Original Research Article

Comparative study on DPPH free radical scavenging activity of 25 kinds of Traditional Chinese Medicines

Abstract

Aims: To determine and compare the antioxidant activity of water and ethanol extract of 25 kinds of Traditional Chinese Medicines.

Results: The ethanol extract of 4 kinds of medicinal herbs had the strongest scavenging activity. They were *Magnolia officinalis*, *Rheum officinale*, *Psoralea corylifolia* and *Radix Bupleuri*. In addtion, *Rheum laciniatum*, *Chrysanthemum morifolium*, *Magnolia officinalis* and *Salvia miltiorrhiza* had the strongest scavenging activity of their water extract. On the basis of the above comparison, we evaluated the EC_{50} and total phenolic content of their ethanol extract. They were *Magnolia officinalis* (2.75mg·mL^{-1} and 4.80μg·L^{-1}), *Rheum officinale* (11.82mg·mL^{-1} and 1.19μg·L^{-1}), *Psoralea corylifolia* (25.22mg·mL^{-1} and 1.07μg·L^{-1}) and *Radix Bupleuri* (42.67mg·mL^{-1} and 0.75μg·L^{-1}).

Conclusion: The results showed the correlation between the antioxidant activity of DPPH and the total phenol content. Furthermore, the reaction time of the DPPH test affected the free radical scavenging, which reflected the difference of the extract component would impact the test method.

Keywords: Antioxidant activity; DPPH; EC_{50}; Total phenol content

1 Introduction

Free radicals are atoms or groups with unpaired electrons produced by the splitting of simple substances or compounds, which are closely related to the occurrence of various diseases [1]. Effectively inhibiting the production of free radicals can also effectively prevent, delay or even cure diseases such as cancer [2], emphysema [3] and retinal vein occlusion [4]. Therefore, the antioxidant active substances which are capable of suppressing the generation of free radicals, particularly the natural-derived compounds, have become a hot topic in the scientific field in recent years. Among them, the antioxidants from natural traditional chinese medicinal materials have drawn more and more attention. A large number of cosmetics add plant antioxidants to confront the skin aging caused by free radicals [5]. Many natural foods or drugs such as seabuckthorn [6], blackcurrant [7], *etc*., have been used as dietary supplements for health and disease prevention for a long time.

In order to promote the development of traditional chinese herbal using in the filed of natural antioxidant health food and medicine. DPPH free radical scavenging method was used to evaluate and compare the antioxidant activities of 25 chinese medicine herbs, while the EC_{50} and polyphenol content of strongest herbs were determined. These results provided a theoretical basis for their further research as a natural antioxidant.

2 Material and methods

2.1 Material and Chemicals

25 kinds of chinese medicine materials were purchased from Tongjunge Pharmacy, Mianyang, Sichuan in March 2018. The production place of these samples were as follows: *Magnolia officinalis* (Santai County), *Rheum officinale* (Santai County), *Psoralea corylifolia* (Jintang county), *Coptis chinensis* (Hongya County), *Radix Bupleuri* (Jiange County), *Ligusticum* (Chengdu City), *Cortex Phellodendri Chinensis* (Hongya County), *Dolomiaea berardioidea* (Aba Tibetan Autonomous Prefecture), *Chrysanthemum morifolium* (Cangxi County), *Acorus tatarinowi*i (Santai County), *Aconitum carmichaelii* (Jiangyou City), *Eriobotrya japonica* (Santai County), *Platycodon grandiflorum* (Zitong County), *Salvia miltiorrhiza* (Pingwu County), *Pericarpium Citri Reticulatae* (Jiangyou City), *Flos magnoliae* (Beicuan County), *Aconitum carmichaeli Debx* (Jiangyou City), *Eucommia ulmoides* (Santai County), *Radix Aconiti* (Jiangyou City), *Dioscorea opposita* (Jiangyou City), *Tamarindus indica* (Jiangyou City), *Codonopsis pilosula* (Jiuzhaigou County), *Gastrodia elata* (Pingwu County), *Fritillaria cirrhosa* (Aba Tibetan Autonomous Prefecture), *Lilium*

brownii (Jiangyou City). All materials were dried at 60 □ to constant weight, smashed through a 50 mesh sieve, stored at 4 □ and protected from light. The whole experiment was completed within 1 month.

DPPH (1,1-diphenyl-2-bitracinium free radical) was purchased from TCI (Shanghai) Chemical Industry Development Co., Ltd. Folin-Ciocalteu was purchased from Nanjing Oddfoni Biological Technology Co., Ltd.. BHA (Butyl hydroxyanisole) was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. Anhydrous sodium carbonate and other reagents were purchased from Chengdu Kelon Chemical Reagent Factory. All chemicals used were of analytical grade. Pure water was self-made (electrical resistivity was 18 MΩ•cm).

Comment [N1]: Pls 2,2-diphenyl-1-picrylhydrazyl

2.2 Sample Preparation

500mg of the dry sample was added into a 50 mL conical flask. After 10 mL absolute ethanol or 10mL distilled water were added, the solution weight was recorded. After being vortexied for 10min and sonicated in an ultrasonic bath for 5min or 30 min, the corresponding solvent was weighed and supplied according to the recorded weight. The sample preparation was accomplished by obtaining the supernatant after the solution filtration. Three sets of parallel samples were prepared for each sample.

2.3 DPPH radical scavenging activity

7.5mg DPPH was accurately weighed and dissolved in a 250mL volumetric flask with anhydrous ethanol. The solution was prepared into a concentration of 0.03mg·mL^{-1}, which was stored in dark for later use. The absorbance value of the solution at 517nm was around 0.8.

Antioxidant activity of extraction was measured using Vishya's method [8] with slight modification. 0.2 mL supernatant of various extracts were added into 4mL of DPPH solution separately. The mixture was vigorously shaken and incubated for 5min or 30min in the dark at room temperature. Then the supernatant were transferred to the cuvette and the absorbance of the sample was measured at a wavelength of 517nm. The decrease of the absorbance indicated the

radical-scavenging activity. The antioxidant capacity of the sample can be expressed by the scavenging rate (SR %) and calculated using the following formula:

$$SR\ \% = (1-(A_i - A_j)/A_0) \times 100\%$$

A_i: Absorbance of 0.2 mL test solution mixed with 4 mL DPPH solution;

A_j: Absorbance of 0.2 mL test solution mixed with 4 mL anhydrous ethanol solvent;

A_0: Absorbance of 0.2 mL of the solvent used in preparing the test solution after mixing with 4 mL of DPPH solution.

After preliminary screen of 25 kinds of traditional chinese medicines, EC_{50} (concentration of the extract for 50% scavenging rate of DPPH) of four strongest antioxidant activity samples were determined for better evaluation. L-Ascorbic acid was used as the reference compound.

2.4 Total Phenolic Content Determination

The Folin-Ciocalteu method was used to measure the total phenolic contents of these plants. This method relied on the transfer of electrons from phenolic compounds to the Folin-Ciocalteu reagent in alkaline media, the reaction product had maximum absorption at 760 nm and the absorbance value was linear with polyphenol content.

1.5mg gallic acid was dissolved in 10 mL absolute ethanol to prepare the stock solution. And sample solutions were made by dilutioning the stock solution to five different concentrations including 50, 75, 100, 125, and 150μg • mL^{-1}. 0.2 mL of sample solution were mixed with 0.5 mL Folin-Ciocalteau reagent and 4.0 mL of pure water in 10 mL volumetric flasks. Then, 200 μL of 20% sodium carbonate solution was added in and the final volume was 10.0 mL with distilled water. The absorbance of the reaction mixtures was measured at 760 nm after incubation for 30 minutes at room temperature. The total phenolic content was expressed as micrograms of gallic acid equivalent per milligram of crude extract (μg GAE/mg CE).

3 Results and Discussion

3.1 Comparison of DPPH antioxidant activity

In this study, the antioxidant ability of 25 chinese medicine herbals was compared and the extraction solution and reaction time were investigated. The SR% values were shown in Table 1 and Figure 1. It could be seen that when anhydrous ethanol was used as the extraction solvent, at the test time of 5 min and 30 min. *Magnolia officinalis* showed the highest clearance (94.21%, 94.90%), followed by *Rheum officinale* (90. 41%, 87. 37%) , *Psoralea corylifolia* (66. 69%, 65. 69%) and *Radix Bupleuri* (59. 51%, 52. 83%). When extracted by water, the four chinese herbal medicines with higher clearance rate were *Chrysanthemum morifolium* (96.98%, 93.80%), *Salvia miltiorrhiza* (90.27%, 90.42%), *Rheum officinale* (95.35%, 94.82%) and *Magnolia officinalis*(93.36%, 91.36%). These showed that using water as extraction solution had a slightly better effect on the extraction of antioxidant active substances than anhydrous ethanol. In addition, the test reaction time also had a great influence on some chinese herbal medicines.

Table 1 Results of the scavenging rate of 25 kinds of traditional chinese medicines

Plant	Absolute ethanol		Water	
	5min	30min	5min	30min
Magnolia officinalis	94.90%	94.21%	91.36%	93.36%
Rheum officinale	87.37%	90.41%	94.82%	95.35%
Psoralea corylifolia	65.69%	66.69%	11.93%	14.78%
Coptis chinensis	57.90%	30.28%	91.10%	40.54%
Radix Bupleuri	52.83%	59.51%	88.21%	87.51%
Ligusticum chuanxiong	52.11%	45.88%	93.82%	39.81%
Cortex Phellodendri Chinensis	51.07%	16.61%	52.36%	65.50%
Dolomiaea berardioidea	50.16%	15.56%	43.89%	48.97%
Chrysanthemum morifolium	47.47%	43.84%	93.80%	96.98%
Acorus tatarinowii	37.88%	15.41%	52.73%	10.35%
Aconitum carmichaelii	28.64%	6.36%	30.05%	15.02%

Eriobotrya japonica	15.46%	18.50%	88.66%	90.81%
Platycodon grandiflorum	12.84%	14.48%	17.83%	18.14%
Salvia miltiorrhiza	12.82%	17.30%	90.42%	90.27%
Pericarpium Citri Reticulatae	11.92%	14.03%	35.05%	46.18%
FlosMagnoliae	11.95%	13.51%	66.23%	74.88%
Aconitum carmichaeli Debx	10.66%	11.54%	13.24%	11.36%
Eucommia ulmoides	9.17%	12.34%	62.82%	67.75%
Radix Aconiti	6.95%	5.39%	75.20%	16.25%
Dioscorea opposita	6.12%	6.21%	1.89%	1.27%
Tamarindus indica	5.93%	5.84%	13.77%	18.29%
Codonopsis pilosula	5.61%	6.87%	61.13%	67.83%
Gastrodia elata	4.31%	8.02%	3.61%	5.84%
Fritillaria cirrhosa	4.35%	5.19%	4.44%	20.28%
Lilium brownii	5.19%	5.99%	3.97%	5.65%

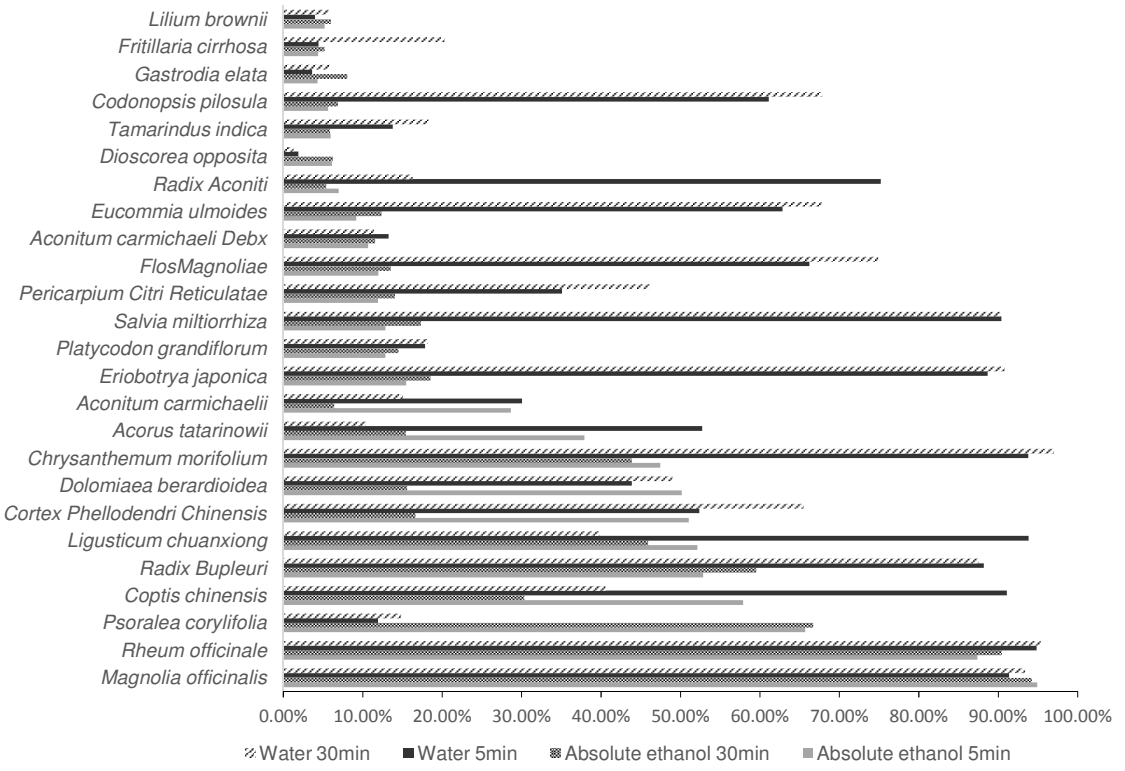

Figure 1 Results of antioxidant activity of 25 kinds of traditional chinese medicines

3.2 Determination of EC_{50}

The cost of subsequent development of water extract may be much higher than that of alcohol extract due to the former contained more impurities. Therefore, EC_{50} of ethanol extract from four kinds of plants(*Magnolia officinalis, Rheum officinale,Psoralea corylifolia and Radix Bupleuri*) with strong antioxidant activity were determined for further evaluation after screening. EC_{50} of ethanol extract of *Magnolia officinalis* was 2.75mg•mL^{-1}, which was close to the standard BHA (1.30mg•mL^{-1}), following was *Rheum officinale, Psoralea corylifolia* and *Radix Bupleuri*. On account of the similar EC_{50} to BHA, *Magnolia officinalis* was most suitable for development as a source of natural antioxidant active compounds.

3.3 Determination of total phenols content

Based on the results of EC_{50}, the content of polyphenols in above four chinese herbal medicines (*Magnolia officinalis*, *Rheum officinale*, *Psoralea corylifolia* and *Radix Bupleuri*) were determined by FoLin-CiocaLteu method which can evaluate flavonoid and non-flavonoid phenolic compounds. Table 2 showed that *Magnolia officinalis* had the highest content, followed by *Rheum officinale, Psoralea corylifolia* and *Radix Bupleuri*. This result had a significant correlation with the EC_{50} value which speculated that the presence of total phenol contributed to antioxidant activity.

Table 2 Determination of polyphenols content in 4 kinds of chinese medicinal materials

Type	Average	Average concentration of gallic acid(ug/mL)
Magnolia officinalis	1.33	4.80
Rheum officinale	0.24	1.19
Psoralea corylifolia	0.20	1.07
Radix Bupleur	0.10	0.75

4 Conclusion

The compounds of the extract can scavenge the stable DPPH free radicals and cause the difference in absorbance at 517nm. The results showed that 25 kinds of chinese herbal medicine extracts all had some antioxidant activities, but the differences were significant. In terms of extraction solvent, water had a slightly higher extraction effect than absolute ethanol. The reason may be that the antioxidant active substances in medicinal materials are mostly flavonoid glycosides or polyphenols with stronger water solubility, which are more easily soluble in water. In addition, different test time of antioxidant activity also resulted in different test results. Some samples showed better activity of 30 min than 5 min, probably due to some macromolecular antioxidants needed longer time to fully contact DPPH.. This phenomenon reminded us that we must pay attention to the test time in the preliminary of screen of antioxidant substances to avoid the occurrence of analysis omissions.

Li fuhua *et al.* [9] reported that phenolic compounds in the extracts of chinese herbal medicines were the main antioxidant components and their total content was proportional to the antioxidant activity. This was also observed and verified that the antioxidant activity and total phenolic content had a very strong correlation in this experiment.

In this paper, the antioxidant activities of 25 chinese herbal medicines were evaluated, and EC_{50} and polyphenol content were determined. The antioxidant activity of the ethonal extract from *magnolia officinalis* was strongest in all samples, followed by *Rheum officinale, Psoralea corylifolia* and *Radix Bupleuri*. These results provided a reference for the further study of these medicinal materials, especially *magnolia officinalis* can be explored as an antioxidant food.

REFERENCES

1. Phaniendra A, Jestadi DB, Periyasamy L. Free radicals: properties, sources, targets, and their implication in various diseases. Indian journal of clinical biochemistry. 2015; 30(1):11-26. DOI:10.1007/s12291-014-0446-0.

2. Poprac P, Jomova K, SImunkova M, Kollar V, Rhodes CJ,Valko M, *et al*. Targeting Free Radicals in Oxidative Stress-Related Human Diseases. Trends in pharmacological sciences. 2017;38(7):592-607. DOI:10.1016/j.tips.2017.04.005.

3. Thatcher TH, Hsiao H-M, Pinner E, Laudon M, Sime PJ, Phipps RP, *et al*. Neu-164 and Neu-107, two novel antioxidant and anti-myeloperoxidase compounds, inhibit acute cigarette smoke-induced lung inflammation. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2013; 305(2):L165-L174.

4. Masuda T, Shimazawa M, Hara H.Retinal Diseases Associated with Oxidative Stress and the Effects of a Free Radical Scavenger (Edaravone). Oxidative Medicine and Cellular Longevity.2017; 1-14.

5. Zeng MF. Application research of Several Natural Antioxidants in Cosmetics.Research on synthetic materials and aging. 2014;43(04):73-77.

6. Ding J, Guan Y, Pei CJ, Shan JY, Bao YH, Wu YQ. Optimization of Extraction Process of Seabuckthorn Fruit Oil by Orthogonal Test and Determination of Fatty Acid Composition. Food Science. 2016;37(02):13-18.

7. Jia LL, Lu JC. Progress in Medicinal Research of Blackcurrant. Chinese Journal of Information on Traditional Chinese Medicine. 2008;15(S1):110-113.

8. Wei XY. Research progress in evaluation of antioxidant activity by DPPH method. Food Science. 2014;35(09):317-322.

9. Li FH, Guo XH, Xia CY, Chen L, Ling B, Ming J. Research progress on antioxidant activity of phenolic compounds in whole grains. Food Science. 2012;33(13):299-304.