Original Research Article

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

Abstract

Aims: To determine and compare the antioxidant activity of water and ethanol extract of 25 kinds of traditional chinese medicinal plants.

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 EC_{50} and total phenolic content of their ethanol extract. The EC_{50} of *Magnolia officinalis*, *Rheum officinale*, *Psoralea corylifolia* and *Radix Bupleuri* were 2.75mg·mL^{-1}, 11.82mg·mL^{-1}, 25.22mg·mL^{-1}and 42.67mg·mL^{-1}. The total phenlic content of them were 4.80μg·L^{-1} , 1.19μg·L^{-1}, 1.07μg·L^{-1} and 0.75μg·L^{-1}, respectively.

Conclusion: The results showed the correlation between the antioxidant activity 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 effectively prevent, delay or even cure diseases such as cancer [2], emphysema [3] and retinal vein occlusion [4]. Therefore, the antioxidant 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. Some herbs, like *ganoderma lucidum, ginkgo biloba L, salvia miltiorrhiza* are considered as affluent sources of free radical scavenging molecules[5], such as vitamin E, vitamin C, phenolic compounds, carotene, phospholipids, terpenes, *etc*. In addtion, a large number of plant antioxidants are added into cosmetics to confront the skin aging caused by free radicals [6]. Many natural foods or drugs such as seabuckthorn [7], blackcurrant [8], *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 activity of 25 chinese medicinal plants, while the EC_{50} and phenol content of four kinds of herbs with strongest antioxidant activity 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 medicinal plants 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 tatarinowii* (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 (2,2-Diphenyl-1-picrylhydrazyl(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 were analytical grade. Pure water was self-made (electrical resistivity was 18 MΩ•cm).

2.2 Sample Preparation

500mg of the dry sample was mixed with 10 mL absolute ethanol or 10mL distilled water in a 50 mL conical flask and vortexied for 10min followed by an ultrasonic treatment for 5min or 30 min. Then, the obtained supernatant by filtration was conducted to analysis process.

2.3 DPPH Radical Scavenging Activity

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

Antioxidant activity of extract was measured using Vishya's method [9] 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 5 and 30min in the dark at room temperature. Then the supernatant were transferred to the cuvette and the absorbance was measured at 517nm. The decrease of the absorbance indicated the radical-scavenging activity. The antioxidant capacity of the sample could be expressed by the scavenging rate (SR(%)) and calculated using the following formula:

$$SR(\%)=\left(1-\frac{A_i-A_j}{A_0}\right)\times 100\%$$

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 screening of 25 kinds of traditional chinese medicinal plants, 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

The Folin-Ciocalteu method [10] 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 phenol content.

Sample solutions were made by diluting the stock solution (1.5mg gallic acid was dissolved in 10 mL absolute ethanol) to five different concentrations including 50, 75, 100, 125 and 150μg·mL^{-1}. 0.2 mL of sample solution was mixed with 0.5 mL Folin-Ciocalteau reagent and 4.0 mL of pure water in 10 mL volumetric flask. Then, 200 μL of 20% sodium carbonate solution was added in and the final volume was made 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.

3 Results and Discussion

3.1 Comparison of DPPH Antioxidant cctivity

In this study, the antioxidant ability of 25 chinese medicinal plants was compared and the extraction solution and reaction time were investigated. The SR values were shown in Table 1 and Figure 1. As anhydrous ethanol was used as the extraction solvent, *Magnolia officinalis* had the highest clearance (94.21% of 5min, 94.90% of 30min), followed by *Rheum officinale* (90.41%, 87.37%) , *Psoralea corylifolia* (66.69%, 65.69%) and *Radix Bupleuri* (59.51%, 52.83%). While extracted by water, the four plants with highest 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%), respectively. These results showed that using water as extraction solvent had a slightly better effect on the extraction of antioxidant substances than anhydrous ethanol. In addition, the test reaction time also had a great influence on some chinese herbal medicines.

Table 1 The scavenging rate of 25 kinds of traditional chinese medicinal plants

Plants	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%

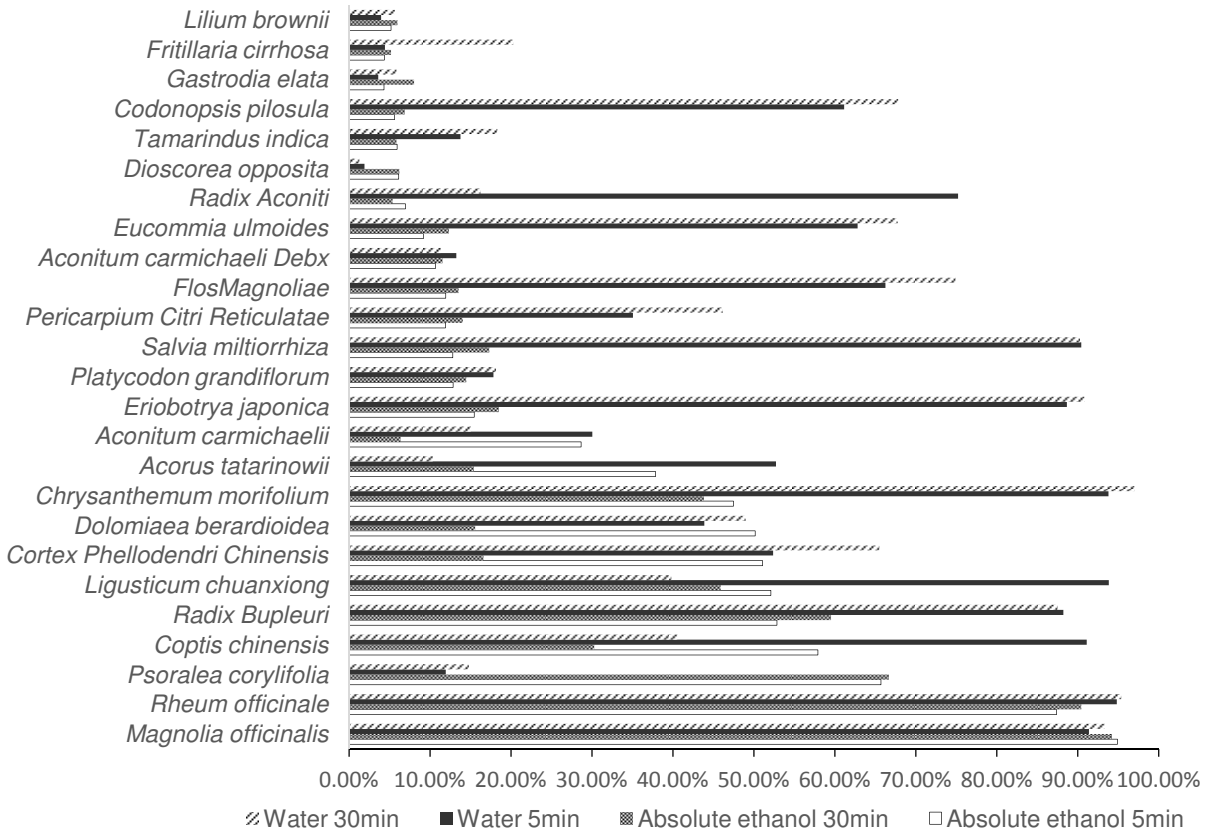

Figure 1 Antioxidant activity of 25 kinds of traditional chinese medicine plants

3.2 Determination of EC_{50}

The cost of subsequent development of water extract may be much higher than ethanol 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 strongest antioxidant activity were determined for further evaluation. The lower EC_{50} value measured, the higher antioxidant activity was. As shown in Table 2, *Magnolia officinalis* had the lowest value of 2.75 mg·mL^{-1}, followed by *Rheum officinale (*11.82mg·mL^{-1}), *Psoralea corylifolia* (25.22mg·mL^{-1}) and *Radix Bupleur (*42.67mg·mL^{-1}), respectively. This sort was consistent with the previous results of part 3.1.

What kind of components made these plants showed higher antioxidant activity? Generally speaking, typical compounds that exhibit antioxidation mainly included vitamins, flavones, polyphenolic and tannin. In Ding Haodong's report [11], *Magnolia officinalis* polyphenols had stronger antioxidant effects when used KM mice as test materials for *in vivo* antioxidant tests. And Yan Juan *et al.* [12] demonstrated that phenolic substances in *Rheum officinale* can

significantly scavenge superoxide radicals, DPPH free radicals and hydroxyl radicals *in vitro*. Besides phenolic compounds, cumarins and flavonoids were the major antioxidant in *Psoralea corylifolia*l [13] and *Radix Bupleur* [14]. These findings indicated that the species of antioxidant substances were diversity.

Furthermore, the EC_{50} of *Magnolia officinalis* (2.75mg·mL^{-1}) was slightly higher than that of BHA(1.30mg·mL^{-1}) which implied *Magnolia officinalis* was suitable as a source of natural antioxidant active compounds for development.

Table 2 The EC_{50} of ethanol extracts from 4 kinds of traditional chinese medicinal plants

Name	EC50
Magnolia officinalis	2.75mg·mL^{-1}
Rheum officinale	11.82mg·mL^{-1}
Psoralea corylifolia	25.22mg·mL^{-1}
Radix Bupleur	42.67mg·mL^{-1}
BHA (positive control)	1.30mg·mL^{-1}

3.3 Determination of total phenols content

According to Ren-You Gan's report [15] and our previous result, a positive linear correlation between antioxidant capacities and total phenolic contents indicated that phenolic compounds in plants could be the main components contributing to the observed activities. Hence, based on the results of EC_{50}, the content of polyphenol in above four chinese herbas (*Magnolia officinalis*, *Rheum officinale*, *Psoralea corylifolia* and *Radix Bupleuri*) were determined by Folin-Ciocalteau method which can evaluate flavonoid and non-flavonoid phenolic compounds. Table 3 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. It illustrated a viewpoint that the presence of total phenol mainly contributed to antioxidant activity which had been verified by Li fuhua *et al.* [16].

Table 3 The polyphenols content of 4 kinds of chinese medicinal materials

Type	Average concentration of gallic acid(ug/mL)

Magnolia officinalis	4.80
Rheum officinale	1.19
Psoralea corylifolia	1.07
Radix Bupleur	0.75

4 Conclusion

The antioxidant activities of 25 chinese medicine plants were evaluated, as well as the EC_{50} and polyphenol content of several well performed herbs. The result showed that the antioxidant activity of the ethanol extract from different plants were significant variance. M*agnolia officinalis* was the strongest in all samples, followed by *Rheum officinale, Psoralea corylifolia* and *Radix Bupleuri,* respectively. In terms of extraction solvent, water had a slightly higher extraction effect than absolute ethanol. The reason might be that the antioxidant active substances were mostly flavones or polyphenols with stronger water solubility, which were easily soluble in water. In addition, different test time of antioxidant activity also resulted in different results. Some samples showed better activity of 30 min than 5 min, probably due to some macromolecular antioxidants needed longer time to completely contact DPPH. This phenomenon reminded us pay enough attention to the test time to avoid the occurrence of analysis omissions. Above results provided a reference for the further study of these medicinal materials, especially *magnolia officinalis* can be explored as an antioxidant food.

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