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Full Length Research Paper

Free radical scavenging activity of Chinese traditional four substances decoction (FSD) extracts

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This article examined the interactive effect among herbs extracts in combined use, compared with the effect of individual herbal extracts on 1,1-diphenyl picryl hydrazyl (DPPH). The Chinese traditional prescription, four substances decoction (FSD) consisted of 25 g of *Angelica sinensis* (Umbelliferae, AS), 6 g of *Ligusticum chuanxiong* Hort. (Umbelliferae, LCH), 10 g of *Paeonia loactiflora* Pall. (Paeoniaceae, PCP), and 15 g of *Radix rehmanniae preparata* (Scrophulariaceae, RRP). Each herb was quantitatively mixed according to the FSD prescription ratio. The interaction between the herbal extract in two-two combination way were estimated using a spectrophotometer. The results showed the strongest synergistic effect in LCH and PLP system, with IC_{50} 0.25 mg/ml, and the additive effect was obvious in the AS and LCH combined system. The other combined system showed additive effect or sub-additive effect. This implied that the combined system effect have a close relationship with the single component activity.

Key words: Compatibility, 1,1-diphenyl picryl hydrazyl (DPPH) free radical, four substances decoction, antioxidant activity.

INTRODUCTION

Herbal remedies are popular in many countries. There is evidence that some of the most commonly used herbs can interact with each other, sometimes with potentially serious consequences. It is of no surprise therefore that some herbal remedies have the potential to interact with conventional drugs.

The interactive effect includes additive, antagonism and synergistic (McFadden and Peterson, 2011). The additive effect means that two drugs, with similar therapeutic action, when used together, can summate and result in unexpected effects. Antagonistic effect means that when two drugs with opposing actions, are taken concurrently, their actions can compete. The synergy effect is defined that drug combination effect is better than single drug effect. Such interactions have been tested in limited fashion in herbal combinations and have the possibility of antagonism as well as synergism effect.

Four substances decoction (FSD) prescription early found by Compendium of Materia Medica in China, is composed of four kinds of Chinese herbal medicine, consisted of 25 g of *Angelica sinensis* (Umbelliferae, AS), 6 g of *Ligusticum chuanxiong* Hort. (Umbelliferae, LCH), 10 g of *Paeonia lactiflora* Pall. (Paeoniaceae, PLP), and 15 g of *Radix rehmanniae preparata* (Scrophulariaceae, RRP). From the purpose of prescription of FSD, the individual component has its own role, for example, *R. rehmanniae preparata* supplement the role of the blood and enhanced renal function (Zhang et al., 2009). *A. sinensis* has the main functions of promoting blood circulation (Yang et al., 2008; Yu et al., 2008; Gao et al., 2008; Wang et al., 2007). *L. chuanxiong* Hort. mainly acts on the liver and bile with the function of calming liver, rheumatism pain, and relieving stasis (Yuan et al., 2008; Ding et al., 2008; Xiao et al., 2010). *P. lactiflora* Pall. can alleviate the vessels by spasms, and increase arterial blood supply (Mao et al., 2009; Zhang et al., 2008). The prescription core of FSD focused on the blood supply. But when individual herb was combined, the interactions among herbal ingredients are not clear. Consequently, the effect of compatibility of herbs is the recent focus of attention.

The aim of this present research work is to assess the interactions of compatibility of herbal extracts from FSD on DPPH free radical scavenging activities.

MATERIALS AND METHODS

A. sinensis, *L. chuanxiong* Hort., *P. lactiflora* Pall., and *R. rehmanniae preparata* were purchased from local pharmacy (YanCheng, Jiangsu, China).

DPPH was obtained from TCI Co. Ltd (Japan). All other chemicals with analytic grade were purchased from Sinopharm Chemical Reagent Co. Ltd (ShangHai, China). Ultrasonic cleaner (KQQ 2200, 40 KHz) from Kunshan City Ultrasonic Instrument Co. Ltd, ShangHai, China and UV-Vis Spectrophotometer from SPECORD-50, JENA, Germany.

Preparation of herbs extracts

The four individual herbs of traditional FSD prescriptions were pulverized into fine powder using a common grinder and was sieved through a No. 60 mesh sieve and stored in an air-tight container, respectively. Each sample (1.00 g of dry weigh, dw) were accurately weighed and firstly extracted in the ultrasonic instrument with 50 ml ethanol for 30 min, followed by a vacuum filtration. Ethanol extracts were collected, and the residues were repeated twice according to the aforementioned method. The ethanol extract of herbs was at a final volume of 100 ml and was stored in the dark until used. Finally, the 10 mg dw/ml stock liquor was obtained.

DPPH assay of individual herb extract of FSD

The potential antioxidant activity of the extracts was assessed on the basis of the scavenging activity of the stable DPPH free radical (Shun et al., 2008) with minor modifications. The reaction mixture contained 2 ml of 5×10^{-3} mol/1 of DPPH ethanol solution, 1 ml of 50 mmol/1 of Tris-HCl buffer (pH 7.4) and 1 ml of the sample extracts. The mixture was shaken and allowed to be placed for 30 min at room temperature. The absorbance of the reaction mixture was measured at 520 nm against ethanol as blank in the spectrophotometer.

The scavenging rate (DPPH%) values were calculated according to the following equation:

$$DPPH\ (\%) = (A_0 - A_s) \times 100\%/A_0$$

where A_0 is the absorbance of control solution, A_s is absorbance of sample. The antioxidant ability of the fraction was expressed as inhibitory concentration (IC_{50}). The IC_{50} value was defined as the extract concentration providing 50% inhibition of dry power sample per ml.

DPPH assay of two-two combined system of extracts of FSD

In combination experiment, the two-two combined system was prepared into a single herb system in three manners, which was first quantitatively mixed from the stock liquor according to the prescription ratio, then extracted in ethanol. Secondly, it was made from mixed stock liquor at equal quantity. Mixture system was finally formulated with an inverse ratio volume of prescriptions. The scavenging rate (DPPH%) values and IC_{50} values of a single combined herb system were determined according to the methods mentioned earlier. Antagonism, additive or synergistic effect were assessed with the interaction index from the data derived experimentally according to the method by Tallarida (2002) and Prakash et al. (2009) with some changes.

Interaction index

The interaction index was originated from the literature (Prakash et al., 2009) and was modified by the consideration that the interaction effect is not same as the ratio of each herb is different. Consequently, the mass percent and IC_{50} were added into the formula and it was denoted by γ. It is calculated by using the following equation.

$$\gamma = \left(\frac{w_1\%}{IC_{50,1}} + \frac{w_2\%}{IC_{50,2}} \right) * IC_{50,12}$$

where $IC_{50,1}$ is IC_{50} value of herb 1 alone, $IC_{50,2}$ is IC_{50} value of herb 2 alone, w_1% is the mass percentage of herb 1 in combination, w_2% is mass percentage of herb 2 in combination, $IC_{50,12}$ is IC_{50} value of herb 1 and 2 in the combination system.

If γ=1, the interaction is additive, if γ<1, it is synergistic, and if γ>1, it is subadditive, even antagonism.

Statistical analysis

Experimental results were given as mean value ± standard deviation (SD) of three separate experiments. Statistical analysis was conducted using Microsoft Excel software. Differences at $P < 0.05$ using student's *t*-test were considered to be significant.

RESULTS AND DISCUSSION

DPPH assay of individual herb extract of FSD

The DPPH assay of individual component extract of FSD in ethanol was investigated. The IC_{50} values obtained from the regression curve of DPPH scavenging rate with each herbal concentration are listed in the Table 1.

DPPH assay of two-two combined system of extracts of FSD

The experiment of any two combined system was performed according to FSD prescription. When any two herbs were combined into a single treatment, DPPH assay of the combination system is same with method of individual component extract of FSD. DPPH radical scavenging activities of the combined systems are as shown in Figure 1. The IC_{50} values of the combined system, obtained from the regression curve of DPPH scavenging activity with herbal concentrations, are listed in Table 1.

The experimental results (Table 2) showed that the combined system exhibited some degree of inhibitory activities in the DPPH assay. The strong synergistic effect was observed in LCH and PLP combined system. The ethanol extract from LCH and PLP system have most small IC_{50} value with 0.25 mg/ml, which is beyond the

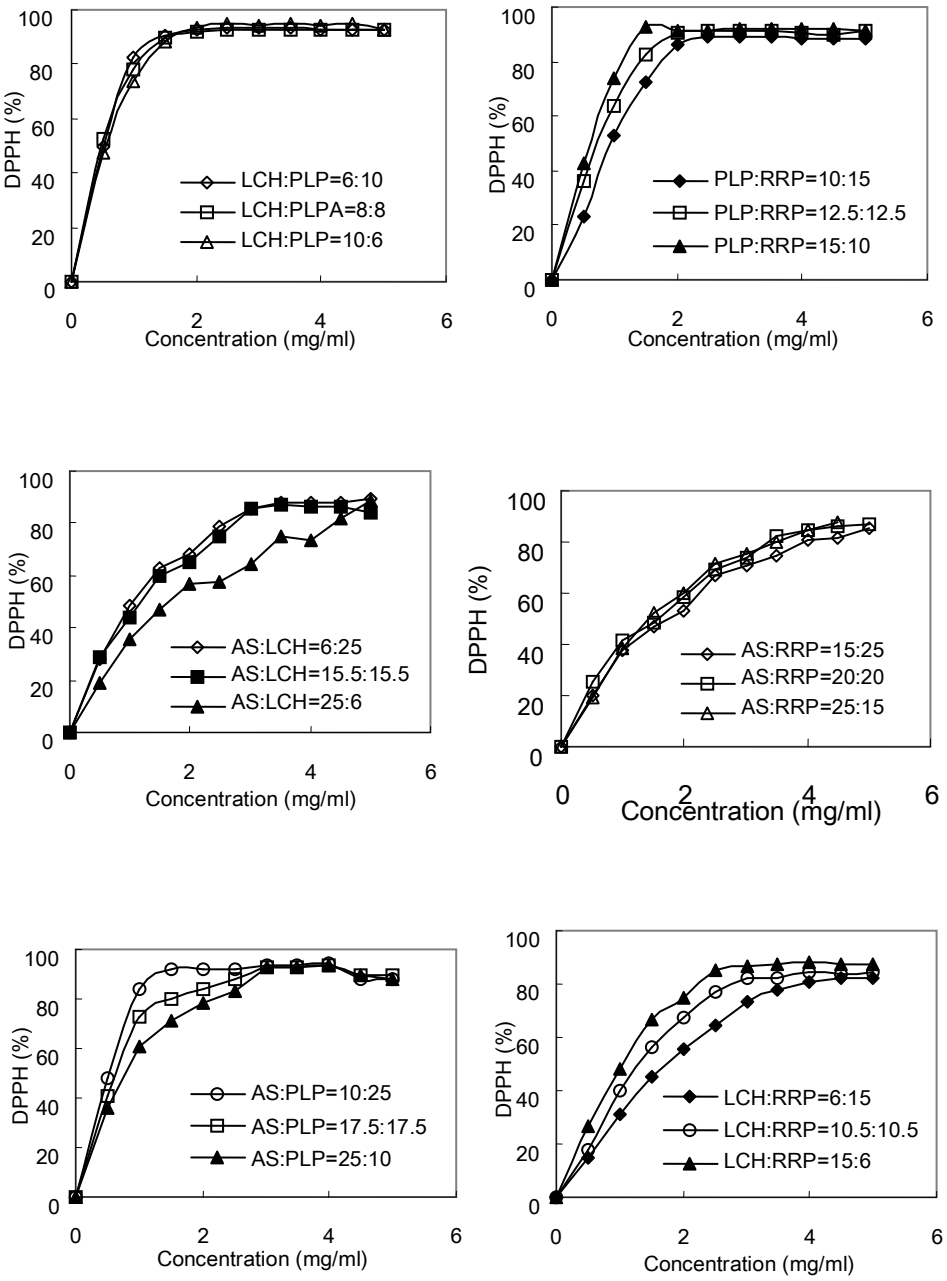

Figure 1. DPPH radical scavenging activities of the combined systems of ethanol extract of four substances.

IC_{50} value range of individual herbs. Meanwhile, the interaction index of the system is also low, only 0.2280. In addition, other systems also have a certain degree of synergy effects such as PLP and RRP system, AS and PLP system.

The vast majority of the system seems to be more of additive effect, such as AS and LCH system, LCH and RRP system. The IC_{50} value of such combined system is always between the IC_{50} value range of individual herbs, and the interaction index seems to be close to 1, while the interaction effect of AS and RRP system was divided into the sub-additive. Few systems seem to display antagonistic effect.

The main role of the famous FSD is that it can effectively make the female skin smooth and delicate in high spirit by regulating endocrine system in the body, scavenging free radicals and making up for the insufficiency of blood. It can fully exert pharmacological activity, when these four herbs can produce the additive effect and synergistic effect in the combined system.

Table 1. *In vitro* antioxidant activity of FSD extracts (n=3).

Herbal	IC_{50} values (mg dw/ml) in ethanol
A. sinensis (AS)	$1.67 \pm 0.11_a$
L. chuanxiong Hort (LCH)	1.04 ± 0.45
P. lactiflora Pall (PLP)	1.16 ± 0.09
R. rehmanniae preparata (RRP)	2.06 ± 0.65

Results showed mean ± SD.
Means along the same column are significantly different at $p < 0.05$ as analyzed by student's t-test. FSD consist of *A. sinensis*, *Li. chuanxiong* Hort., *P. lactiflora* Pall. and *R.x rehmanniae preparata*.

Table 2. Antioxidant activity and interaction index of the combined system of FSD extracts.

Combination system (m_1: m_2)		IC_{50} values (mg dw/ml) in ethanol	Interaction index in ethanol
	6:25	1.05 ± 0.03	0.9360
AS+ LCH	15.5:15.5	1.32 ± 0.02	1.0299
	25:6	1.65 ± 0.03	1.1039
	10:25	0.53 ± 0.02	0.4170
AS+ PLP	17.5:17.5	0.64 ± 0.01	0.4680
	25:10	0.78 ± 0.01	0.5257
	15:25	1.73 ± 0.02	0.9123
AS+ RRP	20:20	1.58 ± 0.04	0.8465
	25:15	1.42 ± 0.02	0.7895
	6:10	0.51 ± 0.01	0.4586
LCH+ PLP	8:8	0.25 ± 0.01	0.2280
	10:6	0.55 ± 0.01	0.5084
	6:15	1.75 ± 0.02	1.1197
LCH+RRP	10.5:10.5	1.31 ± 0.02	0.9478
	15:6	1.05 ± 0.01	0.8474
	10:15	0.95 ± 0.01	0.6042
PLP+RRP	12.5:12.5	0.75 ± 0.03	0.5053
	15:10	0.62 ± 0.01	0.4411

Results showed mean ± SD. Means along the same column are significantly different at $p < 0.05$ as analyzed by student's t-test. FSD consist of AS, LCH, PLP and RRP.

These results demonstrated that the two-two combined effects of the four individual herbs have been observed on antioxidant activity *in vitro*. The interactions between the two herbs each from FSD indeed have influence on the activity of scavenging DPPH free radical. However, these interactions may depend upon the complementary nature of different families of compounds in each of the herb extracts. All two-two combined system show the synergistic and additive effect from FSD. The results were confirmed by the interaction index. This implied that the modified interaction index formula may be used to interpret the experimental results.

Conclusions

Compatibility effects of FSD extracts was investigated on DPPH free radical scavenging activity. When two-two mixed way was used, the compatibility effects were found. These four herbs in FSD can produce the additive effect, even synergistic effect in combined system. This imply that interactions between each of the two herbs of

FSD indeed have influence on the activity of scavenging DPPH free radical, and may affect pharmacological activity.

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