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Herb pairs containing *Angelicae Sinensis Radix* (Danggui): A review of bio-active constituents and compatibility effects

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ABSTRACT

Ethnopharmacological relevance: Herb compatibility is one of the most important characteristics of traditional Chinese medicine (TCM). Rather than being used singly, Chinese herbs are often used in formulae to obtain synergistic effects or to diminish possible adverse reactions. Herb pair, the most fundamental and simplest form of multi-herb formulae, is a centralized representative of herb compatibility. Danggui (*Angelicae Sinensis Radix*), a widely used Chinese medicine, is usually combined with another herb to treat women's diseases in the clinic. A series of herb pairs containing Danggui have gradually become a focus of modern research, and they exhibit encouraging prospects for development.

Materials and methods: A systematic search for studies related to herb pairs containing Danggui was performed via a library search (books, theses, reports, newspapers, magazines, and conference proceedings) and an electronic search (Web of Science, PubMed, and Google Scholar). These sources were scrutinized for information on Danggui herb pairs.

Results: Based on a previous statistical analysis, a database containing 16,529 formulae of Danggui from the "Dictionary of Traditional Chinese Medicine Formulae" was reviewed. The results showed a high frequency of compatibility between Danggui and other 22 herbs. The most common ratio among these chosen herb pairs was 1:1, and a majority of the pairs were applied for the treatment of diseases in internal medicine. The present paper reviews ethnopharmacology and advances in variations of the bio-active components and compatibility effects of the herb pairs containing Danggui, especially Danggui-Huangqi, Danggui-Chuanxiong, and Danggui-Shaoyao, which are used at high frequency. It was also observed that there were fewer studies of Danggui-Fuzi, Danggui-Huanglian, Danggui-Gancao, Danggui-Fangfeng and Danggui-Ganjiang, although they have been recorded in classical books as commonly used herb pairs. Moreover, some herb pairs such as Danggui-Niuxi and Danggui-Chaihu have been used at high frequency according to the statistical analysis, however, they were not recognized as herb pairs in many relevant books.

Conclusions: Recently, several TCM researchers have become interested in investigating the bio-active constituents and compatibility effects of herb pairs. Thus, some methods for in-depth study of herb pairs are essential to be established. The *in vitro* or *in vivo* bio-active constituents of

herb pairs may differ from those of the single herbs. Additionally, comparative methods should be applied to study not only the bio-active constituents but also the effects of herb pairs. Study of component compatibility may be considered when the bio-active constituents and effects of an herb pair have been definitively demonstrated. Overall, the goal of our basic study of herb pairs should be their clinical application and the development of new drugs.

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References

1. Introduction

Traditional medicine—a system of ancient medical practices that differs in substance, methodology and philosophy from modern medicine—plays an important role in health maintenance for the peoples of Asia and is becoming more frequently used in Western countries (Corson and Crews, 2007; Xue and Roy, 2003). Traditional medicine has formed its own distinct culture not only in China but also in other Asian countries, such as Japan, South Korea, Malaysia and Vietnam (Wang et al., 2010). In ancient times, many medical practitioners realized that the pathogenesis and progression of diseases are so complicated that the therapeutic effect of a single drug may be modest and hampered by various side effects or resistance in the clinic (Keith et al., 2005). However, the compatibility of two or more herbs could be much more effective; thus, formulae have since been gradually developed, and have shown significance in long-term clinical practice (Li and Zhang, 2008). As the marrow of traditional Chinese culture, traditional Chinese medicine (TCM) has accumulated more than 100,000 formulae over the past 2000 years, which is quite different from other traditional herbal therapies across the world (Qiu, 2007).

As a note of concern, scientists pay attention to unique combinations of two relevant herbs in clinics (Wang et al., 2012). Synergistic effects of herb pairs can be achieved by using a pair of herbs with some ingredients of special pharmacokinetic profile; for example, one ingredient enhances the therapeutic effect of another active ingredient by regulating its absorption, distribution, metabolism or excretion (ADME) (Gilbert and Alves, 2003; Li et al., 2006; Ung et al., 2007). These delicate combinations are named herb pairs. Without altering the basic therapeutic features of multi-herb formulae, herb pairs as the basic composition units of Chinese herbal formulae are of special clinical significance in TCM and have gradually become a focus of modern research (Deng et al., 2008). Although composed of only two herbs, herb pairs ingeniously express the core idea of the multi-herb prescription. Shennong's Herbal Classic, the earliest existing monograph of TCM, first summarized the interactions between pairs of herbs and identified them as “seven compatibilities”. The seven aspects of herbal compatibility, namely single action, mutual reinforcement, mutual assistance, mutual restraint, mutual incompatibility, mutual inhibition and mutual antagonism, are a comprehensive summation of long-term clinical experience, that still affect perspectives on drug interactions in modern medicine (Zhang, 2008). Among those aspects, mutual reinforcement and mutual assistance are the most common

compatibilities.

A series of herb pairs containing Danggui represent aspects of mutual reinforcement and mutual assistance. Recently, we reviewed a database containing 16,529 formulae of Danggui from the “Dictionary of Traditional Chinese Medicine Formulae”. The results showed that combinations of Ganciao-Danggui and Shaoyao-Danggui were the most frequent (Table 1), and 1:1 was the most common ratio among these chosen herb pairs. Furthermore, a majority of these herb pairs were applied for treating diseases in internal medicine (Jin et al., 2015). It was also found that there were large numbers of modern studies on herb pairs containing Danggui, such as Danggui-Chuanxiong and Danggui-Huangqi (Tang and Duan, 2014). This review focuses on variations of the bio-active components and compatibility effects of several main herb pairs containing Danggui, and it provides a comprehensive overview that could serve as a reference for researchers interested in herb pairs.

2. Main herb pairs containing Danggui

2.1. Danggui and Huangqi

Danggui (*Angelicae Sinensis Radix*), the dried radix of *Angelica sinensis* (Oliv.) Diels (Umbelliferae), is used to invigorate the blood circulation in the treatment of menstrual disorders. Danggui exhibits many bio-activities such as the inhibition of smooth muscle contraction (Du et al., 2006), immunomodulation (Ma et al., 2011), anti-tumor (Cao et al., 2006), anti-radiation (Sun et al., 2005), anti-inflammation (Sakai et al., 1999), and cardio-cerebro vascular effects (Liu et al., 2010). Aromatic acids (Yi et al., 2009), phthalides (Lao et al., 2004), and polysaccharides (Cao et al., 2006) were found to be responsible for the main bio-activities of Danggui. Huangqi (*Astragali Radix*), the dried radix of *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao, *Astragalus membranaceus* (Fisch.) Bge. (Leguminosae), is a well-known Chinese traditional medicine with the function of "invigorating qi". Huangqi has functions such as immunomodulation (Zhao et al., 2011), anti-aging (Li et al., 2009), anti-tumor (Yu et al., 2007), and cardio-cerebro vascular protection (Wang et al., 2013). Saponins (Yu et al., 2007) and polysaccharides (Zhu et al., 2011) have been recognized as the main bio-active components of Huangqi. We reviewed a database containing 3,110 formulae of Danggui and Huangqi, and it was found that “1:1” appeared to be the most frequent ratio (Jin et al., 2015). Huangqi and Danggui combined in a 5:1 ratio is a classical TCM formulae named Danggui Buxue Tang (DBT), which

was traditionally used in China as a remedy for menopausal symptoms.

2.1.1. *Bio-active components of Danggui-Huangqi*

2.1.1.1. *Bio-active component variation of Danggui-Huangqi*

The variation of the bio-active components in Danggui and Huangqi combinations is not simply the sum of those in the single herbs. Wang et al. (2005) detected the components of DBT using HPLC-DAD, and found 18 chromatographic peaks, of which 10 peaks belonged to Danggui, 11 peaks belonged to Huangqi, 5 peaks belonged to Danggui and Huangqi, and 2 peaks were attributed to new components which were not present in the single herbs. Additionally, a quantitative analysis revealed that the content of ferulic acid in DBT was higher than that in the single herbs (Liang et al., 2003). In a later study, additional chemical components of Huangqi and Danggui with different ratios (1:1, 2:1, 3:1, 4:1, 5:1, 7:1, 10:1, 0:1, and 1:0) were determined. Significantly, higher amounts of Huangqi-derived astragaloside IV, calycosin, and formononetin, as well as Danggui-derived ferulic acid, were found in the ratio of 5:1 (Dong et al., 2006).

2.1.1.2. *Bio-active component variation of Danggui-Huangqi in vivo*

The ingredients of TCM are complicated and abundant, and the pharmacokinetics of a single ingredient do not represent those of the TCM. Wang et al. (2009) observed changes in the pharmacokinetic parameters in animal plasma of astragaloside IV and ferulic acid when Danggui and Huangqi were used solely or in combination. It was found that Danggui in combinations increased the activity of astragaloside IV in the plasma, and it raised the effectiveness of astragaloside IV, which embodies the adjuvant contribution of the ministerial drug of Danggui. Furthermore, Shi et al. (2014a) compared the *in vivo* pharmacokinetic properties of seven major bioactive components (ferulic acid, caffeic acid, butylphthalide, ligustilide, calycosin-7-*O*- β -glucoside, ononin and astragaloside IV) in normal rats and rats with acute blood deficiency after the oral administration of DBT. The results showed that the C_{max} , T_{max} and MRT_{0-T} of astragaloside IV; the C_{max} , $T_{1/2Z}$, AUC_{0-T} and MRT_{0-T} of calycosin-7-*O*- β -glucoside; the $T_{1/2Z}$ and AUC_{0-T} of ferulic acid; the $T_{1/2Z}$, AUC_{0-T} and MRT_{0-T} of ononin; and the MRT_{0-T} of ligustilide, butylphthalide, and caffeic acid in the blood deficiency rats were significantly different from the normal rats. Moreover, the tissue distribution profiles of five major bio-active constituents (ferulic acid, caffeic acid, calycosin-7-*O*- β -glucoside, ononin and astragaloside IV) were also investigated. The contents of these compounds in the liver were greater than those in

other tissues in both the normal and blood deficiency rats. Compared to the normal rats, the partial contents of these components were significantly different in the tissues of the acute blood deficiency rats at different time points (Shi et al. 2014b).

2.1.2. *Compatibility effects of Danggui-Huangqi*

2.1.2.1. *Effect on hematological system*

Blood deficiency is a common syndrome characterized by a sallow complexion, palpitation or feeble pulse, and it is considered the root of many gynecological disorders. Shi et al. (2012) explored the interactions between the nourishing and tonifying blood effects of combinations of Danggui-Huangqi (0:1, 1:5, 2:5, 2:3, 1:1, 3:2, 5:2, 5:1, and 1:0) using a response surface method to observe the changes of the peripheral blood indexes and organ indexes. The results showed that most combination ratios of Danggui-Huangqi had synergetic effects. Within the range of 1:5-5:1, all of the extracts of Danggui-Huangqi showed synergic effects, with high doses exhibiting better synergic effects than low doses (Fig. 2). The combination of Danggui-Huangqi also had effects on activating blood. Nine different proportions were found to significantly reduce blood viscosity in the acute blood stasis rats, and the best ratio was 3:2 (Teng et al., 2003).

When DBT was applied to cultured Hep3B human hepatocellular carcinoma cells, it induced the mRNA expression of erythropoietin (EPO) in a dose-dependent manner, which peaked at ~2.5-fold induction (Gao et al., 2008). Additionally, treatment with DBT was shown to increase the mRNA and protein expression of hypoxia-inducible factor-1 α (HIF-1 α). The activation of the Raf/MEK/ERK signaling pathway by DBT could also enhance the translation of HIF-1 α , suggesting dual actions of DBT in stimulating the EPO expression in kidney cells (Zheng et al., 2010). Moreover, improvements were obtained in the formation of capillaries and the numbers of blood vessels after treatment of DBT (Lei et al., 2003a).

2.1.2.2. *Effect on immune cells*

As a single herb, Danggui or Huangqi can boost immunity of organ systems. Similarly, Wang et al. (2007) found that Danggui-Huangqi combination could effectively decrease the excessive expression of CD54 in vessels endothelial cells and hepatic sinusoid endothelial cells of Qi deficiency and blood stasis rats. In cultured HUVECs used as a model to examine cell proliferation with the MTT assay, Danggui-Huangqi showed more significant effects on promoting the growth of endothelial cells and increasing population of the cells in S phase when

used in combination (Lei et al., 2003b). In addition, DBT could stimulate the proliferation of T-lymphocytes, and further research demonstrated that the polysaccharide-enriched fraction of DBT exhibited a marked response on cultured T-lymphocytes suggesting important role of DBT polysaccharides in triggering such immune responses (Gao et al., 2007a). Moreover, Huangqi-Danggui (5:1) produced the best effects on stimulating osteoblast proliferation compared with other ratios (1:1, 2:1, 3:1, 4:1, 7:1, 10:1, 0:1, and 1:0), and the application of ethanol-treated Danggui enhanced its efficacy (Dong et al., 2006).

2.1.2.3. *Anti-fibrosis effect*

Fibrosis has an extremely poor prognosis and can affect many organs, and there is currently no effective treatment for this condition. Fortunately, different proportions of Danggui and Huangqi have shown good anti-hepatic fibrosis effects in rats. The classical combination at a 5:1 proportion had the best general effects, the mechanism of which may be related to the alleviation of hepatic lipid peroxidation damage (Tao et al., 2008). In addition, DBT was effective in the treatment of renal fibrosis, and Meng et al. (2010) discovered that the anti-renal fibrosis effects of DBT in obstructive nephropathy were mediated through multiple pathways, and novel mechanisms may be involved in increasing degeneration of ECM, decreasing ROS reaction, and regulation of the calcium-phosphate metabolism.

2.1.2.4. *Other pharmacological activities*

As mentioned above, Danggui-Huangqi has ideal effects on the hematological system, immune system and fibrosis of tissue and organs. This herb pair also exhibited anti-hyperglycemic activity, and estrogen-like effects, and other effects. Liu et al. (2011) found that DBT could improve insulin sensitivity through increased post-receptor insulin signaling mediated by enhancements in insulin receptor substrate-1-associated phosphatidylinositol 3-kinase step and glucose transporter subtype 4 translocation in the soleus muscles of animals with insulin resistance. Furthermore, DBT could serve as a novel therapeutic treatment to replace estrogen to relieve menopausal symptoms (Gao et al., 2007b). Lastly, Danggui-Huangqi also showed significantly greater scavenging capacity than would be expected from the theoretical sum of the activities of the respective constituent herbs (Yang et al., 2009).

2.1.3. *Clinical studies*

Clinical studies of the Danggui-Huangqi herb pair have demonstrated promising results

(Table 2). A combination of the Danggui-Huangqi herb pair and the Danggui-Rougui herb pair was used in the Huangqi Buqi Tang formulae recorded in *Fuqingzhu Nvke*, the formulae was used to treat miscarriage resulted from aversion to cold. A combination of the herb pair with the Dihuang-Maidong herb pair formed the Songzi Dan formulae used for treating hematasthenic dystocia. The Danggui-Huangqi herb pair was combined with the Ganciao-Jinyinhua herb pair to create the Gui Qi Yin formulae described in *Zhangshi Yitong*, which was suitable for treating the syndrome of carbuncle in the middle nape and back. Because TCM is constantly developing, it is essential that we correctly recognize, evaluate and make full use of this accumulated clinical experience as the basis for innovation (Liu, 2009).

2.1.4. Remarks on Danggui-Huangqi

Modern researchers have explored the variation in the chemical components, pharmacological activities and clinical practice of the combination of Danggui and Huangqi. The variation in the contents of their bio-active components and the improved synergetic effects after combination may reveal why ancient physicians prefer to boil Danggui and Huangqi at a particular ratio (1:5) for oral administration.

2.2. Danggui and Chuanxiong

Chuanxiong (Chuanxiong Rhizoma), the dried rhizome of *Ligusticum chuanxiong* Hort. (Umbelliferae), is one of the most popular plant medicines in the world. Chuanxiong, which is commonly used in TCM, is warm in property and pungent in flavor, with the functions of activating qi, promoting blood circulation, expelling wind and alleviating pain (Li et al., 2012a). The main pharmacological effects of Chuanxiong are vasorelaxation (Chen et al., 2007), immunomodulation (Cai et al., 2011), anti-inflammation (Huang et al., 2013), and analgesia (Du et al., 2007). Organic acids (Li et al., 2002), phthalides (Yan et al., 2005) and alkaloids (Yang et al., 2011) were reported to be the bioactive ingredients in Chuanxiong. By reviewing a database containing 5623 formulae of Danggui and Chuanxiong, we found that a 1:1 ratio was the most frequent and that the combination was commonly used in gynecology according to the statistical analysis (Jin et al., 2015). A combination of Danggui and Chuanxiong with a ratio of 3:2 is an ancient and classic formulae named Foshousan, which was first recorded by Xu Shuwei in *Puji Benshi*, published in the Song Dynasty (AD 1132) of China. Foshousan is mainly prescribed to treat women's ailments, especially obstetric diseases, including dystocia, vaginal bleeding with

fetal movement, dead fetus in uterus, and postpartum anemic fainting.

2.2.1. *Bio-active components of Danggui-Chuanxiong*

2.2.1.1. *Bio-active component variation of Danggui-Chuanxiong*

The supercritical CO₂ extraction technique is a promising alternative to conventional extraction methods. This technique has gained increased attention in Chinese medicine industries for obtaining high-added-value compounds. To study regularity using this technique, Yu et al. (2002) compared the extraction rates of the combination of Danggui-Chuanxiong and the single herb. The results showed the rates of Danggui-Chuanxiong were clearly greater than the sum of the rates of single Danggui or Chuanxiong. Furthermore, the results of investigation of variation in the extractable contents of the main phthalides in Foshousan, Siwutang, Danggui and Chuanxiong revealed that the extractable content of the phthalides in Foshousan was higher than total of that in Danggui and Chuanxiong, whereas the content of the phthalides in Siwutang was lower than the total of those in Danggui and Chuanxiong because Dihuang and Shaoyao possibly affected the extraction of the phthalides. Furthermore, the extractable contents of every phthalide in Foshousan and Siwutang were different from those of the single herbs. This finding is a possible reason for the differences in their therapeutic action (Tang et al., 2010). Moreover, water extracts of Danggui-Chuanxiong could better increase the dissolution of aromatic acids and phthalides compared to a single herb, and the contents of total acids in ethanol and water-alcohol extracts of Danggui, Danggui-Chuanxiong and Chuanxiong increased as the proportionality coefficient of Chuanxiong increased. The contents of total phthalides in water extracts of Danggui-Chuanxiong increased as the proportionality coefficient of Chuanxiong increased, whereas the contents of total phthalides in ethanol and water-alcohol extracts of Danggui-Chuanxiong changed erratically. The same extraction method caused the contents of total phthalides in Danggui, Danggui-Chuanxiong and Chuanxiong to be higher than the contents of total acids (Li et al., 2012b). Based on previous research, a study was developed to simultaneously quantify three aromatic acids and six phthalides in Danggui, Chuanxiong and Foshousan. The results showed that the observed contents of 9 analytes in Foshousan were slightly higher than those in Danggui, but significantly lower than those in Chuanxiong. This result was likely because the contents of the compounds were relatively high in the single herb of Chuanxiong. The contents of each component in the formulae were not merely the simple sum of their contents in the single herbs (Li et al., 2014a).

2.2.1.2. *Bio-active component variation of Danggui-Chuanxiong in vivo*

The differences *in vivo* are noteworthy because the since Danggui-Chuanxiong herb pair is orally administrated via decoction in clinics. After oral administration, the pharmacokinetic properties of ferulic acid were compared between normal and blood deficiency model rats. Both the $AUC_{(0-t)}$ and C_{max} of ferulic acid in rats treated with Danggui-Chuanxiong increased significantly, whereas clearance decreased significantly. Among three blood deficiency model groups, $t_{1/2\alpha}$, V_d , $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ all increased significantly in the Danggui-Chuanxiong group compared with the Danggui group. The results indicated that ferulic acid was better absorbed and more slowly eliminated in the blood deficiency rats. Furthermore, Chuanxiong could significantly prolong the half-life of distribution, and increase the volume of distribution and the absorption amount of ferulic acid of Danggui in the blood deficiency rats, which might be due to the synergetic effects of Danggui and Chuanxiong when they were used together to treat the syndrome of blood deficiency (Li et al., 2012c).

2.2.2. *Compatibility effects of Danggui-Chuanxiong*

2.2.2.1. *Effect on nourishing and tonifying blood*

When multiple herbs are used together, the original physicochemical properties of a single herb changed due to mutual influences between them, thus generating variation in pharmacological effects, similar to herb pairs. Compared with the single herbs, the Danggui-Chuanxiong herb pair presented blood-enriching effect to different extents, with a strengthening trend in regulating invigorating blood indexes, immune organs and energy metabolic enzymes (Li et al., 2011). Furthermore, the same research team revealed that Foshousan extracted with different concentrations of ethanol had total enriching blood effects in the following order: 40%>30%>20%>50%>15%>70%>10%>5%>95%>0% (Huang et al., 2011). Interactions with different proportions (0:1, 1:5, 2:5, 2:3, 1:1, 3:2, 5:2, 5:1, and 1:0) were also analyzed using the response surface method. Most compatibility ratios of Danggui and Chuanxiong exhibited synergistic actions, some showed additive actions, and few showed obvious antagonistic actions. Proportions of Danggui and Chuanxiong from 4:1 to 2:1 and doses from low to high showed additive actions, whereas the other proportions showed obvious additive actions at low doses and synergistic actions at high doses (Huang et al., 2013a).

2.2.2.2. *Effect on activating blood circulation and dissolving blood stasis*

Blood stasis plays an important role in the pathogenesis and development of many diseases, such as angina pectoris and acute myocardial infarction. Li et al. (2011b, 2012d) measured prothrombin time (PT), activated partial thromboplastin time (APTT), and plasma fibrinogen (FIB) to observe the effects of Danggui and Chuanxiong on the blood coagulation function of blood stasis rats. Compared with the normal group, hemorheological indexes obviously increased, PT and APTT were obviously shortened, and the contents of FIB increased in the model group. Compared with the model group, the water extracts, alcohol extracts, and water-alcohol extracts of Danggui, Chuanxiong, Danggui and Chuanxiong (1:1, 1.5:1) decreased hemorheological indexes, prolonged the PT and APTT, and significantly reduced the content of FIB. Danggui-Chuanxiong showed better total effects of activating blood circulation and dissipating blood stasis. Using the response surface method to analyze pharmacodynamic interactions with different proportions (0:1, 1:5, 2:5, 2:3, 1:1, 3:2, 5:2, 5:1, and 1:0). Huang et al. (2012) found that proportions from 2:1 to 1.3:1 had synergetic action. Moreover, a study based on anti-oxidative activity indicated that a water preparation of Danggui-Chuanxiong exhibited the strongest scavenging activity against OH generated in a Fenton reaction system. Ethanol preparation had the weakest activity against OH, whereas it exerted the strongest activity on damaged HUVECs (Ma et al., 2010). Additionally, Danggui-Chuanxiong significantly inhibited the proliferation and protein synthesis of vascular smooth muscle cells (VSMC) induced by blood serum in a dose- and time-dependent manner, and it markedly inhibited VSMC proliferation by arresting G1 to S progression (Hou et al., 2004, 2005). Moreover, Foshousan could benefit endothelial function through increased activity of Akt kinase and eNOS, and this effect was caused by an increase in intracellular Ca^{2+} and a reduction in ROS (Bi et al., 2012).

2.2.2.3. *Effect on regulating menstruation and relieving pain*

Dysmenorrhea is the leading cause of recurrent short-term school absence in adolescent girls and a common problem in women of reproductive age. Wang et al. (2010) observed dysmenorrhea model rats administrated Danggui-Chuanxiong at different proportions (1:0, 2:1, 1.5:1, 1:1, 1:1.5, 1:2, and 0:1) and different extraction methods. The results demonstrated that Danggui-Chuanxiong (1.5:1) and a solution extracted first with alcohol and then with water showed the strongest effects on primary dysmenorrhea by reducing the writhing times, increasing the nitric oxide (NO) concentration and reducing the calcium ion (Ca^{2+}) concentration in uterine tissue. An evaluation of

the effects of different ratios and extraction methods on rat uterine smooth muscle contractions *in vitro* revealed that the most remarkable effects occurred when the herbs were extracted with water followed by 50% alcohol and when the ratios were 1.5:1 and 1:1 (Li et al., 2010). Lastly, Danggui-Chuanxiong exhibited better activity on the proliferation of rat ovarian granulosa cells than the single herbs (Yu et al., 2011).

2.2.2.4. *Correlations between the efficacy and chemical constituents of Danggui-Chuanxiong*

Our group evaluated the overall effects of the Danggui-Chuanxiong herb pair at different proportions and formulas on nourishing and tonifying blood (NTB), activating blood circulation and dissolving blood stasis (ADBS), regulating menstruation and relieving pain (RMRP) with the goal of examining correlations between the effects and constituents and their degrees. Using an artificial neural network, we found that water extracts of Danggui-Chuanxiong (1.5:1) had the best effects on total NTB, alcohol extracts of Danggui-Chuanxiong (1:1) had the best effects on total ADBS, and alcohol extracts of Danggui-Chuanxiong (1.5:1) had the best effects on RMRP. Using the same extraction methods, extracts of Danggui had better effects on total NTB than Chuanxiong, whereas extracts of Chuanxiong had better effects on total ADBS and RMRP than Danggui. At the same prescription proportions, water extracts of Danggui-Chuanxiong had the best effects on total NTB, whereas alcohol extracts of Danggui-Chuanxiong had the best effects on total ADBS and RMRP. Additionally, aromatic acids were the main effective components for NTB, especially chlorogenic acid and caffeic acid. Both aromatic acids and phthalide lactones were active components for ADBS, and chlorogenic acid and senkyunolides I and H in particular showed greater effects on ADBS. Both aromatic acids and phthalide lactones were also the important active constituents for the effect of RMRP, in particular, ligustilide, caffeic acid, ferulic acid, and senkyunolide I showed greater effects on RMRP (Fig. 3) (Li et al., 2012e).

2.2.2.5. *Metabolomic studies of the mechanism of the nourishing blood effect of Foshousan*

Metabolomics (alternately, metabonomics), an emerging field of biochemical research, is a complementary technique to genomics, transcriptomics, and proteomics. Metabolomics is the comprehensive assessment and simultaneous profiling of endogenous metabolic changes in living systems. UPLC-QTOF/MS was used to analyze the metabonomics of Foshousan. Eleven potential biomarkers were identified using a multivariate statistical analysis of urine metabolite profiles, and these biomarkers were used to analyze phenylalanine, tryptophan and sphingolipid

metabolism. Those disturbed metabolic pathways in acute blood stasis rats could be returned to a nearly normal state by the administration of Foshousan (Huang et al., 2013b). Additionally, global metabolic profiling combined with pattern recognition method was performed to reveal the underlying hematopoietic regulatory mechanisms of Danggui, Chuanxiong and Danggui-Chuanxiong in rats with hemolytic and aplastic anemia (HAA). Thirteen endogenous metabolites that differentiated the model group from the control group were tentatively identified. The levels of LPCs including lysoPC (18:0), lysoPC (20:4), lysoPC (16:0) and lysoPC (18:2), sphinganine, nicotinic acid, thiamine pyrophosphate, phytosphingosine, and glycerophosphocholine increased significantly in the HAA rats, whereas the levels of oleic acid, 8,11,14-eicosatrienoic acid, ceramides (d18:1/14:0) and 17 α -hydroxypregnenolone decreased significantly compared with the control rats. Those endogenous metabolites are chiefly involved in thiamine metabolism and sphingolipid metabolism. The metabolic deviations were returned to closer to normal levels after Danggui, Chuanxiong and Danggui-Chuanxiong interventions. In terms of hematopoietic function, Danggui-Chuanxiong was the most effective as shown by the relative distance in PLS-DA score plots and relative intensity of metabolomics strategy, reflecting synergetic actions between Danggui and Chuanxiong. The relative distance calculation was first used in metabolomics for semi-quantization (Fig. 4) (Li et al., 2014b).

2.2.3. *Clinical studies*

Numerous clinical trials of the Danggui-Chuanxiong herb pair are now underway in China and many other countries (Table 2). The Danggui-Chuanxiong herb pair is applied for the syndrome of depletion of Chong and Ren meridians at a ratio of 3:2; for example, in Jiao Ai Tang, the doses of Danggui and Chuanxiong are 9 g and 6 g. When used to treat the syndrome of impairment of both Yin and Yang, the herb pair is applied at a ratio of 5:3. Using Shu Yu Wan as an illustration, the doses of Danggui and Chuanxiong are 30 g and 18 g. When used to treat the syndrome of deficiency of both heart and spleen, the ratio of the herb pair is 1:1 and the same doses of Danggui and Chuanxiong in Houshi Hei San are used (Tang and Duan, 2014).

2.2.4. *Remarks on Danggui-Chuanxiong*

Studies of the synergetic effects of the Danggui-Chuanxiong herb pair have demonstrated that aromatic acids and phthalide lactones were active components for the effects of NTB, ADBS and RMRP, representing great progress. However, it is unclear whether the synergetic activities of

these components exist in Danggui-Chuanxiong alone or in any other herbs containing these components; hence, this question requires further relevant research.

2.3. *Danggui and Shaoyao*

Shaoyao has historically been described in classical books with Baishao and Chishao. Baishao (*Radix Paeoniae Rubra*) and Chishao (*Radix Paeoniae Alba*), from the dried roots of the common plant *Paeonia lactiflora* Pall (Ranunculaceae), have been widely used to treat inflammatory conditions according to the traditional Chinese medical system. Scientifically, the dried root of wild-grown *Paeonia lactiflora* Pall is *Radix Paeoniae Rubra*, and the dried peeled and water-boiled root of domestically grown *Paeonia lactiflora* Pall is *Radix Paeoniae Alba*. Shaoyao has been reported to exhibit pharmacological actions, such as antitumor (Xu et al., 2012), immunomodulatory (Wang et al., 1997), and liver-protective effects (Liu et al., 2006). Paeoniflorin, a monoterpene glucoside, is the bioactive component of Shaoyao (Wang et al., 2008). Danggui-Chishao and Danggui-Baishao both are recognized as herb pairs. Danggui-Baishao is used to nourish blood, whereas Danggui-Chishao tends to activate blood; they all have unique clinical applications.

2.3.1. *Bio-active components of Danggui-Shaoyao*

2.3.1.1. *Bio-active component variation of Danggui-Shaoyao*

Extraction methods play an important role on the effects of extraction. Using the same extraction method, it was found that the contents of ferulic acid and paeoniflorin in the Danggui-Baishao herb pair were higher than those in the single herbs, as was senkyunolide (Zhu et al., 2013). Furthermore, another study focusing on Danggui-Chishao revealed that different extraction solvents could affect the dissolution rates of phenolic acids, monoterpenes and phthalide lactones in this herb pair, with most being relatively high in water and low-concentration alcohol than in other solvents (Ding et al., 2012a).

2.3.1.2. *Bio-active component variation of Danggui-Shaoyao in vivo*

Chinese herbs are often used in formulae instead of being used singly. As a result, changes in herb compatibility lead directly to variation in metabolic pathways *in vivo*. Ding et al. (2012b) analyzed the metabolites of ferulic acid and gallic acid in rats' plasma and urine after the oral administration of Danggui-Chishao. The results indicated that the *in vivo* metabolites of ferulic acid were in the form of methylation products, sulfate conjugation products and glucuronidation

conjugation products and other forms. Furthermore, gallic acid was mainly transformed into reduction products and methylation products. After the oral administration of Danggui-Baishao to rats, the pharmacokinetics features of seven bio-active components (ferulic acid, caffeic acid, vanillic acid, ligustilide, paeoniflorin, albiflorin and oxypaeoniflorin) could be altered by the compatibility of Danggui and Baishao. Phenolic acids in Danggui-Baishao, such as ferulic acid and caffeic acid, were absorbed less than in the Danggui single herb, and the elimination of all these phenolic acids could be delayed by this herb pair. Ligustilide in Danggui-Baishao was absorbed more than in the Danggui single herb and was eliminated more rapidly. Furthermore, the monoterpene glycosides of paeoniflorin, albiflorin and oxypaeoniflorin were absorbed more rapidly and eliminated more slowly in Danggui-Baishao than in the single herb of Baishao (Luo et al., 2014).

2.3.2. *Compatibility effects of Danggui-Shaoyao*

When a certain herb is combined with other herbs, these herb pairs may have greater efficacy. A study revealed that Danggui-Baishao increased HGB, increased HCT, reduced SI and had better blood-nourishing effects, whereas Danggui-Chishao reduced whole blood low reduction viscosity, blood sedimentation equation K, and AI in addition to lengthening APTT and having a better blood-activating effect (Luo et al., 2013; Zhu et al., 2011a). In a comparative study of the effects of Siwutang and its herb pairs, Danggui-Baishao showed a significant promotion of rat ovarian granulosa cell proliferation (Yu et al., 2011). Additional research indicated that Danggui-Baishao may be the most effective herb pair in Siwutang for isolated rat uterine contractions (Zhu et al., 2011b).

2.3.3. *Clinical studies*

Clinical experience has shown that when Danggui-Baishao is used to treat the syndrome of blood deficiency with heat, the Baishao dose should be large (30 g). Furthermore, when Danggui-Baishao is used for the syndrome of blood deficiency with cold, the Danggui dose should be large (20-30 g) (Tang and Duan, 2014). Unfortunately, there have been few clinical studies of Danggui-Chishao; thus, more attention should be paid to the therapeutic mechanism of the Danggui-Chishao herb pair.

2.3.4. *Remarks on Danggui-Shaoyao*

Although there have been some valuable studies of the chemical components and

compatibility effects of Danggui-Shaoyao, we believe that more pharmacokinetic studies on Danggui-Chishao are needed because the components should be absorbed into plasma to play their role in the hematological system. Thus, it is necessary for relevant researchers to further analyze which components in plasma are active and which are inactive.

2.4. *Danggui and Renshen*

Renshen (Ginseng Radix et Rhizoma), the dried radix and rhizome of *Panax ginseng* C.A. Meyer (Araliaceae), is an “imperial” herb because of its nontoxic and rejuvenating properties. Renshen has been used as an herbal remedy in ancient China, Korea, Japan and the Far East for more than 5,000 years. Renshen has been shown to affect various biological processes, including tumor metastasis and the central nervous system, as well as prevention of the aging process and diabetes (Chen et al., 2015). Ginsenosides are the major constituents of Renshen (Wang et al., 1999). The total ginsenoside content was decreased by the combination of Danggui and Renshen at different proportions in alcohol; the 1:1 proportion caused the greatest reduction (Bei et al., 2013). In addition, Danggui-Renshen had ideal effects on blood stasis rats by effectively reducing blood viscosity and decreasing the amount of plasma endothelin. Another study also showed that the proportion of 1:1 could improve the immune function of immunocompromised rats by obviously raising the spleen index and carbon clearance index (Yang et al., 2013). During the process of clinical application, it was demonstrated that when the Danggui-Renshen herb pair was used for benefiting Qi to control blood, the doses of Danggui and Renshen should be 6 g and 30 g. When the herb pair was used to invigorate Qi and activate blood, the best doses were 10 g and 15g (Tang and Duan, 2014). Based on the above analysis, it was found that the synergetic effects of different compatibility ratios of Danggui-Renshen had different synergetic effects, but this result was not sufficient to explain the association between ratio and efficacy. As a result, more data are needed in terms of chemistry and pharmacology.

2.5. *Danggui and Dahuang*

Dahuang (Rhei Radix et Rhizoma), the dried radix and rhizome of *Rheum palmatum* L., *Rheum tanguticum* Maxim. Ex Baif, *Rheum officinale* Baill. (Polygonaceae), is bitter in flavour and cold in nature. The herb pair of Dahuang and Danggui could nourish blood and purge fire. The total contents of anthraquinones in Dahuang injection increased in the combination, which was directly proportional to the added amount of Danggui. Furthermore, the contents of free and

glycoside-combined anthraquinones in Dahuang decreased, which might contribute to reducing the toxicity of Dahuang (Qin et al., 2010). The combination of Danggui-Dahuang presented better antineoplastic activity in Lewis lung carcinoma, sarcoma-s180 and Ehrlich ascites tumor cells. It will be worthwhile to consider the optimal clinical dosage to exert synergetic anti-tumor effects.

2.6. Danggui and Baizhu

Baizhu (*Atractylodes Macrocephalae Rhizoma*), the dried rhizome of *Atractylodes macrocephala* Koidz., has been used to invigorate spleen and eliminate dampness according to the theory of TCM science. Danggui and Baizhu were combined to nourish both blood and Qi. In clinical application, Danggui-Baizhu made a great contribution to the antidepressant effect of Xiaoyao San, a famous Chinese prescription for the treatment of depression (Zhou et al., 2012). Unfortunately, there is a lack of research which will demonstrate whether the combination of Danggui-Baizhu has potential synergetic effects.

2.7. Danggui and Baizhi

Baizhi (*Angelicae Dahuricae Radix*), the radix of *Angelica dahurica* (Fisch. ex Hoffm.) Benth. et Hook. f., and *Angelica dahurica* (Fisch. ex Hoffm.) Benth. et Hook. f. var. *formosana* (Boiss.) Shan et Yuan, has traditionally been used for the treatment of headache caused by the common cold, asthma, coryza, hypertension, vitiligo, psoriasis and photodynamic therapy. Water decoctions of Danggui-Baizhi were examined for antibacterial action using a suspension quantitative bactericidal test. As a result, killing rates for *Escherichia coli* and *Staphylococcus aureus* were higher than 99.9% (Liu et al., 2000). Although the antibacterial action of Danggui-Baizhi was significant, it is a pity that the study of Danggui-Baizhi was too limited to discover the regularity of their compatibility.

2.8. Danggui and Chenpi

Chenpi (*Citri Reticulatae Pericarpium*), the dried pericarp of *Citrus reticulata* Blanco or its cultivars, is used to treat indigestion and inflammatory syndromes of the respiratory tract, such as bronchitis and asthma. As adjuvant drugs in the formulae of Buzhong Yiqi Tang, Danggui-Chenpi had marked effects on the contents of glycyrrhizic acid (Ke et al., 2009). However, there are no reports on the relationship between variation in the contents of glycyrrhizic acid and pharmacological effects. Additionally, there has also been scant research examining whether the combination of Danggui and Chenpi has impacts on other components of Gancan.

3. Concluding remarks

Herb pairs, as an important link between single herbs and formulae, are used in TCM clinics with great success. Many famous herb pairs, such as Danggui-Huangqi and Danggui-Chuanxiong, have attracted considerable attention. These herb pairs have been studied by modern scholars, which may be related to their wide use in such well-known TCM formulae as DBT and Foshousan. It was found that fewer studies focused on Danggui-Fuzi, Danggui-Huanglian, Danggui-Gancao, Danggui-Fangfeng and Danggui-Ganjiang, although they were recorded in classical books as commonly used herb pairs. Moreover, some combinations, such as Danggui-Niuxi and Danggui-Chaihu, have not been recognized as herb pairs in many relevant books although they were combined at high frequency according to the statistical analysis. Further research is urgently needed to gain a better understanding of the compatibility law of these herb pairs and offer better service for TCM clinics.

Recently, some TCM researchers have been interested in investigating the bio-active constituents and compatibility effects of herb pairs. Thus, some methods for in-depth study of herb pairs are essential to be established. First, the *in vitro* or *in vivo* bio-active constituents of herb pairs may differ from those of the single herbs. Complicated physical and chemical reactions are involved in the decoction process of herb pairs, and these chemical interactions result in quantitative or qualitative changes in bio-active constituents, which influences the variation in the overall effects of herb pairs. Furthermore, the different components of herb pairs may promote or inhibit the absorption, distribution, metabolism and excretion *in vivo* each other. Therefore, studies on the bio-active constituents of herb pairs are needed to establish a foundation for revealing the regularity of herb pair compatibility. Second, comparative research methods ought to be used for the effect evaluation of herb pairs. These methods could include effect comparisons between herb pairs and single herbs; between herb pairs and formulae; among single herbs, herb pairs, and formulae; and among single compounds, single herbs, herb pairs, and formulae. Comparisons of different compatibility ratios, different extraction methods, and different processed products of the same herb pair might also be useful. Third, component compatibility may be considered when the bio-active constituents and effects of an herb pair have been clearly demonstrated, which may lead directly to the development of medicines for clinical application. Of course, the goal of our basic study of herb pairs should be their clinical application and the development of new drugs. By

means of many basic studies, we might demonstrate the dosages and ratio of two herbs as an herb pair and the synergetic extent when an herb pair has the synergetic effects, and we could explain why the herb pair has the synergetic effects and reveal the relevant effect mechanisms, which should be utilized by TCM physicians in clinical practice. Furthermore, abundant experimental studies and clinical observations of herb pairs and the relevant formulae may provide clues for the development of new drugs; in turn, this will provide better clinical service in the future.

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Legends of Figures

Fig. 1. The correlation between major chemical constituents and pharmacological effects of Danggui and Huangqi

Fig. 2. Three-dimensional response surface diagram (A), Sectional response surface diagram (B,C) of Danggui-Huangqi compatibility

Fig. 3. The correlation between major chemical constituents and pharmacological effects (A. nourishing and tonifying blood, B. activating blood circulation and dissipating blood stasis, C. regulating menstruation and relieving pain) of Danggui and Chuanxiong

Fig. 4. PLS-DA score plot (A1 and B1) and 3-D PLS-DA score plot (A2 and B2) classifying the control, model, Danggui, Chuanxiong and Gui-Xiong groups on day 7 both in positive and negative modes

Fig. 5. The PLS score plot (A) and VIP plot (B) of blood-deficiency indexes after oral administration (1. normal group 2. model group 3. Danggui-Shudihuang 4. Danggui-Huangqi 5. Danggui-Baishao 6. Danggui-Chishao 7. Danggui-Chuanxiong 8. Danggui-Taoren)

Table 1. The composition, function and related books of herb pairs containing Danggui

(compatibility frequency >1500)

No.	Herb pairs	Compositions	Frequency	Functions and clinical uses	Disease category	The related recorded books
1	Danggui and Shaoyao	Angelicae Sinensis Radix, Paeoniae Radix Alba/Paeoniae Radix Rubra	7938	Nourishing blood, Cultivating heart and softening liver (Baishao) Clearing heat, activating blood and dispersing swelling (Chishao)	Internal medicine	Shishi Milu (Baishao) 石室秘录(白芍) Yifang Leiju (Chishao) 医方类聚(赤芍)
2	Danggui and Gancao	Angelicae Sinensis Radix, Glycyrrhizae Radix et Rhizoma	7571	Palate carbuncle (悬痈)	Internal medicine	Yangke Jiejin (疡科捷径)
3	Danggui and Chuanxiong	Angelicae Sinensis Radix, Chuanxiong Rhizoma	5623	Nourishing blood, Activating blood and resolving stasis	Gynecology	Puji Benshi Fang (普济本事方)
4	Danggui and Renshen	Angelicae Sinensis Radix, Ginseng Radix et Rhizoma	4894	Tonifying heart Qi, Benefiting heart blood	Internal medicine	Xueshi Yian (薛氏医案)
5	Danggui and Fuling	Angelicae Sinensis Radix, Poria	3951	---	Internal medicine	---
6	Danggui and Baizhu	Angelicae Sinensis Radix, Atractylodis Macrocephalae Rhizoma	3918	Strengthen the spleen-Qi, Benefiting heart blood	Internal medicine	Yixue Rumen (医学入门)
7	Danggui and Rougui	Angelicae Sinensis Radix, Cinnamomi Cortex	3520	---	Internal medicine	---
8	Danggui and Dihuang	Angelicae Sinensis Radix, Rehmanniae Radix/ Rehmanniae Radix Praeparata	3402	Nourishing blood,liver and kidney, Nourishing Chong and Ren meridians	Internal medicine	Yixue Rumen 医学入门(地黄) Puji Benshi Fang 普济本事方 (熟地黄)
9	Danggui and Fangfeng	Angelicae Sinensis Radix, Saposhnikoviae Radix	3241	hemorrhage of pregnancy	Internal medicine	Yichao Leibian (医钞类编)
10	Danggui and Huangqi	Angelicae Sinensis Radix, Astragali Radix	3110	Invigorating Qi and generating blood	Internal medicine	Neiwaishang Bianhuo Lun (内外伤辨惑论)
11	Danggui and Muxiang	Angelicae Sinensis Radix, Aucklandiae Radix	2457	---	Internal medicine	---

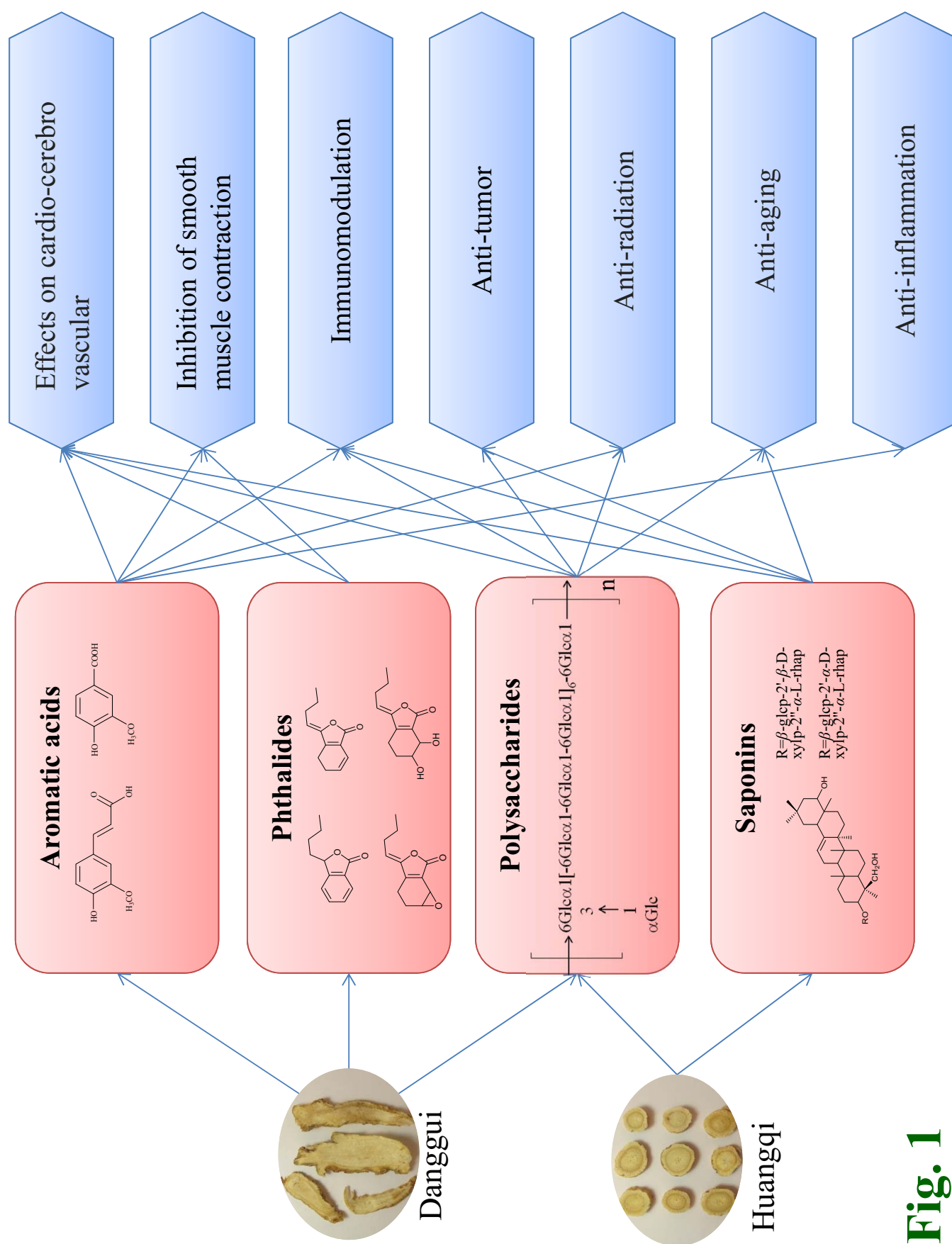
12	Danggui and Huangqin	Angelicae Sinensis Radix, Scutellariae Radix	2439	Nourishing blood and clearing heat, Stopping menstrual blood	Internal medicine	Gujin Yijian (古今医鉴)
13	Danggui and Baizhi	Angelicae Sinensis Radix, Angelicae Dahuricae Radix	2198	Stopping bleeding	Internal medicine	Xiannian Ji (仙拈集)
14	Danggui and Chenpi	Angelicae Sinensis Radix, Citri Reticulatae Pericarpium	2110	Moving Qi to activate blood	Internal medicine	Zhushi Jiyan Fang (朱氏集验方)
15	Danggui and Niuxi	Angelicae Sinensis Radix, Achyranthis Bidentatae Radix	2036	---	Internal medicine	---
16	Danggui and Huanglian	Angelicae Sinensis Radix, Coptis Rhizoma	2014	Nourishing blood and clearing heat	Internal medicine	Shenghui Fang (圣惠方)
17	Danggui and Dahuang	Angelicae Sinensis Radix, Rhei Radix et Rhizoma	1992	Nourishing blood and purging fire, Activating blood	Internal medicine	Hejiju Fang (和剂局方)
18	Danggui and Fuzi	Angelicae Sinensis Radix, Aconiti Lateralis Radix Praeparata	1944	Nourishing blood and eliminating dampness	Internal medicine	Xiuzhen Fang (袖珍方)
19	Danggui and Qianghuo	Angelicae Sinensis Radix, Notopterygii Rhizoma et Radix	1911	---	Internal medicine	---
20	Danggui and Chaihu	Angelicae Sinensis Radix, Bupleuri Radix	1797	---	Internal medicine	---
21	Danggui and Ganjiang	Angelicae Sinensis Radix, Zingiberis Rhizoma	1793	Nourishing blood and eliminating dampness	Internal medicine	Zhengzhi zhunsheng (证治准绳)
22	Danggui and Zhiqiao	Angelicae Sinensis Radix, Aurantii Fructus	1586	---	Internal medicine	---

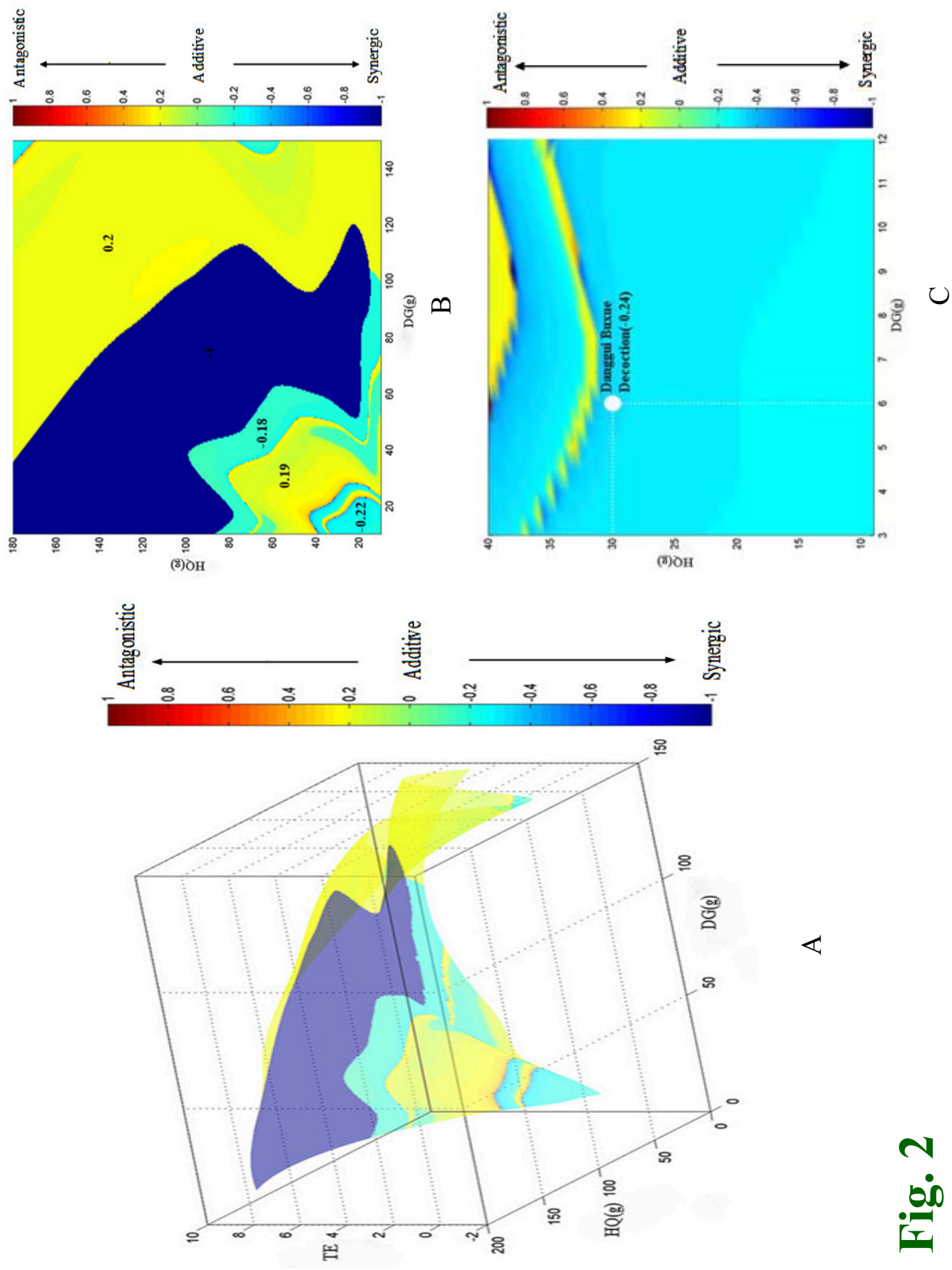
Note: “---” is no record.

Table 2. Clinical studies of herb pairs containing Danggui

Herb 1	Herb 2	Ratio(Herb 1: Herb 2)	Patients	Treatment	Response (Effective Rate)	Reference
	Huangqi (Astragali Radix)	1:5	100 cases of anemia	10 ml oral solution, bid, for 2 months	91%	(Zong et al., 1999)
	Chuanxiong (Chuanxiong Rhizoma)	2:1	60 cases of primary dysmenorrhea	300 ml decoction, bid, for 21 days	98%	(Tong, 2010)
Danggui (Angelicae Sinensis Radix)	Shaoyao (Paeoniae Radix Alba) Renshen (Ginseng Radix et Rhizoma)	3:2 1:1	30 cases of acute myocardial Infarction 32 cases of allergic purpura	200 ml decoction, tid, for 3 weeks 500ml decoction, bid, for 5 weeks	80% highly significant decrease in the number of urinary protein	(Li and Tang, 2014) (Li and Liu, 2008)
	Dahuang (Rhei Radix et Rhizoma)	3:2	300 cases of chronic pelvic inflammatory disease	100 ml decoction per rectum, sid, for 10	94%	(Yu and Ji, 2011)

			days		
Baizhu	1:2	73 cases of	200 ml	92%	(Han et
(Atractylodis		alcoholic	decoction,		al., 2011)
Macrocephalae		fatly liver	bid, for 3		
Rhizoma)			months		
Baizhi	2:1	50 cases of	2000ml	90%	(Liu et
(Angelicae		perianal	decoction,		al., 2013)
Dahuricae		abscess	sitz bath,		
Radix)			bid, for 3		
			weeks		
Chenpi (Citri	2:3	15 cases of	500 ml	80%	(Dong et
Reticulatae		myasthenia	decoction,		al., 2011)
Pericarpium)		gravis	bid, for 3		
			months		

**Fig. 1**



	W-1	A-1	WA-1	W-2	A-2	WA-2	W-3	A-3	WA-3	W-4	A-4	WA-4	W-5	A-5	WA-5	W-6	A-6	WA-6	W-7	A-7	WA-7
C1	0.07	0.06	0.08	0.20	0.31	0.11	0.19	0.48	0.13	0.36	0.33	0.15	0.30	0.46	0.17	0.53	0.49	0.31	0.17	0.79	0.22
C2	0.14	0.09	0.17	0.23	0.12	0.19	0.18	0.15	0.24	0.53	0.18	0.30	0.24	0.15	0.31	0.51	0.16	0.37	0.24	0.20	0.46
C8						0.01		0.12		0.05	0.11	0.01	0.03	0.13		0.12	0.02	0.06	0.03	0.06	0.03
C7							0.09		0.06		0.06	0.05		0.06	0.01			0.06			0.10
C6						0.06		0.02									0.05			0.05	
C9						0.02														0.01	
C5																					
C4																					
C3																					

A

	W-1	A-1	W/A-1	W-2	A-2	W/A-2	W-3	A-3	W/A-3	W-4	A-4	W/A-4	W-5	A-5	W/A-5	W-6	A-6	W/A-6	W-7	A-7	W/A-7	W-8	A-8	W/A-8
C1	0.14	0.30	0.18	0.32	0.88	0.62	0.94	1.19	1.15	0.47	1.16	0.72												
C4	0.04	0.07	0.08	0.30	0.33	0.46	0.30	0.39	0.55	0.21	0.40	0.34												
C5	0.03	0.02	0.06	0.28	0.12	0.46	0.32	0.34	0.40	0.21	0.29	0.33												
C8	0.05	0.01	0.02	0.14	0.09	0.11	0.24	0.06	0.04	0.16		0.25												
C9								0.09	0.14		0.07													
C6		0.01							0.12															
C7																								
C3																								
C2																								

B

	C6	C9	C2	C3	C8	C4	C7	C1	C5
W-1									
A-1	0.51	0.38	0.12	0.28	0.22	0.03	0.05	0.01	
WA-1	0.42	0.26	0.48	0.39	0.32	0.01			
W-2		0.05	0.40	0.03	0.18	0.09			
A-2	0.65	0.63	0.36	0.65	0.31	0.09	0.18		
WA-2	0.05	0.50	1.03	0.41	0.41	0.09		0.01	
W-3		0.05	0.31		0.15	0.11			
A-3	1.12	0.85	0.41	0.75	0.49	0.15	0.08		
WA-3	0.46	0.39	0.55	0.29	0.35	0.11			
W-4	1.14	0.99	0.71	0.15	0.30	0.13		0.05	
A-4	0.51	0.62	0.56	1.10	0.55	0.12	0.41		
WA-4	0.35	0.07		0.46	0.23	0.12			
W-5	1.27	0.35	0.22		0.15	0.19			
A-5	0.35	0.35	0.63	1.60	0.79	0.05	0.26	0.07	
WA-5		0.13	0.52	0.23	0.20	0.10		0.05	
W-6		0.06	0.11	0.23	0.36	0.14			
A-6	1.30	0.30	0.29	0.75	0.23	0.21			
WA-6	0.30	0.30	0.29	0.30	0.20	0.09	0.09		
W-7			0.14		0.09	0.15			
A-7	0.89	0.50	0.07		0.21			0.13	
WA-7	1.39	1.01		0.92	0.50		0.47		

C

Fig. 3

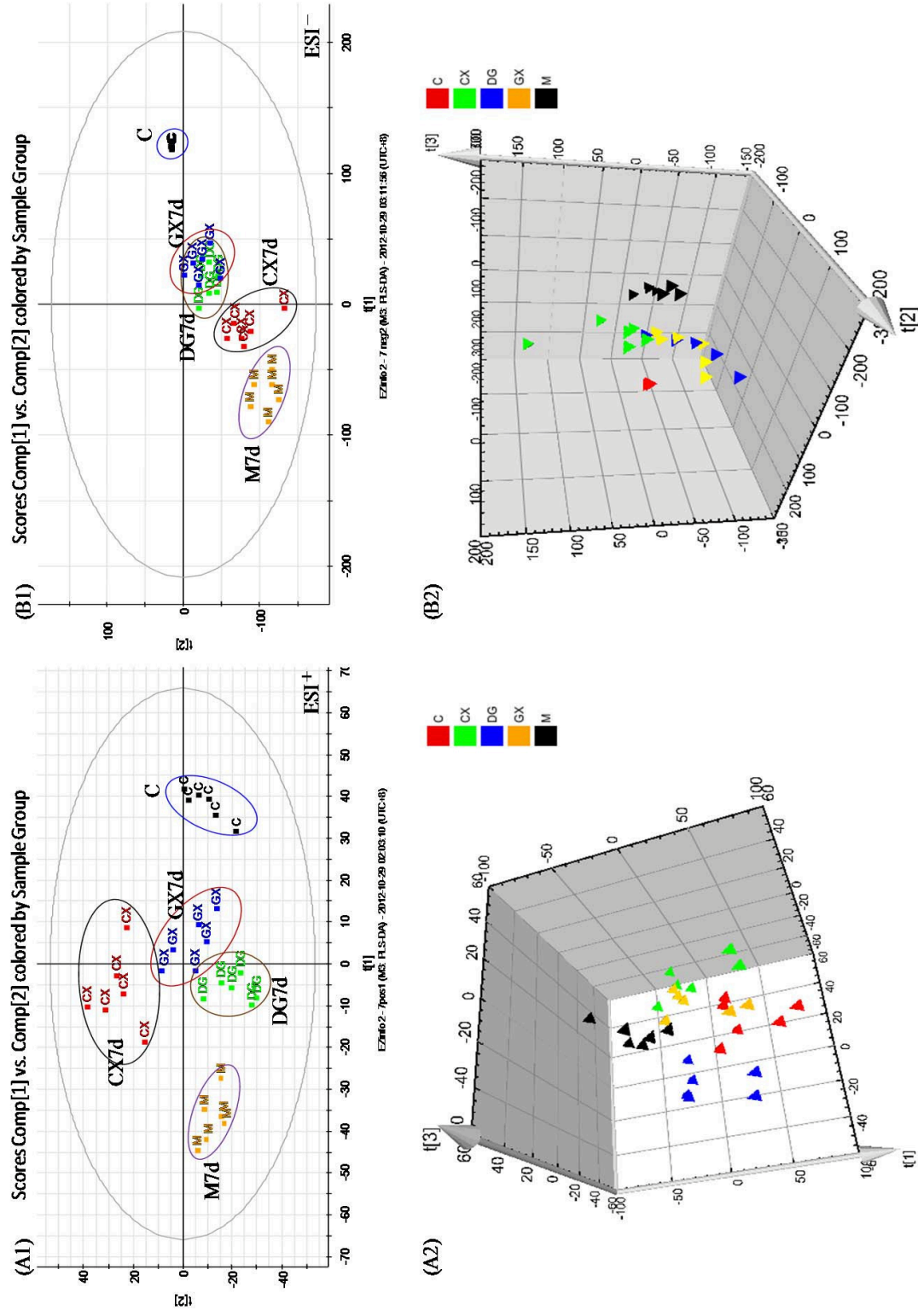
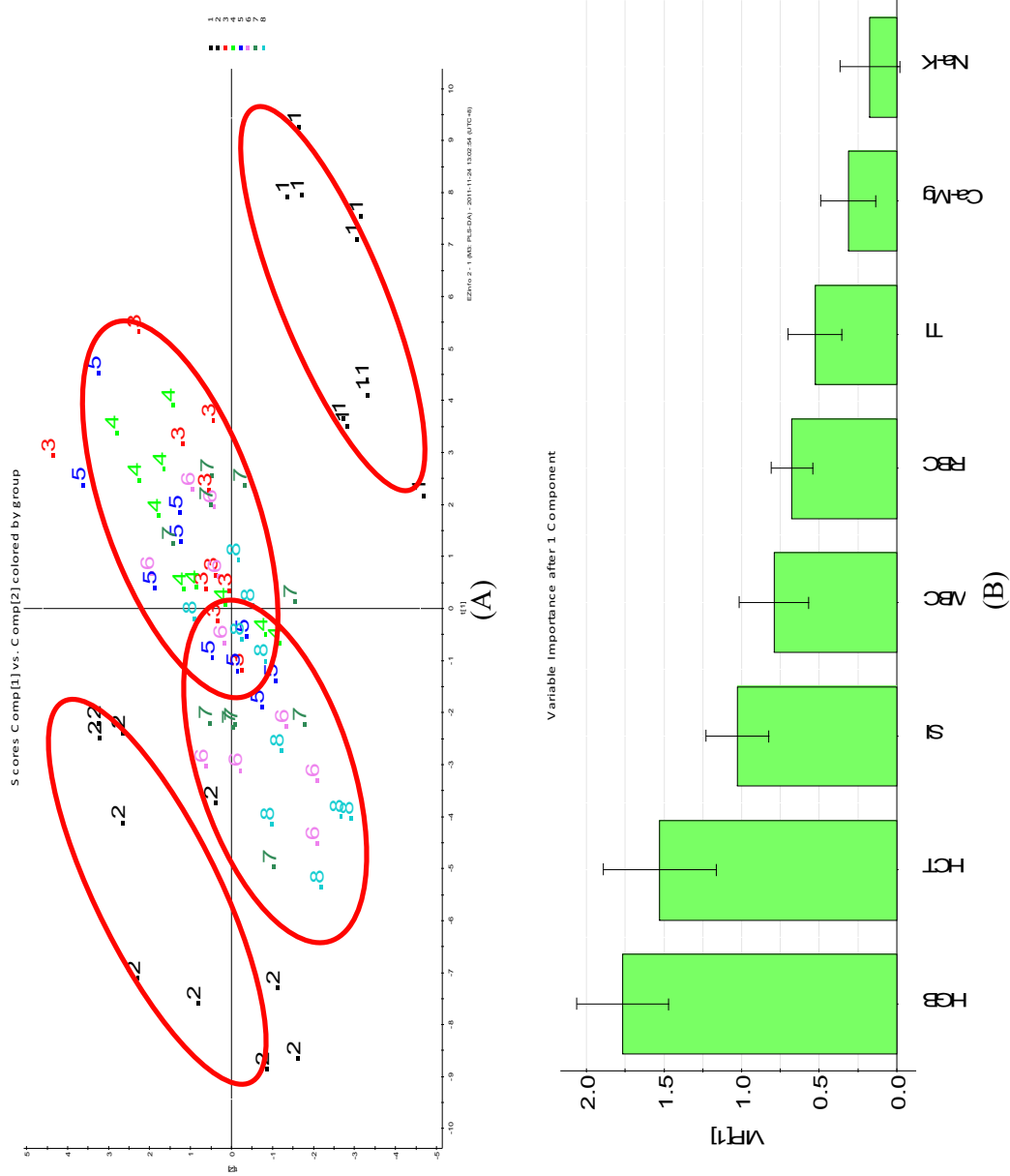


Fig. 4



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Fig. 5

