www.elsevier.com/locate/jep

A Systems pharmacology approach to investigate the Mechanisms of action of *semen Strychni* and Tripterygium wilfordii Hook F for treatment of rheumatoid arthritis

Yan Li, Jinghui Wang, Yuanchun Xiao, Yonghua Wang, Sushing Chen, Yinfeng Yang, Aiping Lu, Shuwei Zhang

PII: S0378-8741(15)30140-9
DOI: http://dx.doi.org/10.1016/j.jep.2015.09.016
Reference: JEP9739

To appear in: *Journal of Ethnopharmacology*

Received date: 12 June 2015
Revised date: 2 September 2015
Accepted date: 13 September 2015

Cite this article as: Yan Li, Jinghui Wang, Yuanchun Xiao, Yonghua Wang, Sushing Chen, Yinfeng Yang, Aiping Lu and Shuwei Zhang, A Systems pharmacology approach to investigate the Mechanisms of action of *seme Strychni* and Tripterygium wilfordii Hook F for treatment of rheumatoid arthritis *Journal of Ethnopharmacology,* http://dx.doi.org/10.1016/j.jep.2015.09.016

A Systems Pharmacology Approach to Investigate the Mechanisms of Action of *Semen Strychni* and Tripterygium wilfordii Hook F for Treatment of Rheumatoid Arthritis

Yan Li[1,*,†], Jinghui Wang[1,†], Yuanchun Xiao[1,†], Yonghua Wang[2,*], Sushing Chen[3], Yinfeng Yang[1], Aiping Lu[4,*], Shuwei Zhang[1]

1 Key laboratory of Industrial Ecology and Environmental Engineering (MOE), Faculty of Chemical, Environmental and Biological Science and Technology, Dalian University of Technology, Dalian, Liaoning 116024, P R China.
2 Center of Bioinformatics, Northwest A&F University, Yangling, Shaanxi, 712100, China.
3 Department of Computer Information Science & Engineering, Systems Biology Lab, University of Florida-Gainesville, FL 32611.
4 School of Chinese Medicine, Hong Kong Baptist University, Kowloon Tong 999077, Hong Kong.
† Contributing equally to this work.
*Corresponding authors: Tel.: +86-411-84986062; E-mail address: yanli@dlut.edu.cn.

Ethnopharmacology relevance: The angiogenesis control at the initiation of rheumatoid arthritis (RA) that mainly blocks the inflammatory cascades expects to attenuate the action of angiogenic mediators, synovial angiogenesis, and to partially reverse the erosive bone damage. Two typical Chinese herbs, *Semen Strychni* and Tripterygium wilfordii Hook F (TwHF) have been used as a remedy to treat RA since ancient time. However, their functioning mechanisms are still unknown. Thus it is necessary to exploit their underlying mechanism for the treatment of RA.
Methods: This study was undertaken to analyze their underlying mechanism based on a systems biology platform. Firstly, active components of the two herbs were screened out from TcmSP database based on their OB and DL values. Then their potential targets were predicted by using Random Forest, Support Vector Machine, and validated via docking process. Finally, a network of compound-target was constructed.
Results: In this work, 27 and 33 active compounds were screened out from *Semen Strychni* and TwHF, targeting 28 and 32 potential proteins, respectively. The results show that the two herbs modulate the angiogenesis mediators through both direct and indirect pathways, and 21 common targets shared by *Semen Strychni* and TwHF bear major responsibility for treating RA.
Conclusions: The underlying mechanism of *Semen Strychni* and TwHF in treatment of RA is through multiple targets interaction by their blocking of the angiogenesis mediator cascades. This may provide us a better understanding of the function of the two herbs for the treatment of RA, as well as a clue to unveil their possible treatment effects of other systemic diseases, and in this way, hopefully the screening models may facilitate the discovery of novel combined drugs.

Keywords: Rheumatoid arthritis, angiogenesis, Chinese herbs, *Semen Strychni*, Tripterygium wilfordii Hook F

Rheumatoid arthritis (RA) is a heterogeneous and chronic systemic disease, whose inflammatory progression could be joint welling, joint tenderness, joint destruction, functional impairment and premature mortality (Aletaha et al., 2010; Combe, 2009). For being highly associated with the perpetuation of new vessel and capillaries formation, RA could be regarded as

an "angiogenic disease" (Szekanecz and Koch, 2009). Actually, angiogenesis seems to play key roles in triggering and promoting the rheumatoid diseases which can be strategically inhibited to reduce the inflammation (Lainer-Carr and Brahn, 2007; Woods et al., 2003). Up to date, series of angiogenic mediators have been identified relevant to RA, such as the growth factors, chemokines, pro-inflammatory cytokines, extracellular matrix molecules, cellular adhesion molecules, as well as matrix-degrading proteolytic enzymes (Combe, 2009; Lainer-Carr and Brahn, 2007; Szekanecz and Koch, 2009). As dozens of mediators are involved in the complex regulatory network of synovial angiogenesis, therapeutic strategies with multiple targets may be more efficacious when hindering the angiogenic cascade, compared with the regulation of only one single target (Szekanecz and Koch, 2009).

Traditional Chinese Medicine (TCM), including hundreds of herbs that have been widely used in many Asian countries, has drawn great amount of attentions worldwide as a significant contributor to human health maintenance beyond its traditional boundaries (Ehrman et al., 2007). The reason may be due to the increasing amount of available information online about the TCM herbs' chemical structures, pharmacological activity data, traditional use, as well as their specificity descriptions against those known molecular targets, which all facilitate the virtual screening and experimental design of TCMs. Being innately complex, TCM medical herbs contain dozens of ingredients in which usually more than one active components are included (Wang et al., 2013c; Wang et al., 2012). Thus, given their multiple components, herbal phytocomplexes can interact simultaneously with multiple targets, thus exerting their pharmacological actions in at least three ways: 1) tackling multiple major pathogens or pathologies of a disease; 2) producing overall therapeutic synergy based on mutual complements; 3) interfering with multiple pathological avenues (Miyazaki et al., 2012).

For treating RA, *Semen Strychni* and Tripterygium wilfordii Hook F (TwHF) are two typical TCM herbs, the investigation of whose active compounds and underlying mechanisms has always been a research hotspot. As a matter of fact, for a long history, the dried seed of Strychnos nux-vomica L. (Loganiaceae), nux-vomica (*Semen Strychni*), has been clinically used for improving the blood circulation, reducing the swelling, relieving the rheumatic pain, easing the allergic symptoms and treating cancers (Chen et al., 2012; Li et al., 2013). Abundant evidences have shown that its main bioactive ingredients may be alkaloids, which, to a great extent, may account for the pharmacological and toxic properties like the analgesic and anti-inflammatory activities (Chen et al., 2012; Li et al., 2013). For instance, the brucine N-oxide and brucine significantly inhibit the release of prostaglandin E2 (PGE2) in inflammatory tissue which leads to the suppression of the synovial angiogenesis, and ultimately exhibits the inhibitory effects in carrageenan-induced rat paw edema (Kakinuma et al., 2010).

As for TwHF, a deciduous twining shrub in East Asia, it has also been historically used as TCM to treat a broad spectrum of autoimmune and inflammatory diseases, including RA, systemic lupus erythematosus (SLE), psoriatic arthritis, and Behcet's disease (Ma et al., 2007). The extracts from the root of TwHF have been proven being capable of inhibiting the expression of pro-inflammatory cytokine, adhesion molecules and metalloproteinases, and thus have demonstrated immunosuppressive, cartilage protective and anti-inflammatory effects in the treatment of RA (Bao and Dai, 2011; Zhang et al., 2013). Of these extracts, diterpinoids, including especially the triptolide, tripdiolide and triptonide, are the most abundant components which may contribute to most of the activities (Bao and Dai, 2011).

As shown above, the therapeutic effects of Chinese herbs of *Semen Strychni* and TwHF for the treatment of RA have been evaluated and validated. However, simple quantitative analysis of one or several ingredients of the herbs is of little help for identifying the herbs' targets as well as for explaining the underlying function mechanism and synergistic therapeutic effects. Meanwhile, for the multiple components-multiple targets interaction model of TCM, conventional experimental research faces a situation of long-term investment to investigate the interaction mechanism. All this makes a comprehensive method which integrates the systems biology and computational technologies a necessity in the development of novel TCM drugs.

Thus, the aim of the present work is to investigate the mechanism of *Semen Strychni* and TwHF in treating RA based on a systems biology platform we newly developed (Wang et al., 2013c; Wang et al., 2012). This platform was built based on the knowledge of pharmacokinetics by a combinational use of series of techniques including the oral bioavailability evaluation, multiple drug targets prediction and validation, as well as the network pharmacology analysis (Wang et al., 2012). With the help of this powerful tool, this study aims to determine the active ingredients of *Semen Strychni* and TwHF, and the herbs' corresponding proteins and therapeutic functions, in order to facilitate the drug discovery and development of combination therapies for the treatment of RA.

Materials and methods

As *Semen Strychni* and TwHF contain considerable chemical compounds, the ingredient database of each herb was built firstly. After that, a series of approaches were integrated to screen out the active components from the database, followed by the prediction and validation of the potential targets. Finally, based on the active components and predicted targets, a network of compound-target was constructed.

Construction of database. The ingredients of both TwHF and *Semen Strychni* were obtained from TcmSP database (Traditional Chinese Medicines Systems Pharmacology Database and Analysis Platform, http://sm.nwsuaf.edu.cn/lsp/). As a result, 174 and 64 ingredients were obtained for the TwHF and *Semen Strychni* respectively, whose molecular structures were saved as mol2 format for subsequent analysis.

Oral bioavailability prediction. Oral bioavailability (OB) is an important indicator which determines whether orally administered drugs could overcome several barriers, like the intestinal epithelium and gut wall, to reach their target sites. The ingredients with poor OB might show relatively low efficiency when entering blood, and thus may perform less beneficial therapeutic effects. The OB values of the 238 ingredients (seen in Table S1 and S2) of the two herbs were calculated by using a robust *in silico* model which integrated the metabolism (P450 3A4) and transport (P-glycoprotein) information (Wang et al., 2013c; Wang et al., 2012). Based on the docking score, the set of the ingredient compounds were grouped into four subsets. Then the descriptors of the subsets were calculated by DRAGON professional (version 5.6; Talete SRL: Milano, Italian, 2006). High predictability was achieved by using Support Vector Machine (SVM), evidenced by the regression coefficients of internal training and external test data $R_{training}$=0.89 and R_{test}=0.85, and the standard errors of estimate $SEE_{training}$=0.35, SEE_{test}=0.42, respectively.

Drug-likeness prediction. To distinguish drugs from nondrugs, quantum mechanically derived descriptors which depict the physical and partition properties of molecules, were adopted to calculate the drug-likeness (DL) index, which could help us estimate the absorption,

distribution, metabolism, and excretion (ADME) properties of the chemicals in study (Brustle et al., 2002). Those molecules with lower DL values are considered to be less likely to be drug. The DL index was predicted by Tanimoto similarity formulated as follows:

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

where A indicates the new compound and B represents the average DL index of all 6,511 molecules in DrugBank database (access time: June 1st, 2011, http://www.drugbank.ca/).

In the present work, of all 226 molecules of the database, the average value (0.18) together with the OB value (50%) was set as threshold to screen out the active compounds.

Target prediction. A systematic model established based on Random Forest (RF) and SVM approaches, which integrates chemical, genomic and pharmacological information, was applied in the searching of candidate targets. The novel model calculated the probability of interactions between each compound and its targets from DrugBank database (http://drugbank.ca/). The proteins with the values predicted by both methods of RF and SVM larger than 0.8 and 0.7 respectively are chosen as targets.

Target validation. The binding orientation and affinity of molecules to their targets with known 3D structures could be predicted by using molecular docking program GOLD, which generates poses and GOLD Score to rank: the higher the score, the better the binding affinity (Wang et al., 2013a). In the candidate targets, the ones with known crystallographic structures were obtained from RCSB Protein Data Bank (http://www.pdb.org), and the ones without were modeled using the Swiss-Model Automated Protein Modeling Server (http://swissmodel.expasy.org/). Then the original ligands were extracted from the crystallographic structure of target proteins and mixed into the docking database for re-docking. For the unknown function of water-mediated hydrogen bonds, all water molecules in the crystallographic structures were retained. Finally, all the candidate compounds were docked into the binding pocket, and 20 conformations with different possible scores were obtained.

Network construction. Network analysis facilitates the scientific interpretation of the complex relationship between compounds and targets. Based on Cytoscape v2.8.3, a powerful bioinformatics package for data visualization and integration, the active compound-potential target network was generated (Smoot et al., 2011), which provides information about the relationship between biological components and RA. In this net, the biological components (i.e., compounds, targets) are marked as nodes, and the interactions between the biological components are marked as edges.

Results

OB prediction and DL calculation. As for multi-compound medicinal herbs, many compounds in the mixture that lack appropriate pharmaceutical (including especially the OB) properties are believed to fail in reaching the cellular targets, and thus exhibit little efficacy and should be neglected. To evaluate the human OB for the structurally diverse ingredients of the two herbs, in present work, a robust in-house system OBioavail 1.2 we developed previously (Wang et al., 2013c; Wang et al., 2012) was applied. Likewise, the DL index was predicted by Tanimoto equation. Thus, by this OB prescreening and DL modeling, the ingredients of *Semen Strychni* and TwHF with favorable pharmacokinetic property were screened out as shown in Tables 1 and 2.

Semen Strychni. Strychnos nux-vomica is grown widely in southern Asian countries, whose dried seed is also officially listed in Chinese Pharmacopoeia. Presently, a total of 64 ingredients are identified in the seed of Strychnos nux-vomica. As seen from Table 1, 24 of them demonstrate good oral bioavailability (with OB≥50%), which are mostly alkaloids and organic acids.

Major alkaloids that are present in the seed of Strychnos nux-vomica were effective against SMMC-7721 cells proliferation, and induced cell apoptosis partially due to the activation of caspase 3 as well as the inhibition of cyclooxygenase 2 (COX-2) (Eisermann et al., 2013). In these alkaloids, the most typical one is brucine which has been proven of many beneficial pharmacological effects. Firstly, brucine significantly inhibited the release of PGE2 in inflammatory tissue, and reduced both the acetic acid-induced vascular permeability and the content of 6-keto-PGF1α and 5-hydroxytryptamine (5-HT) in Freund's complete adjuvant (FCA)-induced arthritis rat's blood plasma (Kakinuma et al., 2010). In addition, brucine itself also inhibited the hypoxia-inducible factor 1 (HIF-1) pathway for its anti-metastasis activity and attenuated HIF-1 target genes, i.e., fibronectin, matrix metallopeptidase 2 (MMPs-2), and cathepsin D, and downregulated the expression levels of HIF-1 responsive genes *in vivo* (Chisholm et al., 2012). Thirdly, it is also reported that brucine inhibited the VEGF-induced neovascularization and the downstream protein kinases of vascular endothelial growth factor 2 (VEGF2), and then reduced the production of VEGF, nitric oxide (NO), interleukin-6 (IL-6), IL-8, as well as the tumor necrosis factor-α (TNF-α) in HUVECs (Saraswati and Agrawal, 2013). Besides, some other alkoids we predicted as active compounds of *Semen Strychni* are also proven with RA-associated pharmacological effects. A good illustration is strychnine which actively interacted with the epidermal growth factor receptor (EGFR) and thus inhibited the cell proliferation of HEK293/EGFR cells as well as the Erk phosphorylation (Murohara, 2012).

In addition, some reported experimentally therapeutically compounds with inadequate OB are also chosen as candidate compounds due to the following reasons: 1) some active compounds are abundant in *Semen Strychni*, such as isostrychnine, (-)-Brucine, ursolic acid (UN, 2007). Therefore, their large quantity of content, to some extent, compensates for their low OB; 2) Chlorogenic acid though exhibits poor OB of 11.93%, displayed functions of anti-tumor and inflammation (Wang et al., 2013c). Actually, it is a conjugate of quinic (OB＝59.20%) and caffeic (OB＝66.70%) acids, while the latter two molecules both exert properties of anti-tumor and anti-thrombosis (Wang et al., 2013c). This implies that chlorogenic acid may exert pharmacodynamics effects in the form of its catabolites.

In summary, 27 ingredients from *Semen Setrychni* are chosen as active compounds for further study which are assumed playing pivotal role in the treatment of RA.

TwHF. TwHF belongs to the Celastraceae tripterygium plant, whose official part is roots, the extracts of which are usually therapeutically active. As a matter of fact, the roots have been demonstrated of not only significantly relieving the symptom of collagen-induced arthritis (CIA) rats, down-regulating the production of IL-6, IL-8, TNF-α in serum, the expression of IL-6, IL-8, nuclear factor kappa B (NF-κB) and TNF-α in synovial tissue, but also potently inhibiting the induction of MMPs-13 and aggrecanases by suppressing the principal inflammatory cytokines in chondrocytes (Liacini et al., 2005; Zhang et al., 2013).

Table 1. All candidate compounds of *Semen Strychni*

ID	CAS	Name	OB (%)	DL
ST-1	1373445-33-0	5-Oxopseudostrychnine	91.82	0.49
ST-2	1373445-35-2	10-Hydroxyicajine	53.08	0.74
ST-3	1373445-36-3	5-Hydroxyvomicine	56.44	0.71
ST-4	1374238-33-1	Stryvomicine	79.27	0.45
ST-5	1374238-35-3	Isopseudostrychnine	97.55	0.79
ST-6	154-23-4	Catechin	52.48	0.24
ST-7	11053-97-7	Holstiine (8CI)	64.57	0.83
ST-8	17948-42-4	Venoterpine (8CI)	53.92	0.14
ST-9	22029-94-3	21,22α-Epoxyvomicine	61.00	0.48
ST-10	22029-96-5	14-Hydroxyicajine	85.73	0.75
ST-11	118-71-8	Larixic acid	52.62	0.12
ST-12	22029-99-8	14-Hydroxy-21,22α-epoxyicajine	83.87	0.50
ST-13	30333-81-4	Cantleyine	67.73	0.18
ST-14[§]	327-97-9	Chlorogenic acid	11.93	0.33
ST-15	331-39-5	Caffeic acid	66.70	0.19
ST-16[§]	357-57-3	Brucine	42.41	0.41
ST-17	38752-93-1	Strychnine chloromethochloride	55.15	0.47
ST-18	465-62-3	ψ-Strychnine	100.00	0.53
ST-19	5096-72-0	16-Methoxystrychnine	64.85	0.50
ST-20	545-47-1	Clerodol	51.45	0.78
ST-21	5525-31-5	N-Methylpseudostrychnine	80.18	0.77
ST-22	57-24-9	Strychnine	57.23	0.57
ST-23	639-34-9	Spermostrychnin	60.19	0.79
ST-24[§]	77-52-1	Ursolic acid	17.73	0.75
ST-25	130641-44-0	Isostrychnine N-oxide	55.26	0.80
ST-26	1373445-32-9	5-Oxobrucine	57.18	0.38
ST-27[§]	467-16-3	(+)-Isostrychnine	9.22	0.80

[§] Compounds with OB＜50%, yet validated pharmaceutically.

Table 2. All candidate compounds of TwHF

ID	CAS	Name	OB (%)	DL
TW-1	37239-51-3	Wilfordine	100.00	0.14
TW-2	38647-11-9	Triptonide	68.07	0.68
TW-3	38748-32-2	Triptolide	51.02	0.68
TW-4[§]	478-01-3	Nobiletin (6CI)	25.24	0.52
TW-5	488-93-7	3-Furanoic acid	53.53	0.02
TW-6[§]	508-02-1	Oleonolic acid	28.57	0.76
TW-7	53990-48-0	Caesalpinine C	95.18	0.59
TW-8[§]	559-74-0	Friedelan-3-one	29.16	0.76
TW-9	56121-42-7	Sarapeptate	58.02	0.52
TW-10	58-08-2	Cafeina	89.89	0.08
TW-11[§]	77-52-1	β-Ursolic acid	17.73	0.75
TW-12	79548-61-1	Triptonolide	51.34	0.49
TW-13[§]	83-46-5	β-Sitosterol	36.91	0.75
TW-14	853783-85-4	1-Phenanthrenecarboxylic acid	53.31	0.35
TW-15	98618-76-9	Wilforidine	51.76	0.22
TW-16	99694-86-7	Triptolidenol	59.93	0.66
TW-17	132368-08-2	Tripchlorolide	68.24	0.72
TW-18	134306-16-4	Isowilfordine	100.00	0.14
TW-19	139122-81-9	Hypodiolide A	76.81	0.49
TW-20	1416696-25-7	2α-Hydroxytriptonide	77.52	0.66
TW-21	144539-79-7	(14α)-Triptolide	55.62	0.68
TW-22	149249-32-1	Neotripterifordine	59.68	0.49
TW-23	151636-98-5	Zhepiresinol	64.54	0.19
TW-24	161127-57-7	3-O-Methyl-22β,23-dihydroxy-6-oxotingenol	56.59	0.74
TW-25	110-15-6	Succinic acid	79.13	0.01

TW-26	21453-69-0	Syringaresinol,(+)-	52.22	0.72
TW-27	260999-77-7	16-Hydroxy-19,20-epoxy-kaurane	63.58	0.44
TW-28	26488-24-4	Cyclo-(S-Pro-R-Phe)	72.79	0.15
TW-29	329775-38-4	Tetrahydro-6,6'-dimethoxy-(3R,3'S,4R,4'S)-rel-	52.11	0.54
TW-30[§]	34157-83-0	Celastrol	14.57	0.78
TW-31	36238-67-2	Cyclo-L-prolyl-D-leucine	74.08	0.08
TW-32[§]	940909-08-0	Tripterygiol	4.03	0.57
TW-33[§]	38647-10-8	Tripdiolide	7.89	0.67

[§] compounds with OB＜50%, yet validated pharmaceutically.

Table 3. The candidate compounds of *Semen Strychni* with their corresponding targets and downstream angiogenesis mediators.

Candidates compounds	Target proteins		Downstream angiogenesis mediators[$]	Reference
	Name	ID[&]		
ST-23^	5-Hydroxytryptamine 1B receptor	5-HT-1B	eNOS	(Fujita et al., 2004; Hoyer et al., 1994; Iwabayashi et al., 2012)
ST-21^,ST-22, ST-23^, ST-27^	5-Hydroxytryptamine 2A receptor	5-HT-2A	eNOS	(Fujita et al., 2004; Hoyer et al., 1994; Iwabayashi et al., 2012)
ST-23	5-Hydroxytryptamine 2C receptor	5-HT-2C	eNOS	(Fujita et al., 2004; Hoyer et al., 1994; Iwabayashi et al., 2012)
ST-21^, ST-23^	5-Hydroxytryptamine 3 receptor	5-HT-3	eNOS	(Fujita et al., 2004; Hoyer et al., 1994; Iwabayashi et al., 2012)
ST-5, ST-7, ST-15, ST-23, ST-27	Acetylcholinesterase	AChE	TNF, IL-1, IL-1β, MIF, NF-κB, HIF-1α, VEGF, NO	(Kakinuma et al., 2010; Miyazaki et al., 2012; UN, 2007)
ST-5, ST-6^, ST-7^, ST-20, ST-23, ST-24, ST-25^, ST-27^	Androgen receptor	AR	VEGF, IGF-1	(Eisermann et al., 2013; Svensson et al., 2010)
ST-15	Beta-1 adrenergic	ADRB1	VEGF, NO,	(Chisholm et al., 2012;

	receptor		COX-2, PGE2	Wong et al., 2007b)
ST-8, ST-13, ST-15, ST-23^	Beta-2 adrenergic receptor	ADRB2	VEGF, NO, COX-2, PGE2	(Chisholm et al., 2012; Wong et al., 2007b)
ST-6^, ST-13, ST-15	Cell division protein kinase 2	CDK2		

VEGF: vascular endothelial growth factor; bFGF: basic fibroblast growth factor; HIF: hypoxia-inducible factors; TNF-α: tumor necrosis factor α; PGE2: prostaglandin E2; VCAM: vascular cell adhesion molecule; ICAM-1; intercellular adhesion molecule-1; PDGF: platelet-derived growth factor; COX-2: cyclooxygenase-2; MIF: macrophage migration inhibitory factor; EGF: epidermal growth factor; MMPs: matrix metalloproteinases; IL: interleukin; SDF-1: stromal cell-derived factor-1; TGF-β: transforming growth factor-β; NF-κB: nuclear factor kappa-B; PGI2: prostaglandin I2; IGF-1: insulin-like growth factor 1; iNOS: inducible nitric oxide synthase; eNOS: endothelial nitric oxide synthase; AP-1: activator protein-1. [&]The abbreviation name of the protein. [#]The target proteins modulating corresponding downstream angiogenesis mediators that are associated with the rheumatoid arthritis. ^The compounds with ≥ 50% score when docked with corresponding target proteins.

To be continued.

Candidates compounds	Target proteins		Downstream angiogenesis mediators[$]	Reference
	Name	ID[&]		
ST-6^, ST-13, ST-15^	Dipeptidyl peptidase 4	DPP4	SDF-1, substance P, Nitric oxide (NO)	(Murohara, 2012)
ST-23^	5-Hydroxytryptamine 1B receptor	5-HT-1B	eNOS	(Fujita et al., 2004; Hoyer et al., 1994; Iwabayashi et al., 2012)
ST-21^, ST-22, ST-23^, ST-27^	5-Hydroxytryptamine 2A receptor	5-HT-2A	eNOS	(Fujita et al., 2004; Hoyer et al., 1994; Iwabayashi et al., 2012)
ST-23	5-Hydroxytryptamine 2C receptor	5-HT-2C	eNOS	(Fujita et al., 2004; Hoyer et al., 1994; Iwabayashi et al., 2012)
ST-21^, ST-23^	5-Hydroxytryptamine 3 receptor	5-HT-3	eNOS	(Fujita et al., 2004; Hoyer et al., 1994; Iwabayashi et al., 2012)
ST-5, ST-7, ST-15, ST-23, ST-27	Acetylcholinesterase	AChE	TNF, IL-1, IL-1β, MIF, NF-κB, HIF-1α, VEGF, NO	(Kakinuma et al., 2010; Miyazaki et al., 2012; UN, 2007)
ST-5, ST-6^, ST-7^, ST-20, ST-23, ST-24,	Androgen receptor	AR	VEGF, IGF-1	(Eisermann et al., 2013; Svensson et al.,

ST-25^, ST-27^				2010)
ST-15	Beta-1 adrenergic receptor	ADRB1	VEGF, NO, COX-2, PGE2	(Chisholm et al., 2012; Wong et al., 2007b)
ST-8, ST-13, ST-15, ST-23^	Beta-2 adrenergic receptor	ADRB2	VEGF, NO, COX-2, PGE2	(Chisholm et al., 2012; Wong et al., 2007b)

VEGF: vascular endothelial growth factor; bFGF: basic fibroblast growth factor; HIF: hypoxia-inducible factors; TNF-α: tumor necrosis factor α; PGE2: prostaglandin E2; VCAM: vascular cell adhesion molecule; ICAM-1; intercellular adhesion molecule-1; PDGF: platelet-derived growth factor; COX-2: cyclooxygenase-2; MIF: macrophage migration inhibitory factor; EGF: epidermal growth factor; MMPs: matrix metalloproteinases; IL: interleukin; SDF-1: stromal cell-derived factor-1; TGF-β: transforming growth factor-β; NF-κB: nuclear factor kappa-B; PGI2: prostaglandin I2; IGF-1: insulin-like growth factor 1; iNOS: inducible nitric oxide synthase; eNOS: endothelial nitric oxide synthase; AP-1: activator protein-1. [&]The abbreviation name of the protein. [#]The target proteins modulating corresponding downstream angiogenesis mediators that are associated with the rheumatoid arthritis. ^The compounds with ≥ 50% score when docked with corresponding target proteins.

To be continued.

Candidates compounds	Target proteins		Downstream angiogenesis mediators[$]	Reference
	Name	ID[&]		
ST-2^, ST-3^, ST-5^, ST-6^, ST-7^, ST-9^, ST-10, ST-12^, ST-14^, ST-20, ST-21, ST-23, ST-24, ST-25^, ST-27^	Estrogen receptor	ER	VEGF, NO, IGF-1	(Losordo and Isner, 2001; Svensson et al., 2010)
ST-6^, ST-25^, ST-27^	Estrogen receptor beta	ER beta	HIFα	(Lim et al., 2009)
ST-6	Glycogen synthase kinase-3 beta	GSK-3 beta	β-catenin,suvivin	(Bergmann et al., 2004; Gunin et al., 2003; Kaga et al., 2006)
ST-6^	Heat shock protein HSP 90-alpha	HSP 90α	IL-8, Ap-1, NF-kB, VEGFR	(Park et al., 2008; Sanderson et al., 2006; Yeo et al., 2004)
ST-6^	Mitogen-activated protein kinase 14	MAPK14 (MAPK 38α)	TNF-α, NO, iNOS, NF-kB	(Matsumoto et al., 2000; Park et al., 2002)
ST-2, ST-5, ST-7,	Muscarinic	CHRM1	NOS,VEGF	(Fong et al., 2013;

ST-8, ST-10, ST-13, ST-16, ST-19, ST-21, ST-22, ST-23, ST-25, ST-27	acetylcholine receptor M1			Negroni et al., 2010)
ST-13^, ST-18^, ST-21^, ST-22, ST-23^, ST-27^	Muscarinic acetylcholine receptor M2	CHRM2	NOS,VEGF	(Fong et al., 2013; Negroni et al., 2010)

VEGF: vascular endothelial growth factor; bFGF: basic fibroblast growth factor; HIF: hypoxia-inducible factors; TNF-α: tumor necrosis factor α; PGE2: prostaglandin E2; VCAM: vascular cell adhesion molecule; ICAM-1; intercellular adhesion molecule-1; PDGF: platelet-derived growth factor; COX-2: cyclooxygenase-2; MIF: macrophage migration inhibitory factor; EGF: epidermal growth factor; MMPs: matrix metalloproteinases; IL: interleukin; SDF-1: stromal cell-derived factor-1; TGF-β: transforming growth factor-β; NF-κB: nuclear factor kappa-B; PGI2: prostaglandin I2; IGF-1: insulin-like growth factor 1; iNOS: inducible nitric oxide synthase; eNOS: endothelial nitric oxide synthase; AP-1: activator protein-1. [&]The abbreviation name of the protein. [#]The target proteins modulating corresponding downstream angiogenesis mediators that are associated with the rheumatoid arthritis. ^The compounds with ≥ 50% score when docked with corresponding target proteins.

To be continued.

Candidates compounds	Target proteins		Downstream angiogenesis mediators[$]	Reference
	Name	ID[&]		
ST-2^, ST-7, ST-10^, ST-13, ST-16^, ST-21^, ST-23^, ST-25^, ST-27^	Muscarinic acetylcholine receptor M3	CHRM3	NOS,VEGF, PGE2	(Fong et al., 2013; Negroni et al., 2010)
ST-21, ST-22, ST-23, ST-27	Muscarinic acetylcholine receptor M4	CHRM4	NOS, VEGF	(Fong et al., 2013; Negroni et al., 2010)
ST-2^, ST-10^, ST-21, ST-23^, ST-27^	Muscarinic acetylcholine receptor M5	CHRM5	VEGF	(Fong et al., 2013; Negroni et al., 2010)
ST-13, ST-23	Neuronal acetylcholine receptor subunit alpha-7	α7 nAChR	VEGF	(Wang et al., 2003; Wong et al., 2007a)
ST-6^, ST-13^, ST-15^	Nitric oxide synthase, inducible	iNOS	PGE2,VEGF,HIF-1α,$\alpha_v\beta_3$, TGF-,substance P	(Bao et al., 2012; Carreau et al., 2011; Cooke, 2003; Kimura and Esumi, 2003)
ST-8^, ST-13^, ST-15^	Nitric-oxide synthase, endothelial	eNOS	PGE2,VEGF,HIF-1α,$\alpha_v\beta_3$, TGF-β, substance P	(Bao et al., 2012; Carreau et al., 2011; Cooke, 2003; Kimura and Esumi, 2003)

ST-6	Nuclear receptor coactivator 2	SRC-2	Interact with ER and AR	(Bourdoncle et al., 2005; Pan et al., 2012)

VEGF: vascular endothelial growth factor; bFGF: basic fibroblast growth factor; HIF: hypoxia-inducible factors; TNF-α: tumor necrosis factor α; PGE2: prostaglandin E2; VCAM: vascular cell adhesion molecule; ICAM-1; intercellular adhesion molecule-1; PDGF: platelet-derived growth factor; COX-2: cyclooxygenase-2; MIF: macrophage migration inhibitory factor; EGF: epidermal growth factor; MMPs: matrix metalloproteinases; IL: interleukin; SDF-1: stromal cell-derived factor-1; TGF-β: transforming growth factor-β; NF-κB: nuclear factor kappa-B; PGI2: prostaglandin I2; IGF-1: insulin-like growth factor 1; iNOS: inducible nitric oxide synthase; eNOS: endothelial nitric oxide synthase; AP-1: activator protein-1. [&]The abbreviation name of the protein. [#]The target proteins modulating corresponding downstream angiogenesis mediators that are associated with the rheumatoid arthritis. ^The compounds with ≥ 50% score when docked with corresponding target proteins.

To be continued.

Candidates compounds	Target proteins		Downstream angiogenesis mediators[$]	Reference
	Name	ID[&]		
ST-6^	Peroxisome proliferator-activated receptor gamma	PPAR-γ	VEGF,VCAM,MMP9,iNOS, endothelin-1,COX-2,TNF-α, ICAM-1	(Arany et al., 2008; Biscetti et al., 2009; Bongartz et al., 2005; Jiang et al., 1998; Jung et al., 2008; Lee et al., 2003)
ST-6^, ST-7, ST-13^, ST-15^	Prostaglandin G/H synthase 1	COX-1		
ST-6^, ST-13^, ST-15	Prostaglandin G/H synthase 2	COX-2		
ST-7, ST-8, ST-13, ST-15	Prothrombin	Thrombin	NO,PGI2,ICAM-1	(Cao et al., 2006; Duarte et al., 2006; Fritzen et al., 2005; Mitsos et al., 2014)

VEGF: vascular endothelial growth factor; bFGF: basic fibroblast growth factor; HIF: hypoxia-inducible factors; TNF-α: tumor necrosis factor α; PGE2: prostaglandin E2; VCAM: vascular cell adhesion molecule; ICAM-1; intercellular adhesion molecule-1; PDGF: platelet-derived growth factor; COX-2: cyclooxygenase-2; MIF: macrophage migration inhibitory factor; EGF: epidermal growth factor; MMPs: matrix metalloproteinases; IL: interleukin; SDF-1: stromal cell-derived factor-1; TGF-β: transforming growth factor-β; NF-κB: nuclear factor kappa-B; PGI2: prostaglandin I2; IGF-1: insulin-like growth factor 1; iNOS: inducible nitric oxide synthase; eNOS: endothelial nitric oxide synthase; AP-1: activator protein-1. [&]The abbreviation name of the protein. [#]The target proteins modulating corresponding downstream angiogenesis mediators that are associated with the rheumatoid arthritis. ^The compounds with ≥ 50% score when docked with corresponding target proteins.

Table 4. The candidate compounds of TwHF with their corresponding targets and downstream angiogenesis mediators.

Candidates compounds	Target proteins		Downstream angiogenesis mediators[$]	Reference
	Name	ID[&]		
TW-3^, TW-12^, TW-14^, TW-25, TW-26^, TW-27, TW-29^, TW-31^, TW-32^	Acetylcholinesterase	AChE	TNF, IL-1, IL-1β, MIF, NF-κB, HIF-1α, VEGF, NO	(Kakinuma et al., 2010; Miyazaki et al., 2012; UN, 2007)
TW-10	Adenosine A1 receptor	ADORA1	VEGF	(Clark et al., 2007; Headrick et al., 2013)
TW-10	Adenosine A2a receptor	ADORA2A	Tie-2, VEGF	(Hara et al., 2009; Headrick et al., 2013; Montesinos et al., 2004)
TW-25	Aldose reductase	AKR1B1	VEGF, VCAM,IL-13,NF-κB	(Jiang et al., 2012; Yadav et al., 2012)
TW-3^,TW-4^,TW-6,TW-7, TW-8, TW-11, TW-12, TW-13, TW-14^, TW-19^, TW-22^, TW-26, TW-27^, TW-29^, TW-30, TW-32	Androgen receptor	AR	VEGF, IGF-1	(Eisermann et al., 2013; Svensson et al., 2010)
TW-10^, TW-12^, TW-28	Beta-2 adrenergic	ADRB2	VEGF, NO, COX-2,	(Chisholm et al.,

	receptor		PGE2	2012; Wong et al., 2007b)
TW-4^, TW-12, TW-26^, TW-29^, TW-32^	Calmodulin	CaM	TNF-α, EGFR, Type I collagen, tyrosine kinase, E-selectin	(Chen et al., 2002; Li et al., 2004)

VEGF: vascular endothelial growth factor; bFGF: basic fibroblast growth factor; HIF: hypoxia-inducible factors; TNF-α: tumor necrosis factor α; PGE2: prostaglandin E2; VCAM: vascular cell adhesion molecule; ICAM-1; intercellular adhesion molecule-1; PDGF: platelet-derived growth factor; COX-2: cyclooxygenase-2; MIF: macrophage migration inhibitory factor; EGF: epidermal growth factor; MMPs: matrix metalloproteinases; IL: interleukin; SDF-1: stromal cell-derived factor-1; TGF-β: transforming growth factor-β; NF-κB: nuclear factor kappa-B; PGI2: prostaglandin I2; IGF-1: insulin-like growth factor 1; iNOS: inducible nitric oxide synthase; eNOS: endothelial nitric oxide synthase; AP-1: activator protein-1.&The abbreviation name of the protein.#The target proteins modulate corresponding downstream angiogenesis mediators associated with the rheumatoid arthritis. ^The compounds with ⩾ 50% score when docked with corresponding target proteins.

To be continued.

Candidates compounds	Target proteins		Downstream angiogenesis mediators[$]	Reference
	Name	ID[&]		
TW-25	Cathepsin D	CTSD	bFGF	(Berchem et al., 2002)
TW-12, TW-28	Cell division protein kinase 2	CDK2		
TW-4^, TW-32^	Coagulation factor VII	F7	Thrombin	(Schaub, 2011)
TW-4^, TW-7^, TW-9^, TW-12, TW-26^, TW-32^	Coagulation factor X	F10	Thrombin	(Schaub, 2011)
TW-4^, TW-10, TW-12^, TW-25, TW-28, TW-29^, TW-31	Dipeptidyl peptidase 4	DPP4	SDF-1, substance P, Nitric oxide(NO)	(Murohara, 2012)
TW-3, TW-4^, TW-7^, TW-8, TW-9^, TW-11, TW-12, TW-13^, TW-14^, TW-19^, TW-22, TW-26^, TW-27^, TW-28, TW-29^, TW-32^	Estrogen receptor	ER	VEGF, NO, IGF-1	(Losordo and Isner, 2001; Svensson et al., 2010)
TW-4, TW-12, TW-26, TW-29,	Estrogen receptor beta	ER beta	HIFα	(Lim et al., 2009)
TW-3, TW-7^, TW-12, TW-29^, TW-32^	Glycogen synthase kinase-3 beta	GSK-3 beta	β-catenin, suvivin	(Bergmann et al., 2004; Gunin et al.,

				2003; Kaga et al., 2006)
TW-4^, TW-26, TW-29, TW-32^	Heat shock protein HSP 90-alpha	HSP 90α	IL-8, Ap-1, NF-kB, VEGFR	(Park et al., 2008; Sanderson et al., 2006; Yeo et al., 2004)

VEGF: vascular endothelial growth factor; bFGF: basic fibroblast growth factor; HIF: hypoxia-inducible factors; TNF-α: tumor necrosis factor α; PGE2: prostaglandin E2; VCAM: vascular cell adhesion molecule; ICAM-1; intercellular adhesion molecule-1; PDGF: platelet-derived growth factor; COX-2: cyclooxygenase-2; MIF: macrophage migration inhibitory factor; EGF: epidermal growth factor; MMPs: matrix metalloproteinases; IL: interleukin; SDF-1: stromal cell-derived factor-1; TGF-β: transforming growth factor-β; NF-κB: nuclear factor kappa-B; PGI2: prostaglandin I2; IGF-1: insulin-like growth factor 1; iNOS: inducible nitric oxide synthase; eNOS: endothelial nitric oxide synthase; AP-1: activator protein-1.&The abbreviation name of the protein.#The target proteins modulate corresponding downstream angiogenesis mediators associated with the rheumatoid arthritis. ^The compounds with ⩾ 50% score when docked with corresponding target proteins.

To be continued.

Candidates compounds	Target proteins		Downstream angiogenesis mediators[$]	Reference
	Name	ID[&]		
TW-3, TW-14^, TW-19^, TW-22, TW-27	Mineralocorticoid receptor	MR	VEGFR-2	(Fujii et al., 2012)
TW-12, TW-28	Muscarinic acetylcholine receptor M1	CHRM1	NOS, VEGF	(Fong et al., 2013; Negroni et al., 2010)
TW-12^, TW-28^	Muscarinic acetylcholine receptor M2	CHRM2	NOS, VEGF	(Fong et al., 2013; Negroni et al., 2010)
TW-12^	Muscarinic acetylcholine receptor M3	CHRM3	NOS, VEGF, PGE2	(Fong et al., 2013; Negroni et al., 2010)
TW-12^	Muscarinic acetylcholine receptor M5	CHRM5	VEGF	(Fong et al., 2013; Negroni et al., 2010)
TW-12	Neuronal acetylcholine receptor subunit alpha-7	α7 nAChR	VEGF	(Wang et al., 2003; Wong et al., 2007a)
TW-3^, TW-4^, TW-7^, TW-10, TW-12^, TW-14^, TW-25, TW-26^, TW-29^, TW-31, TW-32^	Nitric oxide synthase, inducible	iNOS	PGE2, VEGF, HIF-1α,$\alpha_v\beta_3$, TGF-β, substance P	(Bao et al., 2012; Carreau et al., 2011; Cooke, 2003; Kimura and Esumi, 2003)

TW-10^, TW-25, TW-29^, TW-31	Nitric-oxide synthase, endothelial	eNOS	PGE2, VEGF, HIF-1α, $\alpha_v\beta_3$, TGF-β, substance P	(Bao et al., 2012; Carreau et al., 2011; Cooke, 2003; Kimura and Esumi, 2003)
TW-12, TW-14	Nuclear receptor coactivator 1	SRC-1	Interact with ER and AR	(Bourdoncle et al., 2005; Pan et al., 2012)

VEGF: vascular endothelial growth factor; bFGF: basic fibroblast growth factor; HIF: hypoxia-inducible factors; TNF-α: tumor necrosis factor α; PGE2: prostaglandin E2; VCAM: vascular cell adhesion molecule; ICAM-1; intercellular adhesion molecule-1; PDGF: platelet-derived growth factor; COX-2: cyclooxygenase-2; MIF: macrophage migration inhibitory factor; EGF: epidermal growth factor; MMPs: matrix metalloproteinases; IL: interleukin; SDF-1: stromal cell-derived factor-1; TGF-β: transforming growth factor-β; NF-κB: nuclear factor kappa-B; PGI2: prostaglandin I2; IGF-1: insulin-like growth factor 1; iNOS: inducible nitric oxide synthase; eNOS: endothelial nitric oxide synthase; AP-1: activator protein-1.&The abbreviation name of the protein.#The target proteins modulate corresponding downstream angiogenesis mediators associated with the rheumatoid arthritis. ^The compounds with ⩾ 50% score when docked with corresponding target proteins.

To be continued.

Candidates compounds	Target proteins		Downstream angiogenesis mediators[$]	Reference
	Name	ID[&]		
TW-3, TW-4, TW-12, TW-14, TW-26, TW-32	Nuclear receptor coactivator 2	SRC-2	Interact with ER and AR	(Bourdoncle et al., 2005; Pan et al., 2012)
TW-4^, TW-7^, TW-9^, TW-12^, TW-29^, TW-32^	Peroxisome proliferator-activated receptor gamma	PPAR-γ	VEGF, VCAM, MMP9, iNOS, endothelin-1, COX-2, TNF-α, ICAM-1	(Arany et al., 2008; Biscetti et al., 2009; Bongartz et al., 2005; Jiang et al., 1998; Jung et al., 2008; Lee et al., 2003)
TW-12^, TW-13	Progesterone receptor	PR	VEGF, eNOS	(Chwalisz et al., 2000)
TW-10^, TW-28^	Prostaglandin G/H synthase 1	COX-1		
TW-3^, TW-4^, TW-7^, TW-9^, TW-10, TW-12^, TW-14^, TW-26^, TW-28^, TW-29^, TW-31, TW-32^	Prostaglandin G/H synthase 2	COX-2		
TW-4^, TW-7, TW-9^, TW-10, TW-12, TW-14,	Prothrombin	Thrombin	NO, PGI2, ICAM-1	(Cao et al., 2006; Duarte et al., 2006; Fritzen et al., 2005;

TW-25, TW-26^,TW-28^, TW-29, TW-31, TW-32^				Mitsos et al., 2014)
TW-25	Proto-oncogene tyrosine-protein kinase Src	c-Src	PDGF	(Amanchy et al., 2009)

VEGF: vascular endothelial growth factor; bFGF: basic fibroblast growth factor; HIF: hypoxia-inducible factors; TNF-α: tumor necrosis factor α; PGE2: prostaglandin E2; VCAM: vascular cell adhesion molecule; ICAM-1; intercellular adhesion molecule-1; PDGF: platelet-derived growth factor; COX-2: cyclooxygenase-2; MIF: macrophage migration inhibitory factor; EGF: epidermal growth factor; MMPs: matrix metalloproteinases; IL: interleukin; SDF-1: stromal cell-derived factor-1; TGF-β: transforming growth factor-β; NF-κB: nuclear factor kappa-B; PGI2: prostaglandin I2; IGF-1: insulin-like growth factor 1; iNOS: inducible nitric oxide synthase; eNOS: endothelial nitric oxide synthase; AP-1: activator protein-1.&The abbreviation name of the protein.#The target proteins modulate corresponding downstream angiogenesis mediators associated with the rheumatoid arthritis. ^The compounds with $\geqslant$ 50% score when docked with corresponding target proteins.

In this work, 174 compounds of various types have been identified in the root of TwHF, including diterpinoids, triterpinoids, sesquiterpinoids, alkaloids, β-sitosterol, dulcitol, glycosides and diterpinoids (Zhang et al., 2013), out of which 25 molecules demonstrate good bioavailability (≥50%). Among these ingredients, triptolide, triptonide, tripchlorolide and triptolidenol exhibited immunosuppressive activity and significant inhibitory property for the proliferation of T and B lymphocytes. For instance, triptolide could reduce PGE2 production via COX-2 gene suppression, and interfere with the CIA-augmented expression of MMPs-13 and -3, and simultaneously augment the CIA-reduced tissue inhibitors of metalloproteinases-1 and -2 expressions in the joints (Lin et al., 2007).

Some reported therapeutically active compounds with low OB value, such as tripterygiol, celastrol and nobiletin, were also treated as candidate compounds due to their abundant quantity (Hoyer et al., 1994). Tripterygiol demonstrated its anti-inflammation activities by down-regulating the COX-2, inducible nitric oxide synthase (iNOS), as well as the IL-1b genes' expression in LPS-elicited mouse macrophages. As to the triterpenoid celastrol, it could inhibit the production of pro-inflammatory cytokines IL-1b and TNF-α (Ma et al., 2007). Nobiletin demonstrated novel anti-inflammatory actions on human synovial fibroblasts, and it could also down-regulate the COX-2 and IL-1-induced production of PGE2, as well as interfere the gene expression of pro-inflammatory cytokines, e.g., IL-1α, IL-1e, TNF-e, IL-6 in mouse macrophages (Losordo and Isner, 2001).

In brief, 33 ingredients from TwHF are assumed playing major roles in the treatment of RA, and thus are chosen as active compounds for further study.

Target prediction and validation. As mentioned above, for the two herbs totally 60 compounds are screened out, but their specific acting targets as well as the interaction mechanism still remain a mystery. These questions are quite difficult to be answered by solely the experimental methods due to the limitations of large expense, long-term investment, etc. Besides, up to date a systematic and overall investigation report is still unavailable. Thus, a screening method based on the combinational use of RF and SVM (Wang et al., 2013c) was applied to the active ingredients presently.

As a result, a total of 118 targets are predicted interacting with the 60 ingredients of the two herbs. To validate the relevance of these proteins with RA, every protein was searched and verified to determine whether they relate with the angiogenesis mediators directly or indirectly. It turns out that 39 proteins have high relevance with angiogenesis, acting as intermediate to regulate various angiogenesis mediators, as shown in Tables 3 and 4.

To validate the reliability of these targets, docking analyses were further conducted to explore the ligand-protein interactions. In fact, all targets were docked with corresponding active compounds to estimate the ligand-protein interactions except for four proteins with no crystallographic structures available. The results are listed as follows: 1) 28 proteins achieve high docking score (≥50%) when docked with most of their corresponding ligands, which inspires us to go further investigations. As seen from Tables 3 and 4, 15 targets exhibit high binding affinity with above 70% of their corresponding filtered ligands. It is worth mentioning that triptolide, nobiletin and trypterygiol exhibit relatively high binding affinity with COX-2 with docking score 63.2%, 70.1% and 50.0%, respectively. In addition, triptolide and trypterygiol interact with iNOS with docking scores of 63.2% and 75.7%, respectively. These ligand-protein interactions correlate well with those experimental results as mentioned above. The simulation results, to some extent,

indicate good filtered and predicted accuracy as well.

2) 7 proteins, c-Src, cathepsin D, ADRB1, AKR1B1, ADORA2A, ADORA1 and 5-HT-2C, obtain low docking scores that are all less than 50%, indicating weak binding affinity with corresponding ligands. This may be due to the non-human protein source or the rigid pocket of protein crystal structure.

To summarize and visualize, some important mediators and inhibitors of angiogenesis, which are deemed to be crucial for the RA treatment, are shown in Tables 3 and 4 with corresponding protein and compounds. For example, triptolide (TW-3), triptonolide (TW-12), syringaresinol (TW-26) and tripterygiol (TW-32) may interact with protein acetylcholinesterase, and in this way further regulate the angiogenesis mediators such as IL-1, HIF-1 α, NF-κB and VEGF, and achieve the therapeutic effect. That means the ingredients from the two herbs not only directly act on the predicted targets, but also indirectly regulate the angiogenesis mediators to affect the RA microenvironment through the targets.

Discussion

RA starts with chronic and systemic inflammation and angiogenesis in synovial cavity, the situation of which, if uncontrolled, would lead to synovial pannus formation and osteoporosis, and then finally to joint destruction and bone resorption, as seen in Figure 1. At the early stage of RA, synovial inflammation, the generalized osteoporosis, has already perturbed the balance between the osteoclast-mediated bone resorption and osteoblast-mediated bone formation (Karmakar et al., 2010). Thus we focus on the angiogenesis control at the initiation of RA, which mainly involves the blockage of inflammatory cascades, expecting to attenuate the actions of angiogenic mediators, synovial angiogenesis, and thus to partially reverse the erosive bone damage. This has drawn attention to the need for effective disease-suppression therapy to prevent the destructive progression of RA.

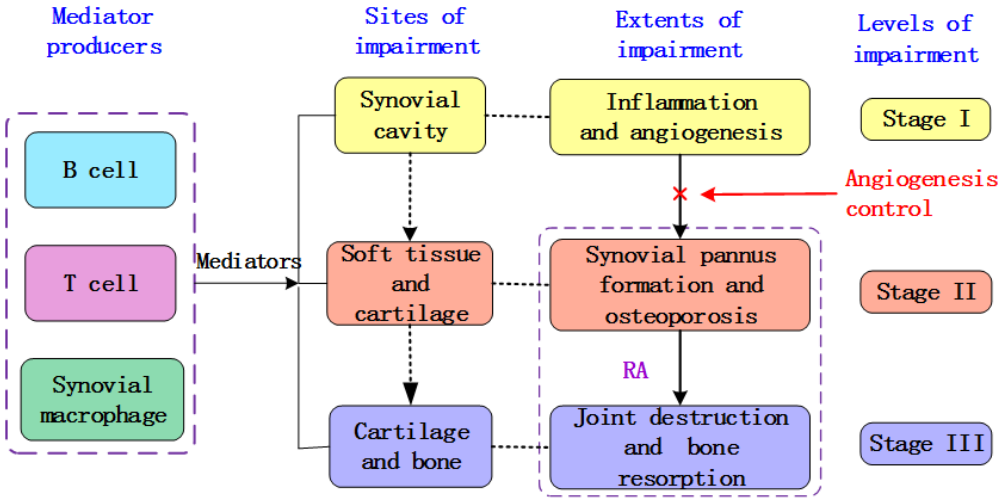

Figure 1. The destructive progression of RA and the focus of angiogenesis control

Fortunately, thousands of years' clinical practices have proven the *in vivo* efficacy and safety of traditional Chinese herbs (Ehrman et al., 2007), which show great prospect in the treatment of system diseases for their synergistic effects through the strategies of multi-targeting. However, it is difficult to identify the potential therapeutic targets of the Chinese herbs as well as

to understand the functional mechanism of botanic drugs. Thanks to the recent advances in systems biology, computational technologies and medicines, it is possible to predict the potential targets, to unveil the synergistic effects on the molecular mechanism, as well as to assist the subsequent development of network-based multi-component drugs.

The target analysis. From the perspective of direct or indirect functions, the pathways that involve those candidate compounds for treating RA can be classified into two types, i.e., the direct and indirect pathways. In former type, the active compounds directly act on some angiogenesis mediators, followed by the affection of the RA progression. Whereas, in the latter one, the active compounds work on certain protein targets which further indirectly regulate the downstream angiogenesis mediators, and in this way achieve the final therapeutic effect.

As mentioned above, a total of 39 proteins are screened out associated with the angiogenesis targeted by *Semen Strychni* and/or TwHF, among which 21 are overlapped targets. Interestingly, out of them, 4 proteins belong to direct pathway, and thus are analyzed firstly in the present work.

Direct pathway: targeting the proteins directly associated with RA. Some compounds directly target pro-inflammatory mediators, such as Prostaglandin G/H synthase 1 (COX-1), Prostaglandin G/H synthase 2 (COX-2) and prothrombin, and they play pivotal role for the promotion of angiogenesis and the development of arthritis (2). Compared to them (COX-1/2 and prothrombin), peroxisome proliferator-activated receptor γ (PPARγ) also acts as a direct overlapped target of TwHF and *Semen Strychni*, but exhibits an opposite effect. This indicates that though these targets all get involved in direct modulation of the RA development, their specific functions may be opposite, i.e., either by promoting or suppressing the angiogenesis process.

As a target protein with promotion effect on the angiogenesis, prothrombin (thrombin or coagulation factor 2) stimulates the cell proliferation and motility via indirectly regulating and organizing angiogenic molecules, and plays an important role in the initiation of angiogenesis. (Gunin et al., 2003; Lim et al., 2009). In addition, prothrombin also increases the NO generation, and the IL-8 and PGI2 release without the treatment of Lopap, which is a prothrombin activator (Gunin et al., 2003).

Similar to prothrombin, COX-1 and COX-2 also promote the angiogenesis process, which express abundantly in joints of RA patients. Researchers found that the genetic deletion of COX-1 is associated with a significant reduction of PGE2 levels, which suppresses the mouse mammary tumor growth and angiogenesis (Kaga et al., 2006). Additionally, studies show that COX-2 null mice suffer less from the pathogenesis of arthritis (Kaga et al., 2006). Besides, COX-2 inhibitors also exhibited selective inhibition of PGE2 and basic fibroblast growth factor (bFGF), and demonstrated functions in relieving both pain and inflammation with less adverse side effects when being compared to those nonsteroidal anti-inflammation drugs (Bergmann et al., 2004; Woods et al., 2003). Amazingly, the down-regulation of COX-2 by TwHF was experimentally proven, e.g., triptolide (TW-3) (Ma et al., 2007), tripterygiol (TW-32) (Ma et al., 2007), and nobiletin (TW-4) (Losordo and Isner, 2001), as shown in Table 4.

As a protein with suppressing effects on angiogenesis, PPAR γ is also capable of regulating and organizing angiogenesis and inflammation. The activation of PPAR γ could significantly reduce the expression of proinflammatory mediators, such as MMP9, iNOS and COX-2, and inhibit the angiogenesis which may further inhibit the chronic inflammation, e.g. RA (Jung et al., 2008). Furthermore, PPAR γ together with PGC-1 α (PPAR γ coactivator 1α) are also involved in HIF-independent regulatory pathway (Yeo et al., 2004).

In short, though both two herbs target angiogenesis mediators through direct pathway, their specific functions are not exactly the same. In addition, the curative effects of the two herbs also vary, which may be due to the difference of those downstream angiogenesis mediators regulated through the indirect pathway.

Indirect pathway: targeting those proteins indirectly associated with RA via downstream angiogenesis mediators. Out of the 39 targets of the two herbs, as shown in Tables 3 and 4, 35 proteins are associated with angiogenesis via an indirect interaction with certain downstream angiogenesis mediators. To illustrate this specific function, some proteins targeted by more than 10 candidate compounds, like iNOS and CHRMs, are discussed in details as below.

iNOS mediates the production of NO, which modulates the adhesion molecule expression and regulates angiogenesis by modifying cell adhesion (Carreau et al., 2011). As to NO, it mediates both the upstream and downstream of VEGF-mediated angiogenesis. And the reciprocal regulation between NO and VEGF is involved in HIF-1 mediated pathway, where iNOS plays a pivotal role (Kimura and Esumi, 2003).

In the proposed cellular pathway of acetylcholine (ACh)-induced proliferation of human tendon cells, ACh stimulates the muscarinic ACh receptors (mAChR or CHRMs). Then mAChR activates MMPs, which cleaves a cell-surface-associated EGFR ligand. Through the phosphorylation of mitogen-activated protein kinases and extracellular-signal-regulated kinases 1 and 2, the ligand binds to EGFR and then increases the cell proliferation (Park et al., 2008). In addition, mAChR activation triggers the PGE2 synthesis and VEGF production, which are angiogenesis mediators, and promotes the neovascularization (Park et al., 2002). Besides those candidate targets as discussed above, other 31 targets with their downstream mediators are also summarized in Tables 3 and 4.

Network construction and analysis. RA is a heterogeneous and chronic systemic disease with high association with angiogenesis, which is a programmed sequence of angiogenesis mediators that are produced by endothelial cells in synovium. Thus, angiogenesis control would stop or ameliorate this negative trend during the development of RA, which finally leads to the bone resorption and joint destruction. The multi-targeting strategy of herbs may provide drug researchers a new insight that it may be more efficacious in jamming angiogenic cascades, complex regulatory network of synovial angiogenesis, by affecting enough mediators to produce a therapeutic benefit yet without serious side effects. In this work, the multi-targeting mechanism of the two herbs is revealed by the techniques of pharmacological network, which may offer in-depth exploration into biologically relevant patterns.

As mentioned above, 118 predicted targets are sorted out, out of which 39 proteins are finally screened out as related to RA. The other 79 proteins are found irrelevant to RA and thus are not involved in the building of the networks for further analysis, though most of them may still be the therapeutic targets for treating other diseases (like SLE, ankylosing spondylitis and psoriasis).

Additionally, 60 active compounds are screened out of all ingredients of TwHF and *Semen Strychni*. Further study shows that 17 active compounds target those proteins that have little relevance with angiogenesis, thus only 43 active compounds may account for the curative effects of TwHF and *Semen Strychni*. Thereby, based on these outcomes, an active compound-potential target network (C-T net) related to the treatment of RA is built (as shown in Fig.2), with purpose to investigate the pharmacological mechanisms of *Semen Strychni* and TwHF for the treatment of RA.

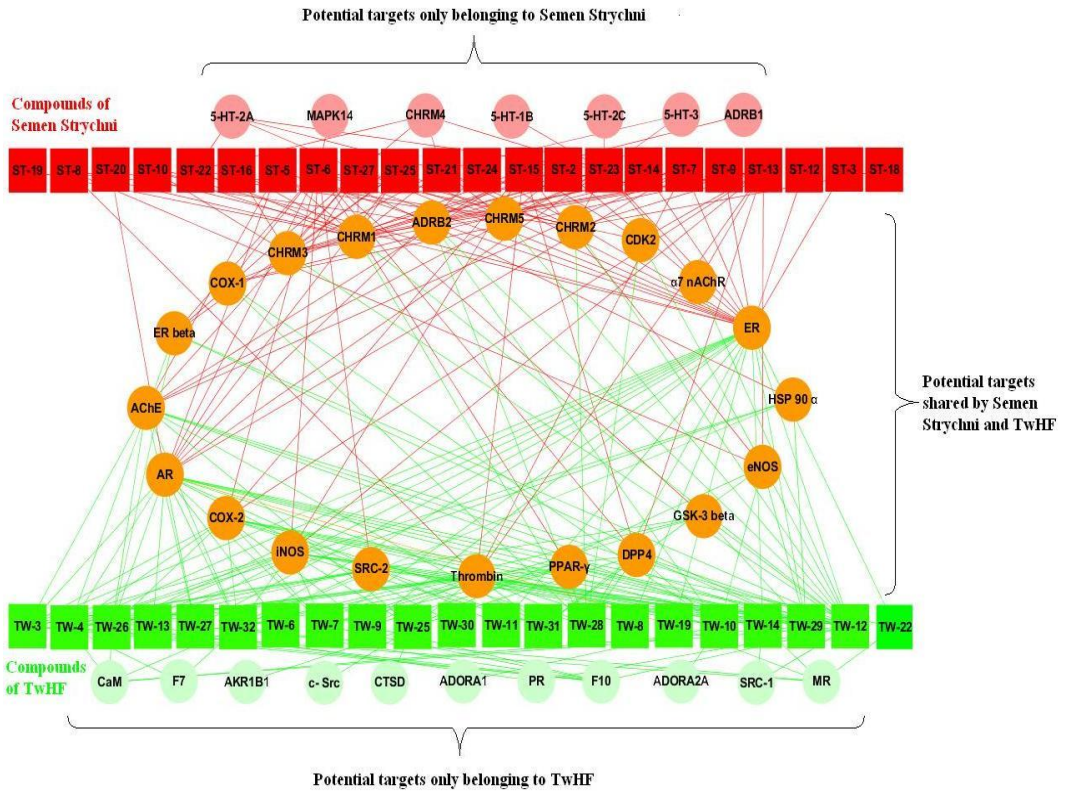

Figure 2. Network of the candidate compounds and candidate targets. 43 active compounds (ST-/TW-, rectangle) map 39 potential protein targets (short name referred in Tables 3 and 4, circle). The 22 red rectangles are active compounds from *Semen Strychni* and the 21 bright green ones represent those from TwHF. The 7 dark pink circles on the upper side are the potential proteins hit by the compounds of *Semen Strych*ni and 11 tea green ones on the bottom side are the potential proteins only targeted by the compounds of TwHF. The 21 orange circles which form anellipse are the proteins targeted by those compounds identified from both the *Semen Strychni* and TwHF.

Since *Semen Strychni* and TwHF are both typical Chinese herbs for treating RA, they are analyzed separately, and later a conjoint analysis is also carried out.

***Semen Strychni* C-T analysis.** As seen from Fig. 2, for *Semen Strychni*,

1) 22 active compounds are screened out, which may take major responsibility for the treatment of RA. Interestingly, only one component, ursolic acid (ST-24 or TW-11), is shared by *Semen Strychni* and TwHF. It is reported that ursolic acid could suppress LPS-induced cytokine production, such as iNOS and IL-8, and dramatically inhibit the PGE2 production and radiological changes in joint of CFA-induced arthritic rats (Wang et al., 2013b). Other 21 active compounds, though diverse in both structures and chemical properties, can mostly be classified as organic acids, alkaloids, flavonoids and flavanols, among which brucine and strychnine are the main active compounds in *Semen Strychni*.

2) 28 targets are predicted getting involved in the treatment of RA, in which 7 proteins belong to *Semen Strychni* solely, other 21 ones belong to both *Semen Strychni* and TwHF.

The 7 targets owned by *Semen Strychni* alone are 5-HT-2A, 5-HT-1B, 5-HT-2C, 5-HT-3,

MAPK14, CHRM4 and ADRB1, in which the first four belong to 5-HT receptors. In human EC, 5-HT significantly stimulates the eNOS expression and increases the tubule formation. In the vasculature, vasoconstriction could be induced by 5-HT through 5-HT2A, while vasodilatation is believed to be mediated through eNOS production via 5-HT1B. Selective blockage of 5-HT1B may lead to a decrease in NO production via the Akr/eNOS pathway and arrest angiogenesis (Hoyer et al., 1994; Iwabayashi et al., 2012). Among other 21 targets that are shared with TwHF, 4 of them play a role via direct pathway, while the other 17 targets function via indirect pathway.

3) As shown in Fig.2, 22 compounds hit 28 targets, and 5 active compounds have relatively higher degree distribution with each hitting more than 10 potential targets. They are ST-6 (catechin), ST-13 (cantleyine), ST-15 (caffeic acid), ST-23 (spermostrychnin) and ST-27 ((+)-isostrychnine), which may be more pharmacologically important for targeting multiple proteins (Jeong et al., 2001).

4) *Semen Strychni* has been demonstrated with pharmacological properties for relieving rheumatic pain and reducing swelling. However, the possible poison it may create such as the nephrotoxicity (Matsumoto et al., 2000) also draws attention for its safe use. In recent years, the cases of serious bad effects caused by this herb orally administrated in decoction are though rare, but not nonexistent (Fong et al., 2013; Matsumoto et al., 2000). The reasons may be as follows:

a) Strychnine, which is abundant in *Semen Strychni*, is well known to be a deadly poison, and its lethal dose for human being varies between 30 and 120 mg (Chen et al., 2012). Compared to brucine alone treatment, relatively higher pharmacological activity has been achieved by removing most strychnine from *Semen Strychni* via detoxification processing (Eisermann et al., 2013), which may be due to the synergism between the significant decrease of strychine and the relative increase of brucine. Therefore, the detoxification processing of *Semen Strychni* is necessary (Eisermann et al., 2013), which can greatly reduce the toxicity and improve corresponding anti-inflammatory activity (Chen et al., 2012). Actually, there are many detoxification processing ways to treat the *Semen Strychni*, such as deep frying, vinegar pickling, baking and hot sand stirred frying. Compared to unprocessed *Semen Strychni*, the content of strychnine significantly declines but varies during these different processing procedures. As a matter of fact, even using the same processing method, like the hot sand stirred frying which is included in Pharmacopoeia, the content still varies, because different workers perform the processing solely based on their own individual, and also different experiences (Negroni et al., 2010).

b) The dosage of *Semen Strychni* is crucial for the safe use, but hard to measure precisely in practical use. As described above, the contents of alkaloids, like strychnine and brucine, in processed *Semen Strychni* vary. Moreover, the different age, gender and physical conditions of patients also increase the uncertainty of safe dosage which patients can tolerate.

In fact, it has already been found that the high dose of several active components from *Semen Strychni*, like strychnine, may do harm to the renal tubular epithelial cell, which contributes to the kidney failure and uremia (Wang et al., 2003). Once consumed, strychnine could produce uncontrollable muscle contractions, severe lactic acidosis and rhabdomyolysis, that eventually results in death (Matsumoto et al., 2000; Negroni et al., 2010). As shown in Table 3 and Figure 2, *Semen Strychni* specifically targets some proteins such as 5-HT receptors, which are actually associated with the vasoconstriction. And the temporary vasoconstriction of intra-renal arteries could lead to reversible renal ischemia, which may in turn cause acute renal failure (Wong et al., 2007a).

TwHF C-T analysis. For TwHF, also seen in Figure 2,

1) 21 ingredients from TwHF are screened out as active compounds, in which just one component, i.e., ursolic acid (ST-24 or TW-11), is shared by TwHF and *Semen Strychni*. Other 20 candidate components are organic acids, alkaloids, flavanols, terpenes, sterols, etc.

2) These active compounds target 32 proteins which may get involved in the treatment of RA. Among them, 21 proteins are also targeted by *Semen Strychni*, and the rest 11 ones are TwHF solely-owned targets, including CaM, F7, F10, AKR1B1, c-Src, CTSD, ADORA1, PR, ADORA2A, SRC-1 and MR.

It is reported that F7, F10, together with thrombin, are all important players in inflammation, angiogenesis and vascular development (Schoenmakers et al., 2005). Moreover, PR, together with AR and ER, are the targeted proteins hit by compounds from TwHF. They three, also called sex hormones or steroids, all belong to the nuclear receptor subfamily 3 which play a pivotal role in female reproduction. In Fig.2, only two compounds, TW-39 (triptonolide) and TW-40 (β-sitosterol), map to PR out of which the former one achieves high docking score with this protein, while AR and ER are each extensively targeted by 16 compounds of TwHF. This indicates that AR and ER play important roles in the treatment of RA, but whether their interactions with TwHF play a dominant role still remains unknown, which would be elaborated in the following part.

3) As a matter of fact, several experimental reports have proven the close correlation between RA and sex hormones:

i) In many women, the RA activity diminishes during pregnancy and becomes worse after delivery (Ostensen et al., 1983).

ii) The peak incidence of RA is also reported being coincide with menopause (Islander et al., 2011; Ostensen et al., 1983), which is associated with the decreased production of sex steroids (estrogen, progesterone and adrenal androgen) (Islander et al., 2011). Actually, these sex hormones have long been proven crucial for the development and maturation of the reproductive system.

iii) The key role of estrogen playing in the pathogenesis and progression of RA has also been confirmed that in the last decades of past century, hormone replacement therapy (HRT) (using estrogens or combination of estrogen and progesterone) was once recommended for the treatment of postmenopausal osteoporosis; though, the application of HRT was decreased later due to side effects (Islander et al., 2011).

Thus, the level of sex steroids is assumed being tightly connected with the RA development and treatment. In fact, the treatment of another disease, SLE, is also greatly affected by the sex steroid level that, during pregnancy many patients with SLE get worse symptom, which may also be due to the increased sex hormone levels like estrogen (Petri, 2008).

Therefore, a question arises: does TwHF also exert its treatment function of RA by targeting the sex hormone-related proteins? If so, are these proteins the sole targets for RA treatment?

During the exploration of the answer to this question, two contradictive phenomena emerge: i) Many patients with RA during pregnancy got improved symptom (Islander et al., 2011; Ostensen et al., 1983; Petri, 2008), which might be due to the increased sex hormone levels, like estrogen; ii) When patients took TwHF at reproductive age, there are some reports of decreased estrogen level (and malfunction of male and female reproductive system) (Bao and Dai, 2011). However, it has been well recognized that TwHF is a good remedy for RA treatment, and from our studies it is known that AR, ER and PR are all potential targets of components of TwHF. Thus, we

assume that sex hormonal disorder may result from the sex hormone receptors that are targeted by compounds of TwHF, but this disorder itself is not sufficient for the remission or cure of RA, which process should also involve some other non-sex steroid receptors. So we have reason to believe that the underlying mechanism of TwHF must be through multiple compounds-multiple targets interaction.

4) As for TwHF, 21 components target 32 proteins. And among them, 5 candidate compounds hit more than 10 potential targets with relatively higher degree distribution. They are TW-53 (syringaresinol, (+)-), TW-56 ([3, 3'-Bi-2H-1-benzopyran]-4, 4'-diol, 3', 4, 4'-tetrahydro-6, 6'-dimethoxy-, 3R, 3'S, 4R, 4'S)-rel-), TW-39 (triptonolide), TW-59 (tripterygiol) and TW-31 (nobiletin (6CI)). For triptonolide and tripterygiol, they both target two groups of proteins, i.e., the pro-inflammatory proteins such as COX-2 and iNOS, and nuclear receptors like PR, ER and AR. The pro-inflammatory proteins match the experimental results, and the sex hormones disorder may explain the infertility for patients taking TwHF for long period at reproductive age.

***Semen Strychni* and TwHF network analysis.** In C-T network, biological species (components and proteins) are represented as nodes, and compound-protein interactions are represented as edges between the nodes. As seen in Fig. 2， the net comprises 82 nodes (including 43 active compounds and 39 potential targets) and 264 edges, with a mean number of edge per potential target of 6.77. In this figure, 21 proteins targeted by both TwHF and *Semen Strychni* form an ellipse between the compound lines of the two herbs, comprising 64 nodes (including 43 active compounds and 21 potential targets) and 223 edges, with a mean number of edges per potential target of 10.62 which is much higher than the average value of 6.77. So the 21 shared targets show much more interactions with active compounds than those in the outside compound lines of TwHF and *Semen Strychni*. This indicates that the overlapped targets may bear major responsibility for the treatment of RA.

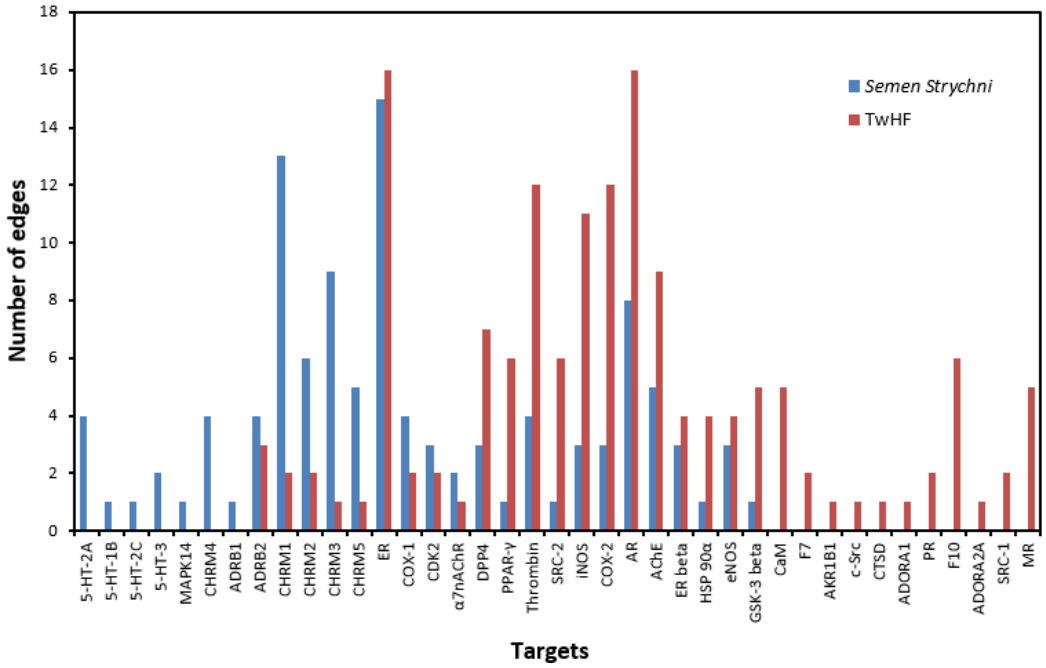

Figure 3. The comparison of proteins targeted by two herbs with their edge number

Since all active compounds of TwHF and *Semen Strychni* only share one component, ursolic acid (ST-24 or TW-11), while the two herbs both exhibit desirable therapeutic effects for the

treatment of RA, it is difficult to explain this by the theory of one component-one target mechanism, which indicates that recipes with multi-component may be the answers to RA treatment. In addition, apparently, it is difficult to design certain drugs to interfere with one system while without upsetting other control circuits, especially for the treatment of heterogeneous and systemic disease like RA. All this further verifies that *Semen Strychni* and TwHF may achieve therapeutic effects through a multi-targeting strategy.

Conclusions

TCM is a heritage that is thousands of years old and is still used by millions of people all over the world-even after the development of modern scientific medicine. Herbal medicine has been widely used for disease treatment and is fast becoming a very popular form of alternative medicine worldwide. In this work, we have constructed an integrated model of systems pharmacology by combining the knowledge of chemistry, biology and the theoretical background of TCM to investigate the mechanisms of action of Chinese herbs related to RA. Presently, a systems pharmacology methodology integrating drug-likeness evaluation, oral bioavailability prediction, multiple drug targets prediction and network analysis has been applied to the investigation of the therapeutic mechanism of *Semen Strychni* and TwHF. Our main findings are as following:

1) 60 components out of 238 ingredients (25%) in *Semen Strychni* and TwHF are identified as active substances through oral bioavailability and drug-likeness screening. These include many reported active components as brucine, strychnine, triptolide, triptonide, tripchlorolide and triptolidenol, which further validate the reasonability of our screening model. In addition, we also predict some molecules such as catechin, cantleyine, spermostrychnin, (+)-isostrychnine, chlorogenic acid, nobiletin (6CI), triptonolide, syringaresinol and tripterygiol as potential bioactive compounds, which might serve to guide our further study of this botanical drug.

2) 21 common targets shared by *Semen Strychni* and TwHF may bear major responsibility for RA treatment. This would be explained by the following reasons: i) the active components of the two herbs are almost completely different except one compound, ursolic acid, which doesn't relieve RA alone; ii) the number of edge of overlapped targets outnumber the one of the targets solely owned by the two herbs; iii) 4 targets themselves being angiogenesis mediators, which work in a direct pathway, are all shared potential targets of *Semen Strychni* and TwHF. Based on the present target prediction and network analysis, it is assumed that the active compounds of both *Semen Strychni* and TwHF realize their curative effects by modulating angiogenesis mediator cascades.

Despite these potentially interesting associations, cautious interpretation is warranted as these findings mainly relied on statistical analysis. Further experimental testing of these hypotheses will be required to support further assessments of potential clinical applications. Hopefully, the present work has provided a new systems pharmacology framework to study herbal drugs and understanding of the chemical and pharmacological basis of TCM, which will promote drug discovery from herbal medicines.

Acknowledgements

Thanks for the financial support given by the National Natural Science Foundation of China (Grant No.11201049).

Reference

Aletaha, D., Neogi, T., Silman, A.J., Funovits, J., Felson, D.T., Bingham, C.O., 3rd, Birnbaum, N.S., Burmester, G.R., Bykerk, V.P., Cohen, M.D., Combe, B., Costenbader, K.H., Dougados, M., Emery, P., Ferraccioli, G., Hazes, J.M., Hobbs, K., Huizinga, T.W., Kavanaugh, A., Kay, J., Kvien, T.K., Laing, T., Mease, P., Menard, H.A., Moreland, L.W., Naden, R.L., Pincus, T., Smolen, J.S., Stanislawska-Biernat, E., Symmons, D., Tak, P.P., Upchurch, K.S., Vencovsky, J., Wolfe, F., Hawker, G., 2010. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum 62, 2569-2581.

Amanchy, R., Zhong, J., Hong, R., Kim, J.H., Gucek, M., Cole, R.N., Molina, H., Pandey, A., 2009. Identification of c-Src tyrosine kinase substrates in platelet-derived growth factor receptor signaling. Molecular oncology 3, 439-450.

Arany, Z., Foo, S.Y., Ma, Y., Ruas, J.L., Bommi-Reddy, A., Girnun, G., Cooper, M., Laznik, D., Chinsomboon, J., Rangwala, S.M., Baek, K.H., Rosenzweig, A., Spiegelman, B.M., 2008. HIF-independent regulation of VEGF and angiogenesis by the transcriptional coactivator PGC-1alpha. Nature 451, 1008-1012.

Bao, F., Wu, P., Xiao, N., Qiu, F., Zeng, Q.P., 2012. Nitric oxide-driven hypoxia initiates synovial angiogenesis, hyperplasia and inflammatory lesions in mice. PloS one 7, e34494.

Bao, J., Dai, S.M., 2011. A Chinese herb Tripterygium wilfordii Hook F in the treatment of rheumatoid arthritis: mechanism, efficacy, and safety. Rheumatol Int 31, 1123-1129.

Berchem, G., Glondu, M., Gleizes, M., Brouillet, J.P., Vignon, F., Garcia, M., Liaudet-Coopman, E., 2002. Cathepsin-D affects multiple tumor progression steps in vivo: proliferation, angiogenesis and apoptosis. Oncogene 21, 5951-5955.

Bergmann, M.W., Rechner, C., Freund, C., Baurand, A., El Jamali, A., Dietz, R., 2004. Statins inhibit reoxygenation-induced cardiomyocyte apoptosis: role for glycogen synthase kinase 3beta and transcription factor beta-catenin. J Mol Cell Cardiol 37, 681-690.

Biscetti, F., Straface, G., Pitocco, D., Zaccardi, F., Ghirlanda, G., Flex, A., 2009. Peroxisome proliferator-activated receptors and angiogenesis. Nutrition, metabolism, and cardiovascular diseases : NMCD 19, 751-759.

Bongartz, T., Coras, B., Vogt, T., Scholmerich, J., Muller-Ladner, U., 2005. Treatment of active psoriatic arthritis with the PPARgamma ligand pioglitazone: an open-label pilot study. Rheumatology 44, 126-129.

Bourdoncle, A., Labesse, G., Margueron, R., Castet, A., Cavailles, V., Royer, C.A., 2005. The nuclear receptor coactivator PGC-1alpha exhibits modes of interaction with the estrogen receptor distinct from those of SRC-1. Journal of molecular biology 347, 921-934.

Brustle, M., Beck, B., Schindler, T., King, W., Mitchell, T., Clark, T., 2002. Descriptors, physical properties, and drug-likeness. J Med Chem 45, 3345-3355.

Cao, H., Dronadula, N., Rao, G.N., 2006. Thrombin induces expression of FGF-2 via activation of

PI3K-Akt-Fra-1 signaling axis leading to DNA synthesis and motility in vascular smooth muscle cells. American journal of physiology. Cell physiology 290, C172-182.

Carreau, A., Kieda, C., Grillon, C., 2011. Nitric oxide modulates the expression of endothelial cell adhesion molecules involved in angiogenesis and leukocyte recruitment. Exp Cell Res 317, 29-41.

Chen, J., Wang, X., Qu, Y.G., Chen, Z.P., Cai, H., Liu, X., Xu, F., Lu, T.L., Cai, B.C., 2012. Analgesic and anti-inflammatory activity and pharmacokinetics of alkaloids from seeds of Strychnos nux-vomica after transdermal administration: effect of changes in alkaloid composition. J Ethnopharmacol 139, 181-188.

Chen, K.H., Chang, B.H., Younan, P., Shlykov, S.G., Sanborn, B.M., Chan, L., 2002. Increased intracellular calcium transients by calmodulin antagonists differentially modulate tumor necrosis factor-alpha-induced E-selectin and ICAM-1 expression. Atherosclerosis 165, 5-13.

Chisholm, K.M., Chang, K.W., Truong, M.T., Kwok, S., West, R.B., Heerema-McKenney, A.E., 2012. beta-Adrenergic receptor expression in vascular tumors. Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc 25, 1446-1451.

Chwalisz, K., Brenner, R.M., Fuhrmann, U.U., Hess-Stumpp, H., Elger, W., 2000. Antiproliferative effects of progesterone antagonists and progesterone receptor modulators on the endometrium. Steroids 65, 741-751.

Clark, A.N., Youkey, R., Liu, X., Jia, L., Blatt, R., Day, Y.J., Sullivan, G.W., Linden, J., Tucker, A.L., 2007. A1 adenosine receptor activation promotes angiogenesis and release of VEGF from monocytes. Circulation research 101, 1130-1138.

Combe, B., 2009. Progression in early rheumatoid arthritis. Best Pract Res Clin Rheumatol 23, 59-69.

Cooke, J.P., 2003. NO and angiogenesis. Atherosclerosis. Supplements 4, 53-60.

Duarte, M., Kolev, V., Soldi, R., Kirov, A., Graziani, I., Oliveira, S.M., Kacer, D., Friesel, R., Maciag, T., Prudovsky, I., 2006. Thrombin induces rapid PAR1-mediated non-classical FGF1 release. Biochem Biophys Res Commun 350, 604-609.

Ehrman, T.M., Barlow, D.J., Hylands, P.J., 2007. Phytochemical databases of Chinese herbal constituents and bioactive plant compounds with known target specificities. J Chem Inf Model 47, 254-263.

Eisermann, K., Broderick, C.J., Bazarov, A., Moazam, M.M., Fraizer, G.C., 2013. Androgen up-regulates vascular endothelial growth factor expression in prostate cancer cells via an Sp1 binding site. Molecular cancer 12, 7.

Fong, G., Backman, L.J., Andersson, G., Scott, A., Danielson, P., 2013. Human tenocytes are stimulated to proliferate by acetylcholine through an EGFR signalling pathway. Cell and tissue research 351, 465-475.

Fritzen, M., Flores, M.P., Reis, C.V., Chudzinski-Tavassi, A.M., 2005. A prothrombin activator (Lopap) modulating inflammation, coagulation and cell survival mechanisms. Biochem Biophys Res Commun 333, 517-523.

Fujii, M., Inoki, I., Saga, M., Morikawa, N., Arakawa, K., Inaba, S., Yoshioka, K., Konoshita, T., Miyamori, I., 2012. Aldosterone inhibits endothelial morphogenesis and angiogenesis through the downregulation of vascular endothelial growth factor receptor-2 expression subsequent to peroxisome proliferator-activated receptor gamma. The Journal of steroid biochemistry and molecular biology 129, 145-152.

Fujita, M., Minamino, T., Sanada, S., Asanuma, H., Hirata, A., Ogita, H., Okada, K., Tsukamoto, O., Takashima, S., Tomoike, H., Node, K., Hori, M., Kitakaze, M., 2004. Selective blockade of serotonin

5-HT2A receptor increases coronary blood flow via augmented cardiac nitric oxide release through 5-HT1B receptor in hypoperfused canine hearts. J Mol Cell Cardiol 37, 1219-1223.

Gunin, A.G., Emelianov, V.U., Tolmachev, A.S., 2003. Expression of estrogen receptor-alpha, glucocorticoid receptor, beta-catenin and glycogen synthase kinase-3beta in the uterus of mice following long-term treatment with estrogen and glucocorticoid hormones. European journal of obstetrics, gynecology, and reproductive biology 107, 62-67.

Hara, Y., Kuroda, N., Inoue, K., Sato, T., 2009. Up-regulation of vascular endothelial growth factor expression by adenosine through adenosine A2 receptors in the rat tongue treated with endotoxin. Archives of oral biology 54, 932-942.

Headrick, J.P., Ashton, K.J., Rose'meyer, R.B., Peart, J.N., 2013. Cardiovascular adenosine receptors: expression, actions and interactions. Pharmacology & therapeutics 140, 92-111.

Hoyer, D., Clarke, D.E., Fozard, J.R., Hartig, P.R., Martin, G.R., Mylecharane, E.J., Saxena, P.R., Humphrey, P.P., 1994. International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). Pharmacol Rev 46, 157-203.

Islander, U., Jochems, C., Lagerquist, M.K., Forsblad-d'Elia, H., Carlsten, H., 2011. Estrogens in rheumatoid arthritis; the immune system and bone. Mol Cell Endocrinol 335, 14-29.

Iwabayashi, M., Taniyama, Y., Sanada, F., Azuma, J., Iekushi, K., Kusunoki, H., Chatterjee, A., Okayama, K., Rakugi, H., Morishita, R., 2012. Role of serotonin in angiogenesis: induction of angiogenesis by sarpogrelate via endothelial 5-HT1B/Akt/eNOS pathway in diabetic mice. Atherosclerosis 220, 337-342.

Jeong, H., Mason, S.P., Barabasi, A.L., Oltvai, Z.N., 2001. Lethality and centrality in protein networks. Nature 411, 41-42.

Jiang, C., Ting, A.T., Seed, B., 1998. PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. Nature 391, 82-86.

Jiang, D., Li, Q., Kolosov, V.P., Zhou, X., 2012. The inhibition of aldose reductase on mucus production induced by interleukin-13 in the human bronchial epithelial cells. International immunopharmacology 12, 588-593.

Jung, Y., Song, S., Choi, C., 2008. Peroxisome proliferator activated receptor gamma agonists suppress TNFalpha-induced ICAM-1 expression by endothelial cells in a manner potentially dependent on inhibition of reactive oxygen species. Immunol Lett 117, 63-69.

Kaga, S., Zhan, L., Altaf, E., Maulik, N., 2006. Glycogen synthase kinase-3beta/beta-catenin promotes angiogenic and anti-apoptotic signaling through the induction of VEGF, Bcl-2 and survivin expression in rat ischemic preconditioned myocardium. J Mol Cell Cardiol 40, 138-147.

Kakinuma, Y., Furihata, M., Akiyama, T., Arikawa, M., Handa, T., Katare, R.G., Sato, T., 2010. Donepezil, an acetylcholinesterase inhibitor against Alzheimer's dementia, promotes angiogenesis in an ischemic hindlimb model. J Mol Cell Cardiol 48, 680-693.

Karmakar, S., Kay, J., Gravallese, E.M., 2010. Bone damage in rheumatoid arthritis: mechanistic insights and approaches to prevention. Rheum Dis Clin North Am 36, 385-404.

Kimura, H., Esumi, H., 2003. Reciprocal regulation between nitric oxide and vascular endothelial growth factor in angiogenesis. Acta Biochim Pol 50, 49-59.

Lainer-Carr, D., Brahn, E., 2007. Angiogenesis inhibition as a therapeutic approach for inflammatory synovitis. Nat Clin Pract Rheumatol 3, 434-442.

Lee, T.W., Chen, G.G., Xu, H., Yip, J.H., Chak, E.C., Mok, T.S., Yim, A.P., 2003. Differential expression of inducible nitric oxide synthase and peroxisome proliferator-activated receptor gamma in non-small

cell lung carcinoma. European journal of cancer 39, 1296-1301.

Li, H., Ruano, M.J., Villalobo, A., 2004. Endogenous calmodulin interacts with the epidermal growth factor receptor in living cells. FEBS letters 559, 175-180.

Li, X., Wang, K., Wei, W., Liu, Y.Y., Gong, L., 2013. In vitro metabolism of brucine by human liver microsomes and its interactions with CYP substrates. Chem Biol Interact 204, 140-143.

Liacini, A., Sylvester, J., Zafarullah, M., 2005. Triptolide suppresses proinflammatory cytokine-induced matrix metalloproteinase and aggrecanase-1 gene expression in chondrocytes. Biochem Biophys Res Commun 327, 320-327.

Lim, W., Cho, J., Kwon, H.Y., Park, Y., Rhyu, M.R., Lee, Y., 2009. Hypoxia-inducible factor 1 alpha activates and is inhibited by unoccupied estrogen receptor beta. FEBS letters 583, 1314-1318.

Lin, N., Liu, C., Xiao, C., Jia, H., Imada, K., Wu, H., Ito, A., 2007. Triptolide, a diterpenoid triepoxide, suppresses inflammation and cartilage destruction in collagen-induced arthritis mice. Biochem Pharmacol 73, 136-146.

Losordo, D.W., Isner, J.M., 2001. Estrogen and angiogenesis: A review. Arteriosclerosis, thrombosis, and vascular biology 21, 6-12.

Ma, J., Dey, M., Yang, H., Poulev, A., Pouleva, R., Dorn, R., Lipsky, P.E., Kennelly, E.J., Raskin, I., 2007. Anti-inflammatory and immunosuppressive compounds from Tripterygium wilfordii. Phytochemistry 68, 1172-1178.

Matsumoto, M., Sudo, T., Maruyama, M., Osada, H., Tsujimoto, M., 2000. Activation of p38 mitogen-activated protein kinase is crucial in osteoclastogenesis induced by tumor necrosis factor. FEBS letters 486, 23-28.

Mitsos, S., Koletsis, E.N., Katsanos, K., Bravou, V., Kolonitsiou, F., Marinos, E., Flordellis, C.S., Dougenis, D., 2014. Intramyocardial thrombin promotes angiogenesis and improves cardiac function in an experimental rabbit model of acute myocardial infarction. The Journal of thoracic and cardiovascular surgery 147, 1376-1383.

Miyazaki, R., Ichiki, T., Hashimoto, T., Ikeda, J., Kamiharaguchi, A., Narabayashi, E., Matsuura, H., Takeda, K., Sunagawa, K., 2012. Acetylcholinesterase inhibitors attenuate angiogenesis. Clinical science 123, 241-249.

Montesinos, M.C., Shaw, J.P., Yee, H., Shamamian, P., Cronstein, B.N., 2004. Adenosine A(2A) receptor activation promotes wound neovascularization by stimulating angiogenesis and vasculogenesis. The American journal of pathology 164, 1887-1892.

Murohara, T., 2012. Dipeptidyl peptidase-4 inhibitor: another player for cardiovascular protection. Journal of the American College of Cardiology 59, 277-279.

Negroni, M.P., Fiszman, G.L., Azar, M.E., Morgado, C.C., Espanol, A.J., Pelegrina, L.T., de la Torre, E., Sales, M.E., 2010. Immunoglobulin G from breast cancer patients in stage I stimulates muscarinic acetylcholine receptors in MCF7 cells and induces proliferation. Participation of nitric oxide synthase-derived nitric oxide. Journal of clinical immunology 30, 474-484.

Ostensen, M., Aune, B., Husby, G., 1983. Effect of pregnancy and hormonal changes on the activity of rheumatoid arthritis. Scand J Rheumatol 12, 69-72.

Pan, C., Liu, Y.P., Li, Y.F., Hu, J.X., Zhang, J.P., Wang, H.M., Li, J., Xu, L.C., 2012. Effects of cypermethrin on the ligand-independent interaction between androgen receptor and steroid receptor coactivator-1. Toxicology 299, 160-164.

Park, J.H., Kim, S.H., Choi, M.C., Lee, J., Oh, D.Y., Im, S.A., Bang, Y.J., Kim, T.Y., 2008. Class II histone deacetylases play pivotal roles in heat shock protein 90-mediated proteasomal degradation of vascular

endothelial growth factor receptors. Biochem Biophys Res Commun 368, 318-322.

Park, S.Y., Lee, H., Hur, J., Kim, S.Y., Kim, H., Park, J.H., Cha, S., Kang, S.S., Cho, G.J., Choi, W.S., Suk, K., 2002. Hypoxia induces nitric oxide production in mouse microglia via p38 mitogen-activated protein kinase pathway. Brain research. Molecular brain research 107, 9-16.

Petri, M., 2008. Sex hormones and systemic lupus erythematosus. Lupus 17, 412-415.

Sanderson, S., Valenti, M., Gowan, S., Patterson, L., Ahmad, Z., Workman, P., Eccles, S.A., 2006. Benzoquinone ansamycin heat shock protein 90 inhibitors modulate multiple functions required for tumor angiogenesis. Molecular cancer therapeutics 5, 522-532.

Saraswati, S., Agrawal, S.S., 2013. Brucine, an indole alkaloid from Strychnos nux-vomica attenuates VEGF-induced angiogenesis via inhibiting VEGFR2 signaling pathway in vitro and in vivo. Cancer Lett 332, 83-93.

Schaub, R.G., 2011. Recent advances in the development of coagulation factors and procoagulants for the treatment of hemophilia. Biochem Pharmacol 82, 91-98.

Schoenmakers, S.H., Reitsma, P.H., Spek, C.A., 2005. Blood coagulation factors as inflammatory mediators. Blood Cells Mol Dis 34, 30-37.

Smoot, M.E., Ono, K., Ruscheinski, J., Wang, P.L., Ideker, T., 2011. Cytoscape 2.8: new features for data integration and network visualization. Bioinformatics 27, 431-432.

Svensson, J., Moverare-Skrtic, S., Windahl, S., Swanson, C., Sjogren, K., 2010. Stimulation of both estrogen and androgen receptors maintains skeletal muscle mass in gonadectomized male mice but mainly via different pathways. Journal of molecular endocrinology 45, 45-57.

Szekanecz, Z., Koch, A.E., 2009. Angiogenesis and its targeting in rheumatoid arthritis. Vascul Pharmacol 51, 1-7.

UN, D., 2007. Acetylcholinesterase and butyrylcholinesterase as possible markers of low-grade systemic inflammation. Med Sci Monit 13, 8.

Wang, H., Yu, M., Ochani, M., Amella, C.A., Tanovic, M., Susarla, S., Li, J.H., Wang, H., Yang, H., Ulloa, L., Al-Abed, Y., Czura, C.J., Tracey, K.J., 2003. Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. Nature 421, 384-388.

Wang, J., Li, F., Li, Y., Yang, Y., Zhang, S., Yang, L., 2013a. Structural features of falcipain-3 inhibitors: an in silico study. Mol Biosyst 9, 2296-2310.

Wang, Q., Kuang, H., Su, Y., Sun, Y., Feng, J., Guo, R., Chan, K., 2013b. Naturally derived anti-inflammatory compounds from Chinese medicinal plants. J Ethnopharmacol 146, 9-39.

Wang, X., Xu, X., Li, Y., Li, X., Tao, W., Li, B., Wang, Y., Yang, L., 2013c. Systems pharmacology uncovers Janus functions of botanical drugs: activation of host defense system and inhibition of influenza virus replication. Integr Biol (Camb) 5, 351-371.

Wang, X., Xu, X., Tao, W., Li, Y., Wang, Y., Yang, L., 2012. A systems biology approach to uncovering pharmacological synergy in herbal medicines with applications to cardiovascular disease. Evid Based Complement Alternat Med 2012, 519031.

Wong, H.P., Yu, L., Lam, E.K., Tai, E.K., Wu, W.K., Cho, C.H., 2007a. Nicotine promotes cell proliferation via alpha7-nicotinic acetylcholine receptor and catecholamine-synthesizing enzymes-mediated pathway in human colon adenocarcinoma HT-29 cells. Toxicology and applied pharmacology 221, 261-267.

Wong, H.P., Yu, L., Lam, E.K., Tai, E.K., Wu, W.K., Cho, C.H., 2007b. Nicotine promotes colon tumor growth and angiogenesis through beta-adrenergic activation. Toxicological sciences : an official journal of the Society of Toxicology 97, 279-287.

Woods, J.M., Mogollon, A., Amin, M.A., Martinez, R.J., Koch, A.E., 2003. The role of COX-2 in angiogenesis and rheumatoid arthritis. Exp Mol Pathol 74, 282-290.

Yadav, U.C., Srivastava, S.K., Ramana, K.V., 2012. Prevention of VEGF-induced growth and tube formation in human retinal endothelial cells by aldose reductase inhibition. Journal of diabetes and its complications 26, 369-377.

Yeo, M., Park, H.K., Lee, K.M., Lee, K.J., Kim, J.H., Cho, S.W., Hahm, K.B., 2004. Blockage of HSP 90 modulates Helicobacter pylori-induced IL-8 productions through the inactivation of transcriptional factors of AP-1 and NF-kappaB. Biochem Biophys Res Commun 320, 816-824.

Zhang, Y., Xu, W., Li, H., Zhang, X., Xia, Y., Chu, K., Chen, L., 2013. Therapeutic effects of total alkaloids of Tripterygium wilfordii Hook f. on collagen-induced arthritis in rats. J Ethnopharmacol 145, 699-705.