidate targets, respectively, by using the AutoDock software (Cosconati et al., 2010) with default settings. All ligand atoms were allowed to move during the docking simulation. The Lamarckian genetic algorithm (Morris et al., 1998) was applied to deal with the compound–target interactions. The docking reliability was validated using the known X-ray structure in complex with a small molecular ligand. The targets and compounds with docking score ≤ -5.0 kcal/mol were selected as the potential targets for *Radix Curcumae* formula.

2.5. Network construction

To facilitate scientific interpretation of complex relationships between medicines involved in formulae orchestration, network analysis was performed. The procedure for network construction was as following: (1) the "candidate compound–candidate target network" (cC–cT network) was constructed by linking the candidate compounds and all their candidate targets. (2) After the validation by docking analysis, the "potential compound–potential target network" (pC–pT network) was established by connecting the potential compounds and their validated potential targets which are related to CCVD. (3) In the "Target–Disease network" (T–D network), the potential targets were connected with those related diseases which were obtained from the PharmGKB database (Klein et al., 2001), and Therapeutic Targets Database (http://bidd.nus.edu.sg). All networks were generated by Cytoscape (http://www.cytoscape.org/) (Shannon et al., 2003) which is an open source software project for integrating biomolecular interaction networks with high-throughput expression data and other molecular states into a unified conceptual framework. In this graphic network, molecular species (compounds and proteins) or diseases were represented as nodes and intermolecular interactions (compound–target or target–disease interactions), which were indicated as links, i.e., edges between nodes.

3. Results and discussion

TCMs might simultaneously target multiple physiological processes to arouse the whole body's potentiality to recover to health. Unfortunately, the potential targets of the Chinese herbs are difficult to be identified. In addition, the TCM compatibility emphasizes jun (monarch), chen (minister), zuo (adjuvant), shi (messenger) with proper herbs to synergize the desirable effects and minimize side effects integrally (Chan, 1995; Fan et al., 2006). The mission of "jun" is directly to treat the main disease; "chen" is to potentiate the "monarch" drug curing the main disease, or treat the accompanying diseases or symptoms; "zuo" can enhance the therapeutic effects and modulate the side effects of 'monarch' and/or 'minister' agents; and "shi" guides the active ingredients to reach the target organs and to harmonize the actions of these agents (Kong et al., 2009). Despite the efficiency

of "jun–chen–zuo–shi" for building herbal medicine prescriptions, its underlying mechanism is still illusive (Wang et al., 2008; Kong et al., 2009). Thus in this work, we combined several techniques including polypharmacology and network biology to uncover the mechanism of the TCM rule from a molecular/systematic level.

3.1. OB prediction

Oral route is the most convenient and predominant way to deliver drugs to the systemic circulation for patients in the TCM therapy. For natural ingredients with poor aqueous solubility, they will exhibit low efficiency in entering blood after oral intake, and thereby provide few beneficial therapeutic effects in patients (Ekins et al., 2005). Thus in this section, the OB prescreening, as a prerequisite, is used to determine whether a compound is pharmaceutically active in a TCM prescription. The OB calculation for the *Radix Curcumae* formula shows that 74 of 475 (454+21) chemicals (15.6%) possess satisfactory OB (Table 1).

3.1.1. Radix Curcumae

Of the 74 compounds with good OB, 45 are involved in the Chinese herb *Radix Curcumae*, many of which were reported as active ingredients, such as curcumol (M223, OB=100%), curcumenol (M217, 91.1%) and dihydrocurcumin (M243, 65.5%) (Yoshioka et al., 1998; Lü et al., 2011). For aerugidiol (M121, 67.2%) and zedoarondiol (M452, 64.0%), they presented potent protective effect on D-galactosamine/lipopolysaccharide-induced liver injury in mice (You et al., 2005), and thus probably have therapeutic effects on the CCVD. Furthermore, Sesquiterpenoids such as zedoarolide B, zedoarol and 4-Epi-curcumenol are identified as the biological active compounds in *Radix Curcumae* which are probably used to further evaluate their validity.

Interestingly, although curcumin has been reported as one of the important ingredients of *Radix Curcumae* (Pan et al., 1999), it has a relatively poor OB (33.4%). In fact, this ingredient enables to be biotransformed into various metabolites such as dihydrocurcumin (M243, OB=65.5%) under the influence of enzymes *in vivo* (Pan et al., 1999). The metabolite with good pharmacokinetic properties and activities explains why curcumin exhibits pharmacological activities despite its poor OB.

3.1.2. Fructus Gardeniae

Generally, orally delivered pharmacologically active compounds must have favorable absorption and clearance properties and satisfactory metabolic stability to provide adequate systemic exposure to elicit a pharmacodynamic response. However, for *Fructus Gardeniae*, we have found that most active components involved in the Chinese herb are impermeable through intestine membrane (Hou et al., 2008). For example, the OBs of geniposide, asperuloside and gardenoside only range from 4.5% to 20%. Indeed, human commensal intestinal microbiota can modulate the host intestinal glycosylation and other biochemical processes, thus has a major impact on the metabolism of drugs or toxicants and ultimately on their oral bioavailability (Németh et al., 2003). After disposed with deglycosylation, the products of the active compounds in *Fructus Gardeniae* become more orally bioavailable, especially for caryptoside_DG (the product of caryptoside) and gardenoside_DG (the product of gardenoside) with OB of 100%. Similarly, the active compound of chlorogenic acid (OB=39%) could be hydrolyzed by intestinal microflora into various aromatic acid metabolites, such as caffeic acid with good OB of 65.7% (Gonthier et al., 2003).

In addition, we have found that the *Fructus Gardeniae* possess germacrone, curdione, and neocurdione with relative poor OB (about 40%) as the same as *Radix Curcumae*. Since the three sesquiterpene compounds have comparatively high contents in *Radix Curcumae* formula (Zhang et al., 2006; Hu and Shao, 2007), it is reasonable to believe that *Fructus Gardeniae* and *Radix Curcumae* have similar pharmacological effects for the prevention of CCVD, which explains why *Fructus Gardeniae* serves as an adjuvant to assist the effects of *Radix Curcumae* in *Radix Curcumae* formula to the extent.

3.1.3. Moschus

For *Moschus*, since this animal medicine contains multiple hormones with high activity and poor OB, we have determined the threshold of OB in *Moschus* is 30%. As shown in Table 1, Allantoin (with the highest value of predicted OB=98.7%) is the catabolic end product of purines in mammals, thus the small amounts of allantoin detected in human serum may provide a marker of free radical activity *in vivo* (Pavitt et al., 2002). Muscone (OB=31%) was identified to have favorable absorption properties *in vivo* of rat and fast clearance in its brain and serum (Li et al., 2000). Thus this compound has been used as the quality control markers for *Moschus*. Besides, Stanolone, Muscopyridine, androst-4-one-3, 17-dione, Hydroxymuscopyridine and cholic acid also have good predicted OBs.

3.1.4. Borneolum

Borneolum (the crystal of extraction of *Dryobalanops aromatic Gaertn.* f. or *Blumea balsamifera* DC) is commonly used in TCM as an absorption enhancing agent to facilitate other drugs to the target organs (The State Pharmacopoeia Commission of China, 2005). In fact, this medicine is thought to alter the permeability of the intestinal mucous membrane, or loosen the intercellular gap junction, or even block the efflux protein P-glycoprotein (P-gp), and thus enhances the concomitant compound efficiency (Wu et al., 2003). Only 3 compounds in *Borneolum* have good OBs, including borneol (81.9%), isoborneol (87.4%) and camphor (68.8%). Among them, isoborneol and camphor are also the ingredients of *Radix Curcumae* as mentioned above.

3.2. Target prediction and validation

Based on our previous developed target prediction model (Yu et al., 2012), we have analyzed the binding of *Radix Curcumae*, *Fructus Gardeniae*, *Moschus* and *Borneol* to targets of interest in the CCVD. The 74 candidate compounds yielded 328 candidate targets (Table S1) and the connections between them reach up to 3577.

We further applied docking method to validate the reliability of the candidate targets. Only 32 targets were reserved, which linked with 58 ingredients. The amount of potential targets hit by *Radix Curcumae*, *Fructus Gardeniae*, *Moschus* and *Borneolum* is 24, 21, 11 and 1, respectively. There is a significant target overlap between *Radix Curcumae* and *Fructus Gardeniae* (8 potential targets), but less overlap between *Radix Curcumae*, *Fructus Gardeniae* and *Moschus* (6 potential target). Interestingly, no target overlap is found among the *Radix Curcumae*, *Fructus Gardeniae* and *Borneolum*.

The above results indicate the different roles of these medicines in prevention of CCVD. Generally, a formula of TCM is composed of one or more medicines, each possessing its functions and mixed together in proper amounts in accordance with the specific principle, i.e., "Jun–Chen–Zuo–Shi". This implies that the four Chinese medicines in the *Radix Curcumae* formula probably represent monarch, minister, assistant, and messenger, respectively. Detailed analysis about the connections between the drugs and targets are referred in Section 3.3.

Table 1
74 compounds from *Radix Curcumae* formula, and corresponding predicted oral bioavailability (OB), and drug-likeness (DL).

No.	Compound	OB	DL	Medicine
M10*	(1E,4E,6E)-1,7-bis-(4-Hydroxyphenyl)-1,4,6-heptatrien-3-one	80.7	0.25	yujin
M22*	(E)-5-Hydroxy-7-(4-hydroxyphenyl)-1-phenyl-1-heptene	64.7	0.19	yujin
M38*	1,7-Diphenyl-3-acetoxy-6(E)-hepten	62.9	0.22	yujin
M63*	3,7-Dimethyl-5-indanecarboxylic acid	67.8	0.12	yujin
M68*	3-Hexadecenoic acid	67.4	0.12	yujin
M79*	4-Epi-curcumenol	89.6	0.27	yujin
M82*	4s-Dihydrocurcumenone	65.2	0.22	yujin
M117	a-Cadinol	64.8	0.18	yujin
M121*	Aerugidiol	67.2	0.22	yujin
M125*	Alismoxide	66.3	0.19	yujin
M135	Aromadendrene oxide	64.8	0.31	yujin
M179	Calarenepoxide	70.7	0.42	yujin
M196*	Cinnamic acid	79.8	0.15	yujin
M215*	Curcumanolide A	79.0	0.19	yujin
M217*	Curcumenol	91.1	0.27	yujin
M223*	Curcumol	109.8	0.27	yujin
M228*	Curzerenone	63.5	0.20	yujin
M243*	Dihydrocurcumin	65.5	0.44	yujin
M254	Endobornyl acetate	65.5	0.19	yujin
M255	Epicurcumenol	87.8	0.27	yujin
M256*	Epicurzerenone	63.0	0.20	yujin
M258*	Ethyl ferulate	87.8	0.11	yujin
M314	Isoaromadendrene epoxide	64.7	0.34	yujin
M317	Isocurcumenol	100.0	0.27	yujin
M321*	Isospathulenol	81.9	0.26	yujin
M322*	Isozedoarondiol	64.0	0.21	yujin
M329	Ledenoxide	80.5	0.40	yujin
M353*	Neocurcumenol	86.5	0.27	yujin
M379*	Oxycurcumenol	67.2	0.44	yujin
M398*	Procurcumadiol	68.3	0.23	yujin
M412	Spathulenol	79.6	0.26	yujin
M435*	Turmeronol A	67.6	0.12	yujin
M444*	Zedoalactone A	100.0	0.27	yujin
M445*	Zedoalactone B	100.0	0.34	yujin
M446*	Zedoalactone C	71.3	0.23	yujin
M447*	Zedoalactone D	100.0	0.34	yujin
M449*	Zedoarol	100.0	0.26	yujin
M450*	Zedoarolide A	87.2	0.52	yujin
M451*	Zedoarolide B	100.0	0.33	yujin
M452*	Zedoarondiol	60.3	0.21	yujin
M183	Camphor	68.9	0.17	yujin&bingpian
M315	Isoborneol	87.4	0.17	yujin&bingpian
M224*	Curdione	38.5	0.11	yujin&zhizi
M292*	Germacrone	41.1	0.10	yujin&zhizi
M354	Neocurdione	36.5	0.11	yujin&zhizi
M137*	Ascorbic acid	78.6	0.11	zhizi
M190*	Chlorogenic acid	39.0	0.39	zhizi
M202*	Crocetin	16.5	0.30	zhizi
M244*	Dimethoxydurene	63.4	0.12	zhizi
M278*	Gardenic acid	41.1	0.62	zhizi
M279*	GardeninA	40.1	0.56	zhizi
M438	Ursolic acid	19.4	0.88	zhizi
M455*	Asperuloside_DG	91.2	0.23	zhizi
M457*	6″-p-coumaroylgenipingentiobioside_DG	73.5	0.16	zhizi
M459*	6′-O-sinapoyljasminoside C_DG	60.5	0.10	zhizi
M463*	10-acetyl geniposide_DG	60.4	0.20	zhizi
M466*	Picrocrocinicacid_DG	55.3	0.12	zhizi
M468*	Jasminoside I_DG	61.7	0.11	zhizi
M470*	Gardoside_DG	83.0	0.16	zhizi
M471*	Gardenoside_DG	100.0	0.20	zhizi
M475*	Caryptoside_DG	100.0	0.21	zhizi
M64	3-a-hydroxy-4-androsten-17-one	35.5	0.44	shexiang
M94*	5-cis-14-methyl-cyclopentadecenone	32.3	0.11	shexiang
M126*	Allantoin	98.7	0.10	shexiang
M128*	Androst-4-one-3,17-dione	47.2	0.49	shexiang
M131*	Androsterone	36.8	0.44	shexiang
M193*	Cholic acid	37.9	0.73	shexiang
M238*	Dehydroepiandrosterone	33.9	0.44	shexiang
M313*	Hydroxymuscopyridine	42.3	0.15	shexiang
M345*	Muscone	31.5	0.11	shexiang
M346	Muscopyridine	47.5	0.13	shexiang
M351	N-docosane	63.5	0.28	shexiang
M414*	Stanolone	32.7	0.44	shexiang
M167*	Borneol	82.9	0.17	bingpian

* Represent potential compounds.

3.3. Network construction and analysis

To highlight the efficiency of TCM and uncover the synergistic effects of *Radix Curcumae* formula related to CCVD, we have constructed two Compound–Target networks and one Targets–Diseases network.

3.3.1. cC–cT and pC–pT networks for Radix Curcumae formula

Fig. 1 illustrates the cC–cT network for the candidate compounds and their candidate targets. In total, this network consists of 402 nodes (74 candidate compounds and 328 candidate targets) and 3577 edges. The targets in the outer circle show much less interactions with candidate compounds than those in the inner circles. This indicates that many candidate targets are hit by only one candidate compounds, but some can be modulated by multiple ligands rather than single compound.

To further clarify the relationships between the active ingredients and their targets, the pC–pT network was generated by connecting the potential compounds (58) and the potential targets (32) associated with the CCVD (Fig. 2). The mean number of potential targets per potential compound is 2.9. Among the 58 potential compounds, 9 have high degree distributions and each of them hits more than 10 potential targets. 7 of them come from *Radix Curcumae*, i.e. M10 ((1E,4E,6E)-1,7-bis-(4-Hydroxyphenyl)-1,4,6-heptatrien-3-one), M435 (Turmeronol A), M38 (1,7-Diphenyl-3-acetoxy-6(E)-hepten), M243 (dihydrocurcumin), M22 ((E)-5-Hydroxy-7-(4-hydroxyphenyl)-1-phenyl-1-heptene), M258 (ethyl ferulate), M82 (4s-Dihydrocurcumenone). Generally, the potential compounds that have higher degree are more pharmacologically important (Jeong et al., 2001), which indicates a bias toward specific drug compounds in pC–pT network.

The potential compounds (triangle) and the potential targets (circle) with the same color suggest the interactions between

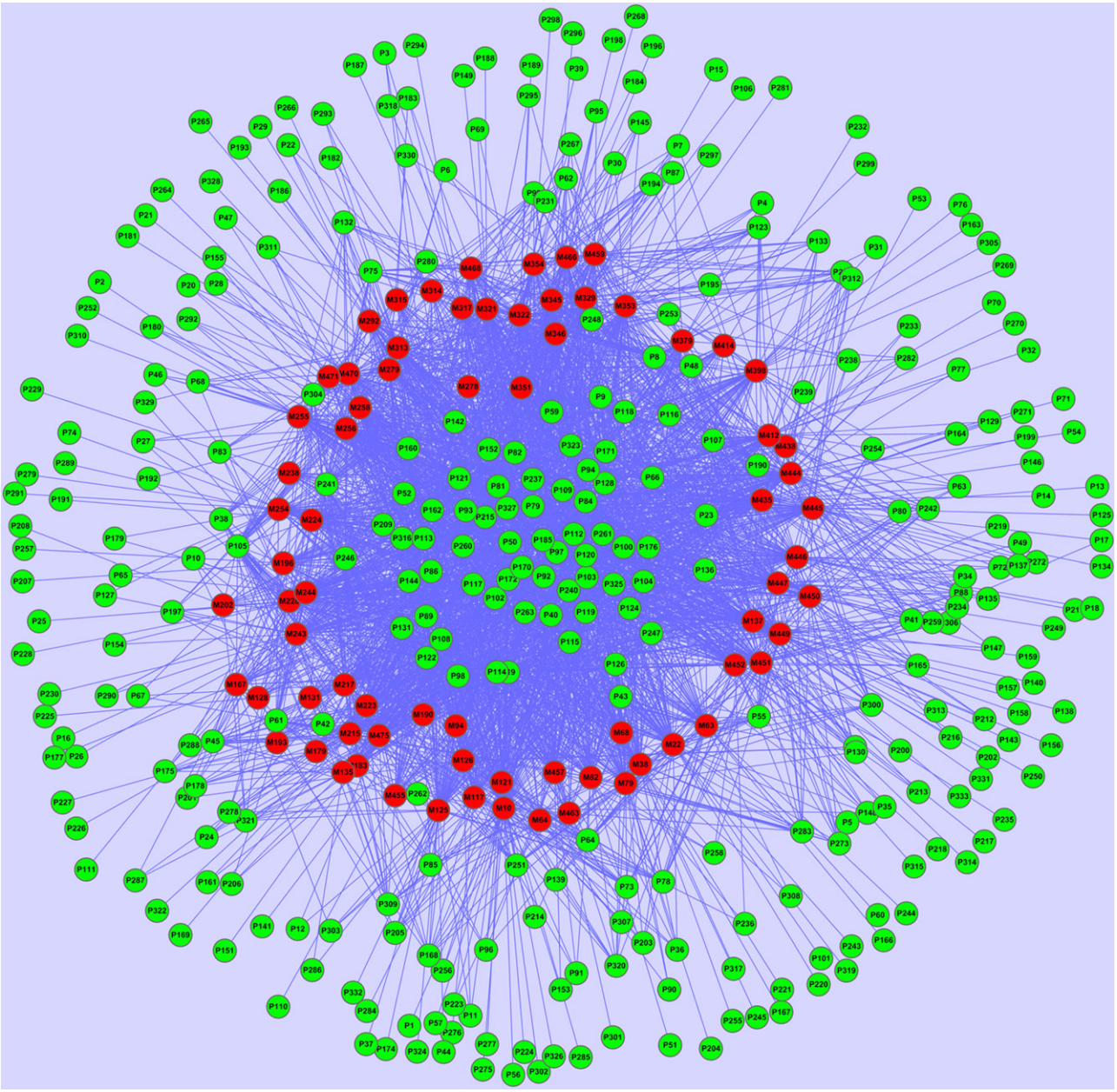

Fig. 1. Network of 74 candidate compounds (M) predicted to have 328 candidate protein targets (P). The red circle represents the compounds, while green circles delineate the proteins. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

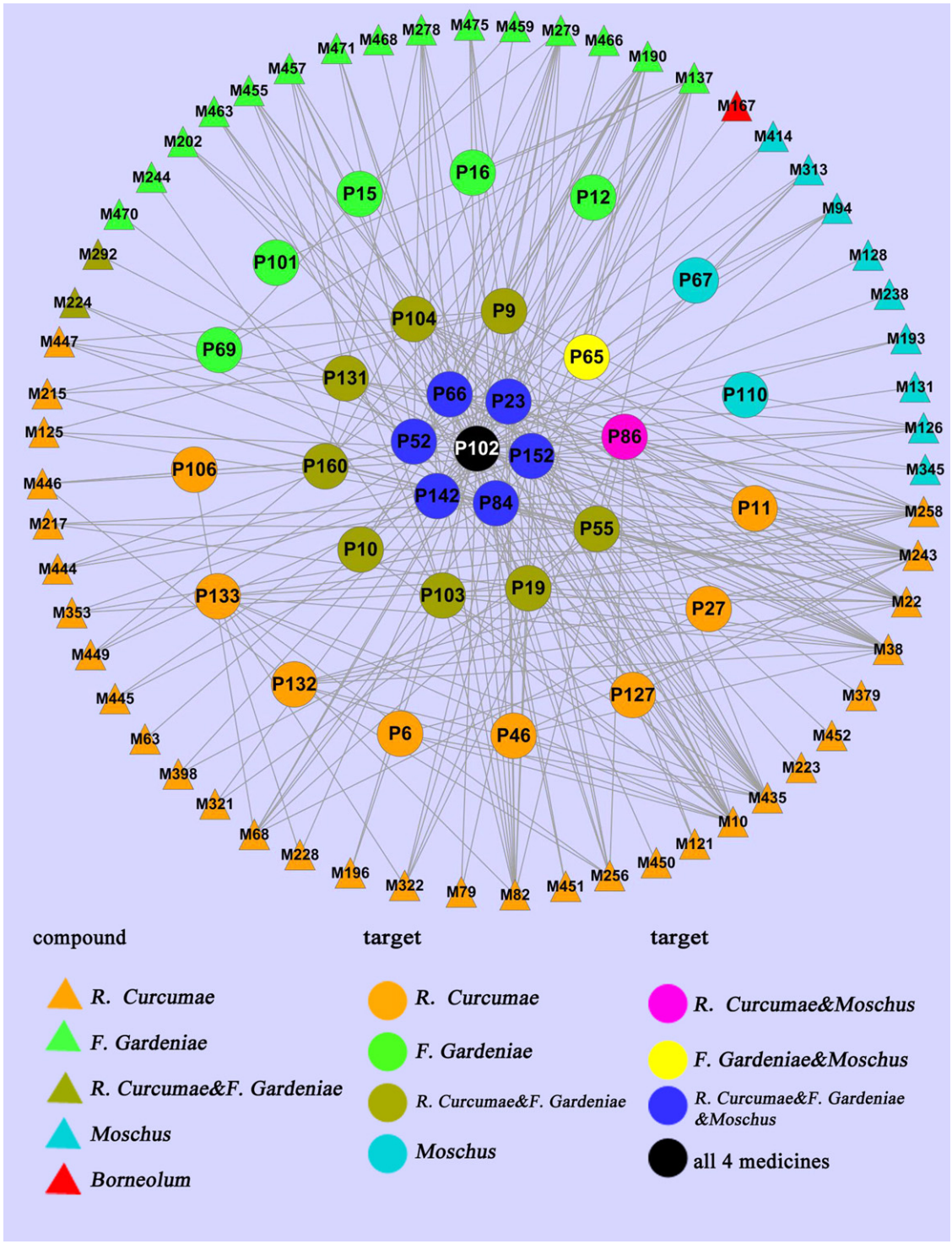

Fig. 2. Network of 58 potential compounds (M, triangle) predicted to have 32 potential protein targets (P, circle) after molecular docking validation. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

the molecules and the proteins (Fig. 2). For examples, 33 active ingredients (orange) of *Radix Curcumae* possess 8 specific potential targets (orange). While 17 active ingredients (green) of *Fructus Gardeniae* possess 5 specific potential targets (green). Of the 16 common targets (the inner three layers in Fig. 2), P9 (Carbonic anhydrase 1), P10 (Dihydroorotate dehydrogenase, mitochondrial), P19 (Prothrombin), P55 (Stromelysin-1), P103 (Prostaglandin G/H synthase 2), P104 (Sodium channel protein type 5 subunit alpha), P131 (Macrophage metalloelastase) and P160 (5-hydroxytryptamine 1D receptor) are the common targets of *Radix Curcumae* and *Fructus Gardeniae*; P86 (Beta-2 adrenergic receptor) the common target of *Fructus Gardeniae* and *Moschus*; P65 (Angiotensin-converting enzyme) the common target of *Radix Curcumae* and *Moschus*. Particularly, we also observed that *Radix Curcumae*, *Fructus Gardeniae* and *Moschus* share 6 common targets (blue), i.e., P23 (Peroxisome proliferator-activated receptor

gamma), P52 (Estrogen receptor), P66 (Cell division protein kinase 2), P84 (Nitric-oxide synthase, endothelial), P142 (Arachidonate 5-lipoxygenase) and P152 (Beta-1 adrenergic receptor). And the four Chinese medicines have one common target (black), P102 (Prostaglandin G/H synthase 1).

CCVD etiology is incriminated for multiple intermediate risk factors like hypertension, diabetes, abnormal blood lipids, and obesity (Hickey, 1972), which might be induced by the difference of expression of proteins. Thus the single drug or herb component is probably insufficient to CCVD therapy. While the common targets shared by multi-medicines imply that the *Radix Curcumae* formula can exert synergistic therapeutic effects on CCVD, which is probably more effective than single drug. In addition, since *Radix Curcumae* possesses the largest number of active ingredients (33) and related targets (24), we suggest that this herb represents the principal component of *Radix Curcumae* formula and enables to prevent CCVD. While for *Fructus Gardeniae*, it was predicted to have the second largest number of potential targets (21), which indicates its pharmacological importance, and also implies that its function is to enhance the actions of *Radix Curcumae*. As for *Moschus* and *Borneolum*, they share much less common targets with *Radix Curcumae* and *Fructus Gardeniae*, indicating the two medicines might not directly treat the CCVD, but reduce the possible toxic effects of *Radix Curcumae* and *Fructus Gardeniae*, or help deliver the two herbs to the target organs.

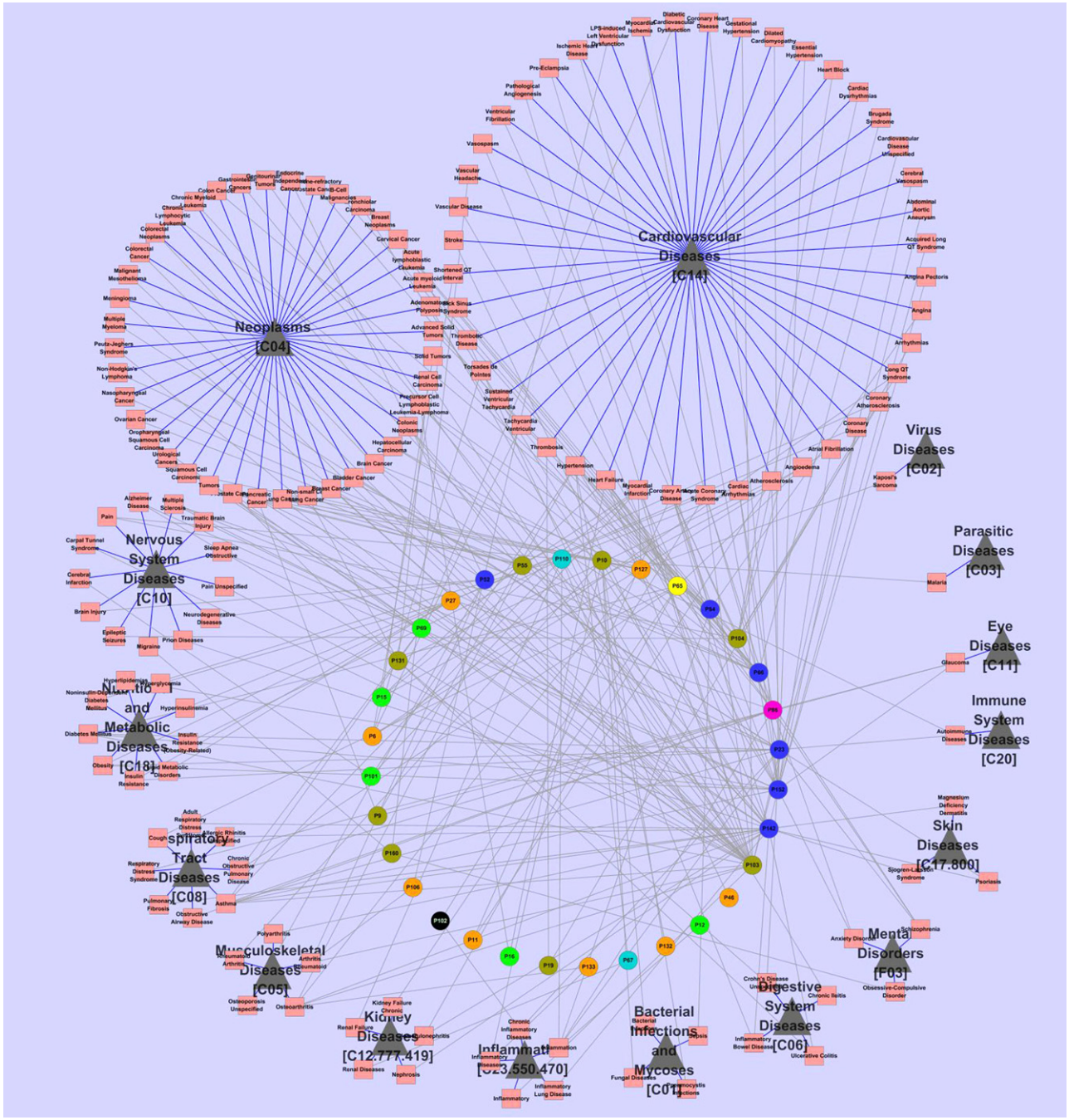

Fig. 3. Network of 32 potential targets (P, circles and the color schemes are the same as Fig. 1b) connected to 147 diseases (red squares) which are classified into 16 groups (C, black triangles) according to Medical Subject Headings. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

In summary, on the basis of the two networks analyses, we find that:

1) 7 of 9 potential compounds with high degree distributions come from *Radix Curcumae*, implying that the monarch serves as the key role in treating the CCVD.
2) Four medicines in the formula have their specific targets. Among them, the monarch *Radix Curcumae* possesses 8 specific targets, followed by minister *Fructus Gardeniae* (5), adjuvant *Moschus* (2) and messenger *Borneolum* (0). This combination probably explores a wider effect at a low concentration, and is evidently safer than a single drug.
3) Four medicines in the formula share the common targets. The *Radix Curcumae* share the most common targets with *Fructus Gardeniae* (15), while less common targets with *Moschus* (8) and *Borneolum* (1). This suggests that TCM offers bright prospects for the control of complex diseases in a synergistic manner.

Taken together, all these results also explain why this formula takes the "jun–chen–zuo–shi" as the rule of prescription.

3.3.2. T–D network

The availability of large phenotypic and molecular networks provides a new opportunity to study the association between diseases and proteomic datasets. In this section, all validated potential targets and their related 147 diseases retrieved from the PharmGKB database and TTD websites (as of April 1st, 2012) were used to construct the T–D network (Fig. 3). The 147 diseases are classified into 16 groups according to Medical Subject Headings (MeSH, http://www.nlm.nih.gov). For examples, Angina Pectoris, Cardiac Arrhythmias, and Hypertension belong to cardiovascular diseases; Hyperlipidemias, Obesity is involved in metabolic diseases.

Interestingly, among these 147 diseases, we find that most of them belong to neoplasms (40/147), nervous system diseases (13/147) and nutritional and metabolic diseases (9/147) apart from cardiovascular disease (44/147). This suggests that *Radix Curcumae* formula probably have demonstrated great effectiveness not only on cardiovascular disease (Yang and Zhuang, 2006) but also on neoplasms (Ouyang et al., 2012), nervous system diseases (Chen et al., 2000), nutritional and metabolic diseases and other diseases. For example, peroxisome proliferator-activated receptor gamma (P23, PPAR-γ) that was predicted to be the potential target of M190 (chlorogenic acid) in this work is a protein target for the treatment of various diseases, including diabetes, atherosclerosis, cancer and inflammation (Vamecq and Latruffe, 1999). Previous experiment has reported that chlorogenic acid could increase glucose uptake through PPAR-γ activation and insulin signaling (Kim et al., 2011), which implies that such compound probably could prevent CCVD by targeting PPAR-γ. This thus validates the good performance of our target prediction model to some extent. To further elucidate the relationship among the compounds, the targets and the correlated diseases, experimental studies are hoped to be carried out in future.

The Beta-1 adrenergic receptor (P152, ADRB1) is found connected to the cardiovascular diseases, respiratory tract diseases, nervous system diseases etc. Indeed, this receptor mediates the physiological effects of the hormone epinephrine and the neurotransmitter norepinephrine, which are common therapeutic targets for the treatment of hypertension, renal disease, and heart failure (Kraja et al., 2011). Thus, it is reasonable to believe that ADRB1 is linked to cardiovascular disease. Similarly, tight regulation of ADRB1 in adipose tissue contributes to increased lipolytic rate, which verifies the close association of the receptor with nutritional and metabolic disease. These results confirm the reliability of our T–D network, and meanwhile, emphasize the connection between the ingredients of the Chinese medicines and their related diseases via the T–D connections.

It is worth to note that since the computational models can only give hints that need to be verified by real experimental data, we plan to incorporate experiments into our systems to facilitate the development of candidate herbal drugs in the future work.

4. Conclusions

TCM, as one of the most important parts in complementary and alternative medicine, significantly improves the efficiency for the treatment of diseases, especially the cardiovascular disease. Why do the Chinese medicines produce therapeutic effects on the special disease? What are the active substances involved in the medicines and the mechanism of action? What combination formulae can exert synergistic therapeutic efficacies on a disease and how? To address these, we have constructed a novel modeling system by integrating OB screening, targets prediction and validation, and network pharmacology to investigate the molecular mechanisms of action of the representative Chinese herbal *Radix Curcumae* formula. Our results show:

1. The potential targets of the Chinese medicines *Radix Curcumae*, *Fructus Gardeniae*, *Moschus*, *Borneolum* involved in the *Radix Curcumae* formula have been predicted, which provides clues to explore the mechanisms of action of medicines for the prevention of CCVD, and for the development of novel drugs and TCM modernization.
2. We have distinguished the auxiliary and conducting ingredients of the *Radix Curcumae* formula, and found that the herbal and animal combination enables to improve the pharmacodynamic action and ensure the administration safety, which clarifies the theory of "jun–chen–zuo–shi" in TCM on a systematic level. Thus our integrated approach has potential to provide insights into the synergetic effect of TCM formula.
3. The integrated network constructed in our work validates the efficiency of the *Radix Curcumae* formula for the treatment of CCVD, which provides clues to translate ancient interpretations of disease treatment into those used in modern medicine.
4. The target–disease network shows the *Radix Curcumae* formula does not only have great efficiency for the treatment of CCVD but also for other diseases such as neoplasms, and nutritional and metabolic diseases, suggesting that distinct diseases (according to modern diagnostics) can be treated with the same formula.
5. Our system, as a new tool to re-evaluate herbal and animal formulae that contain many species of medicines, would provide professional advice to support development of recipe optimization of TCM, which will promote drug discovery.

Competing interest

The authors declare 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.jep.2012.09.051.

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