Evaluation of Antioxidant Ability for Four Traditional Chinese Herbs with Detoxification Function

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Abstract

Because of "natural herb plant extract" being attention in cosmetics in recent year. In this study, we prepared water extracts and ethanol extracts from 4 traditional Chinese herbs(TCHs)- Artemisia capillaris (Ac), Lygodium japonicum (Lj), Pyrrosia lingua (Pl), Alisma plantago-watertica (Apa) that all of them with the detoxification function mentioned from literatures. These extracts were subsequently evaluated for their antioxidation effects by radical scavenging activity and the reducing power assay and the total phenol and total flavonoid contents measurement. Results showed whether water or ethanol extracts of Artemisia capillaris (Ac) that the reducing ability of them were about the same as BHA and the DPPH radical scavenging activity of water extracts was higher than that of Vitamin C. The water extract of Pyrrosia lingua (Pl) showed the strong antioxidant ability that is due to the higher total flavonoid content than other water extracts. Our conclusions are that Artemisia capillaris and Pyrrosia lingua had the dual effects of antibacterial and antioxidant and they had great potential to be chosen as the creative raw materials in skin care products preparation.

Key words: Antioxidant Ability, Traditional Chinese Herbs, Detoxification Function

Introduction

Some synthetic antioxidants, such as BHT and BHA, were often seen in cosmetic formulations of the skin care products. They were confirmed to be toxic or carcinogenic in animal models. Therefore, it is important to identify new sources of safe and inexpensive antioxidants of natural origin. In recent years, Chinese herbs have already been reported as having antioxidant effects. The objectives of this study are to identify that four traditional Chinese herbs (Artemisia capillaris, Lygodium japonicum, Pyrrosia lingua, Alisma plantago-watertica) that have the detoxification function whether also have the antioxidant ability.

Materials and Methods

Materials

The materials used in this study include Chinese herbal medicines, chemicals and instruments etc. which are described as follows:

Chinese herbal medicine: Artemisia capillaris (Ac), Lygodium japonicum (Lj), Pyrrosia lingua (Pl), Alisma plantago-watertica (Apa).

Chemicals: 95% Ethanol, DPPH (2,2-diphenyl-1-picrylhydrazyl), BHA (Butylated hydroxyanisole), PBS (Phosphate Buffer Saline), Potassium hexacyanoferrate(III), Iron(III) chloride hexahydrate, TCA (trichloroacetic acid), Folin-Ciocalteus phenol reagent, Sodium carbonate, Gallic acid, Sodium nitrite, aluminum chloride, Sodium hydroxide, Quercetin, etc. are purchased from Sigma–Aldrich (Jingming Chemical).

Instruments: Electronic balance scale coarse scale:(Sartorius/SA07-15US12R, fine scale: SHIMADZU /SA-121A2F-1), Electromagnetic heating stirrer (Thermo/SP88857100), pH meter (model: EUTECH pH-510), Pulverizer (Model: RT-04), Rotary Decompression Concentrator (Brand Yamato, Model: RE 200), Manifold Type Freeze Dryer (Brand: UNISS, Freeze-Drying Host Model: FDM-5-50℃ , Vacuum Helper Pu model: VP-200), Spectrophotometer (Perkin Elmer® precisely/Lambda 25).

Methods

This Research projects were completed following the flow chart (Figure 1).

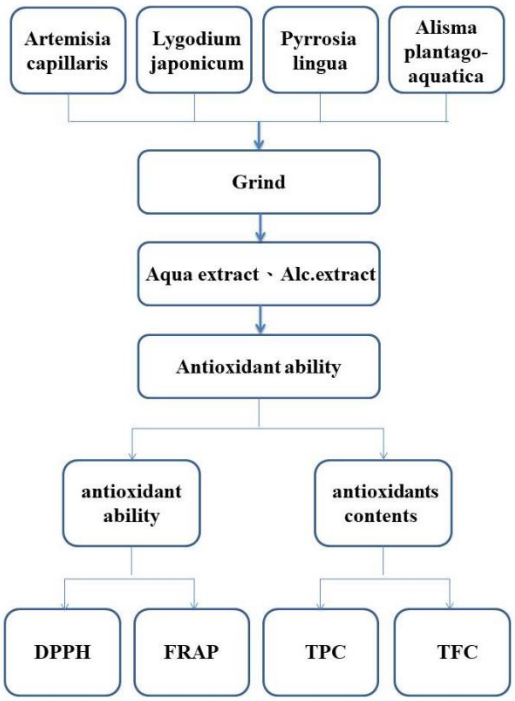

Figure 1 Flow chart

Methods

1. Sample preparation

Four dry traditional Chinese herbs (TCHs) were extracted with water by (microwave extraction) and 95% ethanol (steeped for 3 days at room temperature). Then, the residues were further extracted separately twice with water (volume ratio is 1:1.2 each time) and 95% ethanol (steeped for 1 days at room temperature) and the supernatants were combined. The water extracts were treated through suction filtration, concentration under reduced pressure, and freeze-drying to obtain powdered crude extracts, which were stored in a drying box at 4˚ C before use. These crude extracts were directly used in the further antioxidant assay.

2. Determination of pH

pH meter was used to measure and record the pH value of 4 TCHs extracts.

3. DPPH free radical scavenging capacity assay

In this assay, the purple radical 2,2-diphenyl1-picrylhydrazyl (DPPH•) is reduced by antioxidant to the corresponding pale yellow hydrazine. The procedure involves measurement of decrease in absorbance of DPPH at its absorption maxima of 517 nm wavelength, which is proportional to concentration of free radical scavenger added to DPPH reagent solution. BHA (Butylated hydroxyanisole) and Vitamin C were used as the standard substance.

Capability to capture DPPH radical (%)=[1-($A_{517nm.sample}$ / $A_{517nm.blank}$)]×100

4. Ferric reducing antioxidant power (FRAP) assay

The FRAP assay is simple, inexpensive, and may offer a putative index of antioxidant capacity. In this assay, BHA was used as the standard to make a calibration line, and the ethanol substitute sample was used as the blank control group.

Weigh 0.025g crude extract to quantify to 25mL to prepare sample solution. Mix 1mL pH6.6 PBS、1mL of 1% red blood salt and 1mL of sample well, and heat to 50℃ for 30 minutes in a water bath. After cooling, add 1 mL of 10% TCA into above solution then centrifuge at 3000 RPM for 10 minutes. Take 2 mL of the supernatant clear liquid, add 2 mL of deionized water and 0.4 mL of 0.1% ferric chloride, and mix for 10 minutes. The maximum absorption wavelength is 700.0 nm, and is determined by the formula Calculate its reducing power. The higher the absorbance, the stronger the reducing power of the sample.

5. Determination of Total phenol content (TPC)

For the determination of total phenolic content, gallic acid was used as the standard to make calibration curve.

Weigh 25mg of extract to 25mL, and take 0.5mL sample, add 2.5mL 10% phenol reagent (Folin-Ciocalteus phenol reagent) and 2.0mL 10% sodium carbonate solution and mix well. After 30 minutes of coloring, the maximum absorption wavelength was determined by water extraction at 750 nm.

6. Determination of Total flavonoid content (TFC)

The total flavonoid content was measured using Quercetin as a standard to prepare a calibration curve. Weigh 50 mg of the extract and quantify it to 100 mL. Take 0.5mL sample, add 2mL deionized water and 0.15mL 5% Sodium Nitrite and let it stand for 5 minutes, add 0.15mL 10% aluminum chloride, mix and let stand for 5 minutes, then add 1.2mL Deionized water and 1 mL of 1M sodium hydroxide were mixed for 10 minutes, and the color was determined by water extraction at the maximum absorption wavelength of 355 nm, and the measurement was completed within 15 minutes.

Results

The extraction ratio, pH and antioxidative power were measured and evaluated for Artemisia capillaris, Lygodium japonicum, Pyrrosia lingua, Alisma plantago-watertica. These results were shown as following:

1. Extraction ratio

The extraction ratio of water and alcohol were shown (as Table 1.) that water extraction was higher than alcohol extraction.

Table 1 The extraction ratio of 4 TCHs extracts

Extraction method	Ac	Lj	Pl	Apa
Water extract	22.90	4.97	10.02	10.34
Ethanol extract	8.41	24.11	2.29	4.36

2. pH value

The pH values of Ac, Lj, Pl, Apa water extracts and alcohol extracts are measured, and the results are shown in Table 2.

The pH of the ethanol extract and water extract of the 4 TCHs fall within the range of 5.56-6.53.

Table 2 The pH value of 4TCHs extracts

Extraction method	Ac	Lj	Pl	Apa
Water extract	5.65	6.53	6.26	6.45
Ethanol extract	5.74	5.80	5.56	5.58

3. DPPH free radical scavenging efficacy assay

Use spectrophotometer to measure the change of absorbance value after DPPH radical reacts with the sample to observe the antioxidant capacity of the sample.

The R^2 value of calibration curve for the determination of free radical scavenging was 0.9995.

From Table 3 can be observed that the scavenging free radical activity of Artemisia capillaris was the strongest and were same as BHA.

Table 3 DPPH free radical scavenging ability of 4 TCHs extracts (%)

Extraction method	BHA	Vit C	Ac	Lj	Pl	Apa
Water extract		70.8	74.6	68.3	71.2	66.3
Ethanol extract	94.5		94.5	62.5	90.6	62.1

4. FRAP reducing power assay

First, a calibration curve was made, and its R^2 was 0.9995. The results of the reducing power of the samples obtained by the redox reaction between iron ions and antioxidants are shown in Table 4. The antioxidant activity of Artemisia capillaris in both extracts were the strongest.

Table 4 Determination of reducing power of 4 TCHs extracts (BHA mg/g)

Extraction method	BHA	Vit C	Ac	Lj	Pl	Apa
Water extract		128.1	106.4	27.4	101.0	12.9
Ethanol extract	128.9		108.8	9.1	45.0	15.3

5. Determination of total phenolic content

The Folin-Ciocalteau reagent will react with the reducing hydroxyl group on the polyphenolic compound to form a blue substance to detect the content of the polyphenolic compound in the sample.

The R^2 value of calibration curve for the determination of total phenolic content was 0.9995. The results presented in Table 5 can be seen that the total phenol content of the 4 kinds of extracts were not enough in either water or alcohol extract.

Table 5 Total phenolic content (GAE mg/g) of 4 TCMs extracts

Extraction method	Ac	Lj	Pl	Apa
Water extract	40.7	9.5	43.9	6.5
Ethanol extract	36.6	1.4	16.5	6.9

6. Determination of total flavonoids content

The total flavonoid content in the extract was converted from the Quercetin standard curve, and the unit was Quercetin Equivalent (QE) mg/g.

The R^2 value of calibration curve for the determination of total flavonoids content was 0.9999. The results are shown in Table 6, ethanol extract of Alisma plantago-watertica contained highest dosage of total flavonoid contents.

Table 6 Total flavonoid content (QE mg/g) of 4 TCHs extracts

Extraction method	Ac	Lj	Pl	Apa
Water extract	77.8	55.4	95.1	18.1
Ethanol extract	131.8	99.1	74.8	137.4

Discussion

1. The *Artemisia capillaris* alcoholic extract was the strongest antioxidant, but its extraction ratio was only 1/3 that of water extract, which is one of worth considering factors in the future application.
2. The ethanol residues may induce the irritation or allergy in the cosmetics preparation, the water extracts will be major certain to be used as the raw materials although the effect of alcohol extracts were more strong than water extracts.

Conclusion

In the four extracts, the extract of Artemisia capillaris had quite high extraction ratio, and its antioxidant power was the strongest, followed by Pyrrosia lingua. Artemisia capillaris and Pyrrosia lingua had the dual effects of antibacterial and antioxidant, they are worth chosen as raw materials of skin care cosmetics preparation.

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Conflict of Interest Statement

NONE.

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