



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



## Original article

## Evaluation of the synergetic effect of Yupingfeng san and *Flos Sophorae Immaturus* based on free radical scavenging capacity



Ruoyue Huang<sup>a,1</sup>, Yuning Zhang<sup>a,1</sup>, Yanli Zhang<sup>a</sup>, Li Zhang<sup>a</sup>, Linlin Pei<sup>a</sup>, Gang Shu<sup>a</sup>, Zhixiang Yuan<sup>b</sup>, Juchun Lin<sup>a</sup>, Guangneng Peng<sup>a</sup>, Wei Zhang<sup>a</sup>, Ling Zhao<sup>a</sup>, Fei Shi<sup>a</sup>, Hualin Fu<sup>a,\*</sup>

<sup>a</sup> Department of Pharmacy, College of Veterinary Medicine, Sichuan Agriculture University, Chengdu, Sichuan, 611130, China

<sup>b</sup> College of Pharmacy, Southwest Minzu University, Chengdu, Sichuan, 610225, China

## ARTICLE INFO

## Keywords:

Yupingfeng san  
*Flos Sophorae Immaturus*  
 Free radical scavenging capacity  
 Antioxidant  
 Optimum extraction process

## ABSTRACT

**Objective:** This study aimed to determine the optimal extraction process and examine whether the combination of *Flos Sophorae Immaturus* (FSI) and Yupingfeng san (YPS) has a synergistic effect on free radical scavenging capacity.

**Design and methods:** The time of immersion and extraction and the ratios (material/solvent) of the combination of YPS and FSI were optimized on the basis of polysaccharide and flavonoid yields via orthogonal design. The optimal result was used in the 1,1-diphenyl-1-picrylhydrazyl (DPPH) assay and animal experiments to test the antioxidant activity, which is reflected by superoxide dismutase, malondialdehyde, glutathione peroxidase, and total antioxidant capacity serum levels. The optimal extraction process was determined using various ingredients to obtain complex extracts with high active ingredient content and antioxidant activity. DPPH assay results showed that the optimized ingredients have antioxidant effects, and the combination had better antioxidation function than YPS in vitro. The combination also showed synergistic antioxidant activity compared with YPS in vivo.

**Conclusions:** The combination of YPS and FSI had a synergistic antioxidant effect in vitro. The optimized extracts had antioxidant effects in vivo. These results indicated that YPS could be used with FSI to improve its antioxidant capacity in the body on the basis of free radical scavenging capacity.

## 1. Introduction

Oxidative stress is caused by excessive free radicals when the body encounters various external damages or stimulations. Highly active and unstable molecules such as reactive oxygen species (ROS) and reactive nitrogen species have a strong oxidation effect. Multiple organ dysfunction and failure are caused by excessive free radicals or the decreased ability of scavenging free radicals [1,2]. Many diseases can cause oxidative stress and accumulation of free radicals, which lead to lipid peroxidation in the cell membrane, changes in its permeability, and damage to structural proteins; they react with intracellular nucleic acids and cause cell death, such as in coronavirus disease 2019 (COVID-19) [3]. When the accumulation of free radical molecules greatly exceed the body's ability to scavenge, the oxidation and antioxidant

system become disordered, further leading to tissue damage and serious diseases such as cancer, hypertension, and neurological disease. Traditional Chinese medicines (TCMs) are especially effective on anti-oxidation on the basis of free radical scavenging capacity, which has the advantages of high efficiency and low toxicity. Therefore, Chinese herbal medicine has become one of the focuses in the field of research.

The unopened flower buds of *Sophora japonica* L. (Leguminosae), also known as *Flos Sophorae Immaturus* (FSI), are commonly used as an antioxidant because of their free radical scavenging, anti-inflammatory, and antibacterial activities in TCM. FSI contains flavonoids, saponins and sterols; it is rich in rutin, which is a flavonoid with antioxidant properties based on free radical scavenging activity.

Yupingfeng san (YPS) is the representative formula of TCM used to eliminate pathogenic factors. It originated from "Danxi Heart Sutra" in

**Abbreviations:** TCM, Traditional Chinese Medicines; *S. japonica*, *Sophora japonica* L.; FSI, *Flos Sophorae Immaturus*; *A. mongholicus*, *Astragalus mongholicus*; *A. macrocephala*, *Atractylodes macrocephala* Koidz; *S. divaricata*, *Saposhnikovia*; MYP, modified Yupingfeng san; YPS, Yupingfeng san; DPPH, 1-diphenyl-1-picrylhydrazyl; T-AOC, total antioxidant capacity; GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; MDA, malondialdehyde; ROS, reactive oxygen species

\* Corresponding author.

E-mail address: [fuhl2005@sohu.com](mailto:fuhl2005@sohu.com) (H. Fu).

<sup>1</sup> These authors contributed equally to this work.

<https://doi.org/10.1016/j.bioph.2020.110265>

Received 4 April 2020; Received in revised form 5 May 2020; Accepted 10 May 2020

0753-3322/ © 2020 The Author(s). Published by Elsevier Masson SAS. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

the Yuan Dynasty. It is composed of *Astragalus mongholicus*, *Atractylodes macrocephala* Koidz. and *Saposhnikovia divaricata* (Trucz.) Schischk. Studies on the ratio of *A. mongholicus*, *A. macrocephala* and *S. divaricata* are diverse. The ratio 3:1:1 is documented in the Pharmacopoeia of People's Republic of China. *A. mongholicus* is traditionally used to improve immunity. Pharmacological experiments showed that *A. Radix*, the dry roots of *Astragalus mongholicus* Bge. [*A. membranaceus* Bge. var. *mongholicus* (Bge.) Hsiao] and *Astragalus membranaceus* (Fisch.) Bge. have anti-inflammatory, anti-tumor, anti-oxidant, and immunoregulatory [4]. The use of *Astragalus* species can be dated back to more than two millenniums [5]. This species contains many components, such as terpenoids, flavonoids, and polysaccharides. Flavonoids were discovered as antioxidant constituents from the roots of *A. mongholicus* which can scavenge free radicals. Rhizoma *A. macrocephala* (baizhu in Chinese) has been used as a TCM for approximately 2000 years [6]. Its polysaccharide has remarkable antioxidant activity and can improve the immune response in chickens [7]. Thus, it is widely used in the breeding industry in China as a feed additive. *S. divaricata* (Trucz.) Schischk, an umbelliferous genus, has been ranked as a top-grade herb by the Pharmacopoeia of People's Republic of China which was reported that *S. divaricata* has obvious anti-inflammatory, anti-pyretic, anti-viral, and anti-oxidant effects on its free radical scavenging activity, and analgesic effects [8,9].

Previous studies have reported the efficacy of modified YPS (MYP) as an anti-allergy for the treatment of respiratory diseases [10–13]. However, the prescription of FSI and YPS and the identification of their antioxidant capacity are rarely reported. YPS is widely used to improve immunity and its antioxidant activity inhibits the generation of free radicals. Basic theories of Chinese medicine indicate a critical viewpoint regarding drug combination. Therefore, in this study, YPS and FSI were combined to determine the synergistic antioxidant activity.

Water extraction technology is the most commonly used method in natural product extraction due to its security and low cost. However, the extract yield of this method depends on various conditions, such as extraction time, times and solid-to-solvent ratio, which could lead to differences in the extraction yield and biological activities of the ingredients.

YPS is an effective prescription of TCM for the prevention of pneumonia caused by the new coronavirus (2019-nCoV) in China [14,15]. The present study investigated the synergistic antioxidant effects of modified yupingfeng san (MYP), which is composed of FSI and YPS, and identified the optimal extraction process to obtain the extracts containing ingredients with high activity. The antioxidant effect of this process was verified in vitro and in vivo, thereby laying a foundation for its clinical application.

## 2. Materials and methods

### 2.1. Materials

All components of MYP are shown in Table 1. *A. mongholicus*, *A. macrocephala*, FSI and *S. divaricata* were purchased from Sichuan Ya'an Huimintang Pharmaceutical Chain Co., Ltd. The authenticity of these herbal medicines was confirmed by Qiaojia Fan, a professor from the Department of Pharmacy of Sichuan Agriculture University. Rutin

**Table 1**  
Components included in the modified Yupingfeng san.

Latin name	Scientific name <sup>a</sup>	Used part	Chinese name
Astragali Radix	<i>Astragalus mongholicus</i> Bunge	Root	Huangqi
Flos Sophorae Immaturus	<i>Sophora japonica</i> L.	unopened flower bud	Huaimi
Atractylodis Macrocephalae Rhizoma	<i>Atractylodes macrocephala</i> Koidz.	Root	Baizhu
Radix Saposhnikoviae	<i>Saposhnikovia divaricata</i> (Turcz.) Schischk.	Root	Fangfeng

<sup>a</sup> <http://www.theplantlist.org>.

standard was provided by National Institutes for Food and Drug Control (batch number: 10080-200707, 92.5 % purity). Aluminum trichloride was purchased from Tianjin Fuchen Chemical Reagent Factory (batch number: 20121102, 97 % purity). In addition, 1,1-diphenyl-1-picrylhydrazyl (DPPH, 97 % purity) was purchased from West Asia Reagent Co., Ltd. Superoxide dismutase (SOD), malondialdehyde (MDA), glutathione peroxidase (GSH-Px), and total antioxidant capacity (T-AOC) ELISA kits were purchased from Nanjing Jiancheng Co., Ltd.

### 2.2. Experimental design for the extraction yield

An orthogonal array design was used to investigate the effect of four variables [ $X_1$ , soaking time,  $X_2$ , water-to-raw material ratio;  $X_3$ , extraction time and  $X_4$ , times (three levels each)] on the basis of polysaccharide and total flavonoid contents from *A. mongholicus*, *A. macrocephala*, FSI and *S. divaricata*. The levels of the four variables are shown in Table 2.

*A. mongholicus*, *A. macrocephala*, *S. divaricata* and FSI samples (the ratio is 3:1:1:3, a total of 24 g) were extracted in distilled water for different soaking times, water-to-raw material ratios, extraction time, and times in accordance with the experimental design. The solvents were concentrated to 0.8 g raw medicine/mL, added with absolute ethanol to obtain a final concentration of 80 %, and stored overnight. Each extract was centrifuged (3500 r/min, 10 min). The supernatant and sediment were taken and stored separately. Then, the precipitation 3 times with absolute ethanol and dissolved in distilled water in a 50 mL volumetric flask to obtain the polysaccharide solution. The supernatant was a total flavonoid solution. All experiments were performed in triplicate.

### 2.3. Determination of the polysaccharide content of the extracts

The total carbohydrate content of the polysaccharide fractions of the extracts obtained in Item 2.2 was determined using the phenol-sulfuric acid method [16]. The polysaccharide concentration was calculated using the following linear equation on the basis of the calibration curve (Fig. 1):

$$y=0.043x+0.107, R^2=0.998, \quad (1)$$

where  $y$  is the absorbance and  $x$  is the polysaccharide concentration. A linear relationship of between 2.054 and 13.011  $\mu\text{g/mL}$  is good. The polysaccharide content was calculated as follows:

$$\text{Polysaccharide content} \left( \frac{\text{mg}}{\text{g}} \right) = \frac{c \times a \times v}{m} \quad (2)$$

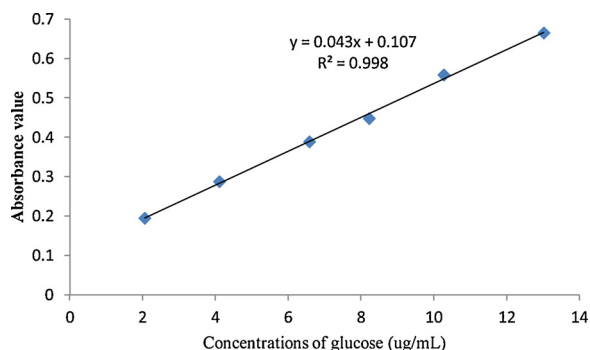
Where  $c$  is the polysaccharide concentration in the sample solution,  $a$  is the dilution factor of the sample solution at the time of determination,  $v$  is the total volume of the sample solution, and  $m$  is the weight of medicinal materials.

### 2.4. Determination of total flavonoid content of the extracts

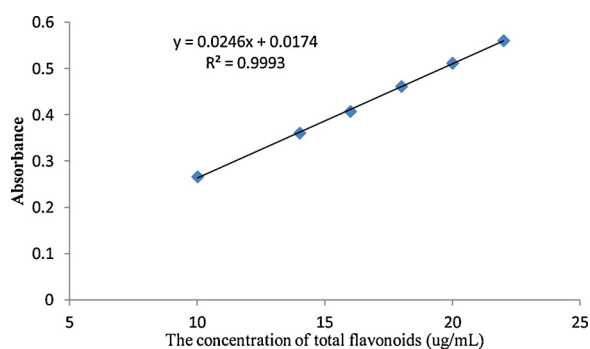
Total flavonoid contents were measured using a modified colorimetric method with rutin as the standard. The prepared sample solution (0.1 mL) was placed in a 10 mL volumetric flask, and 60 % ethanol

**Table 2**  
Factor-Level of MYP.

Experimental Runs	Soaking time/h (A)	Water to ratio of raw material / g/mL (B)	Extraction time/h (C)	Times (D)
1	1	12	2	2
2	2	14	3	3
3	3	16	4	4



**Fig. 1.** The calibration curve of polysaccharides.



**Fig. 2.** The calibration curve of total flavonoids.

solution was used for volume determination and blending. The extract at 1 mL was placed in a 10 mL volumetric flask and added with 0.1 mL of  $\text{AlCl}_3$  (1 mL). Ethanol solution (60 %) was used for volume determination and blending. The absorbance of the mixture was measured against a blank at 415 nm after 15 min. The total flavonoid concentration was calculated using the following linear equation on the basis of the calibration curve (Fig. 2):

$$y = 0.0246x + 0.0174, R^2 = 0.9993, \quad (3)$$

where  $y$  is the absorbance and  $x$  is the total flavonoid concentration. A linear relationship between 10 and 22  $\mu\text{g/mL}$  is good. The total flavonoid content was calculated as:

$$\text{Total flavonoids content} \left( \frac{\text{mg}}{\text{g}} \right) = \frac{c \times a \times v}{m}, \quad (4)$$

where  $c$  is the total flavonoid concentration in the sample solution,  $a$  is the dilution factor of the sample solution at the time of determination,  $v$  is the total volume of the sample solution, and  $m$  is the weight of medicinal materials.

## 2.5. Measurement of the synergistic antioxidant activities of MYP extracts in vitro

DPPH assay was performed in accordance with the methods previously described with some modifications to assess the free radical scavenging activity of the extracts. In brief, the DPPH solution (0.1 mM in ethanol, 1 mL) was added into the sample solution (2 mL, the

concentration was 0.8 g raw medicine /mL). The mixture was shaken and kept in the dark for 30 min at room temperature, and then the absorbance of the reaction mixture was determined at 517 nm by a UV-vis spectrophotometer (UV-2102PC). Ethanol served as the control. The capability of the DPPH radicals to scavenge was calculated as follows:

$$\text{Scavenging activity (\%)} = [A_0 - (A_1 - A_2)/A_0] \times 100, \quad (5)$$

where  $A_0$  is the absorbance of the extracts,  $A_1$  is the control absorbance (ethanol instead of the extracts), and  $A_2$  is the absorbance of the combined reaction reagents (DPPH solution is mixed with ethanol).

The synergistic effect of scavenging activity could be determined by calculating the SE index. Theoretical scavenging activity was calculated, as follows:

$$\text{TSC (\%)} = 100 - [(100 - \text{ESC}_1) \times (100 - \text{ESC}_2)/100], \quad (6)$$

where  $\text{ESC}_1$  and  $\text{ESC}_2$  (Effect of the scavenging capacity) are the scavenging activity of the two samples. The calculation formula is shown in Formula (5).

SE could be calculated, as follows [11]:

$$\text{SE} = \frac{\text{ESC}}{\text{TSC}}, \quad (7)$$

the remaining judgment criteria are as follows: when the  $\text{SE} > 1$ , synergy occurs.

## 2.6. Animal experiment design

Drug was extracted in accordance with Section 1.2.3, and the polysaccharide and total flavonoid contents in YPS, FSI and MYP were measured. Then the drug solution was concentrate to achieve a thick paste, vacuum dried at 50 °C, pulverized and passed through a 100 mesh screen. Subsequently, the sample for animal experiment was obtained.

Healthy male KM mice [No. SCXK (Sichuan) 10-2008], aged 6–8 weeks (weight  $20 \pm 0.2$  g), were purchased from Chengdu dashuo animal co. LTD and kept in microisolator cages with food and water ad libitum. The laboratory temperature was  $24^\circ\text{C} \pm 1^\circ\text{C}$  with 12 h/12 h light–dark cycle for acclimatization, and the relative humidity was 40 %–60 %. Before experiment, the mice were allowed to adapt to the experimental environment for a minimum of 1 week. All experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and National Standard of the People's Republic of China for Laboratory animal-Guideline for ethical review of animal welfare. The mice were randomly divided into five groups ( $n = 10$ ), including normal control (NC) group; and high-, medium-, and low-dose MYP groups (MYP-H, MYP-M, and MYP-L), as well as YPS group. From days 1 to 9, the NC group was treated with normal saline. The YPS group was orally administered with YPS at a dose of 10 g/kg/bw in accordance with the raw medicine. MYP-H, MYP-M, and MYP-L groups were orally administered with MYP daily at doses of 15, 10, and 5 g/kg/bw in accordance with the raw medicine, respectively. At 24 h after the last administration, all the animals in each group were sacrificed.

## 2.7. Measurement of antioxidant activities of the extracts in vivo

At 24 h after the last administration, blood was collected and kept in

**Table 3**

Experimental values of content of polysaccharide and total flavonoids of extracts.

Runs	A	B	C	D	Indexes /(mg/g)	
					Contents of polysaccharide	Contents of total flavonoids
1	2	3	3	1	47.520	76.519
2	1	3	2	3	51.871	84.037
3	3	3	1	2	35.904	76.378
4	1	2	3	2	51.904	87.105
5	3	2	2	1	36.204	69.518
6	2	2	1	3	44.054	73.357
7	3	1	3	3	61.834	91.847
8	2	1	2	2	47.397	80.744
9	1	1	1	1	26.364	67.024
K1	43.380	45.198	35.441	36.696		
K2	46.324	44.054	45.157	45.068		
K3	44.647	45.098	53.753	52.586		
R	2.944	1.144	18.312	15.890		
k1	79.389	79.872	72.253	71.020		
k2	76.873	76.660	78.100	81.409		
k3	79.248	78.978	85.157	83.080		
r	2.516	3.212	12.904	12.060		

K1, K2, and K3 are the means of the first, second, and third levels of this factor in the polysaccharide content, respectively, whereas k1, k2, and k3 are the means of the first, second, and third levels of this factor in the total flavonoid content, respectively. The r is extremum.

the refrigerator at 4 °C. The levels of oxidation factors T-AOC, GSH-Px, SOD, and MDA) in serum were measured using assay kits in accordance with the manufacturer's instruction.

## 2.8. Statistical analysis

All the analyses were carried out in triplicate and all data were expressed as the mean  $\pm$  standard deviation. The development of experimental design and data analysis was performed using SPSS software version 19.0 (SPSS Inc., Armonk, NY, USA). The significance, adequacy and reliability of the suggested model between groups were determined using one-way ANOVA and Duncan's multiple range tests between groups using SPSS software version 19.0. Differences with p values of less than 0.05 were considered statistically significant.

## 3. Results

### 3.1. Extraction process optimization

The orthogonal test results are shown in Table 3. R is extremum, and indicated that the order of the influence of various factors on the polysaccharide content was C > D > B > A, and the optimal extraction process was A<sub>2</sub>B<sub>1</sub>C<sub>3</sub>D<sub>3</sub>. The same was true for the F values of 1.532, 0.274, 59.059, and 44.263, respectively, as well as the total flavonoid content. The order of the factors affecting the total flavonoid content was C > D > B > A, and the optimal extraction process was A<sub>1</sub>B<sub>1</sub>C<sub>3</sub>D<sub>3</sub>, and the F values were 2.282, 3.139, 47.690, and 48.769, respectively. As shown in Table 4, each parameter was extremely significant (p < 0.01) at various combinations of extraction time and times but not significant (p > 0.05) at soaking time and water-to-raw material ratio. The degrees of freedom were both 2. The influence degree of each factor on each active ingredient was the same. Therefore, A<sub>1</sub>B<sub>1</sub>C<sub>3</sub>D<sub>3</sub> was chosen as the optimal extraction process for the animal experiment, because it saved time and materials.

One-way ANOVA showed an extremely statistically significant variation (p < 0.01) in observed values of each parameter at various combinations of extraction time and times. However, no significant difference (p > 0.05) was found in each parameter at soaking time and water-to-raw material ratio, as shown in Table 5.

**Table 4**

Variance analysis of polysaccharide content as indexes.

Source	SS <sup>a</sup>	df <sup>b</sup>	MS <sup>c</sup>	F-value	P-value
A	39.357	2	19.678	1.532	P > 0.05
B	7.029	2	3.514	0.274	P > 0.05
C	1517.227	2	758.613	59.059*	P < 0.01
D	1137.108	2	568.554	44.263*	P < 0.01
Pure error	231.211	18	12.845		

<sup>a</sup> Sum of Squares.

<sup>b</sup> Degree of Freedom.

<sup>c</sup> Mean Square.

\* Means significance (significance level 0.05).

**Table 5**

ANOVA of total flavonoid content as indexes.

Source	SS <sup>a</sup>	df <sup>b</sup>	MS <sup>c</sup>	F-value	P-value
A	35.954	2	17.977	2.282	P > 0.05
B	49.471	2	24.735	3.139	P > 0.05
C	751.49	2	375.748	47.690*	P < 0.01
D	768.497	2	384.248	48.769*	P < 0.01
Pure error	141.820	18	7.879		

<sup>a</sup> Sum of Squares.

<sup>b</sup> Degree of Freedom.

<sup>c</sup> Mean Square.

\* Means significance (significance level 0.05).

**Table 6**

Chi-square analysis of polysaccharide content as indexes.

Source	H-value <sup>a</sup>	P-value
A × B	20.564	P < 0.05
A × C	9.237	P > 0.05
A × D	12.363	P < 0.05
B × C	9.473	P = 0.05
B × D	12.575	P < 0.05
C × D	1.007	P > 0.05

<sup>a</sup> Chi-square values.

The chi-square test showed a correlation (p < 0.05) between soaking time and water-to-raw material ratio, soaking time and times, water-to-raw material ratio and soaking time, and water-to-raw material ratio and soaking time in the polysaccharide content, as shown in Table 6. In addition, no correlation (p > 0.05) was observed between the factors in the total flavone content, as shown in Table 7.

To sum up, the observed values in each parameter changed significantly under different combinations of extraction time and times (p < 0.05). The optimal values of the tested variables, that is, the optimal extraction process were as follows: soaking time of 1 h, water-to-raw material ratio 12 g/mL, extraction time of 3 h, and three times.

### 3.2. Measurement of the antioxidant activities of MYP extracts in vitro

The synergistic antioxidant results of the different concentrations of

**Table 7**

Chi-square analysis of total flavonoid content as indexes.

Source	H-value <sup>a</sup>	P-value
A × B	6.357	P > 0.05
A × C	3.194	P > 0.05
A × D	3.551	P > 0.05
B × C	3.110	P > 0.05
B × D	3.465	P > 0.05
C × D	0.290	P > 0.05

<sup>a</sup> Chi-square values.

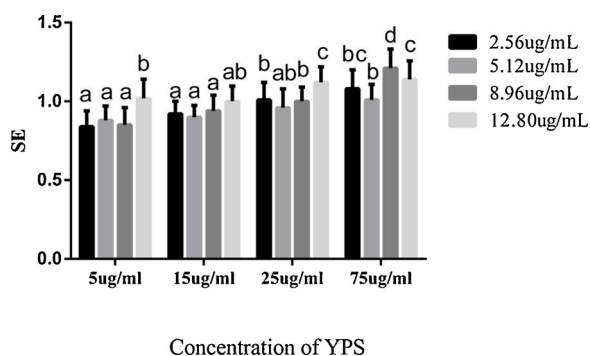


Fig. 3. Synergy results of antioxidant activities of the extracts in vitro.

YPS and FSI in vitro are shown in Fig. 3. With the increase in both concentrations, the anti-oxidative effect in vitro changed from antagonistic to synergistic. The synergistic effect intensified with increasing concentration. When the YPS and FSI concentrations were more than 25 and 2.56 ug/mL ( $P < 0.05$ ), respectively,  $SE > 1$ , thereby indicating that a synergistic antioxidant action possibly existed between the formulas in the DPPH radical-scavenging model. In terms of the dose-effect relationship, a high FSI concentration (2.56–12.8 ug/mL) led to a strong synergistic effect. The same was true for YPS (15–75 ug/mL). The best synergistic effect obtained when FSI and YPS concentration were 8.96 and 75 ug/mL ( $P < 0.05$ ), respectively.

The horizontal axis shows the YPS concentration, and the columns in different colors represent the FSI concentration, respectively ( $n = 6$  absorbance values/group). The values on the same column followed by different letters were significantly different ( $p < 0.05$ ).

### 3.3. Measurement of antioxidant activities of the extracts of MYP in vivo

The polysaccharide and total flavonoid contents in YPS, FSI, and MYP are shown in Table 8.

Treatment with MYP-M and MYP-L showed significant high expression levels of T-SOD and T-AOC ( $P < 0.05$ ). GSH-Px tended to increase when used at all doses. However, this difference was not statistically significant. In addition, the T-SOD, T-AOC, and GSH-Px activities were higher at the different MYP doses than in the YPS and NC groups, and the decrease in MDA was clearly mitigated ( $P < 0.05$ ), as shown in Fig. 4.

A. Statistical results of T-SOD ( $n = 10$  rats/group). B. Statistical results of MDA ( $n = 10$  rats/group). C. Statistical results of GSH-Px ( $n = 10$  rats/group). D. Statistical results of T-AOC ( $n = 10$  rats/group). The values on the same column followed by different letters were significantly different ( $p < 0.05$ ).

## 4. Discussion

Orthogonal design finds the corresponding orthogonal table in accordance with the test on the number of factors, i.e., factors on the number of levels and whether interaction was present. Some representative points were selected from the comprehensive experiment on the basis of the orthogonality of the orthogonal table, which could achieve the equivalent of a large number of comprehensive tests with a minimum number of tests. The orthogonal design is frequently used

Table 8  
The concentration of the active ingredient.

Group	Polysaccharide content (mg/g)	Total flavonoid content (mg/g)
MYP	82.15	91.87
FSI	6.41	60.47
YPS	21.54	1.50

because of its high efficiency, speed, and cost. It considerably reduces the number of experiments. In the present study, the optimal extraction process of MYP was obtained via orthogonal design on the basis of polysaccharide and total flavonoid contents (Table 2).

YPS has antioxidant function; polysaccharide is the active component of this function [17,18]. YPS can scavenge ROS, such as superoxide anion ( $O_2^-$ ), hydroxyl radical ( $-OH$ ), and hydrogen peroxide ( $H_2O_2$ ). It can also complex metal ions which is necessary for the production of living oxygen to achieve antioxidant activity in the body [19]. Flavonoids have antioxidant properties [20], and can inhibit  $-OH$ -induced lipid peroxidation and nonenzymatic glycosylation [21], which are the active ingredients of FSI in terms of oxidation resistance [22]. Thus, polysaccharide and total flavonoid contents were used as indicators (Table 2) to determine the optimal extraction process of undergoing redox reactions in different ways in the organisms. The content of the active ingredient of the extract from the raw material was closely related to the extraction method. The optimal extraction process could improve the contents of the active ingredient of the compound compared with other conventional methods. MYP could obtain more polysaccharides and total flavonoids than FSI and YPS (Table 8) possibly due to the “co-dissolution” phenomenon in the extraction process.

Nitroxide can scavenge free radicals in the tissue, thereby protecting cell macromolecules from oxidative damage. DPPH is a free radical with relative stability and could attract electrons from antioxidant [23] or hydrogen atoms. Thus, the determination of DPPH free radical scavenging activity is based on the reduction of DPPH ethanol solution in the presence of hydrogen supplying antioxidants which form the non-free radical form of DPPH-H because of the formation of stable molecules. The assay could accommodate many samples in a short period and is sensitive enough to detect active ingredients at low concentrations [24]. In the present study, the free radical scavenging capacities of antioxidant compounds present in extracts were determined to measure their hydrogen donating ability against reactive nitrogen (DPPH radical) species. The concluded that the compatibility of the two had a synergistic effect. MYP exhibited a synergistic antioxidant action in vitro (Fig. 3). Most antioxidants protect the organisms through redox reaction by inhibiting the generation of oxygen anions and scavenging free radicals. Lipid peroxidation is a chain reaction of free radical formation in the lipid parts of cellular membranes induced by the addition of hydrogen peroxide and/or  $Cu^{2+}$  ions. Flavonoids protect the membrane from the damage and destruction of lipid molecules; suppressed  $H_2O_2$  provokes changes in cell membrane elasticity and its morphological properties [25]. Flavonoids scavenge free radicals, inhibit the lipid peroxidation induced by hydroxyl radicals, and reduce the transmission of automatic oxidation chain reaction to exert antioxidant effect on the body and protect the structure and function of cell membrane [25]. Polysaccharides can act as electron or hydrogen donors to scavenge hydroxyl radicals [26]. They can also directly remove ROS and complex the metal ions necessary for the ROS production to achieve the antioxidant effect of the organisms. In the present study, MYP could significantly improve the antioxidant function in mice (Fig. 4). The antioxidant activity of FSI and YPS was probably through the polysaccharides and total flavonoids, and their combination could contribute to the easy dissolution and absorption of the effective ingredients. The total flavonoids in *S. japonica* have antioxidant and renal protective functions [20,27], and rutin could protect against ultraviolet B radiation through the alteration of microRNA expression [28].

ROS contain  $O_2^-$ ,  $H_2O_2$ , and  $OH^-$ , which are produced during various cellular aerobic metabolisms as by-products of many enzyme reactions in the mitochondrial respiratory chain [29]. They are highly reactive molecules containing oxygen and modify DNA and proteins [30]. SOD, GSH-Px and catalase (CAT) are important antioxidant enzymes that can adjust the balance in biological systems [31]. ROS are balanced by the antioxidative defense system, which include SOD, CAT and GSH-Px under normal conditions. When an imbalance between oxidants and antioxidants occurs, oxidative stress is reached [32]. SOD

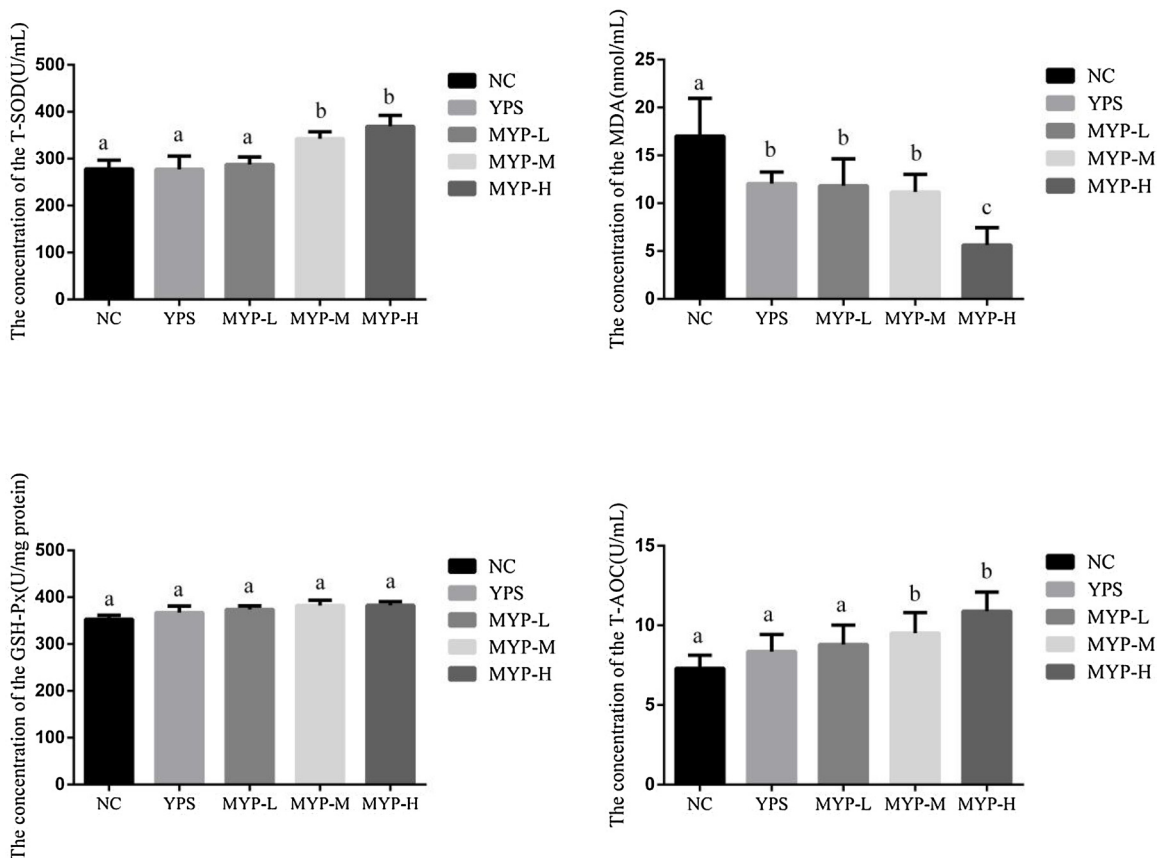


Fig. 4. The influence of antioxidant indexes in mice serum.

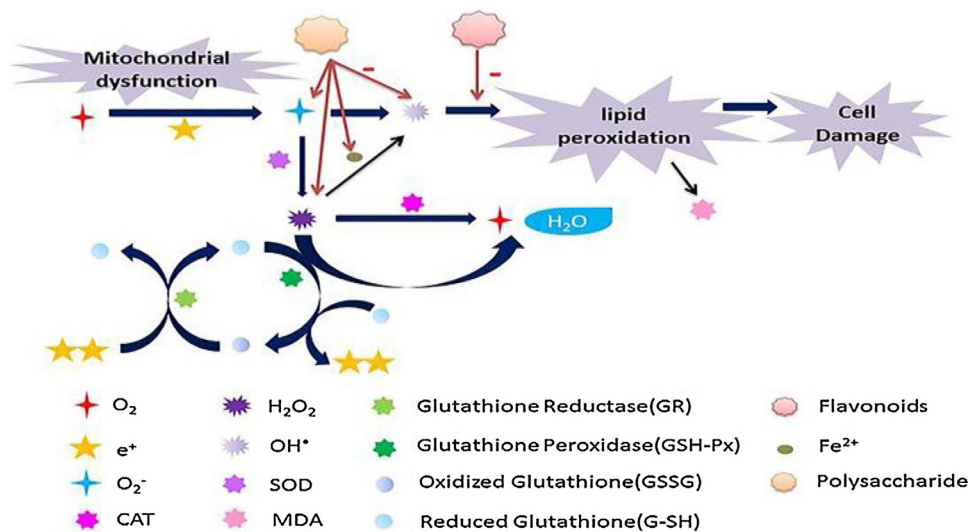


Fig. 5. The diagram of tissue antioxidant process. Symbols represent the reactions of different substances in the body.

and GSH-Px protect the cell from oxidative damage by removing excessive MDA and ROS [33]. The antioxidant process of the effective components of YPS and FSI in tissues is shown in Fig. 5.

Virus infection could cause ROS release, which leads to oxidative stress in the body and increases the sensitivity of host cells to pre-oxidative factors, resulting in extensive damage; it activates phagocytes and releases pre-oxidative cytokines, such as IL-1 [34]. Influenza viral infection could lead to the destruction of body's oxidative balance, thereby damaging the tissues and cells via oxidative stress and resulting

in changes in cell membrane structure and enzyme inactivation, which is conducive to the release of influenza virus [35]. The mechanism of COVID-19 is organ damage caused by a large amount of free radicals produced by an extreme immune response [3,36]. YPS could prevent and cure influenza virus [37]; it has been recognized as a promising antiviral drug against RNA viruses (including influenza virus) [38]. YPS inhibits the replication of influenza virus and inactivates the virus [39]. Rutin could inhibit influenza virus by affecting the monooxygenase activity [40], and it is widely found in flavonoids. MYP may resist

COVID-19 by scavenging free radicals and improving the antioxidant activity of the body to protect the tissues. Therefore, the combination of YPS and FSI may be used to treat viral infections on the basis of free radical scavenging capacity.

In conclusion, the results indicated the therapeutic potential of MYP on the basis of free radical scavenging capacity *in vitro* and *in vivo*. MYP may have a promising potential as a new antioxidant prescription.

#### Author's contributions

RYH and YLZ designed the experiment process, collected data; RYH and YNZ analyzed the data; RYH wrote the manuscript; LZ polished the article; LLP collected the references; ZXY, JCL, GNP, FS and LZ made suggestions; WZ and GS coordinated technical support; HLF participated in the coordination of the study and reviewed the manuscript. All the authors have read and confirmed the final manuscript.

#### Funding

This work was supported by grant from the miao sub-project of Department of Science and Technology of Sichuan Province (No. 2017RZ0041).

#### Declaration of Competing Interest

The authors claimed that there is no conflict of interest.

#### Acknowledgments

The authors thank Mr. Quanjin Liu, Mr. Shiqi Chen, Mr. Haibo Dou and Ms. Jingyuan Fu of Sichuan Agricultural University, China for improving the manuscript.

#### Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.biopha.2020.110265>.

#### References

- [1] L. Chang-xiao, W. Yu-li, Z. Hong-bing, et al., Attach importance to research and development of Chinese materia medica based on prevention and control needs of SARS-CoV-2 infection, *Chin. Tradit. Herbal Drugs* 51 (6) (2020) 1361–1374.
- [2] K.J. Yong, C.P. Gyun, O. Yoonju, et al., The first case of 2019 novel coronavirus pneumonia imported into Korea from Wuhan, China: implication for infection prevention and control measures, *Pubmed* 35 (5) (2020) e61.
- [3] Z. Yan, T. Dezh, S. Bing, et al., Discuss the mechanism of Chinese medicine intervention on new coronavirus pneumonia based on literature, *J. Tradit. Chin. Med.* (2020) 1–8.
- [4] L. Xiaoxia, Q. Lu, D. Yongzhe, et al., A review of recent research progress on the astragalus genus, *Molecules* 19 (11) (2014) 18850–18880.
- [5] H. Jiang, J.M. Yang, G.Z. Jia, et al., Physical and Ecological Impacts of Chromones of Fresh Root of *Saposhnikovia divaricata* Exposure to High Temperature(Article), *Clin. J. Chin. Med.* 120 (5) (2019) 268–273.
- [6] L. Zhang, D. Chen, M. Bai, et al., Antioxidant ability and mechanism of *Rhizoma Atractylodes macrocephala*, *Molecules* 17 (11) (2012) 13457–13472.
- [7] D. Xu, Y. Tian, Selenium and polysaccharides of *Atractylodes macrocephala* Koidz play different roles in improving the immune response induced by heat stress in chickens, *Biol. Trace Elem. Res.* 168 (1) (2015) 235–241.
- [8] J. Kreiner, E. Pang, G.B. Lenon, et al., *Saposhnikovia divaricata*: a phytochemical, pharmacological, and pharmacokinetic review, *Chin. J. Nat. Med.* 15 (4) (2017) 255–264.
- [9] Y. Men, D. Wang, B. Li, et al., Effects of drought stress on the antioxidant system, osmolytes and secondary metabolites of *Saposhnikovia divaricata* seedlings (Article), *Acta Physiol. Plant.* 40 (11) (2018) 1–14.
- [10] Y.-D. Shao, Effects of Modified Yupingfeng powder on clinical symptoms of patients with seasonal allergic rhinitis, *Phytomedicine* 11 (12) (2019) 20–21.
- [11] D. Liu, J. Shi, A.C. Ibarra, et al., The scavenging capacity and synergistic effects of lycopene, vitamin E, vitamin C, and  $\alpha$ -carotene mixtures on the DPPH free radical, *Food Sci. Technol.* 41 (7) (2008) 1344–1349.
- [12] W. Hai-rong, The clinical effect of Yupingfeng Powder on treating body flu, *China Pract. Med.* 14 (15) (2019) 154–155.
- [13] D. Li-gang, Clinical efficacy of Yupingfeng powder in the treatment of allergic rhinitis in children and its effect on immune function, *China Foreign Med. Treat.* 38 (11) (2019) 178–180.
- [14] Traditional Chinese Medicine Treatment Scheme for Pneumonia Infected by 2019-nCoV in Shaanxi Province (Trial Version 2), (2020) 2020-02-02 15:03:00 [http://www.cnr.cn/sxpd/sx/20200202/t20200202\\_524957384.shtml](http://www.cnr.cn/sxpd/sx/20200202/t20200202_524957384.shtml).
- [15] Traditional Chinese Medicine Treatment Scheme for Pneumonia Infected by 2019-nCoV in Hainan Province (Public Version Trial Version 2), (2020) 2020-02-03 18:30 <http://www.hi.chinanews.com.cn/hnnew/2020-02-03/513709.html>.
- [16] E.I. Evstigneyev, Quantification of polysaccharides in vegetable raw materials and lignin preparations, *Russ. J. Bioorg. Chem.* 43 (7) (2017) 732–736.
- [17] H. Lingzi, L. Wenliang, Z. Wenhui, et al., Research progress of prevention and treatment of liver disease by Yupingfeng powder, *World Chin. Med.* 13 (9) (2018) 2357–2361.
- [18] Z. Zhao, Y. Gao, W. Liu, et al., Modern pharmacology of Yupingfeng San and its research progress in dermatology, *Chin. J. Dermatovenereol. Integr. Tradit. Western Med.* 17 (2) (2018) 187–189.
- [19] Y. Mingyong, Z. Zhonglin, W. Dan, et al., Study on the antioxidant activity of Yupingfeng powder, *Hebei Med.* 20 (6) (2014) 901–904.
- [20] M.S. Miao, Y. Li, T. Wang, et al., Effect of total flavonoids of *Flos Sophorae* on glucose levels, serum lipid and antioxidation ability in diabetic rats model, *Adv. J. Food Sci. Technol.* 12 (12) (2016) 663–666.
- [21] J.F. Yang, C.H. Yang, C.C. Wu, et al., Study on Antioxidant Activity of Chinese Drugs Based on Ultraviolet and Fluorescence Spectra 35 (2015), pp. 680–683.
- [22] X. He, Y. Bai, Z. Zhao, et al., Local and traditional uses, phytochemistry, and pharmacology of *Sophora japonica* L.: a review, *J. Ethnopharmacol.* 187 (2016) 160–182.
- [23] M.N. Khan, M. Ahmed, M.W. Khan, et al., *In vitro* pharmacological effects of *Astragalus eremophilus* and *Melilotus parviflora*, *Acta. Biol. Hung.* 69 (4) (2018) 411–422.
- [24] S. Hongmei, W. Menghan, L. Ran, et al., Extraction condition optimization and effects of drying methods on physicochemical properties and antioxidant activities of polysaccharides from *Astragalus cicer* L, *Sci. Rep.* 8 (1) (2018) 1–12.
- [25] M. Lucija, S. Anja, S. Vida, et al., Enhanced protection of biological membranes during lipid peroxidation: study of the interactions between flavonoid loaded mesoporous silica nanoparticles and model cell membranes, *Int. J. Mol. Sci.* 20 (11) (2019) 2709.
- [26] Y. Qu, C. Li, C. Zhang, et al., Optimization of infrared-assisted extraction of *Bletilla striata* polysaccharides based on response surface methodology and their antioxidant activities, *Carbohydr. Polym.* 148 (2016) 345–353.
- [27] T. Wang, M. Miao, M. Bai, et al., Effect of *sophora japonica* total flavonoids on pancreas, kidney tissue morphology of streptozotocin-induced diabetic mice model, *Saudi J. Biol. Sci.* 24 (3) (2017) 741–747.
- [28] K.S. Lee, H.J. Cha, G.T. Lee, et al., Troxerutin induces protective effects against ultraviolet B radiation through the alteration of microRNA expression in human HaCaT keratinocyte cells, *Int. J. Mol. Med.* 33 (4) (2014) 934–942.
- [29] S.H. Lee, S.J. Kim, S.J. Kim, Anti-oxidant activity with inhibition of osteoclastogenesis by *Atractylodes Rhizoma* extract, *Eur. Rev. Med. Pharmacol. Sci.* 18 (12) (2014) 1806–1812.
- [30] A. Zafar, S. Singh, I. Naseem, Cu(II)-coumestrol interaction leads to ROS-mediated DNA damage and cell death: a putative mechanism for anticancer activity, *J. Nutr. Biochem.* 33 (2016) 15–27.
- [31] H. Elabd, H.P. Wang, A. Shaheen, et al., Feeding *Glycyrrhiza glabra* (licorice) and *Astragalus membranaceus* (AM) alters innate immune and physiological responses in yellow perch (*Perca flavescens*), *Fish Shellfish Immunol.* 54 (2016) 374–384.
- [32] F. Meng, P. Xu, X. Wang, et al., Investigation on the immunomodulatory activities of *Sarcodon imbricatus* extracts in a cyclophosphamide (CTX)-induced immunosuppressed mouse model, *J. Saudi Pharm. Soc.* 25 (4) (2017) 460–463.
- [33] J.Q. Ma, R.Z. Luo, H.X. Jiang, et al., Quercitrin offers protection against brain injury in mice by inhibiting oxidative stress and inflammation, *Food Funct.* 7 (1) (2015) 549–556.
- [34] S. Zhi-hui, S. Xun-long, Z. Hai-yun, et al., The relationship between virus infection and oxidative stress, *Fudan Univ. J. Med. Sci.* 39 (1) (2012) 80–85.
- [35] Z. Qin-yang, C. Wen-tao, C. Yong, New progress on anti-influenza activity and mechanism of Chinese materia medica, *Chin. Tradit. Herbal Drugs* 50 (7) (2019) 1719–1726.
- [36] Z. Yuqing, Z. Zhenhua, Influence of Traditional Chinese Medicine on "Inflammation Storm" in Corona Virus Disease, (2019), <https://doi.org/10.13729/j.issn.1671-7813.Z20200180> 2020-03-19 11:21:58.
- [37] W. Jian, Y. Donghui, T. Siyuan, et al., Yupingfeng powder experimental research overview of the prevention and control of influenza, *Clin. J. Tradit. Chin. Med.* 29 (6) (2017) 762–765.
- [38] Q. Liu, L. Lu, M. Hua, et al., Jiawei-Yupingfeng-Tang, a Chinese herbal formula, inhibits respiratory viral infections *in vitro* and *in vivo*, *J. Ethnopharmacol.* 150 (2) (2013) 521–528.
- [39] G. Jie, Z. Mu-ying, G. Si-bin, et al., The research into influenza treated with jade screen powder, *Henan Tradit. Chin. Med.* 35 (8) (2015) 1842–1844.
- [40] V.M. Savov, A.S. Galabov, L.P. Tancheva, et al., Effects of rutin and quercetin on monoxygenase activities in experimental influenza virus infection, *Exp. Toxicol. Pathol.* 58 (1) (2006) 59–64.