

## Basic Study

# Antioxidant activity and hepatoprotective effect of 10 medicinal herbs on CCl<sub>4</sub>-induced liver injury in mice

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## Abstract

### BACKGROUND

Many natural products confer health benefits against diverse diseases through their antioxidant activities. Carbon tetrachloride (CCl<sub>4</sub>) is often used in animal experiments to study the effects of substances on liver injury and the related mechanisms of action, among which oxidative stress is a major pathogenic factor.

### AIM

To compare antioxidant and hepatoprotective activities of ten herbs and identify and quantify phytochemicals for the one with strongest hepatoprotection.

### METHODS

The antioxidant activity of ten medicinal herbs was determined by both ferric-reducing antioxidant power and Trolox equivalent antioxidant capacity assays. The total phenolic and flavonoid contents were determined by Folin-Ciocalteu method and aluminum chloride colorimetry, respectively. Their effects on CCl<sub>4</sub>-induced oxidative liver injury were evaluated and compared in a mouse model by administrating each water extract (0.15 g/mL, 10 mL/kg) once per day for seven consecutive days and a dose of CCl<sub>4</sub> solution in olive oil (8%, v/v, 10 mL/kg). The herb with the strongest hepatoprotective performance was analyzed for the detailed bioactive components by using high-performance liquid chromatography-electrospray ionization source-ion trap tandem mass spectrometry.

### RESULTS

The results revealed that all tested herbs attenuated CCl<sub>4</sub>-induced oxidative liver injury; each resulted in significant decreases in levels of serum alanine transaminase, aspartate transaminase, alkaline phosphatase, and triacylglycerols.

according to the ARRIVE guidelines.

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In addition, most herbs restored hepatic superoxide dismutase and catalase activities, glutathione levels, and reduced malondialdehyde levels. *Sanguisorba officinalis* (*S. officinalis*) L., *Coptis chinensis* Franch., and *Pueraria lobata* (Willd.) Ohwi root were the three most effective herbs, and *S. officinalis* L. exhibited the strongest hepatoprotective effect. Nine active components were identified in *S. officinalis* L. Gallic acid and (+)-catechin were quantified ( $7.86 \pm 0.45$  mg/g and  $8.19 \pm 0.57$  mg/g dried weight, respectively). Furthermore, the tested herbs displayed a range of *in vitro* antioxidant activities proportional to their phenolic content; the strongest activities were also found for *S. officinalis* L.

### CONCLUSION

This study is of value to assist the selection of more effective natural products for direct consumption and the development of nutraceuticals or therapeutics to manage oxidative stress-related diseases.

**Key Words:** Antioxidant activity; CCl<sub>4</sub>-induced liver injury; Medicinal herbs; Hepatoprotection; *Sanguisorba officinalis* L.; *Coptis chinensis* Franch

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**Core Tip:** Many natural products confer health benefits against diverse diseases through their antioxidant activities. In this study, ten medicinal herbs were selected for an evaluation and comparison of their effects on carbon tetrachloride (CCl<sub>4</sub>)-induced oxidative liver injury. *Sanguisorba officinalis* (*S. officinalis*) L. exhibited the strongest hepatoprotective effect, and the strongest *in vitro* activities were also found for *S. officinalis* L. Our results provided valuable information for the selection of more efficient herbs to protect against CCl<sub>4</sub>-induced liver injury and to support the direct application of herbs or the development of novel therapies for the management of oxidative stress-related diseases.

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## INTRODUCTION

Redox reactions are involved in numerous physiological and pathological processes; moreover, cellular homeostasis depends on the interaction between oxidants and the defense system, which includes reductants and antioxidant enzymes<sup>[1,2]</sup>. The prevalence of free radicals, such as reactive oxygen species and reactive nitrogen species, at a desirable level can contribute to cell growth and differentiation<sup>[2]</sup>. However, the overproduction of free radicals is destructive, resulting in oxidative stress and contributing to various diseases, such as cardiovascular diseases, cancer, diabetes, obesity, neurodegenerative disorders, and liver diseases<sup>[3-6]</sup>.

Many factors can cause liver damage. In addition to physical factors (*e.g.*, radiation) and biological factors (*e.g.*, viruses), some chemicals are hepatotoxic. Carbon tetrachloride (CCl<sub>4</sub>) is often used in animal experiments to study the effects of substances on liver injury and the related mechanisms of action<sup>[7-9]</sup>. The causal link between CCl<sub>4</sub> and liver diseases has been well established<sup>[9,10]</sup>. During the metabolism of CCl<sub>4</sub> in the liver, hepatotoxic metabolites and excessive free radicals such as trichloromethyl radical ( $\cdot\text{CCl}_3$ ) and trichloromethylperoxy radical ( $\cdot\text{OOCCL}_3$ ) are generated, accompanied by other free radicals (*e.g.*, O<sup>2-</sup> and H<sub>2</sub>O<sub>2</sub>). Consequently, reductants (*e.g.*, glutathione, GSH) are depleted, and antioxidant enzymes [*e.g.*, superoxide dismutase (SOD) and catalase (CAT)] are inhibited, inducing oxidative stress<sup>[9]</sup>. Meanwhile, the toxic metabolites and free radicals bind to phospholipid molecules embedded in the membranes of mitochondria, the endoplasmic reticulum, and hepatocytes, which leads to lipid peroxidation and membrane dysfunction or

damage<sup>[9]</sup>. In addition, they can also bind with other macromolecules, such as proteins and DNA, and result in cell damage or death. Such a condition may aggravate hypoxia, induce the accumulation of more lipids, facilitate gut leakage and bacterial translocation, promote cytokine release, and increase hepatic iron accumulation, which exacerbates the production of highly reactive radicals<sup>[4,11]</sup>. Therefore, oxidative stress is a major pathogenic factor for CCl<sub>4</sub>-induced liver injury.

Through their antioxidant activities, many natural products have been shown to exert protective effects against various oxidative stress-related diseases, such as cardiovascular disease, cancer, and liver diseases<sup>[4,12,13]</sup>. Notably, based on observations from many cultures over many years, numerous foods and herbs, including staple foods, vegetables, seasonings, and herbal teas<sup>[14,15]</sup>, have been reported to protect the liver. Subsequently, scientific evidence has shown that many related products and specific bioactive components have protective effects in the liver against oxidative injuries<sup>[8,16]</sup>. In this study, ten medicinal foods and herbs were selected for an evaluation and comparison of their effects on CCl<sub>4</sub>-induced oxidative liver injury. In addition, the herb with the strongest hepatoprotective performance was analyzed for the detailed bioactive components by using high-performance liquid chromatography-electrospray ionization source-ion trap tandem mass spectrometry (HPLC-ESI-ITMS/MS). The *in vitro* antioxidant capacities, total phenolic content (TPC), and total flavonoid content (TFC) of the ten herbs were also determined. This study aimed to provide valuable information for the selection of more efficient herbs to protect against CCl<sub>4</sub>-induced liver injury and to support the direct application of herbs or the development of novel therapies for the management of oxidative stress-related diseases.

## MATERIALS AND METHODS

### Chemicals

CCl<sub>4</sub>, sodium chloride, and acetic acid were obtained from Damao Chemical Reagent Factory (Tianjin, China). Olive oil was purchased from Aladdin Industrial Corporation (Shanghai, China). Methanol (99.9%, HPLC/ACS grade) and formic acid (≥ 90%, guaranteed grade) were purchased from Amethyst Chemicals (Beijing, China) and Kermel Chemical Factory (Tianjin, China), respectively. The phenolic standards, 2,4,6-tripyridyl-s-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), Folin and Ciocalteu's phenol, and 2,2'-azino-bis(3-ethylbenothiazoline-6-sulphonic acid) diammonium salt (ABTS) were purchased from Sigma-Aldrich (St. Louis, MO, United States). Gallic acid (> 98%) and (+)-catechin (> 98%) were obtained from Chengdu Derick Biotechnology Co. Ltd. (Sichuan, China).

All chemicals were of analytical or chromatographical grade. Double-distilled water was used in all experiments. Bifendate was obtained from the Beijing Union Pharmaceutical Factory. Detection kits for total protein, malondialdehyde (MDA), triglyceride (TG), GSH, SOD, and CAT were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

### Preparation of water extract

Ten medicinal foods and herbs, *Acanthopanax senticosus* (Rupr. et Maxim.) harms (root and rhizome), *Amomum villosum* Lour. (fruit), *Amomum kravanh* Pierre ex Gagnep. (fruit), *Artemisia capillaris* Thunb. (herb), *Cimicifuga heracleifolia* Kom. (rhizome), *Coptis chinensis* Franch. (rhizome), *Glycyrrhiza uralensis* Fisch. (root and rhizome), *Pueraria lobata* (Willd.) Ohwi (flower), *P. lobata* (Willd.) Ohwi (root), and *Sanguisorba officinalis* L. (root) were purchased from Tong Ren Tang Chinese Medicine Co., Ltd. (Guangzhou, Guangdong, China). The samples were processed in accordance with a published method<sup>[17]</sup> but with minor modifications. The finely ground powder of each sample was filtered through a 100-mesh sieve. Then, 10.00 g of filtered powder was mixed with 100 mL of water (room temperature, 30 min) before the mixture was decocted in a water bath (98 °C, 30 min). The cooled mixtures were then centrifuged (4200 g, 10 min), and the supernatant was collected. The extraction was conducted twice, and the supernatants were combined for further use.

### Animals and experimental design

Each of the water extracts was freeze dried in a FreeZone Freeze Dryer (Labconco FreeZone®, United States), and the dried crude extract was dissolved in water to give a concentration of 0.15 g/mL. Therefore, when gavaged with 10 mL/kg water extract, each mouse received 1.5 g dried weight (DW)/kg, which is equivalent to the desired

human dose recommended by National Administration of Traditional Chinese Medicine<sup>[18-20]</sup>.

SPF Kunming mice (male, weight 18–22 g) were provided by the Laboratory Animal Center of Sun Yat-Sen University, Guangzhou, China. With free access to water and rodent chow, the animals were kept in a controlled environment (22 °C ± 0.5 °C, 40%–60% relative humidity, and a 12 h light-dark cycle). All animal procedures were approved by the Animal Ethics Committee of the School of Public Health at Sun Yat-Sen University (No. 2017-011). The mice were randomly assigned into 13 groups each containing eight mice: control group, model group, positive control, and ten treatment groups. Each herb extract (15 mg/mL, 10 mL/kg) was administered to the mice by gavage once per day for seven consecutive days; bifendate (150 mg/kg) was administered in the positive control group<sup>[21]</sup>, and an equivalent volume of water was administered in the control and model groups. One hour after the final administration, CCl<sub>4</sub> solution in olive oil (8%, v/v, 10 mL/kg)<sup>[22,23]</sup> was administered by intraperitoneal injection to the mice in the model group, positive control group, and ten treatment groups; an equivalent concentration of olive oil (10 mL/kg) was administered to the control group. After 16 h, the mice were anesthetized, blood samples were taken, and the livers were harvested.

The blood samples were centrifuged twice at 3600 g for 15 min, and the serum was separated and analyzed by using a Beckman Coulter Chemistry Analyzer (AU5821, Tokyo, Japan), and the following serum biomarkers were analyzed: alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total bilirubin (TBIL), and TG. Two slides of liver were sampled from the middle of the left lobe of the liver, one for histopathologic examination (hematoxylin and eosin staining) and the other for biomarker assessment. Liver homogenate was prepared from liver tissue (0.2 g) and physiological saline (ice-cold, 1.8 mL), centrifuged at 2500 g for 10 min and then examined for the activities of SOD and CAT and the levels of GSH, MDA, and TG in accordance with the manufacturer's instructions and as described in our previous publications<sup>[24]</sup>.

### HPLC-ESI-ITMS/MS

The decoction of the herb with the strongest hepatoprotective effect (*S. officinalis* L.) was analyzed by reverse-phase HPLC using a Shim-pack GIS C18 column (5 µm, 4.6 mm × 250 mm; Shimadzu) maintained at 30 °C and a gradient elution (solvent A: H<sub>2</sub>O containing 0.1% (v/v) formic acid; solvent B, methanol containing 0.1% (v/v) formic acid). The phytochemical compounds were separated using a 70-min linear gradient from 20% to 70% solvent B at a flow rate of 1.0 mL/min and detected at 280 nm. The injection volumes were 20 µL (sample without dilution) for HPLC analysis and 50 µL (sample with 10-fold dilution) for HPLC-MS/MS analysis.

The MS analysis was performed by using an Ion Trap Mass Spectrometer (ITMS; Thermo Scientific, United States) equipped with an ESI under the following conditions: ESI source temperature, 320 °C; source voltages, 4.0 kV for positive mode and 3.5 kV for negative mode; capillary voltages, 24.0 V for positive polarity, and -12.0 V for negative polarity; desolvation gas, nitrogen; full scan mass range, *m/z* 50–1500. Tandem mass spectrometry analyses were performed using nitrogen as the collision gas with a collision energy of 35 eV. The MS/MS spectrum was obtained to the tenth most intense ion from ITMS.

### Evaluation of *in vitro* antioxidant activities

The ferric-reducing antioxidant power (FRAP) assay was performed to assess the antioxidant activity (Fe<sup>3+</sup>-reducing capability) of the tested herbs using a slightly modified version of a published method<sup>[25]</sup>. In short, FRAP reagent was freshly prepared and kept in a water bath at 37 °C until use with the following three solutions (10:1:1, v/v/v): (1) Sodium acetate buffer (300 mmol/L, pH 3.6); (2) TPTZ solution (10 mmol/L, solvent: 40 mmol/L HCl); and (3) Ferric chloride solution (20 mmol/L). Then, the mixture of the water extract of each sample (100 µL) and FRAP reagents (3 mL) were incubated at room temperature for 4 min. The absorbance of each mixture at 593 nm was recorded. The results were expressed in the form of µmol Fe<sup>2+</sup>/g DW, with reference to ferrous sulfate as the standard.

The Trolox equivalent antioxidant capacity (TEAC) assay was also conducted to measure the antioxidant activities (free radical, *i.e.*, ABTS<sup>•+</sup>-scavenging capability) of the tested herbs and was performed using a slightly modified version of a published method<sup>[26]</sup>. Briefly, the ABTS free radical (ABTS<sup>•+</sup>) solution was a 1:1 (v/v) solution of ABTS stock solution (7 mmol/L) and potassium persulfate (2.45 mmol/L). The mixture was kept in the dark (room temperature, more than 16 h) for no more than 2 d. The ABTS<sup>•+</sup> solution was diluted with ethanol until the solution had an absorbance



of  $0.710 \pm 0.050$  at 734 nm. Each water extract (100  $\mu$ L) was mixed with diluted ABTS<sup>++</sup> solution (3.8 mL) and allowed to react for 6 min; subsequently, the absorbance was recorded, and the results were presented as  $\mu$ mol Trolox equivalent per gram DW ( $\mu$ mol TE/g DW).

### Determination of TPC and TFC

The TPC was determined in accordance with the Folin-Ciocalteu method<sup>[27]</sup>. Briefly, a properly diluted sample (0.5 mL) was mixed with Folin-Ciocalteu reagent (2.5 mL, 0.2 M). After 4 min, a saturated sodium carbonate solution (2 mL, 75 g/L) was added, and the mixture was kept at room temperature for 2 h. The absorbance at 760 nm was recorded. The results were presented as mg gallic acid equivalent per gram DW (mg GAE/g DW).

The TFC was determined by using a slightly modified version of a published aluminum chloride colorimetric method<sup>[28]</sup>. Specifically, ethanol (1.5 mL, 95%, v/v), aluminum chloride (0.1 mL, 10%, w/v), potassium acetate (0.1 mL, 1 mol/L), and water (2.8 mL) were added to the diluted sample (0.5 mL), and the mixture was kept at room temperature for 30 min. Absorbance was recorded at 415 nm. The results were expressed in terms of mg quercetin equivalent per gram DW (mg QE/g DW).

### Statistical analysis

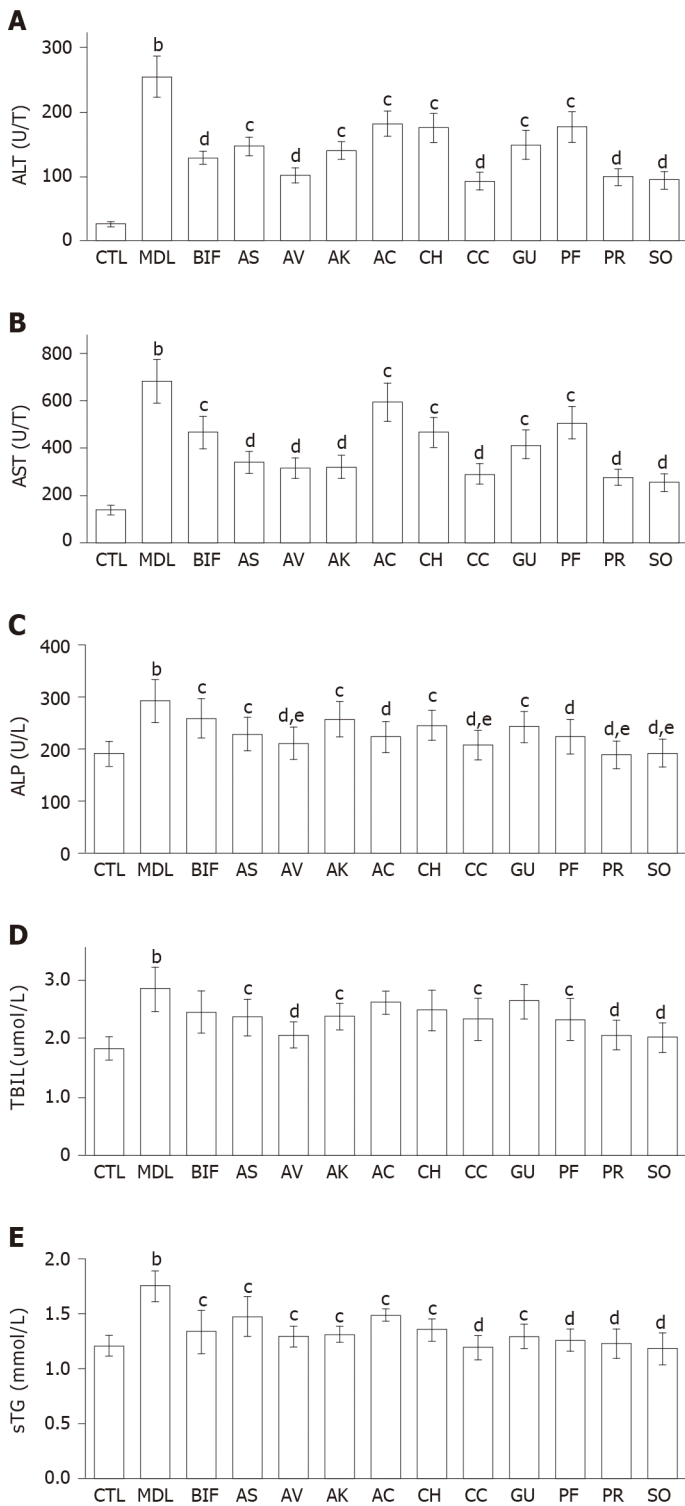
All data were expressed as the mean  $\pm$  standard deviation. IBM SPSS Statistics 20.0 (SPSS Inc., Chicago, IL, United States) was used for statistical analysis. Analysis of variance and the least significant difference test were applied to examine the differences in means. Systematic cluster analysis with online analytical processing was also performed. For all tests,  $P < 0.05$  and  $P < 0.01$  were defined as two levels of statistical significance. The statistical methods of this study were reviewed by Chan-Juan Zhao from Department of Bio-statistics, School of Public Health, Hainan Medical University, Hainan Province, China.

## RESULTS

### Effects of tested foods and herbs on CCl<sub>4</sub>-induced injury

**Effects on serum biomarkers:** Compared with the control group, significantly increased serum levels of ALT, AST, ALP, TBIL, and TG were observed in the model group (all  $P < 0.01$ ), indicating that liver injury was successfully induced by CCl<sub>4</sub> (Figure 1). Bifendate is often used to lower the levels of serum transaminases in the treatment of hepatitis<sup>[21,29]</sup>. Compared with the model group, bifendate significantly decreased ALT, AST, ALP, and TG, and moderately reduced TBIL. As Figure 1A and 1B showed, each herb significantly decreased ALT and AST levels ( $P < 0.05$  or  $P < 0.01$ ). Notably, four herbs (*A. villosum*, *C. chinensis*, *P. lobata* root, and *S. officinalis*) were more effective in reducing both ALT and AST (each  $P < 0.01$ ). Compared with the model group, each herb significantly reduced ALP, with the lowest values found after treatment with *P. lobata* root and *S. officinalis* (Figure 1C). Moreover, four herbs (*A. villosum*, *C. chinensis*, *P. lobata* root, and *S. officinalis*) exhibited better ALP-lowering effects than bifendate (each  $P < 0.05$ ). Furthermore, *A. senticosus*, *A. villosum*, *A. kravanh*, *C. chinensis*, *P. lobata* flower, *P. lobata* root, and *S. officinalis* significantly decreased TBIL compared with the model group (Figure 1D). Specifically, better performance was found for *S. officinalis*, *P. lobata* root, and *A. villosum* (each  $P < 0.01$ ). In addition, *A. capillaris*, *C. heracleifolia*, and *G. uralensis*, only slightly decreased TBIL. All herbs remarkably reduced TG compared with the model group ( $P < 0.05$  or  $P < 0.01$ ) (Figure 1E).

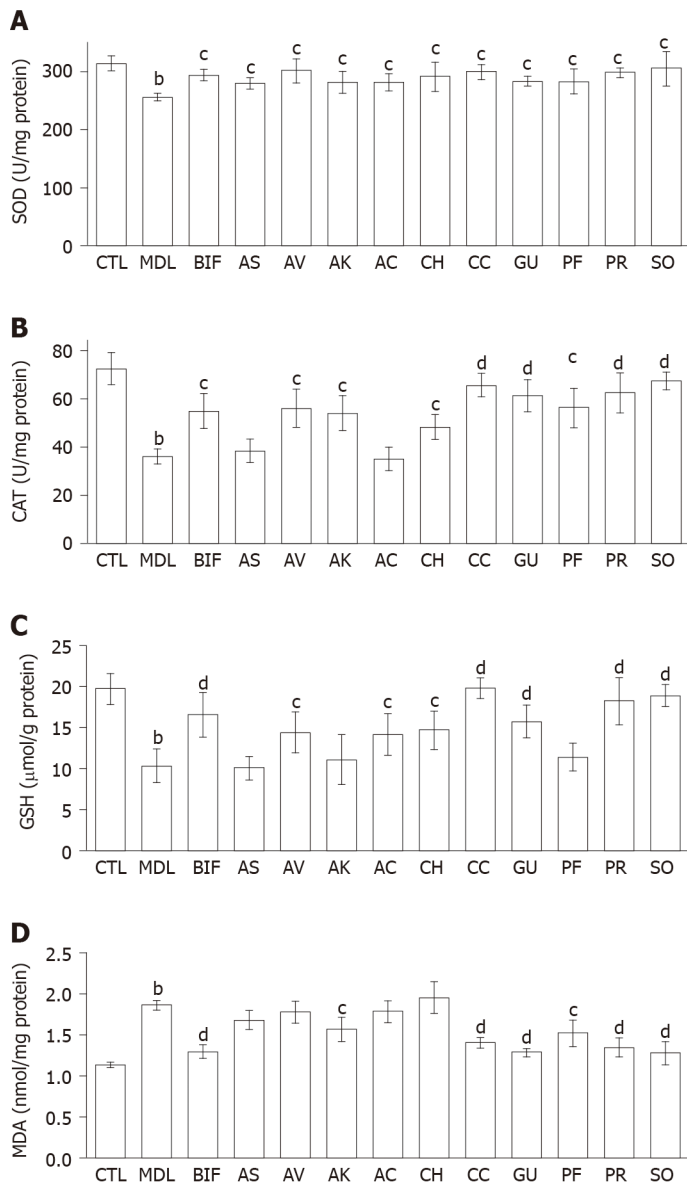
**Effects of herbs on hepatic antioxidant enzymes, GSH, and lipid peroxidation in the liver:** As shown in Figure 2, a significant decrease was observed in SOD and CAT activities and GSH level in the CCl<sub>4</sub> model group (each  $P < 0.01$ ) as well as a significant increase in the MDA level ( $P < 0.01$ ), indicating that oxidative liver injury had been successfully induced. Bifendate and all herbs restored SOD activity compared with the model group (each  $P < 0.05$ ) (Figure 2A). In addition, each herb significantly increased CAT activity ( $P < 0.05$  or  $P < 0.01$ ), except for *A. senticosus* and *A. capillaris* (Figure 2B). Moreover, compared with the model group, significant increases in GSH were found in the *S. officinalis*, *P. lobata* root, *C. chinensis*, *G. uralensis*, *A. capillaris*, *C. heracleifolia*, and *A. villosum* groups and moderate elevations of GSH in the *A. kravanh*, *P. lobata* flower, and *A. senticosus* group (Figure 2C). Overall, *S. officinalis*, *P. lobata* root, and *C. chinensis* were more effective than the other herbs in restoring the hepatic antioxidant



**Figure 1** Effects of ten herbs on serum biomarkers (n = 8). A: Alanine transaminase ; B: Aspartate transaminase; C: Alkaline phosphatase; D: Total bilirubin; E: Triglyceride. The values are presented as the mean ± standard deviation. <sup>a</sup>P < 0.05, <sup>b</sup>P < 0.01 vs control; <sup>c</sup>P < 0.05, <sup>d</sup>P < 0.01 vs model; <sup>e</sup>P < 0.05, <sup>f</sup>P < 0.01 vs bifendate. ALT: Alanine transaminase; AST: Aspartate transaminase; ALP: Alkaline phosphatase; TBIL: Total bilirubin; TG: Triglyceride; CTL: Control; MDL: Model; BIF: Bifendate; AS: *Acanthopanax senticosus*; AV: *Amomum villosum*; AK: *Amomum kravanh*; AC: *Artemisia capillaris*; CH: *Cimicifuga heracleifolia*; CC: *Coptis chinensis*; GU: *Glycyrrhiza uralensis*; PF: *Pueraria lobata* flower; PR: *Pueraria lobata* root; SO: *Sanguisorba officinalis*.

activity in CCl<sub>4</sub>-induced liver injury. As shown in **Figure 2D**, only *S. officinalis*, *G. uralensis*, *P. lobata* root, *C. chinensis*, *P. lobata* flower, and *A. kravanh* ameliorated the increase in MDA. *S. officinalis*, *P. lobata* root, *C. chinensis* and *G. uralensis* showed better lipid peroxidation-reducing effects than the other herbs (each P < 0.01).

**Histopathological analysis:** No visible histological abnormalities were found in the



**Figure 2** Effects of ten herbs on hepatic antioxidant enzymes, glutathione, and malondialdehyde ( $n = 8$ ). A: Superoxide dismutase; B: Catalase; C: Glutathione; D: Malondialdehyde. The values are presented as the mean  $\pm$  standard deviation. <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$  vs control; <sup>c</sup> $P < 0.05$ , <sup>d</sup> $P < 0.01$  vs model; <sup>e</sup> $P < 0.05$ , <sup>f</sup> $P < 0.01$  vs bifendate. SOD: Superoxide dismutase; CAT: Catalase; GSH: Glutathione; MDA: Malondialdehyde; CTL: Control; MDL: Model; BIF: Bifendate; AS: *Acanthopanax senticosus*; AV: *Amomum villosum*; AK: *Amomum kravanh*; AC: *Artemisia capillaris*; CH: *Cimicifuga heracleifolia*; CC: *Coptis chinensis*; GU: *Glycyrrhiza uralensis*; PF: *Pueraria lobata* flower; PR: *Pueraria lobata* root; SO: *Sanguisorba officinalis*.

control group (Figure 3A). The liver pathology of the  $\text{CCl}_4$  model showed necrosis and ballooning degeneration in the perivenular zone as a result of severe cell injury, obvious massive inflammatory cells, and the accumulation of lipid droplets in hepatocytes (Figure 3B). These findings indicated the occurrence of  $\text{CCl}_4$ -induced liver injury. Meanwhile, bifendate and the ten tested materials ameliorated the morphological changes by mitigating necrosis, attenuating inflammatory cell infiltration, and resulting in lower lipid droplet accumulation, as shown below, for *C. chinensis*, *P. lobata* root, and *S. officinalis*, respectively (Figure 3D-F).

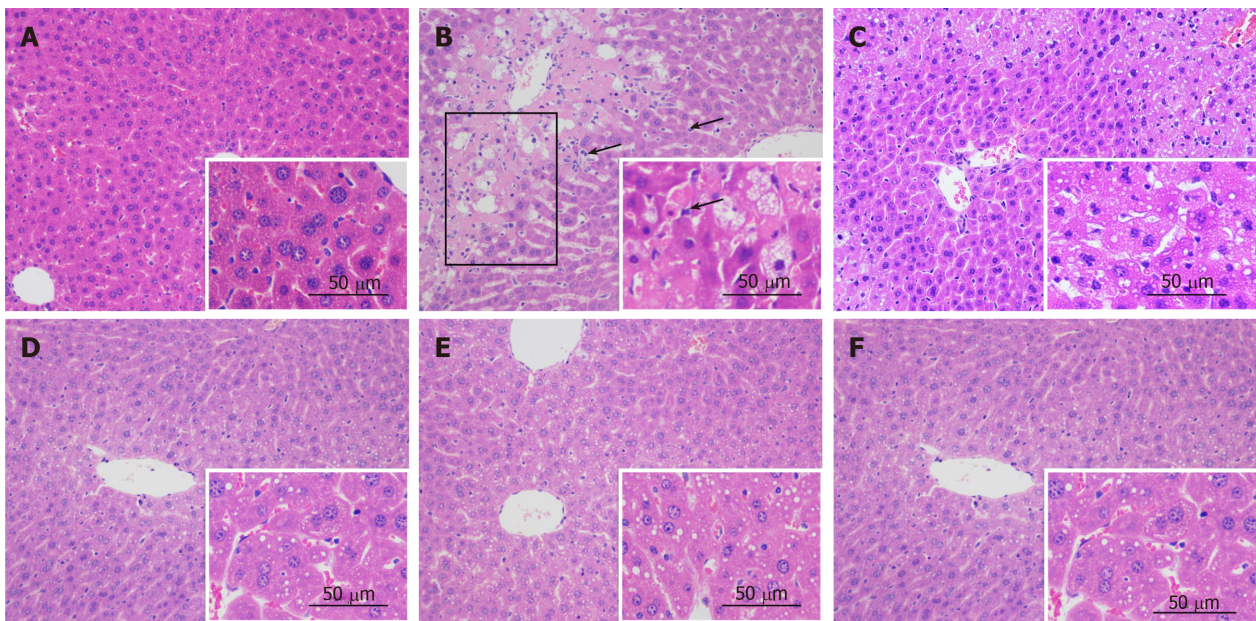
#### Antioxidant activities, TPC, and TFC of tested herbs

**Antioxidant activities of herbs:** The FRAP values of the ten medicinal foods and herbs ranged from  $29.80 \pm 0.29$  to  $1141.88 \pm 81.16 \mu\text{mol Fe}^{2+}/\text{g DW}$ , and the TEAC values varied from  $37.34 \pm 1.02$  to  $1554.48 \pm 68.58 \mu\text{mol TE}/\text{g DW}$  (Table 1). The three highest FRAP values (in decreasing order) were found in *S. officinalis* ( $1141.88 \pm 81.16 \mu\text{mol Fe}^{2+}/\text{g DW}$ ), *C. chinensis* ( $557.04 \pm 4.73 \mu\text{mol Fe}^{2+}/\text{g DW}$ ), and *P. lobata* root ( $554.38 \pm 3.92 \mu\text{mol Fe}^{2+}/\text{g DW}$ ). The lowest two were found in *G. uralensis* and *A. kravanh* ( $29.85 \pm 2.10$  and  $29.80 \pm 0.29 \mu\text{mol Fe}^{2+}/\text{g DW}$ , respectively). Meanwhile, the top three TEAC

**Table 1 Ferric-reducing antioxidant power, Trolox equivalent antioxidant capacity, total phenolic content, and total flavonoid content values of ten medicinal herbs (n = 8)**

| Scientific name of original medicinal plant            | Parts with medicinal properties | FRAP value (μmol Fe <sup>2+</sup> /g DW) | TEAC value (μmol TE/g DW)    | TPC value (mg GAE/g DW)   | TFC value (mg QE/g DW)     |
|--|---------------------------------|--|------------------------------|---------------------------|----------------------------|
| <i>Acanthopanax senticosus</i> (Rupr. et Maxim.) Harms | Root and rhizome                | 53.07 ± 0.23 <sup>c</sup>                | 54.70 ± 3.73 <sup>d</sup>    | 2.05 ± 0.16 <sup>d</sup>  | 0.44 ± 0.01 <sup>e</sup>   |
| <i>Amomum villosum</i> Lour.                           | Fruit                           | 219.85 ± 14.44 <sup>c</sup>              | 210.18 ± 23.88 <sup>c</sup>  | 18.42 ± 0.25 <sup>c</sup> | 1.98 ± 0.07 <sup>c,d</sup> |
| <i>Amomum kravanh</i> Pierre ex Gagnep.                | Fruit                           | 29.80 ± 0.29 <sup>e</sup>                | 37.34 ± 1.02 <sup>d</sup>    | 18.49 ± 0.70 <sup>c</sup> | 0.32 ± 0.01 <sup>e</sup>   |
| <i>Artemisia capillaris</i> Thunb.                     | Herb                            | 146.71 ± 2.91 <sup>d</sup>               | 208.16 ± 1.42 <sup>c</sup>   | 19.06 ± 0.21 <sup>c</sup> | 1.55 ± 0.11 <sup>d</sup>   |
| <i>Cimicifuga heracleifolia</i> Kom.                   | Rhizome                         | 191.25 ± 6.38 <sup>c,d</sup>             | 214.60 ± 25.17 <sup>c</sup>  | 17.07 ± 0.28 <sup>c</sup> | 0.40 ± 0.06 <sup>e</sup>   |
| <i>Coptis chinensis</i> Franch.                        | Rhizome                         | 557.04 ± 4.73 <sup>b</sup>               | 450.36 ± 27.23 <sup>b</sup>  | 50.15 ± 1.14 <sup>b</sup> | 7.39 ± 0.39 <sup>b</sup>   |
| <i>Glycyrrhiza uralensis</i> Fisch.                    | Root and rhizome                | 29.85 ± 2.10 <sup>e</sup>                | 53.09 ± 2.59 <sup>d</sup>    | 5.70 ± 0.04 <sup>d</sup>  | 3.71 ± 0.19 <sup>c</sup>   |
| <i>Pueraria lobata</i> (Willd.) Ohwi                   | Flower                          | 216.53 ± 11.34 <sup>c</sup>              | 213.25 ± 9.65 <sup>c</sup>   | 14.18 ± 0.11 <sup>c</sup> | 2.86 ± 0.35 <sup>c</sup>   |
| <i>Pueraria lobata</i> (Willd.) Ohwi                   | Root                            | 554.38 ± 3.92 <sup>b</sup>               | 454.06 ± 8.14 <sup>b</sup>   | 45.06 ± 0.07 <sup>b</sup> | 6.84 ± 0.08 <sup>b</sup>   |
| <i>Sanguisorba officinalis</i> L.                      | Root                            | 1141.88 ± 81.16 <sup>a</sup>             | 1554.48 ± 68.58 <sup>a</sup> | 91.59 ± 0.00 <sup>a</sup> | 14.93 ± 0.24 <sup>a</sup>  |

<sup>a,b,c,d,e</sup>p < 0.05, different superscript letters indicate statistical significance. DW: dried weight; FRAP: ferric-reducing antioxidant power; GAE: gallic acid equivalent; QE: quercetin equivalent; TE: Trolox equivalent; TEAC: Trolox equivalent antioxidant capacity; TFC: total flavonoid content; TPC: total phenolic content.



**Figure 3 Histopathological findings showing the effects of ten tested materials on carbon tetrachloride-induced liver injury (200 × and 400 × magnification) (n = 8).** A: Control; B: carbon tetrachloride (CCl<sub>4</sub>) model; C: Bifentate + CCl<sub>4</sub>; D: *Coptis chinensis* + CCl<sub>4</sub>; E: *Pueraria lobata* root + CCl<sub>4</sub>; F: *Sanguisorba officinalis* + CCl<sub>4</sub>. Scale bar, 50 μm in 400×; Box, necrotic area; arrow, inflammatory cell. CCl<sub>4</sub>: Carbon tetrachloride.

values were found in *S. officinalis* (1554.48 ± 68.58 μmol TE/g DW), *P. lobata* root (454.06 ± 8.14 μmol TE/g DW), *C. chinensis* (450.36 ± 27.23 μmol TE/g DW), and the lowest two were also found in *G. uralensis* and *A. kravanh* (53.09 ± 2.59 and 37.34 ± 1.02 μmol TE/g DW, respectively).

**TPC and TFC of herbs:** The TPC and TFC of the ten materials were determined (Table 1). The TPC values ranged from 2.05 ± 0.16 to 91.59 ± 0.00 mg GAE/g DW, which was a difference of more than 40-fold. The three highest TPC values were found for *S. officinalis* (91.59 ± 0.00 mg GAE/g DW), *C. chinensis* (50.15 ± 1.14 mg GAE/g DW), and *P. lobata* root (45.06 ± 0.07 mg GAE/g DW). The lowest TPC values were



found for *G. uralensis* and *A. senticosus*; both were below 10 mg GAE/g DW. The highest three TFC values were found for *S. officinalis* ( $14.93 \pm 0.24$  mg QE/g DW), *C. chinensis* ( $7.39 \pm 0.39$  mg QE/g DW), and *P. lobata* root ( $6.84 \pm 0.08$  mg QE/g DW); in contrast, the lowest three TFC values were found in *A. senticosus*, *C. heracleifolia*, and *A. kravanh*, all of which were below 1 mg QE/g DW. Our results revealed that *S. officinalis* showed the strongest *in vitro* antioxidant activities, possibly because it contained the highest contents of phenols and flavonoids.

**Correlations between FRAP, TEAC, TPC, and TFC values:** Correlation analyses were performed to identify the strength of relationships between FRAP, TEAC, TPC, and TFC values (Table 2). A strong correlation between FRAP and TEAC ( $R^2 = 0.9340$ ) indicated that the herbs possessed the ability to reduce  $Fe^{3+}$  to  $Fe^{2+}$  and to scavenge ABTS<sup>•+</sup>. In addition, the FRAP and TEAC values were both found to correlate with the TPC values ( $R^2 = 0.9600$  for FRAP and TPC;  $R^2 = 0.9013$  for TEAC and TPC), implying that the phenolic components contributed to the  $Fe^{3+}$ -reducing and ABTS<sup>•+</sup>-scavenging activities<sup>[30]</sup>. Moreover, a correlation between the TPC and TFC values ( $R^2 = 0.8829$ ) suggested that flavonoids may be the major phenolic compounds but were not the only ones. Correlations were also found between FRAP/TEAC and TFC ( $R^2 = 0.9139$  for FRAP and TFC;  $R^2 = 0.8729$  for TEAC and TFC). In summary, the antioxidants in the tested medicinal herbs were able to both reduce oxidants (*e.g.*,  $Fe^{3+}$ ) and scavenge free radicals (*e.g.*, ABTS<sup>•+</sup>), which was largely dependent on their TPC.

**Relationship between *in vitro* antioxidant activity, effects on liver injury and *in vivo* antioxidant activity:** To investigate the relationship between *in vivo* antioxidant activity of the tested herbs, their effects on liver injury, and *in vitro* antioxidant activity, systematic cluster analyses were performed (range of solutions, 2–6 cluster numbers) for clinical indicators of CCl<sub>4</sub>-induced liver injury and the values of FRAP, TEAC, and TPC (Table 3 and Figure 4). Then, a cluster number of 3 was used for online analytical processing and analysis of variance. With the exception of seven herbs in Cluster 1, Cluster 2 included *P. lobata* root and *C. chinensis*, and Cluster 3 contained *S. officinalis*. Herbs in Clusters 2 and 3 (*P. lobata* root, *C. chinensis*, and *S. officinalis*) showed stronger hepatoprotective effects and *in vivo* antioxidant activity, and they exhibited stronger *in vitro* antioxidant activities, together with higher TPC and TFC.

### Identification and quantification of phytochemical compounds in *S. officinalis*

As *S. officinalis* showed the strongest hepatoprotective effect against CCl<sub>4</sub>-induced liver injury compared with the other tested medicinal foods and herbs, its phytochemical compounds in the decoction were identified by HPLC-ESI-ITMS/MS using external standards and/or according to MS and MS/MS information found in references<sup>[31-33]</sup>. As shown in Figure 5, eight main peaks were observed on the HPLC chromatogram; these corresponded to nine main compounds in the *S. officinalis* decoction (Table 4). The four major peaks (Peak 1-4) were identified as gallic acid (Peak 1), galloyl-methylglucoside, procyanidin C2 (Peak 2), (+)-catechin (Peak 3), and procyanidin B3 3-O-gallate (Peak 4)<sup>[33]</sup>. In addition, the quantification of unambiguously identified compounds was performed by HPLC using corresponding standards; *i.e.*, gallic acid (Peak 1) and (+)-catechin (Peak 3) were quantified as  $7.86 \pm 0.45$  and  $8.19 \pm 0.57$  mg/g DW, respectively.

## DISCUSSION

AST and ALT are distributed predominantly in the liver cells. When the liver is damaged, they enter the bloodstream. Clinically, elevations in serum ALT and AST can be regarded as indicators of liver diseases, although ALT is more specific than AST<sup>[21]</sup>. In our study, all the herbs significantly decreased ALT and AST levels showing their hepatoprotection, with more effectiveness found for *A. villosum*, *C. chinensis*, *P. lobata* root, and *S. officinalis*. Clinically, increased ALP often indicates cholestasis caused by liver injury<sup>[34,35]</sup>. The ALP-lowering effects were also found for each herb. TBIL is also a sensitive indicator of bilirubin metabolic disorders, which are often increased in acute liver injury<sup>[36-38]</sup>. *A. senticosus*, *A. villosum*, *A. kravanh*, *C. chinensis*, *P. lobata* flower, *P. lobata* root, and *S. officinalis* significantly decreased TBIL compared with the model group, particularly *S. officinalis*, *P. lobata* root, and *A. villosum*. Serum TG is another biomarker of liver dysfunction and is found to be elevated if the liver is damaged. In this study, all herbs reduced TG significantly compared with the model group. Histopathological examinations confirmed the hepatoprotective effects of the

**Table 2** Correlation analysis between ferric-reducing antioxidant power, Trolox equivalent antioxidant capacity, total phenolic content, and total flavonoid content values

| Correlation Coefficient ( $R^2$ ) | FRAP value ( $\mu\text{mol Fe}^{2+}/\text{g DW}$ ) | TEAC value ( $\mu\text{mol TE}/\text{g DW}$ ) | TPC value ( $\text{mg GAE}/\text{g DW}$ ) | TFC value ( $\text{mg QE}/\text{g DW}$ ) |
|-----------------------------------|--|---|---|--|
| FRAP value                        | 1  | 0.9340  | 0.9600                                    | 0.9139                                   |
| TEAC value                        | -  | 1   | 0.9013                                    | 0.8729                                   |
| TPC value                         | -  | -   | 1   | 0.8829                                   |
| TFC value                         | -  | -   | -   | 1  |

DW: Dried weight; FRAP: Ferric-reducing antioxidant power; GAE: Gallic acid equivalent; TE: Trolox equivalent; TEAC: Trolox equivalent antioxidant capacity; TFC: Total flavonoid content; TPC: Total phenolic content; QE: quercetin equivalent.

ten tested medicinal foods and herbs by improving the degenerative morphological changes induced by  $\text{CCl}_4$  (Figure 3).

Many chemicals can cause liver injury, and the mechanisms of action involved in  $\text{CCl}_4$ -induced liver disease have been investigated widely<sup>[4]</sup>. Briefly,  $\text{CCl}_4$  is metabolized in the liver by cytochrome P450 enzymes, biotransformed into  $\cdot\text{CCl}_3$ , and then oxygenated to  $\cdot\text{OOCCL}_3$ ; both of these radicals are highly reactive and can induce the depletion of reductants, inhibit antioxidant enzymes, induce lipid peroxidation, hypomethylate proteins, and mutate nucleic acids, resulting in oxidative stress, inflammation, apoptosis, and necrosis<sup>[23]</sup>.

Whether oxidative injury occurs is dependent on the outcome of the interaction between oxidants and the protective system. In the body, there is a complex defense system consisting of antioxidant enzymes that can protect against oxidative damage, such as SOD, CAT, and glutathione peroxidase and some nonenzymatic antioxidants, including GSH, vitamins, and ubiquinone, that can also help to maintain the redox balance<sup>[39]</sup>. The metabolism of  $\text{CCl}_4$  results in the accumulation of reactive oxygen species, mainly  $\text{O}_2^-$  and  $\text{H}_2\text{O}_2$ , which can be scavenged by SOD and CAT, respectively<sup>[40]</sup>. The depletion of reduced endogenous antioxidants (such as GSH) can therefore increase the sensitivity of hepatocytes to oxidative stress<sup>[41]</sup>. Therefore, the activities of SOD and CAT as well as levels of GSH, are often used to evaluate *in vivo* antioxidant activity<sup>[30,42]</sup>. Free radicals are often highly reactive, and they can quickly bind to other molecules or atoms to form reactive metabolites, leading to lipid peroxidation, which results in elevated MDA<sup>[41]</sup>. Thus, MDA is often used to evaluate lipid peroxidation status<sup>[30,42]</sup>.

Compared with the model group, all herbs restored SOD activity, and each herb significantly increased CAT activity, except for *A. senticosus* and *A. capillaris*. Significant increases in GSH were also found in the *S. officinalis*, *P. lobata* root, *C. chinensis*, *G. uralensis*, *A. capillaris*, *C. heracleifolia*, and *A. villosum* groups. Additionally, *S. officinalis*, *G. uralensis*, *P. lobata* root, *C. chinensis*, *P. lobata* flower, and *A. kravanh* ameliorated the increase in MDA. Overall, *S. officinalis*, *P. lobata* root, and *C. chinensis* were more effective than the other herbs in restoring the hepatic antioxidant activity and reducing lipid peroxidation in  $\text{CCl}_4$ -induced liver injury.

The chemical composition of most natural products is complex. Moreover, the antioxidant activities of medicinal herbs are often multifunctional and may be influenced by various factors; as such, more than one method is preferred to evaluate the antioxidant activity of natural products<sup>[43,44]</sup>. In this study, the antioxidant activities of selected medicinal herbs were determined by using the FRAP assay and the TEAC assay. The former indicates the antioxidant activity that is dependent on the capacity to reduce  $[\text{Fe}(\text{TPTZ})_2]^{3+}$  to  $[\text{Fe}(\text{TPTZ})_2]^{2+}$ <sup>[25]</sup>, whereas the latter is based on the ability to scavenge  $\text{ABTS}^{\bullet+}$ <sup>[24,44]</sup>.

The FRAP and TEAC values of the ten medicinal foods and herbs ranged with a nearly 40-fold difference, both of which *S. officinalis* ranked first, and *C. chinensis* and *P. lobata* root were the second or the third. Similar results were found for TPC and TFC. Because bioactive components, especially phenols and flavonoids, are the main contributors to the antioxidant activities of natural products<sup>[17,45]</sup>, our results revealed that *S. officinalis* showed the strongest *in vitro* antioxidant activities, which was possibly because it contained the highest contents of phenols and flavonoids. The correlations between FRAP, TEAC, TPC, and TFC values indicated that the antioxidants in the tested medicinal herbs possessed both oxidant-reducing and free radical-scavenging capability, mainly according to their TPC. Moreover, relationship

Table 3 Online analytical processing cubes based on systematic cluster analysis for indicators of CCl<sub>4</sub>-induced liver injury, antioxidant activity, total phenolic content, and total flavonoid content (cluster number = 3)

|              |                | ALT     | AST     | ALP     | TBIL   | sTG    | SOD     | CAT    | GSH    | MDA    | FRAP    | TEAC    | TPC    | TFC    |
|--------------|----------------|---------|---------|---------|--------|--------|---------|--------|--------|--------|---------|---------|--------|--------|
| <b>Cl1</b>   | <b>Sum</b>     | 1080.59 | 3017.02 | 1640.70 | 16.89  | 9.48   | 1997.54 | 349.62 | 91.24  | 11.58  | 887.06  | 991.32  | 94.97  | 11.26  |
| AV           | <i>n</i>       | 7       | 7       | 7       | 7      | 7      | 7       | 7      | 7      | 7      | 7       | 7       | 7      | 7      |
| AS           | <b>mean</b>    | 154.37  | 431.00  | 234.39  | 2.41   | 1.35   | 285.36  | 49.95  | 13.03  | 1.65   | 126.72  | 141.62  | 13.57  | 1.61   |
| AK           | <b>SD</b>      | 27.92   | 82.89   | 15.86   | 0.20   | 0.09   | 7.67    | 9.92   | 2.17   | 0.22   | 87.09   | 87.42   | 6.89   | 1.33   |
| GU           | <b>% of TS</b> | 78.9%   | 78.6%   | 73.5%   | 72.5%  | 72.0%  | 68.9%   | 64.1%  | 61.6%  | 74.2%  | 28.2%   | 28.7%   | 33.7%  | 27.9%  |
| AC           | <b>% of TN</b> | 70.0%   | 70.0%   | 70.0%   | 70.0%  | 70.0%  | 70.0%   | 70.0%  | 70.0%  | 70.0%  | 70.0%   | 70.0%   | 70.0%  | 70.0%  |
| CH           |                |         |         |         |        |        |         |        |        |        |         |         |        |        |
| PF           |                |         |         |         |        |        |         |        |        |        |         |         |        |        |
| <b>Cl2</b>   | <b>Sum</b>     | 193.77  | 567.29  | 398.53  | 4.39   | 2.46   | 595.96  | 128.15 | 37.99  | 2.74   | 1111.42 | 904.42  | 95.21  | 14.23  |
| CC           | <i>n</i>       | 2       | 2       | 2       | 2      | 2      | 2       | 2      | 2      | 2      | 2       | 2       | 2      | 2      |
| PR           | <b>mean</b>    | 96.89   | 283.65  | 199.27  | 2.20   | 1.23   | 297.98  | 64.08  | 19.00  | 1.37   | 555.71  | 452.21  | 47.61  | 7.12   |
|              | <b>SD</b>      | 4.79    | 9.30    | 13.09   | 0.19   | 0.06   | 1.27    | 2.10   | 1.10   | 0.04   | 1.88    | 2.62    | 3.60   | 0.39   |
|              | <b>% of TS</b> | 14.1%   | 14.8%   | 17.9%   | 18.8%  | 18.7%  | 20.6%   | 23.5%  | 25.7%  | 17.6%  | 35.4%   | 26.2%   | 33.8%  | 35.2%  |
|              | <b>% of TN</b> | 20.0%   | 20.0%   | 20.0%   | 20.0%  | 20.0%  | 20.0%   | 20.0%  | 20.0%  | 20.0%  | 20.0%   | 20.0%   | 20.0%  | 20.0%  |
| <b>Cl3</b>   | <b>Sum</b>     | 95.34   | 256.05  | 192.78  | 2.03   | 1.23   | 304.69  | 67.44  | 18.85  | 1.28   | 1141.88 | 1554.48 | 91.59  | 14.93  |
| SO           | <i>n</i>       | 1       | 1       | 1       | 1      | 1      | 1       | 1      | 1      | 1      | 1       | 1       | 1      | 1      |
|              | <b>mean</b>    | 95.34   | 256.05  | 192.78  | 2.03   | 1.23   | 304.69  | 67.44  | 18.85  | 1.28   | 1141.88 | 1554.48 | 91.59  | 14.93  |
|              | <b>SD</b>      | /       | /       | /       | /      | /      | /       | /      | /      | /      | /       | /       | /      | /      |
|              | <b>% of TS</b> | 7.0%    | 6.7%    | 8.6%    | 8.7%   | 9.3%   | 10.5%   | 12.4%  | 12.7%  | 8.2%   | 36.4%   | 45.1%   | 32.5%  | 36.9%  |
|              | <b>% of TN</b> | 10.0%   | 10.0%   | 10.0%   | 10.0%  | 10.0%  | 10.0%   | 10.0%  | 10.0%  | 10.0%  | 10.0%   | 10.0%   | 10.0%  | 10.0%  |
| <b>Total</b> | <b>Sum</b>     | 1369.70 | 3840.36 | 2232.01 | 23.31  | 13.17  | 2898.19 | 545.21 | 148.08 | 15.60  | 3140.36 | 3450.22 | 281.77 | 40.42  |
|              | <i>n</i>       | 10      | 10      | 10      | 10     | 10     | 10      | 10     | 10     | 10     | 10      | 10      | 10     | 10     |
|              | <b>mean</b>    | 136.97  | 384.04  | 223.20  | 2.33   | 1.32   | 289.82  | 54.52  | 14.81  | 1.56   | 314.04  | 345.02  | 28.18  | 4.04   |
|              | <b>SD</b>      | 36.16   | 101.81  | 22.67   | 0.22   | 0.10   | 9.71    | 11.01  | 3.38   | 0.23   | 348.53  | 449.84  | 27.02  | 4.59   |
|              | <b>% of TS</b> | 100.0%  | 100.0%  | 100.0%  | 100.0% | 100.0% | 100.0%  | 100.0% | 100.0% | 100.0% | 100.0%  | 100.0%  | 100.0% | 100.0% |

|         |        |        |        |        |        |        |        |        |        |        |        |        |        |
|---------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| % of TN | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% |
|---------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|

ALT: Alanine transaminase; AST: Aspartate transaminase; ALP: Alkaline phosphatase; TBIL: Total bilirubin; sTG: Serum triglycerides; SOD: Superoxide dismutase; CAT: Catalase; GSH: Glutathione; MDA: Malondialdehyde; FRAP: Ferric-reducing antioxidant power; TEAC: Trolox equivalent antioxidant capacity; TPC: Total phenolic content; TFC: Total flavonoid content; Cit: Cluster; SD: Standard deviation; TN: Total number; TS: Total sum; AS: *Acanthopanax senticosus*; AV: *Amomum villosum*; AK: *Amomum kravanh*; AC: *Artemisia capillaris*; CH: *Cimicifuga heracleifolia*; CC: *Coptis chinensis*; GU: *Glycyrrhiza uralensis*; PF: *Pueraria lobata* flower; PR: *Pueraria lobata* root; SO: *Sanguisorba officinalis*.

between *in vitro* antioxidant activity, effects on liver injury, and *in vivo* antioxidant activity revealed that *P. lobata* root, *C. chinensis*, and *S. officinalis* performed better hepatoprotection with *in vivo* antioxidant activity; meanwhile, they also showed stronger *in vitro* antioxidant activities containing higher TPC and TFC.

As *S. officinalis* showed the best performance in protecting the liver from CCl<sub>4</sub>-induced injury among the tested herbs, the identification and quantification of phytochemical compounds in its decoction was conducted. Nine main compounds were identified, and (+)-catechin and gallic acid were also quantified. According to the Chinese Pharmacopeia, the gallic acid content was a crucial criterion to evaluate the quality of *S. officinalis*, and it should be no less than 0.60%<sup>[46]</sup>. The gallic acid content quantified in our study was in this desirable range. The phytochemical compounds identified in our study, such as gallic acid and catechin, have been regarded as strong antioxidants that can confer health benefits and may contribute to the hepatoprotective activity<sup>[47-49]</sup>.

*S. officinalis* has a cucumber-like flavor, and many parts are edible. For example, the young leaves and flower buds are often used in salads, the fresh or dried leaves are used to make tea, and the root can be cooked as a constituent in porridge or soups. It is an alpine plant that grows at high elevation and is adapted to harsh conditions, such as low temperatures, strong ultraviolet radiation, and dryness, which result in the altitude-dependent accumulation of antioxidants<sup>[50]</sup>. The phytochemical compounds identified in our study, such as gallic acid and catechin, have been regarded as strong antioxidants that can provide health benefits and may contribute to the hepatoprotective actions and *in vitro* antioxidant activities<sup>[47-49]</sup>.

## CONCLUSION

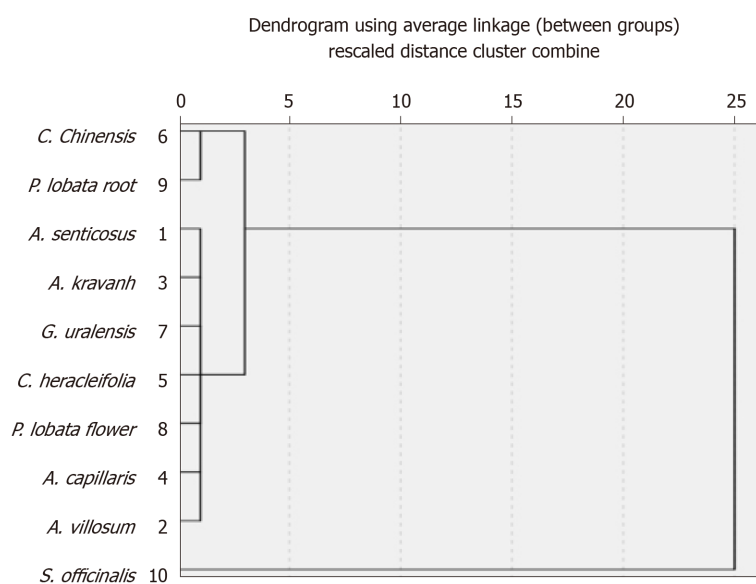
In conclusion, the results of our study indicated that all ten medicinal foods and herbs could individually protect against CCl<sub>4</sub>-induced oxidative liver injury, and *S. officinalis* L., *C. chinensis* Franch., and *P. lobata* (Willd.) Ohwi root were more effective than the others. In addition, *S. officinalis* L. exhibited the strongest hepatoprotective effect, and nine components of its decoction were identified, among which gallic acid and (+)-catechin were quantified. It was also revealed that the tested materials exhibited various *in vitro* antioxidant activities, proportionate to their phenolic content and that the highest values were found in *S. officinalis* L. The results of this study are valuable



**Table 4** Identification of the main phytochemical components in *Sanguisorba officinalis* decoction using high-performance liquid chromatography-electrospray ionization source-ion trap tandem mass spectrometry (positive or negative mode)

| Peak | RT (min) | $\lambda_{\max}$ (nm) | [M+H] <sup>+</sup> | [M-H] <sup>-</sup> | MS/MS fragments                                  | MW       | Putative compound                                  | Ref. |
|------|----------|-----------------------|--------------------|--------------------|--|----------|--|------|
| 1    | 7.10     | 270                   | /                  | 169                | 125  | 170      | Gallic acid <sup>1</sup>                           | [33] |
| 2    | 10.52    | 275                   | /                  | 345; 865           | 313, 169, 151, 125; 739, 695, 577, 543, 407, 287 | 346; 866 | Galloyl-methylglucoside; Procyanidin C2            | [33] |
| 3    | 16.10    | 278                   | 291                | /                  | 273, 165, 151, 139, 123                          | 290      | (+)-Catechin <sup>1</sup>                          | [33] |
| 4    | 20.87    | 275                   | /                  | 729                | 577, 559, 407                                    | 730      | Procyanidin B3 3-O-gallate                         | [33] |
| 5    | 22.35    | 274                   | 563                | /                  | 545, 423, 411, 435, 393, 271                     | 562      | Fisetinidol-(4 $\alpha$ / $\beta$ -8)-(+)-catechin | [33] |
| 6    | 23.65    | 272                   | /                  | 1103               | 1059, 935, 633, 469                              | 1104     | Sanguin H-2  | [31] |
| 7    | 54.30    | 264/362               | /                  | 277                | 197, 182, 111                                    | 278      | Methoxygallic acid methyl ester 5-O-sulfate        | [32] |
| 8    | 68.30    | 243/372               | 345                | /                  | 330, 313   | 344      | 3,3',4'-O-trimethylelagic acid                     | [33] |

<sup>1</sup>Identification of phytochemical compounds by high-performance liquid chromatography, mass spectrometry (MS), and MS/MS using commercial standards or based on published values [31-33]. RT: Retention time;  $\lambda_{\max}$ : Maximum absorbance wavelength; MS: Mass spectrometry; MW: Molecular weight.

**Figure 4** Dendrogram using average linkage (between groups) from systematic cluster analysis of ten medicinal herbs.

for the selection of more effective natural products to be consumed directly or developed into nutraceuticals or therapeutics for the prevention and treatment of oxidative stress-related diseases.

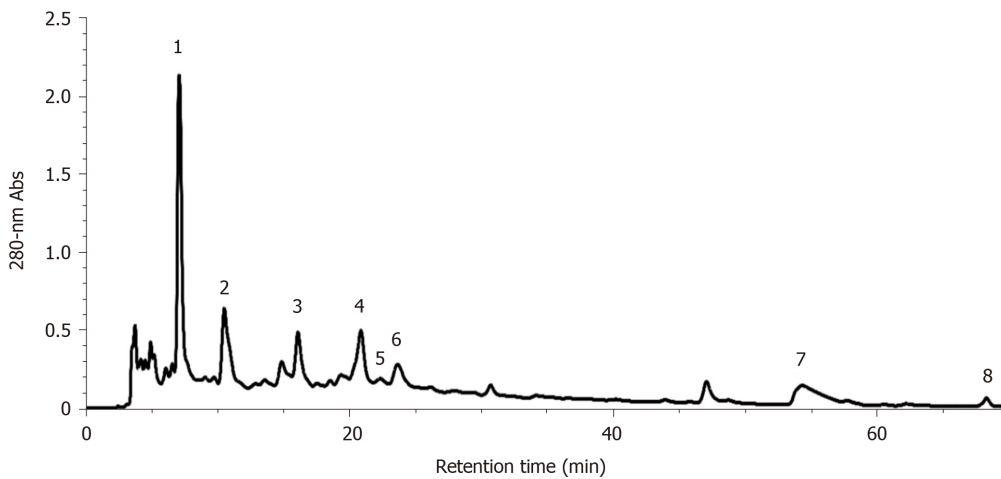


Figure 5 Reverse-phase high-performance liquid chromatography analysis of *Sanguisorba officinalis* decoction.

## ARTICLE HIGHLIGHTS

### Research background

Many natural products confer health benefits against diverse diseases through their antioxidant activities. Carbon tetrachloride ( $\text{CCl}_4$ ) is often used in animal experiments to study the effects of substances on liver injury and the related mechanisms of action, among which oxidative stress is a major pathogenic factor.

### Research motivation

The antioxidant activities of ten herbs were evaluated both *in vitro* and *in vivo*. Their hepatoprotective effects were also evaluated in order to elect more effective natural products for direct consumption and the development of nutraceuticals or therapeutics to manage oxidative stress-related diseases.

### Research objectives

To compare antioxidant and hepatoprotective activities of ten herbs and identify and quantify phytochemicals for the one with strongest hepatoprotection, which could be helpful in the prevention and treatment of oxidative liver injury.

### Research methods

The antioxidant activity of ten medicinal herbs was determined by both ferric-reducing antioxidant power and Trolox equivalent antioxidant capacity assays. The total phenolic and flavonoid contents were determined by Folin-Ciocalteu method and aluminum chloride colorimetry, respectively. Their effects on  $\text{CCl}_4$ -induced oxidative liver injury were evaluated and compared in a mouse model by administrating each water extract [(0.15 g/mL, 10 mL/kg) once per day] for seven consecutive days and a dose of  $\text{CCl}_4$  solution in olive oil (8%, v/v, 10 mL/kg). The herb with the strongest hepatoprotective performance was analyzed for the detailed bioactive components by using high-performance liquid chromatography-electrospray ionization source-ion trap tandem mass spectrometry.

### Research results

The results revealed that all tested herbs attenuated  $\text{CCl}_4$ -induced oxidative liver injury; each resulted in significant decreases in levels of serum alanine transaminase, aspartate transaminase, alkaline phosphatase, and triacylglycerols. In addition, most herbs restored hepatic superoxide dismutase and catalase activities, glutathione levels, and reduced malondialdehyde levels. *Sanguisorba officinalis* (*S. officinalis*) L., *Coptis chinensis* Franch., and *Pueraria lobata* (Willd.) Ohwi root were the three most effective herbs, and *S. officinalis* L. exhibited the strongest hepatoprotective effect. Nine active components were identified in *S. officinalis*, and gallic acid and (+)-catechin were quantified ( $7.86 \pm 0.45$  mg/g and  $8.19 \pm 0.57$  mg/g dried weight, respectively). Furthermore, the tested herbs displayed a range of *in vitro* antioxidant activities proportional to their phenolic content; the strongest activities were also found for *S. officinalis* L.

### Research conclusions

The results of this study indicated that all ten medicinal foods and herbs could individually protect against CCl<sub>4</sub>-induced oxidative liver injury, and *S. officinalis* L., *C. chinensis* Franch., and *P. lobata* (Willd.) Ohwi root were more effective than the others. In addition, *S. officinalis* L. exhibited the strongest hepatoprotective effect, and nine components of its decoction were identified, among which gallic acid and (+)-catechin were quantified. It was also revealed that the tested materials exhibited various *in vitro* antioxidant activities proportionate to their phenolic content, and that the highest values were found in *S. officinalis* L. The results are valuable for the selection of more effective natural products to be consumed directly or developed into nutraceuticals or therapeutics for the prevention and treatment of oxidative stress-related diseases.

### Research perspectives

This study is of value to assist the selection of more effective natural products for direct consumption and the development of nutraceuticals or therapeutics to manage oxidative stress-related diseases. In the future, methods to identify and quantify the phytochemical compounds in medicinal herbs must be investigated more comprehensively. The bioavailability, desirable dose range, and side effects should also be clarified.

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