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Extraction and activity of chemical constituents from *Houttuynia cordata* Thunb by ultrasonic method

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ABSTRACT

Polyphenols and flavonoids are phytochemicals that have essential roles in human nutrition. In this regard, the contents of polyphenols and flavonoids in *Houttuynia cordata* Thunb and their antioxidant activities were evaluated in the current study. Two *Houttuynia cordata* materials with the same chromosome number and chemical type were used to comprehensively assess the contents of total phenols and flavonoids in different parts of *H. cordata*. These chemical components were extracted by the ultrasonic method. The results showed that the total phenols and antioxidant capacity of different parts of *H. cordata* were significantly different. The content of polyphenols in roots and stems was low, the antioxidant capacity was weak, the total phenols in flowers and leaves were high, and the antioxidant capacity was strong. Therefore, different parts of *H. cordata* had different pharmacological and food effects. The whole herb can be used as Chinese herbal medicine, and its young leaves and roots can be used as vegetables. Flavonoids are the main phenolic components, and total phenols are the main components of antioxidant activity. It can explain a very significant positive correlation between total phenols and flavonoids. Therefore, in the further breeding work of *H. cordata*, the procedure can be simplified by determining one of the above indexes to predict the varieties with high total phenolic and antioxidant activity.

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Introduction

Decyl acetaldehyde, the main antibacterial active component of *Houttuynia cordata* Thunb, has an obvious antibacterial effect on a variety of bacteria, acid-fast bacilli and fungi, but its properties are unstable (1-3). The synthetic sodium bisulfite adduct of decanoylacetaldehyde in China is called synthetic houttuynin and has an obvious antibacterial effect on a variety of gram-positive and negative bacteria (4). *Staphylococcus aureus* and its penicillin-resistant strains, pneumococci, Streptococcus A and influenza bacilli are more sensitive, followed by catarrhalis and typhoid bacillus, while *Escherichia coli*, *Pseudomonas aeruginosa* and dysentery bacilli are not very sensitive (5, 6). Houttuyfonate can significantly prolong the survival time of mice infected with Mycobacterium tuberculosis (7, 8). In addition, it also has an obvious inhibitory effect on Candida albicans, Cryptococcus neoformans, Sporothrix, Aspergillus, chromomyces, tinea rubrum, Clavularia, Microsporum gypseum, Microsporum ferrugosum, and shark tinea (9, 10). The synthetic sodium dodecyl acetaldehyde sodium bisulfite adduct is called neohouttuyfonate, which also has an inhibitory effect on pneumococci, typhoid bacillus, Staphylococcus aureus, Escherichia coli and Sporothrix in vitro. The synthesized compound has a strong inhibitory effect on Mycobacterium tuberculosis in vitro and in vivo (11). This paper is based on the ultrasonic method, which is a method to study the generation, reception and application of ultrasound. When the frequency of a sound wave is higher than the frequency limit of human hearing, people will not hear it. This kind of sound wave is called an "ultrasonic wave" (12-14). Therefore, the following content will focus on the extraction of chemical constituents from Houttuynia cordata Thunb and its activity (15).

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Materials and methods

Experimental materials

In this experiment, 16 populations of *Houttuynia cordata* with good comprehensive agronomic traits were selected, including 15 accessions of Michelia and Emei. Since the collection in 2015, it has been planted and preserved in, with the same cultivation conditions and conventional water and fertilizer management as resource materials. In October 2018, the roots were dug out, and each material was planted in three repeated plots with an area of 4 × 2.5m^2 and a seed amount of 3kg roots per plot. Collected in June 2019, dried naturally and crushed, as shown in Table 1.

Table1. Material sources and chromosome numbers

No.	Chromosome number	No.	Chromosome number
1	39	9	83
2	40	10	82
3	82	11	36
4	88	12	88
5	86	13	92
6	57	14	7
7	89	15	86
8	85	16	92

Reagents and instruments

The main experimental reagents of the current study were Sodium tungstate, sodium molybdate, lithium sulfate, phosphoric acid (H_3PO_4 ≥ 85%), sodium carbonate, hydrochloric acid and 30% (V / V) hydrogen peroxide was all analytically pure. Folin ciocalteu chromogenic agent was 20.0g sodium tungstate and 50g sodium molybdate which were put into a round bottom flask, dissolved in 140ml water, added 10ml and 20ml concentrated hydrochloric acid of 85% phosphoric acid solution, refluxed for 10h with slow fire, and then added 3G lithium sulfate and 15ml hydrogen peroxide after cooling, Heat boiling (open boiling) until bright yellow, no green, and cool. Then they were transferred to a 250ml volumetric flask, fixed volume with distilled water, put into a brown bottle, and stored at 4°C.

Also, Folin ciocalteu reagent, DPPH (1,1-diphenylpicophenylhydrazine), β-carotene, Trolox (water-soluble vitamin E), ABTS (2,2-diazo-bis -(3-ethylbenzodihydrothiazole-6-sulfonic acid), gallic acid and rutin were purchased from Sigma Aldrich company. Other reagents were analytically pure and purchased from Xilong chemical company to evaluate polyphenols and antioxidant capacity

The experimental instruments were Re-2000 rotary evaporator (Shanghai Yarong biochemical instrument factory, Shanghai, China), 7200 spectrophotometers, Unocal (Food Co., Ltd, Shanghai), Kq-1008 ultrasonic cleaner (Kunshan Ultrasonic Instrument Co., Ltd), and Bws-20 constant temperature water bath and water bath pot (Shanghai Yiheng Scientific Instrument Co., Ltd).

Sample preparation

In June 2019, the material of *H. cordata* was washed, dried and cut into a small section of about 0.2cm, weighed 5.0g, put into a 100ml triangular flask, add 50ml of ethanol (95%, V / V), methanol, acetone and water respectively, and ultrasonic extraction for 0.5, 1.0, 1.5, 2.0h in the ultrasonic cleaning instrument. Then, filtration, concentration and drying, and removals of organic solvents were done. Then they were transferred to a 100ml volumetric flask and fixed the volume with distilled water.

Extraction solvent and time selection

The 0.5ml of *H. cordata* extract was prepared by different solvents at a different time; 10ml of distilled water and 1ml of Folin ciocalteu chromogenic agent were added. After standing for 1min, 2ml of 20% sodium carbonate buffer solution was added, and the volume was fixed with distilled water to 50ml and reacted for 1.5h at room temperature (25°C). The absorbance at 760nm was determined. The extraction time with the maximum absorbance was selected as the optimum time, and the solvent was used as the optimal extraction solvent.

Standard curve

The 0.050g of gallic acid was added to 100ml of steam water to obtain 500pg/ml gallic acid solution. Then, 0.1, 0.2, 0.3, 0.4 and 0.5ml of the above solutions were added into a 50ml volumetric flask with 10ml of water, and then they were shaking well. 1.5 ml Folin ciocalteu chromogenic reagent and 2.0ml of 20% Na_2CO_3, were added to distilled water and placed in 55°C constant temperature water bath for 15h. After color development, the absorbance was

determined at 760nm wave length to obtain the standard curve.

Evaluation of polyphenols and antioxidant capacity

To prepare the Trolox standard solution, 0.0626g Trolox standard solution was added to 50ml absolute ethanol with a concentration of 5mmol/L to prepare the stock. Before use, it was diluted with distilled water with a concentration of 0, 200, 400, 600, 800, and 1000 μ mol/L.

To prepare Rutin standard solution: 0.010g rutin was dissolved in 75% ethanol and fixed the volume to 100ml volumetric flask to obtain 1.0mg/ml rutin standard solution.

DPH reaction solution was prepared by dissolving 12.5 mg DPPH into methanol solution, constant volume to 100ml, then diluting to 25 mg/ml for using time.

ABTS reaction solution was provided by reacting 7mmol/L ABTS solution with 140mmol/L potassium persulfate solution in dark for 12h and then diluted with ethanol to the light absorption value of 0.70 ± 0.02 under 732mm light. Then 2 ml of a chloroform solution of β - carotene (0.2 mg/ml) was added into the round bottom flask. After chloroform was removed, 40 mg of linolenic acid, 400 mg of Tween 80 emulsifier and 100 ml of deionized water were added into the water bath at 40°C.

Extraction of polyphenols and flavonoids

In June 2019, two portions of *H. cordata* were dried in the shade naturally. Then they were crushed and passed through the 0.315mm sieve. 3.00g *H. cordata* powder was accurately weighed and extracted with 50ml 95% ethanol (V/V) three times. The extract solution obtained from the three times was rotary cut and evaporated to nearly dry. Then it was dissolved in 95% ethanol and the volume was fixed to 100ml volumetric flask to obtain the extract of *H. cordata* (hereinafter referred to as "extract").

DPPH method

The total extract was diluted 10 times, 0.1 ml diluent was added into a 10 ml test tube, and 3.9 ml DPPH reaction solution was added. The same volume of 95% ethanol was used as blank control. After 30 min reaction in the dark, the absorbance was determined under 515 nm light.

The total extract was diluted 10 times, 0.1 ml diluent was added into a 10 ml test tube, and 3.9 ml DPPH reaction solution was added. The same volume of 95% ethanol was used as blank control. After 30 min reaction in the dark, the absorbance was determined under 515 nm light.

Finally, according to the standard curve, the antioxidant capacity of *H. cordata* was expressed as the number of micromol Trolox, i.e. μ m Trolox g^{-1} dw.

Determination of antioxidant capacity

4.8 ml of β-carotene/linolenic acid reaction solution were added to 0.2 ml diluent (1:10) of *H. cordata* extract, shook up, and the absorbance value at 470 nm was measured immediately. The test tube was put in a water bath at 50°C for 2 h, and then the absorbance value was measured at 470 nm, and the value was recorded as abs1. The antioxidant capacity of β-carotene/linolenic acid system of *H. cordata* was calculated as shown in Formula (1):

$$Antioxidant\ activity = \frac{Abs_1}{Abs_0} \times 100\% \quad (1)$$

For determining antioxidant capacity by ABTS method, 0.1 ml of *H. cordata* extract was diluted (1:10) into 10 ml test tube, add 2.3.1 $ABTS^{+}$ reaction solution, and the absorbance value was determined at 734 nm after reaction for 30 min. The standard curve was made with 0, 200, 400, 600, 800 and 1000μm, and the absorbance value was determined according to the above reaction. The standard curve was drawn according to Trolox concentration and absorbance value. Finally, according to the standard curve, the antioxidant capacity of *H. cordata* was expressed as the number of micromoles of Trolox per gram of *H. cordata*, which was μm of Trolox g-1dw.

Results and discussion

Extraction time and solvent selection

Different solvents have different extraction efficiency due to different polarities. It can be seen from Figure 1 that different extraction solvents have different effects on the extraction of polyphenols from Houttuynia cordata Thunb. The extraction rate of

organic solvents (acetone, methanol, and ethanol) is higher than that of water, and the extraction efficiency of methanol is the best. Methanol and ethanol extraction showed the same trend with time, that is, before 2.0 h, it increased with the increase of time, and reached stability after 2.0 h. After the extraction time was more than 3.0 h, the determination value began to decline. After extraction with other solvents for 3.0 h, the measured value also decreased. This may be related to the oxidation of polyphenols in the extraction process. However, compared with ethanol, acetone and methanol have certain toxicity, so ethanol extraction for 2.0 h was the best.

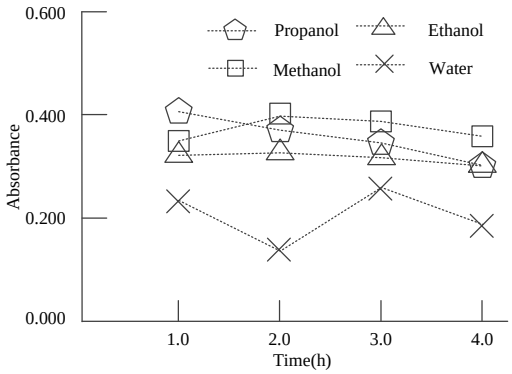

Figure 1. Effect of extraction time and solvent on the determination of the content of polyphenol

Total phenol content

The standard curve for the determination of total phenols in Houttuynia cordata Thunb is shown in Figure 2. In the range of 1.0-5.0 mg/ml gallic acid, there was a good linear relationship between the absorbance and the concentration of gallic acid. The correlation coefficient $R^2 = 0.9987$ (3).

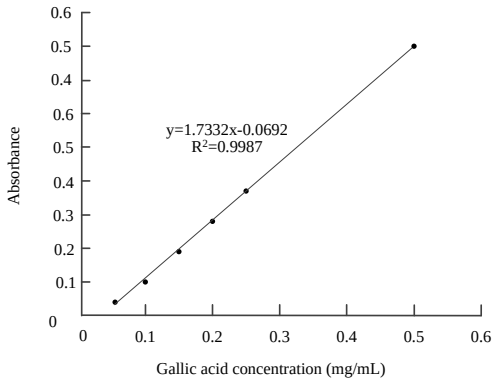

Figure 2. Standard curve for determination of the content of polyphenol

The content of total phenol in different parts of *H. cordata* is as shown in Figure 3. The highest total phenol content is in the leaves of W01-94, which is 10.25 ± 0.888 mg gallic acid g^{-1} DW; the part with the lowest total phenol content is the root of W01-94, which is 1.91 ± 0.030 mg gallic acid g^{-1} DW. In the two populations, the contents of polyphenols in leaves and flowers were similar, and the contents in roots and stems were similar. The content of total phenols in leaves and flowers was about three times that in roots and stems. There was no significant difference in polyphenol content between the two populations.

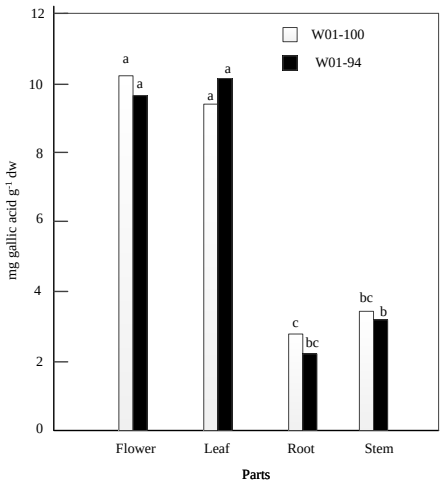

Figure 3. The total polyphenol of different parts of *H. cordata* (Bars carrying different letters are significantly different at $P < 0.05$)

Flavonoid content

The standard curve for the determination of flavonoids in *H. cordata* is shown in Figure 4. In the concentration range of 0.01-0.05 mg/ml, there was a good linear relationship between the absorbance value and the concentration of rutin. Correlation coefficient $R^2 = 0.9990$.

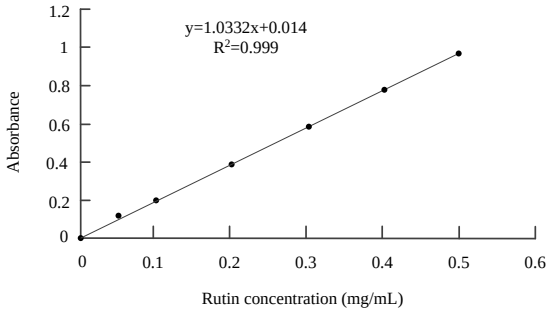

Figure 4. Standard curve for determination of the content of flavonoid

In this experiment, the content of flavonoids in different parts of *H. cordata* was 0.751~12.36mg rutin g^{-1} DW (Figure 5). There was no significant difference in the content of flavonoids between the two populations. The content of flavonoids in leaves and flowers was significantly higher than in roots and stems. Correlation analysis showed that the correlation coefficient between total phenols and flavonoids was 0.948. The difference is that the content of flavonoids in leaves and flowers is 5 times higher than that in roots and stems, and the relative content of flavonoids (flavonoids/total phenolics) in leaves and flowers is higher than that in roots and stems.

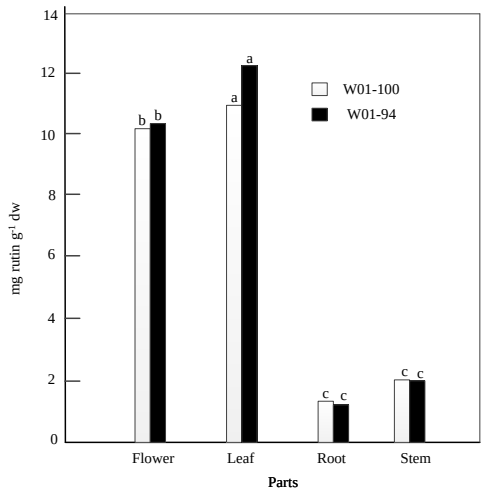

Figure 5. Flavonoid of different parts of H. cordata (Bars carrying different letters are significantly different at $P < 0.05$)

DPPH radical scavenging capacity

The standard curve used for the determination of DPPH is shown in Figure 6. The absorbance value shows a good linear relationship in the range of 100-1000μm Trolox concentration. The regression equation was y = - 0.0004x + 0.634, and the regression coefficient was $R^2 = 0.9986$.

DPPH of different parts of *H. cordata* is shown in Figure 7. The DPPH of the samples tested in this experiment ranged from 14.71μm to 260.8μm Trolox g-1 DW. The DPPH free radical scavenging capacity of flowers and leaves was the highest, and the content of DPPH in leaves of W01-94 was slightly lower, but DPPH in flowers and leaves of both populations exceeded 200μm Trolox g-1 DW. The DPPH free radical scavenging capacity of root and stem was significantly lower than that of flower and leaf, and root was lower than stem. The DPPH free radical scavenging capacity of root and stem were 23.3 and 1.82μm Trolox g-1 DW in W01-100 and W01-94, respectively.

The correlation analysis showed that DPPH radical scavenging ability had a good correlation with total phenol content ($R^2 = 0.934$), which also indicated that DPPH radical scavenging ability was closely related to total phenol content.

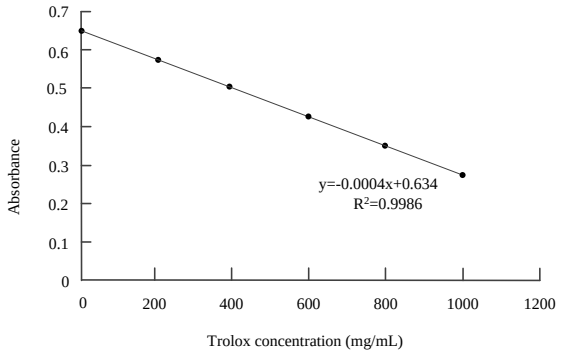

Figure 6. Standard curve for determination of DPPH antioxidant activity

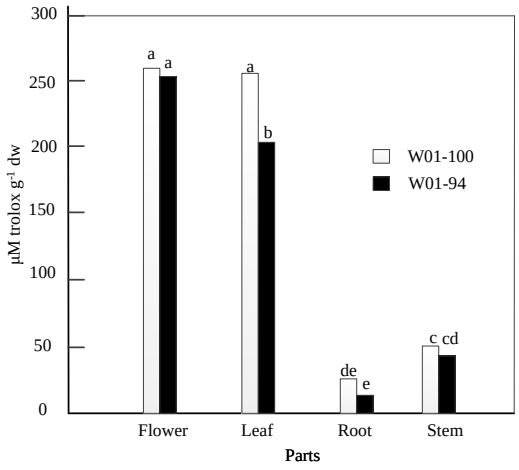

Figure 7. Quantitative determinative of DPPH of different parts of *H. cordata* (Bars carrying different letters are significantly different at $P < 0.05$)

Resistance to beta carotene bleaching

The antioxidant capacity of β-carotene/linolenic acid system is expressed as a percentage, which reflects the percentage of residual β-carotene in the original β-carotene after a period of reaction. The lower degree of β-carotene bleaching had a stronger antioxidant capacity of *H. cordata*.

The results showed that there was no significant difference in the ability of anti-β -carotene bleaching between the two populations (Figure 8). The resistance to β -carotene bleaching of sample W01-94 was the strongest (88.6%), followed by the leaves of sample W01-100 (87.8%), and the root of w01-94 was the weakest (19.0%). However, the antioxidant capacity of different parts is different. In both populations, the antioxidant capacity of the root was the weakest, followed by stem, leaf and flower. The antioxidant capacity of leaves and flowers of W01-94 and W01-100 had no significant difference, which was about 80%. However, it should be pointed out that the absolute value of the results of this method reflects the relative relationship between the antioxidant capacities of the tested materials, and cannot be compared with other values under different conditions.

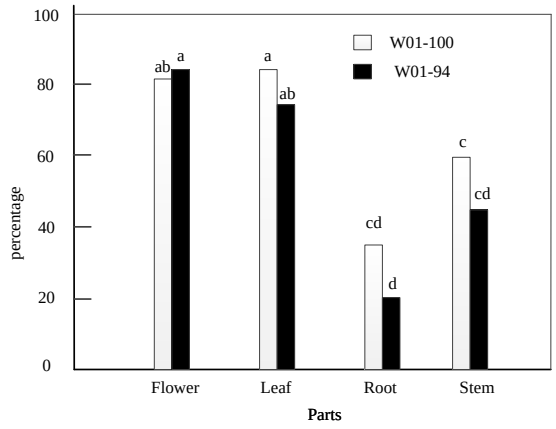

Figure 8. Quantitative determinative of β carotene antioxidant activity of different parts of *H. cordata* (Bars carrying different letters are significantly different at P < 0.05)

ABTS radical scavenging capacity

The standard curve for the determination of ABTS radical scavenging capacity was made with Trolox gradient solution with a concentration range of 100 ~ 1000 μm, as shown in Figure 9. The results showed that there was a good linear relationship between the absorbance and the concentration (R^2 = 0.9994). The regression equation was y = - 0.0007x + 0.7081.

The ABTS free radical scavenging capacity of different parts of *H. cordata* is shown in Figure 10. The ABTS free radical scavenging capacity of different parts of *H. cordata* leaves of W01-94 have the largest ABTS, the value is 209.8μm Trolox g^{-1} DW; the minimum ABTS value in the root of W01-94 is 30.5μm troloxg^{-1}. There was no significant difference in ABTS between the two populations. In general, the ABTS size of different parts of *H. cordata* was flower = leaf > stem > root.

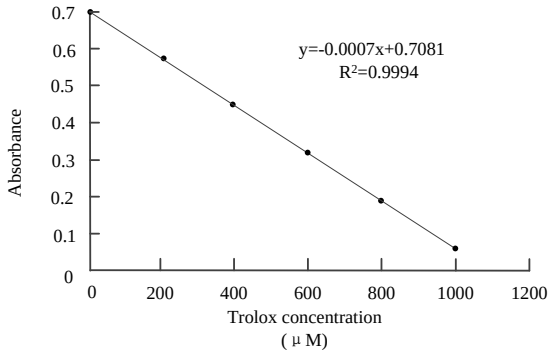

Figure 9. Standard curve for determination of ABTS antioxidant activity

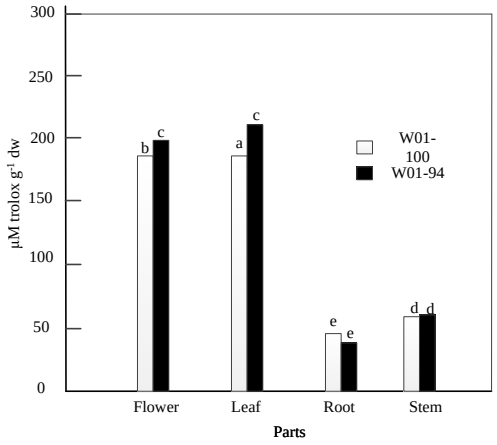

Figure 10. Quantitative determinative of ABTS of different parts of *H. cordata* (Bars carrying different letters are significantly different at P < 0.05 level)

Polyphenols and antioxidant activity of *H. cordata*

The content of total phenols in leaves of *H. cordata* resource materials determined in the experiment is quite different (15), as shown in Table 2. The minimum value was 7.01 mg gallic acid g^{-1} DW in sample 11 and 15.0 mg gallic acid^{-1} DW in *H. cordata* No.9. Among the 16 materials tested, 8 of them reached the value of 10 mg / g. The results showed that the total phenol content of *Houttuynia Emei* was different from other materials. However, the total phenol content of *H. Emei* was not significantly higher than that of samples 2, 3, 4, 6, 7, 8 and 12 (P < 0.01).

Correlation analysis showed that the correlation coefficient between chromosome number and total phenol content of *H. cordata* was 0.4468, but not significant. Therefore, the content of total phenols may not be related to the chromosome number of *H cordata*.

Table 2. The contents of polyphenol and antioxidant activities in vitro of different H. cordata materials

No.	Mg gallic acid g^{-1} dw Total polyphenol	Mg rutin g^{-1} dw Total favonoid	μM trolox| g^{-1} dw DPPH Value	μM trolox| g^{-1} dw ABTS Value
1	10.5^{CD}	5.80^{DE}	149^{D}	168^{BC}
2	8.59^{DEFG}	3.67^{HI}	103^{E}	125^{DE}
3	9.15^{DEFG}	4.26^{GHI}	99^{E}	122^{DE}
4	8.12^{EFG}	3.84^{HI}	91.9^{E}	1 12^{DEF}
5	10.3^{CDE}	5.33^{EF}	128^{D}	139^{CD}
6	8.12^{FG}	4.93^{FG}	139^{D}	102^{DEF}
7	7.82^{FG}	$4.32g^{GH}$	127^{D}	97.0^{EF}
8	9.32^{DEFG}	$6.41d^{D}$	179^{BC}	127^{DE}
9	15.0^{A}	$8.35c^{C}$	177^{C}	196^{AB}
10	10.2^{CDEF}	$5,52e^{EF}$	$136d^{D}$	11^{DEF}
11	7.01^{FG}	3.56^{HI}	102^{E}	78.4^{F}
12	8.67^{DFG}	3.66^{HI}	84.7^{GE}	103^{DEF}
13	13.6^{AB}	7.98^{C}	202^{B}	218^{A}
14	10.6^{CD}	11.0^{A}	248^{A}	186^{AB}
15	12.2^{BC}	9.07^{B}	198^{BC}	186^{AB}
16	13.6^{AB}	11.0^{A}	242^{A}	184^{AB}

Different letters in the same column indicate the significance ($P < 0.01$).

The content of flavonoids in 16 samples ranged from 3.56 mg rutin g-1 DW to 11.0 mg rutin g^{-1} DW. The content of No.16 and No.14 samples was the highest, while that of *H. Emei* No.11 was the lowest. Correlation analysis showed that the flavonoid content of *H. cordata* was higher than that of Houttuynia cordata. The correlation coefficient of total phenols and flavonoids was 0.7886, reaching a very significant level. However, there are some exceptions, such as *H. cordata* sample No.5 and sample No.10 have higher total phenolic content, reaching more than 10 mg gallic acid g^{-1} DW, but their total flavone values are low, which are 5.33 and 5.52 mg rutin g^{-1} DW, respectively.

As shown in Table 2, the DPPH values of 16 samples of Houttuynia cordata are quite different. The DPPH values of samples 13, 14 and 16 were more than 200 μ m Trolox g^{-1} DW, 202, 248 and 242μm Trolox g^{-1} DW, respectively. The DPPH values of three samples were less than 100, which were 95.9μm troloxg^{-1} DW for sample 3, 91.1μm troloxg^{-1} DW for sample 4, and 84.7μm Trolox g^{-1} DW for sample 12, respectively. The DPPH values of the other 10 populations ranged from 100 to 200 μ m troloxg^{-1} DW.

There was no significant correlation between the DPPH value and chromosome number of the Houttuynia cordata population. The correlation coefficients were 0.7200 and 0.9718, respectively.

ABTS value

The ABTS values of different *H. cordata* resource materials are shown in Table 3. The ABTS values of the tested materials ranged from 78.4 to 218μm trolox g^{-1} DW. Among them, the highest sample was 13, and the lowest was sample 11. The correlation analysis showed that ABTS had no significant correlation with the chromosome number of *H. cordata*, but had a significant positive correlation with total phenols, flavonoids and DPPH, with correlation coefficients of 0.9133, 0.8325 and 0.7941, respectively.

Table 3. Correlation analysis among the chromosome number, polyphenol and antioxidant activity of H. cordata populations

Factor	Chromosome number	Total polyphenol	Total flavonoid	DPPH Value
Chromosome number				
Total polyphenol	0.4468			
Total favonoid	0.4666	0.7886*		
DPPHValue	0.3983	0.7200*	0.9718*	
ABTSValue	0.3657	0.9133*	0.8325*	0.7941*

*The difference is very significant. * indicates significantly different at P<0.01 level.

Relationship between total phenols and antioxidant activity of *H. cordata*

16 samples of *H. cordata* were classified into four groups according to the total phenolic content, total flavonoids, DPPH value and ABTS value. The clustering results are shown in Figure 11. If the Euclidean distance is greater than 70, 16 populations can be divided into two groups. Group I includes 11 populations, including sample 1, sample 5, sample 8, sample 2, sample 3, sample 4, sample 12, sample 11, sample 6, sample 10 and sample 7. The total phenolic, flavonoids, DPPH and ABTS values of each population are low; the total phenolic, flavonoids, DPPH and ABTS values of class II were higher than those of sample No. 9, sample 15, sample 13, sample 14 and sample 16. Therefore, further breeding of *H. cordata* with high total phenolic and antioxidant

activity in vitro can be carried out in class II, and the varieties with low total phenolic and in vitro antioxidant activity can be selected in class I. Chen Li et al. Classified *H. cordata* into decanal type (D type) and myrcene type (M type) according to the volatile oil components of *H. cordata* and M type had a higher content of monoterpenoids and sesquiterpenes than D type. In this study, 5 populations of type II were all M-type, and the chromosome numbers were more than 80; class I included D-type and M-type. The results showed that the composition and content of volatile oil from *H. cordata* were related to phenolic compounds.

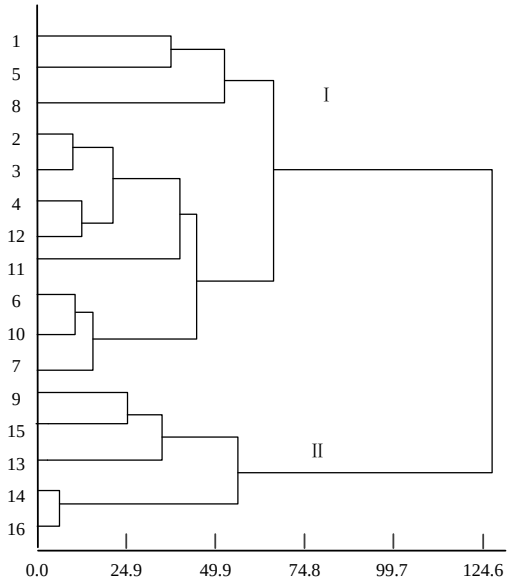

Figure 11. Cluster analysis of polyphenol and antioxidant activity of different *H. cordata* accessions

The amount of phenolic compounds and antioxidant properties of plants plays a vital role in determining their nutritional value (16). *Houttuynia cordata* leaves contain high amounts of soluble sugars, non-volatile acids, and phenolic compounds (17). The antioxidant properties and cytotoxic effects of *H. cordata* leaves and flowers have been demonstrated, and the seeds of this plant have high phenolic and flavonoid materials (18). Its medicinal products in the treatment of insomnia and anxiety have also been proven (19). Numerous studies in genetic diversity have been based on morphological traits and molecular markers (20-22). Morphologically, there is a lot of variety in the leaves of this plant, and essential commercial properties related to different parts of *H. cordata* have been studied (23).

In this study, two *H. cordata* materials with the same chromosome number and chemical type were used to comprehensively evaluate the contents of total phenols and flavonoids in different parts of *H. cordata*, as well as the anti-carotene and other antioxidant activities of *H. cordata*. The results showed that the total phenols and antioxidant capacity of different parts of *H. cordata* were significantly different. The content of polyphenols in roots and stems is low, the antioxidant capacity is weak, and the total phenols in flowers and leaves are high, and the antioxidant capacity is strong. Therefore, different parts of *H. cordata* have different pharmacological and food effects. The whole herb can be used as Chinese herbal medicine for clearing away heat and toxin, eliminating carbuncle and removing pus, diuresis and Tonglin (24), and its young leaves and roots can be used as vegetables (25-28). Flavonoids are the main phenolic components, and total phenols are the main components of antioxidant activity. This can explain that there is a very significant positive correlation between total phenols and flavonoids. Therefore, in the further breeding work of *H. cordata*, the procedure can be simplified by determining one of the above indexes to predict the varieties with high total phenolic and antioxidant activity in vitro.

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Interest conflict

None.

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