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Study on the antioxidant activity of extracts from 18 medicinal plants

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Abstract
Using ABTS· clearance rate as an indicator, 18 medicinal plant extracts were screened. The antioxidant activity of medicinal plant extracts *in vitro* with significant ABTS· clearance rate was evaluated using ABTS· and DPPH· clearance ability, total reduction ability (FRAP), and other indicators. The extracts of *Houttuynia cordata* and *Eleutherococcus senticosus* have the best antioxidant effect, while the extracts of *Stellera chamaejasme*, *Melia azedarach* and *Glycyrrhiza uralensis* have better antioxidant capacity.

Keywords: Medicinal plant polysaccharide, Caenorhabditis elegans, antioxidant stress, anti-aging

Introduction
The generation and development of many diseases are closely related to free radicals in the human body. Currently, most commMon antioxidants are synthetic preparations, and animal experiments have shown that they have potential toxic and side effects on organisms. Therefore, in recent years, the search for safe and side effect free natural antioxidants has gradually become a research hotspot. China is a country with abundant medicinal plant resources, and most medicinal plants can serve as important antioxidants. They have low toxicity and high efficiency in clearing excess free radicals in the body, providing a source for new food additives and precursors of antioxidant drugs, and can also serve as antioxidants for cosmetics and other products.

Materials and Methods
1. Materials
1.1. Medicinal Plants
Coptis chinensis Franch, *Cortex fraxini*, *Gentiana cruciata*, *Pulsatilla chinensis*, *Atractylodes macrocephala*, *Glycyrrhiza uralensis*, *Thradiantha dubia*, *Houpuea officinalis* seed, *Stellera chamaejasme*, *Chaenomes speciosa*, *Floral leaf*, *Melia azedarach*, *Lycium* seed, *Paederia foetida*, *Urtica fissa*, *Houttuynia cordata*, *Eleuther Occus senticosus* and *Portulaca oleracea.*

1.2 Main reagents
ABTS, $K_2S_2O_8$, DPPH, $FeSO_4·7H_2O$, Vc, 70% ethanol, anhydrous ethanol, and purified water.

1.3 Main instruments
Ultraviolet spectrophotometer (Shanghai Yuanxi Instrument Co., Ltd., UV-8000S), Multiskan FC ELISA (Thermo Fisher Instruments Co., Ltd., 51119080), Ultrasonic cleaner (Shanghai Kedao Ultrasonic Instrument Co., Ltd., SK3210LHC), Rotating evaporator (Shanghai Yarong Biochemical Instrument Factory, RE-52AA), 96 hole plate.

2. Method
2.1. Extraction of medicinal plants
18 medicinal plants were extracted using Soxhlet extraction method, with a material to liquid ratio of 1:15 and 70% ethanol added. After heating and refluxing for 6 hours, the solvent was evaporated to obtain the extract.

2.2 Antioxidant preliminary screening
Refer to Kang [1] *et al*.'s method and make improvements. Preparation of ABTS working solution: Prepare ABTS stock solution (7.4 mmol/L) and $K_2S_2O_8$ stock solution (2.6 mmol/L) with distilled water. Mix 7.4 mmol/L ABTS with 2.6 mmoL/L $K_2S_2O_8$ in ratio of 1:1, let stand for 12-16 hours, and prepare ABTS working solution. Take an appropriate amount of ABTS working solution, dilute it with distilled water, and measure the absorbance at 734 nm at room temperature to be 0.7 ± 0.02.
Prepare 18 medicinal plant extracts into a sample solution of 2 mg/mL, and prepare a Vc solution of 0.01 mg/mL as a positive control. Add samples to each test tube separately, mix and shake well, and then let them stand in dark for 40 minutes. Measure the absorbance at 734 nm, and parallel each group three times. The calculation formula is as follows.

$$\text{ABTS·Clearance rate} = [1-\frac{A_1-A_2}{A_0}]\times 100\%$$

A_0: The absorbance of 4 mL of working solution and 200 μL distilled water;
A_1: The absorbance of 4 mL of working solution and 200 μL sample solution (or VC);
A_2: The absorbance of 4 mL of distilled water and 200 μL sample solution (or VC).

2.3 Antioxidant rescreening
Prepare a 1 mg/ml sample solution of medicinal plant extracts with an initial screening ABTS· clearance rate of over 80% for antioxidant activity. The antioxidant operation steps are the same as 1.2.2. Finally, select medicinal plant extracts with a clearance rate of over 80% for antioxidant activity testing at different concentrations.

2.4 ABTS· clearance rate of samples with different concentrations
Prepare the medicinal plant extract (ABTS· clearance rate above 80%) obtained through re screening into different concentration solutions of 1, 0.8, 0.6, 0.4, and 0.2 mg/ml,

with three parallel settings for each concentration. According to the antioxidant operation steps in 1.2.2, determine the ABTS free radical scavenging rate of different concentrations of medicinal plant extracts.

2.5 DPPH· clearance rate of samples with different concentrations

Refer to the methods of Zhao Rong [2] *et al.* and make improvements. Prepare DPPH solution with anhydrous ethanol (0.040 mg/mL) and different concentrations (1, 0.8, 0.6, 0.4, 0.2 mg/mL) were re screened to obtain medicinal plant extract sample solutions, and a 0.01 mg/mL Vc solution was prepared as a positive control and added to 96 well plates, respectively. After reacting in a dark place for 40 minutes, the absorbance was measured at 490 nm using an enzyme-linked immunosorbent assay. The formula for calculating DPPH· clearance rate is as follows:

$$\text{DPPH} \cdot \text{ Clearance rate}=[1-\frac{A_1-A_2}{A_0}]\times 100\%$$

A_0: The absorbance of 100 μL DPPH solution+100 μL anhydrous ethanol;

A_1: The absorbance of 100 μL DPPH solution+100 μL sample/Vc solution;

A_2: The absorbance of 100 μL anhydrous ethanol+100 μL sample/Vc solution.

2.6. Total antioxidant capacity of samples with different concentrations (refer to Wang Rong[3] *et al.*'s method and make improvements)

2.6.1. Drawing of $FeSO_4 \cdot 7H_2O$ Standard Curve

Prepare the $FeSO_4 \cdot 7H_2O$ standard into a mother solution with a concentration of 10 mg/mL and dilute to 9, 8, 7, 6, 5, 4, 3, 2, 1 mg/mL. Measure the absorbance at 593 nm using an enzyme-linked immunosorbent assay. Using the absorbance as the x-axis and the corresponding standard concentration as the y-axis, the $FeSO_4 \cdot 7H_2O$ standard curve is prepared.

1.2.6.2 Determination of total antioxidant capacity of samples

Add the medicinal plant extract sample solution obtained from different concentrations of rescreening to a 96 well plate and measure the absorbance of the sample at 593 nm. Calculate the sample concentration at the same absorbance value as the standard curve. The total antioxidant capacity of the sample is expressed by the concentration of the standard substance $FeSO_4 \cdot 7H_2O$ solution.

Results and Analysis

1. ABTS method

The antioxidant screening and re screening ABTS· clearance rates of 18 medicinal plant extracts are shown in Figure 1. With the ABTS· clearance rate of more than 80% as the standard, 13 extracts such as *Houttuynia cordata*, *Eleutherococcus senticosus* and *Stellera chamaejasme* were screened at the concentration of 2 mg/mL, and the clearance rate of 5 extracts such as *Coptis chinensis Franch*, *Thladiantha dubia* and *Atractylodes macrocephala* was not up to the standard. After diluting the solution prepared with the standard extract to 1 mg/mL, the ABTS· clearance rate of *Houttuynia cordata*, *Eleutherococcus senticosus*, *Melia azedarach*, *Stellera chamaejasme* and *Glycyrrhiza uralensis* extract was still more than 80%. The ABTS· clearance rate of medicinal plant extracts obtained from re screening at different concentrations is shown in Figure 2. The clearance rate of ABTS· is positively correlated with the sample concentration, with *Houttuynia cordata* extract exhibiting the best antioxidant activity.

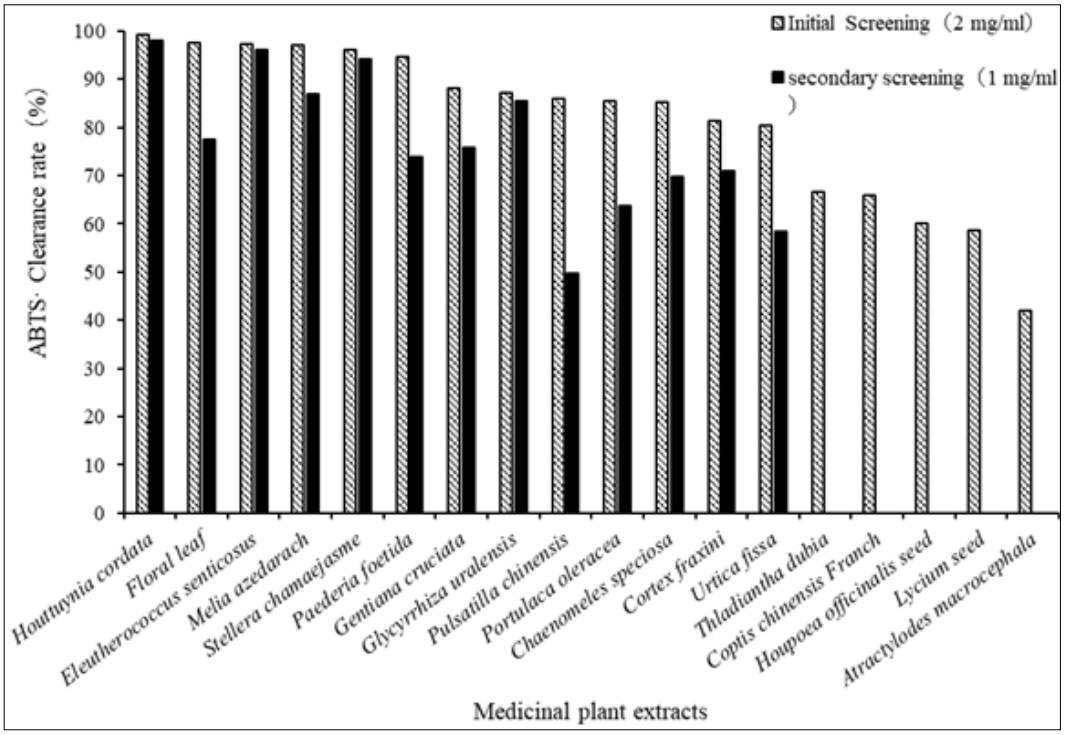

Fig 1: Antioxidant screening and rescreening of 18 medicinal plant extracts ABTS · clearance rate

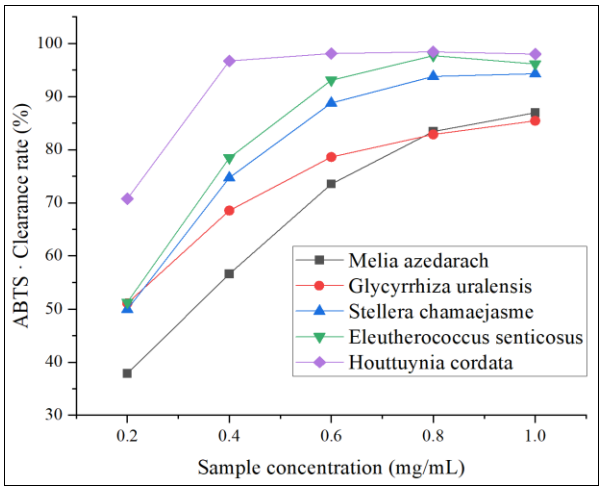

Fig 2: ABTS· Scavenging Activity of Samples with Different Concentrations

2. DPPH method

To explore the DPPH· scavenging capacity of medicinal plants with different concentrations, the results are shown in Figure 3. In general, the DPPH· scavenging capacity of all samples increases with the increase of concentration, and *Houttuynia cordata* has the best DPPH· scavenging capacity, but *Houttuynia cordata* and *Eleutherococcus senticosus* have no obvious growth trend in the range of 0.2~1.0 mg/mL, while *Melia azedarach* and *Stellera chamaejasme* rose gently in the range of 0.4~1.0 mg/mL, However, the scavenging rate of *Glycyrrhiza uralensis* on DPPH· is very low, far below 50%.

Comparing the results of Figure 2 and Figure 3, it was found that at the same concentration, the clearance rate of Melia azedarach extract on ABTS· was lower than that on DPPH·, while the clearance rate of Glycyrrhiza uralensis extract on DPPH· was much lower than that of ABTS·. DPPH· scavenging activity is not related to ABTS· scavenging activity, as the scavenging effect of antioxidants on DPPH· is due to their hydrogen donor ability, while the scavenging effect of antioxidants on ABTS· is the relative ability of antioxidants to scavenge free radicals [4]. Therefore, this study uses multiple free radical scavenging activity indicators to evaluate the antioxidant capacity of medicinal plants, which has certain reliability.

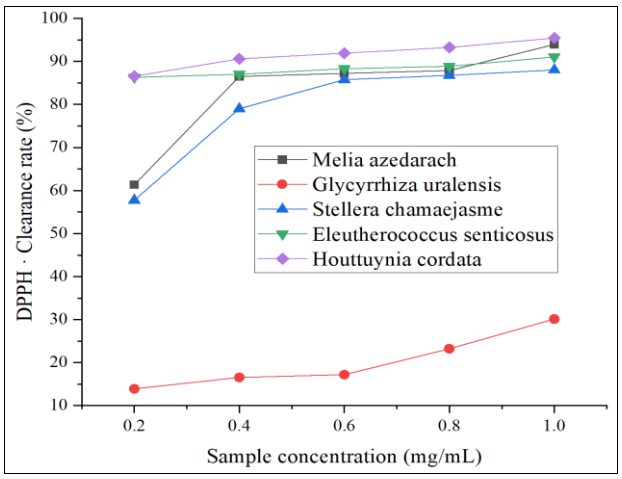

Fig 3: DPPH· Scavenging Activity of Samples with Different Concentrations

3. FRAP method

As shown in Figure 4-1, $FeSO_4 \cdot 7H_2O$ exhibits a good linear relationship between its concentration and absorbance in the range of 1-10 mg/mL, with an equation of y=0.00685x+0.04682 and a correlation coefficient of R^2 of 0.996. Calculate the total antioxidant capacity of the sample at different concentrations (expressed as the concentration of $FeSO_4 \cdot 7H_2O$) based on the standard curve. As shown in Figure 4-2, the total antioxidant capacity of each medicinal plant extract increases with the increase of sample concentration. The total antioxidant capacity of extracts from *Houttuynia cordata* and *Melia azedarach* is generally low, and the difference is small within the range of 0.2~0.8 mg/mL. However, the total antioxidant capacity of *Eleutherococcus senticosus*, *Stellera chamaejasme* and *Glycyrrhiza uralensis* extracts were significantly different in the range of 0.2~1.0 mg/mL.

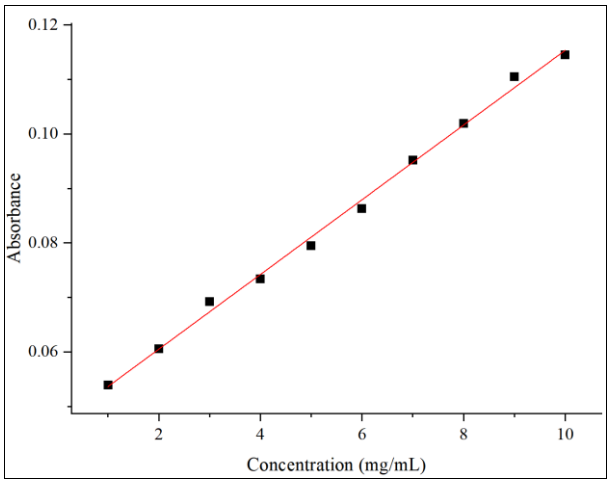

Fig 4-1: FeSO4·7H2O standard curve

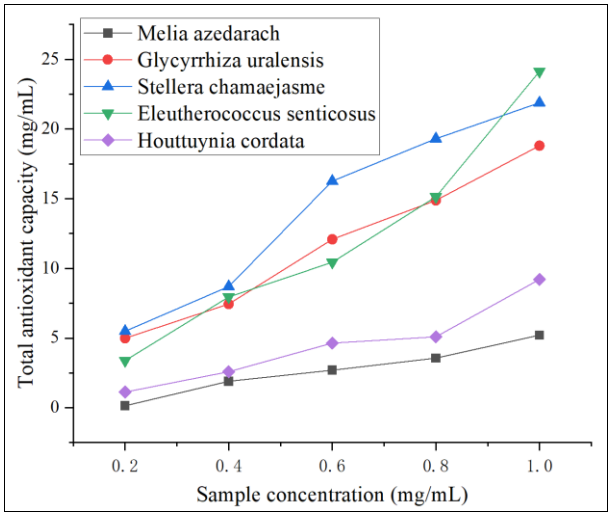

Fig 4-2: Total antioxidant capacity of samples with different concentrations

Discussion

The ABTS method has the advantages of being simple, fast, and does not require complex reaction steps or harsh reaction conditions. However, the oxidation reaction time between ABTS· and different antioxidants is different, so the test results obtained at different reaction times differ significantly [5]. The DPPH method is efficient and simple, with good repeatability of results, and is less affected by

environmental factors. But the color of the tested object itself will have a significant impact on the measurement results. And if there is an overlap between the UV absorption of the tested substance and DPPH, it will also affect the measurement results. The FRAP method is an indirect method for measuring antioxidant activity and needs to be used in conjunction with other antioxidant activity measurement methods. In addition, this method has the problem that different substances have different Redox times, so different reaction times will affect the results [6].

The results of the antioxidant preliminary screening experiment showed that the clearance rates of ABTS· were 60.13% and 42.07%, respectively, with a concentration of 2 mg/mL of *Houpoea officinalis* seed seed and Atractylodes macrocephala extract solution, and the clearance rates were relatively low. However, Tian Qiang's [7] study found that the ethanol extract of *Houpoea officinalis* seed has a strong ability to scavenge ABTS· . When the mass concentration is 0.01 mg/mL, the ethanol crude extract of *Houpoea officinalis* seed has a clearance rate of 89.40% ± 0.41% for ABTS· ; The aqueous extract of the crude methanol extract of *Atractylodes macrocephala* showed a clearance rate of over 50% for ABTS· at 0.08 mg/mL [8]. The experimental results of the DPPH method showed that the clearance rate of 1 mg/mL extract of *Glycyrrhiza uralensis* on DPPH· was very small, much lower than 50%. However, studies have shown that the mass concentration of 60% ethanol crude extract of *Glycyrrhiza uralensis* is 1 mg/mL and 0.8 mg/mL, and its clearance rates for DPPH· are 70.7% ± 1.6% and 63.2% ± 2.4%, respectively, showing good clearance ability for DPPH· [9]. The main reasons for the difference in the above experimental results are the degradation of antioxidant active ingredient in the extracts of *Houpoea officinalis* seed, *Atractylodes macrocephala* and *Glycyrrhiza uralensis*, and the difference in experimental measurement methods and experimental data processing.

Conclusion

Through antioxidant screening and activity determination of 18 kinds of medicinal plant extracts, it was finally concluded that *Houttuynia cordata* and *Eleutherococcus senticosus extracts* had the best antioxidant effect, and the clearance rate of ABTS· and DPPH· was the largest. The extracts of *Stellera chamaejasme*, *Melia azedarach*, and *Glycyrrhiza uralensis* have good antioxidant capacity. In the later stage, it can be purified, separated and identified as an antioxidant active ingredient. As an active ingredient of natural antioxidant, it is widely used in medicine, food, health products, beverages, cosmetics and other fields.

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