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Article

Quality analysis report of Cistanche tubulosa and Cistanche deserticola

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Abstract: The medicinal materials of Cistanche tubulosa (CW) and Cistanche deserticola (CD) were selected to carry out the determination of bioactive substances and antioxidant activity and multivariate statistical analysis. The results of active substance analysis showed that there were significant differences in the content of total polysaccharides between the two varieties, and there were significant differences in the contents of total polyphenols, total flavonoids, total triterpenoids, proanthocyanidins, phenylethanoid glycosides, amino acids, proteins and heavy metals. The total polysaccharides in CW were higher than those in CD. The contents of total polyphenols, total flavonoids, total triterpenes and phenylethanoid glycosides in CW were significantly higher than those in CD. The contents of amino acids, proteins and heavy metals in CD were higher than those in CW. The results of antioxidant analysis showed that the DPPH free radical scavenging ability, ABTS free radical scavenging ability and ORAC superoxide radical scavenging ability of CW were significantly higher than those of CD, and there was a significant difference between the two varieties. Correlation analysis showed that the antioxidant capacity of Cistanches herba was closely related to the contents of total polyphenols, total flavonoids, total triterpenes, total polysaccharides, phenylethanoid glycosides, proanthocyanidins, amino acids, proteins and heavy metals. The samples were divided into two groups by cluster analysis. Four principal components were extracted by principal component analysis, and the cumulative variance contribution rate was 94.15%.

Keywords: Cistanche (Schenk) wight; Cistanche deserticola Y.C.Ma; active substance; antioxidation; principal component analysis

1. Introduction

Cistanche deserticola Y.C.Ma (CD) is a tall herbaceous plant, most of which grows underground, and the stem gradually becomes thinner from bottom to top; the leaves are wide ovate, and the upper part of the stem is sparse and narrow, generally lanceolate; inflorescences are spike-shaped, petal edges are often rolled out, yellowish white or lavender, and often turn brown after drying; fruit ovoid; seeds oval or nearly ovate, outside the mesh, shiny; flowering period from May to June; the fruit period from June to August is a kind of Orobanchaceae plant. It is a perennial parasitic herb that parasitizes on the roots of Chenopodiaceae plants such as Haloxylon ammodendron and Haloxylon persicum.

Cistanche tubulosa (Schenk) Wight (CW)is a perennial parasitic herb belonging to the Cistanche genus of the Orobanchaceae family. The plant is up to 100 cm in height. The stem is not branched, the leaves are milky white, and brown after drying. Triangular, spike-like inflorescences, bracts long-rounded lanceolate or ovate lanceolate, margin pilose, hairless on both sides; bractlets linear lanceolate or spatulate, nearly glabrous. Calyx cylindric, corolla milky white, long ovate triangular

or lanceolate, corolla cylindric funnel-shaped, lobes purple in flower buds, suborbicular, anther ovate, ovary long ovate, capsule oblong, seeds mostly, suborbicular, flowering from May to June, fruiting from July to August. Growing at an altitude of 1200 m in the more abundant water willows and sand dunes; it often parasitizes on the roots of Tamarix plants. The dried scale-leafed stems of Cistanche tubulosa and Cistanche deserticola are widely used in traditional Chinese medicine, both of which are medicinal varieties in the 'Chinese Pharmacopoeia'.

Cistanche deserticola Y.C.Ma (CD) or *Cistanche* (Schenk) Wight (CW) is a kind of dry succulent stem with scaly leaves, which was first recorded in the 'Shennong' Herbal Classic. It is a traditional Chinese medicine with a reputation of 'desert ginseng' [1]. Cistanches herba is sweet, salty, warm in nature, and belongs to the two meridians of kidney and large intestine. It has the effects of tonifying kidney yang, benefiting essence and blood, and moistening intestines and relaxing bowels. It is used to treat kidney yang deficiency, essence and blood deficiency, impotence infertility, soreness and weakness of waist and knees, weakness of muscles and bones, and intestinal dryness and constipation. Cistanches herba is mainly produced in Inner Mongolia (Azuoqi), Gansu (Changma) and Xinjiang. Cistanches herba contains a variety of active substances, such as polyphenols, polysaccharides, phenylethanoid glycosides, iridoids, lignans and other chemical components [2,3]. It has pharmacological effects such as neuroprotection, anti-aging, anti-liver injury, and laxatives [4–13]. It is widely used in clinical practice of traditional Chinese medicine and has a good development prospect.

The Chinese pharmacopoeia (ChP) (2020 edition) mainly uses the content of echinacoside and verbascoside as the index to evaluate the quality of Cistanches herba. However, the bioactive substances in Cistanches herba are complex. In addition to the above two substances, total polyphenols, total flavonoids, total polysaccharides, total triterpenes, total proanthocyanidins and so on have a wide range of pharmacological effects. Therefore, the multi-index quality evaluation method is used to evaluate the quality of Cistanches herba more comprehensively. Based on this, this paper takes different varieties of Cistanches herba as the research object, investigates the types, contents and antioxidant activities of bioactive substances in different varieties of Cistanches herba, and uses correlation analysis, cluster analysis and principal component analysis for comprehensive evaluation, in order to clarify the quality differences of different varieties of Cistanches herba. Partial least squares analysis method was used to establish a predictive analysis model of antioxidant capacity of Cistanches herba, which provided theoretical support and reference for the quality control of Cistanches herba and the selection of Cistanches herba varieties in products.

2. Materials and methods

2.1. Materials and reagents

2.1.1. Major processed materials

The samples of CD and CW from different producing areas are all dry fleshy stems. All samples are wild varieties. They grow and mature in the underground part

of the medicinal materials in autumn, and are harvested when they are just unearthed, or when they are about to be unearthed. All samples information have been identified by Jingpai Chi Zhengtang Pharmaceutical Co., Ltd., The details are shown in **Table 1**.

Table 1. samples information of Cistanches herba.

Number	Species	Batch no	Origin of sample	Climate
1	CW	220300027	Xinjiang, China	Temperate continental climate
2	CW	220500106	Henan, China	Transition zone between temperate monsoon climate zone and subtropical monsoon climate zone
3	CW	220500007	Xinjiang, China	Temperate continental climate
4	CW	220500047	Henan, China	Transition zone between temperate monsoon climate zone and subtropical monsoon climate zone
5	CW	220500063	Henan, China	Transition zone between temperate monsoon climate zone and subtropical monsoon climate zone
6	CW	221200084	Henan, China	Transition zone between temperate monsoon climate zone and subtropical monsoon climate zone
7	CW	220600063	Henan, China	Transition zone between temperate monsoon climate zone and subtropical monsoon climate zone
8	CW	Y21050021	Xinjiang, China	Temperate continental climate
9	CW	211200137	Henan, China	Transition zone between temperate monsoon climate zone and subtropical monsoon climate zone
10	CW	211200229	Henan, China	Transition zone between temperate monsoon climate zone and subtropical monsoon climate zone
11	CW	Y20041036	Xinjiang, China	Temperate continental climate
12	CD	220300013	Xinjiang, China	Temperate continental climate
13	CD	220400019	Xinjiang, China	Temperate continental climate
14	CD	220600029	Xinjiang, China	Temperate continental climate
15	CD	220600049	Xinjiang, China	Temperate continental climate
16	CD	220600079	Xinjiang, China	Temperate continental climate
17	CD	220900001	Xinjiang, China	Temperate continental climate
18	CD	220900035	Xinjiang, China	Temperate continental climate
19	CD	220900049	Qinghai, China	Highland continental climate
20	CD	221200053	Inner Mongolia, China	Temperate continental monsoon climate
21	CD	221200078	Xinjiang, China	Temperate continental climate
22	CD	2301000046	Inner Mongolia, China	Temperate continental monsoon climate

2.1.2. Main reagents

Acetonitrile and phosphoric acid were purchased from Merck (Darmstadt, Germany); echinacea glycoside, Tube anthocyanin A, Tube anthocyanin B, Mulberry glycoside, Isopyranoside and 2 acetyl mullein glycoside were obtained from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China), and the purities of them were all above 98%. Rutin and Qi Dunguo acid purities were 91.9%; purchased from China Institute for Food and Drug Control (Beijing, China). Sodium nitrite, aluminum nitrate, sodium hydroxide, anhydrous ethanol, vanillin, glacial acetic acid, ethyl acetate, perchloric acid, concentrated sulfuric acid, methanol were purchased

from National Pharmaceutical Group Chemical Reagent Co., Ltd. (Shanghai, China), and phenol was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan).

2.1.3. Main instruments

Ultraviolet spectrophotometer (Beijing Puxi General instrument Co., Ltd., model TU-1901), centrifuge (Germany Sigma instrument Company, model 3K15); Ultimate 3000 series high performance liquid chromatograph (HPLC, Semerfei Company, USA); SynergyH1 multi-function enzyme label instrument (Boteng instrument Co., Ltd. China), Electronic balance: Sedorius Company, Germany). Inductively coupled plasma mass spectrometry (ICP-MS), model: iCAP PQ; manufacturer: Semel Fisher Technology Co., Ltd. Amino acid analyzer, model: S443D; manufacturer: Germany Sekham instrument Co., Ltd. Kjeldahl nitrogen meter, model: Kjeltec8400; manufacturer: Denmark Foss Company.

2.2. Determination of bioactive substances

2.2.1. Determination of total polyphenols

According to the method of Zhang Chunling "Extraction and Determination of Polyphenols from Cistanche" [14]. Preparation of sample solution: 0.50 g sample was weighed in a triangular flask, added with 20 mL of 70% ethanol solution, incubated in a water bath at 60 ℃ for 40 min, and then ultrasonically extracted at 250 W for 40 min. After cooling, it was centrifuged at 8000 r/min for 10 min. The supernatant was collected and diluted to 25 mL for later use. Take an appropriate amount of sample solution, add 5 mL of 10% Folin phenol solution, shake well, static 15 min, add 7.5% 4 mL mass fraction of Na_2CO_3 solution, shake well, avoid light static 20 min sutes, determine its absorbance (A765), the results are expressed as Gallic acid equivalent (g GAE/g DW).

2.2.2. Determination of total flavonoids

Take an appropriate amount of the sample solution (as 2.2.1 described above) and dilute it 10 times [15,16]. Add 1 mL of diluted sample solution into a 10 mL brown volumetric flask, add 0.3 mL of 5% $NaNO_2$ solution, shake well, stand for 15 min, and then add 0.3 mL of 10% $Al(NO_3)_3$ solution and 4% 4 mL NaOH solution in turn, dilute to 10 mL with 50% ethanol solution, shake well, and let stand in dark for 20 min. Measure its absorbance (A510), and the result is expressed in rutin equivalent (g RE/g DW).

2.2.3. Determination of total triterpene

0.1 mL sample solution (as 2.2.1 described above) was volatilized dry in a water bath, then the newly prepared 0.4 mL 1.5% vanillin-glacial acetic acid solution and 1.6 mL perchloric acid were added into it and mixed [17]. The mixture was bathed in water at 70 ℃ for 15 min, after cooling, 5 mL ethyl acetate was added and stood for 10 min, and the absorbance was measured (A560). The result is expressed as Oleanolic acid equivalent (g OAE/g DW).

2.2.4. Determination of total polysaccharides

Preparation of sample solution: 0.25 g of sample was weighed and 10 mL of deionized water was added [18]. The sample was bathed in water at 60 ℃ for 50 min, and then extracted by 250 W ultrasonic for 50 min. After cooling, the sample

was centrifuged at 8000 r/min for 10 min. The supernatant was collected, and 40 mL of 95% ethanol solution was added. After standing for 12 h at 4 ℃, the sample was centrifuged at 8000 r/min. The precipitate was collected and diluted to 50 mL for testing. And in turn with acetone, ether, anhydrous ethanol repeatedly washed twice, add the appropriate amount of deionized water after re-dissolution, deproteinization (Sevage method), depigmentation (activated carbon method) treatment, constant volume 10 mL, to be measured. 1 mL of sample solution was added with 0.6 mL of 6% phenol solution and 3 mL of concentrated H_2SO_4 in turn. After shaking and shaking, the sample solution was bathed in boiling water for 10 min. After cooling, the absorbance (A490) was determined, and the results were expressed as glucose equivalent (g DE/g DW).

2.2.5. Determination of proanthocyanidins content

Preparation of sample solution: 0.50 g sample was weighed, 15 mL of 80% ethanol solution was added [19], and the sample was extracted for 40 min in a 250 W ultrasonic device at 50 ℃. After cooling, the sample was centrifuged at 4000 r/min for 10 min. The supernatant was collected and diluted to 50 mL. The 0.5 mL sample solution was added with 3 mL 4% vanillin-methanol solution and 1.5 mL concentrated HCl in turn. After reaction for 15 min, the absorbance (A500) was measured, and the results were expressed as Proanthocyanidin equivalent (g PCE/g DW).

2.2.6. Determination of phenylethanoid glycosides compounds

According to the method of Yang et al. [20] "Study on phenylethanoid glycosides in *Cistanche tubulosa*", The sample was taken about 0.1 g (sieved through the No.3 sieve), precise weighing, adding 50% methanol 20 mL, weighing, soaking 30 min, ultrasonic (500 W, 53 kHz) 40 min, cooling, then weighing, with 50% methanol to make up for the loss of quality, shake well, that is. Using ThermoSyncronis C18 chromatographic column (250 mm × 4.6 mm, 5 μm); Acetonitrile was the mobile phase A, and a 0.1% phosphoric acid solution was the mobile phase B, Gradient elution (0 min–10 min: 10–15% A; 10 min–20 min: 15–18% A; 20 min–45 min: 18–27% A); Flow rate: 1.0 mL/min; Column temperature: 30 ℃; Detection wavelength: 330 nm; Injection volume: 10 μL. Quantitative analysis used external standard method. The results were expressed as percentage content (%) of each compound.

2.2.7. Determination of hydrolyzed amino acid

The sample 1 g was weighed in the hydrolysis tube, and 10 mL of 6 mol/L hydrochloric acid solution was added. Then fill in N_2, and tighten the screw cap in the N_2-filled state. The sealed hydrolysis tube was placed in an electrothermal blast incubator or hydrolysis furnace at 110 ℃ ± 1 ℃. After 22 h of hydrolysis, it was taken out and cooled to room temperature. The hydrolysis tube was opened, and the hydrolysate was filtered into a 50 mL volumetric flask. The hydrolysis tube was washed several times with a small amount of water, and the washing solution was moved into the same 50 mL volumetric flask. Finally, the water was diluted to the scale, and the oscillation was mixed. 0.2 mL of filtrate N_2 was blown to dry, and 1.0 mL of pH2.2 sodium citrate buffer solution was added to shake and mix. After the

solution was passed through the 0.22 μm filter membrane, it was transferred to the instrument injection bottle as the sample determination solution for the instrument determination [21]. The injection volume of the reference solution and the test solution was 50 μL, which was injected into the amino acid analyzer for determination. The mixed amino acid standard working solution and the sample determination solution were injected into the amino acid analyzer with the same volume. The standard curve was established with the reference concentration as the abscissa and the chromatographic peak area as the ordinate. The concentration of amino acids in the sample determination solution was calculated by the peak area using the external standard method. The results were calculated as the total amount of amino acids and expressed as percentage (g/100 g).

2.2.8. Determination of protein

Weigh about 0.5 g (± 0.001 g) of the sample, transfer it into a dry 250 mL Kjeldahl digestion tube, then add 0.5 g of $CuSO_4$, 4.5 g of K_2SO_4 and 10 mL of concentrated H_2SO_4 into the digestion tube, and adjust the temperature of the digestion furnace to 420 ℃, Then cover the waste discharge cover on the sample rack, turn on the cooling water, and heat and digest it in a fume hood for 1 h, After cooling. Turn on the power of the fully automatic Kjeldahl nitrogen analyzer and set the detection method to AN300 mode [22], (The volume of the sample to be diluted in this method was 40 mL, the volume of the alkaline solution was 60 mL, the volume of the receiving solution was 30 mL, and the distillation method was in automatic mode). Determine the nitrogen content in the sample and multiply it by a coefficient of 6.25 to obtain the protein content in the sample, expressed as a percentage (g/100 g).

2.2.9. Determination of total heavy metal

Accurately weigh approximately 0.5 g of the sample, place it in a pressure and high temperature resistant microwave digestion tank, and add 5 mL of nitric acid. Sealed, microwave digestion was performed according to the procedure in **Table 2**. After cooling, take it out, slowly open the digestion tank cover to exhaust, flush the inner cover with a small amount of water, dilute it with water to a 50 mL volumetric flask, shake it up for standby, and conduct a blank test at the same time. Instrument conditions: RF power of 1550 W, sampling depth of 5 mm, cooling airflow rate of 14.0 L/min, atomization airflow rate of 1.14 L/min, auxiliary airflow rate of 0.80 L/min, CCT collision airflow rate of 4.14 L/min [23]. The indicators of sodium, magnesium, aluminum, potassium, calcium, chromium, manganese, iron, cobalt, nickel, copper, zinc, arsenic, selenium, strontium, silver, cadmium, barium, lead, mercury, etc. in the test sample were calculated based on the total amount of each metal element, expressed in g/kg.

Table 2. Microwave digestion procedure.

Digestion method	step	Temperature, ℃	heating time, min	holding time, min
Microwave digestion	1	80	10	3
	2	130	5	3
	3	180	8	15

2.2.10. Determination of in vitro antioxidant activity

1.20 g sample was dissolved in 60% ethanol solution, the ratio of material to liquid was 1:25, and extracted for 40 min in a 250 W ultrasonic device at 60 ℃. After cooling, centrifuge at 4500 r/min for 15 min and collect the supernatant. Take the appropriate amount of supernatant and dilute it with 60% ethanol solution until the sample concentration was 1 mg/mL, 3 mg/mL, 5 mg/mL, 10 mg/mL, 20 mg/mL, 30 mg/mL, and 40 mg/mL, respectively, for testing [24].

DPPH free radical scavenging activity

Take 10 μL sample solution, add 250 μL DPPH solution, mixed well, added to an enzyme calibration plate, kept dark at room temperature for 25 min. Using 60% ethanol solution as the blank control, its absorbance (A517) was measured, and the DPPH radical clearance rate was calculated according to the following equation [25].

$$\text{DPPH radical clearance rate (\%)} = 1 - (A - A_1)/A_0 \times 100\% \quad (1)$$

where A_0 represents the absorbance of 60% ethanol solution instead of the sample solution (blank control); A is the absorbance after mixing the sample solution with DPPH solution; A_1 is originally the absorbance of anhydrous ethanol instead of DPPH solution.

ABTS free radical scavenging activity

The ABTS stock solution was a mixed solution of Potassium persulfate (final concentration: 2.6 mmol/L) and ABTS (final concentration: 7.4 mmol/L) prepared with deionized water, which was kept at 25 ℃ in dark for 16 h. Before using the ABTS stock solution, dilute it with PBS buffer to an absorbance (A734) of 0.70 ± 0.02, which was the ABTS working solution. Sample solution (1 mg/mL–40 mg/mL), with an appropriate amount (5 gradients for each sample), added to the ELISA plate, and an appropriate amount of PBS buffer added (the total volume of buffer and sample was 100 μL) Add another 100 μL ABTS working solution (total reaction volume of 200 μL). At room temperature, avoid light for 30 min, use 60% ethanol solution as the blank control, measure its A734, and calculate the ABTS free radical clearance rate according to the following equation [26].

$$\text{ABTS free radical clearance rate (\%)} = (A_0 - A) \div A_0 \times 100\% \quad (2)$$

A_0 is the absorbance value of the blank sample and A is the absorbance value after adding the sample.

ORAC superoxide radical scavenging ability

Take 25 μL sample solution, add 150 μL Dihydroethylidene fluorescein HE (containing Xanthine) solution, mixed evenly, added to the enzyme plate, incubated at 37 ℃ for 10 min, and immediately added 25 μL of Xanthine oxidase solution, measure the fluorescence intensity every minute (lasting for 45 min). The excitation wavelength was 470 nm, the emission wavelength was 620 nm, the sensitivity was 100–120, and the halogen lamp was the light source. The initial fluorescence intensity value was recorded as f_0, and then measured every minute, denoted as f_1, f_2, ... f_{45}. The data analysis time was determined based on the fluorescence intensity

balance time. The area under the negative control (Neg) curve without antioxidants was deducted from the integral area under the fluorescence increase curve under the action of antioxidants, and the net area under the curve (Net AUC) under the view of antioxidants was obtained [27].

3. Results and analysis

All data were measured three times in parallel, and the results were expressed as (mean ± standard deviation). Using Origin 2019 for principal component analysis and systematic clustering analysis, and SPSS Statistics 26 for analysis of variance, $P < 0.05$ indicates a significant difference, $P < 0.01$ indicated that the difference was extremely significant.

3.1. Analysis of bioactive substances in CW and CD

The results of the content of bioactive substances in CW and CD were listed and analyzed, as shown in **Table 3**. From **Table 3**, it can be seen that there are significant differences in the content of bioactive substances in CW and CD.

Table 3. Comparative table of biological activities in CW and CD herba Cistanches.

Category target	Specific target	CW	CD	*P*-value
Basic physicochemical target	Total polysaccharides	3.948 ± 2.519	2.125 ± 0.599	$P < 0.05$
	Amino acid	2.074 ± 0.205	2.982 ± 0.462	$P < 0.001$
	Protein	3.818 ± 0.502	11.400 ± 1.959	$P < 0.001$
	Total heavy metal content	20.054 ± 3.042	24.946 ± 3.033	$P < 0.01$
Substances with antioxidant activity	Total polyphenol	9.955 ± 2.474	1.618 ± 0.527	$P < 0.001$
	Total flavone	25.070 ± 7.189	3.207 ± 1.446	$P < 0.001$
	Total triterpenes	3.674 ± 0.562	2.801 ± 0.582	$P < 0.01$
	Procyanidine	0.436 ± 0.050	0.593 ± 0.118	$P < 0.01$
Phenylethanol glycosides	Echinacoside	8.638 ± 3.041	1.064 ± 0.579	$P < 0.001$
	Tuphonin A	1.027 ± 0.470	0.097 ± 0.113	$P < 0.001$
	Verbascoside	2.892 ± 1.100	0.172 ± 0.137	$P < 0.001$
	Isoverminoside	0.849 ± 0.334	0.026 ± 0.015	$P < 0.001$
	2’-acetylacteoside	0.089 ± 0.033	0.038 ± 0.033	$P < 0.01$
	Tuphonin B	0.034 ± 0.018	0.011 ± 0.008	$P < 0.001$

The comparative analysis of the content differences of biological activity indexes between CW and CD is shown in **Figures 1–9**. From the diagram, it can be seen that the total polysaccharide in CW is higher than that in CD. The contents of total polyphenols, total flavonoids, total triterpenes and phenylethanoid glycosides in CW are significantly higher than those in CD, while the contents of amino acids, proteins and heavy metals in CD are higher than those in CW. The differences between 11 batches of CW samples and 11 batches of CD samples were analyzed by *t* test. The difference analysis of total polysaccharide content was $p < 0.05$, indicating that there was a significant difference in the total polysaccharide content between CW and CD. The *p* values of other index difference analysis were all < 0.01,

indicating that there were significant differences in the contents of 8 compounds such as total polyphenols in CW and CD. The analysis suggests that these differences may be caused by the differences between the two species of CW and CD.

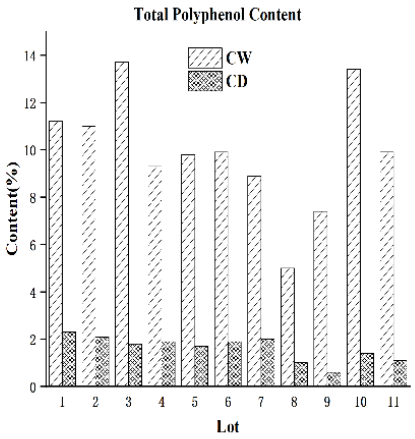

Figure 1. Different cultivars of total polyphenols content.

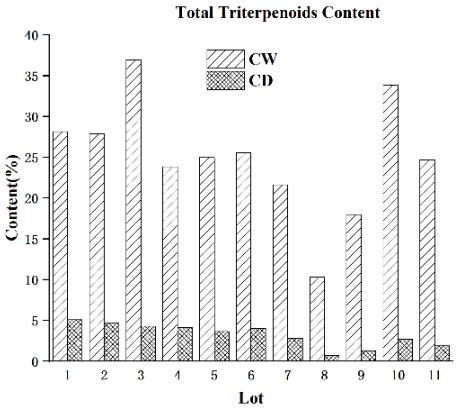

Figure 2. Different cultivars of total flavonoids content.

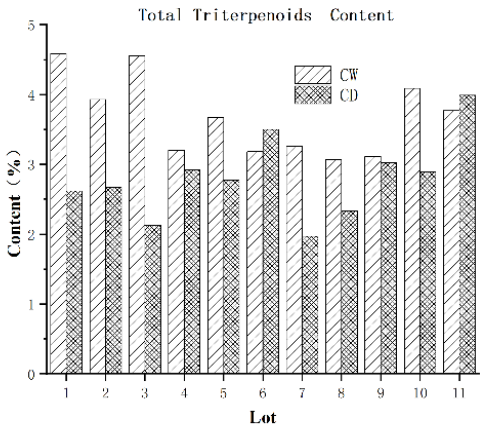

Figure 3. Different cultivars of total triterpenoids content.

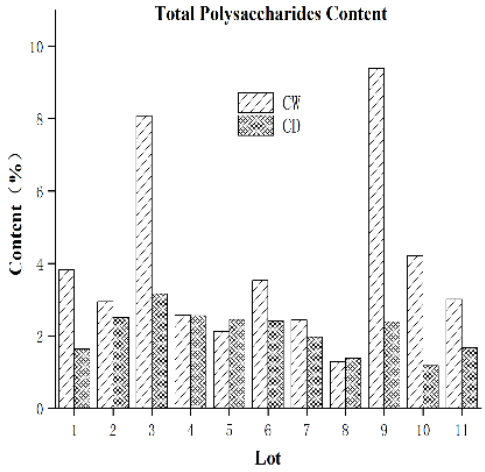

Figure 4. Different cultivars of total polysaccharides content.

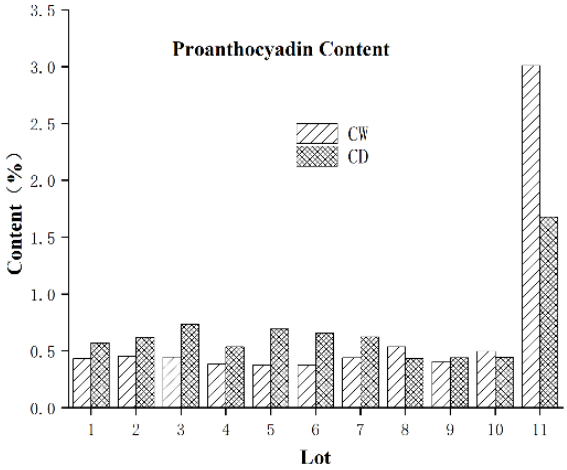

Figure 5. Different cultivars of proanthocyadin content.

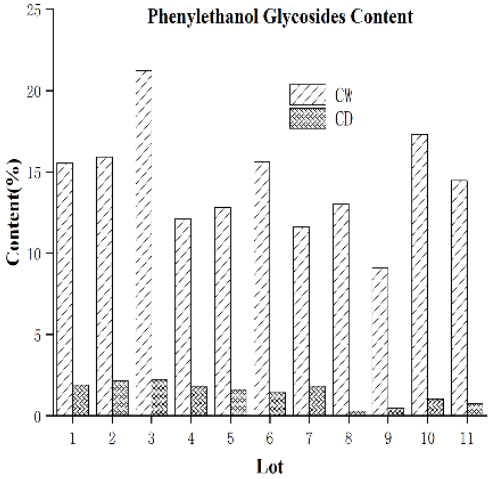

Figure 6. Different cultivars of phenylethanol glycosides content.

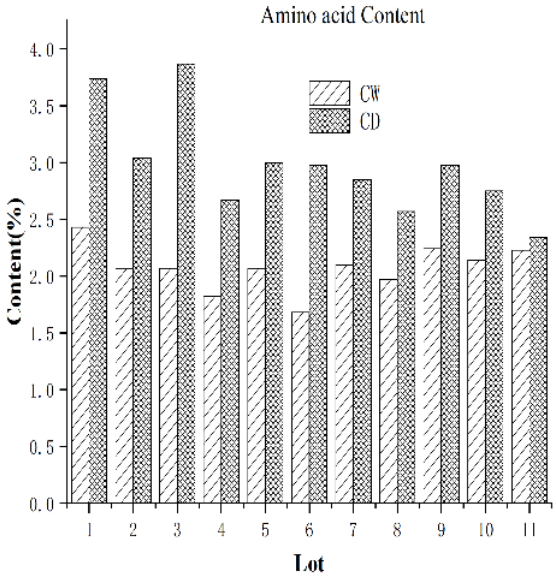

Figure 7. Different cultivars of amino acid content.

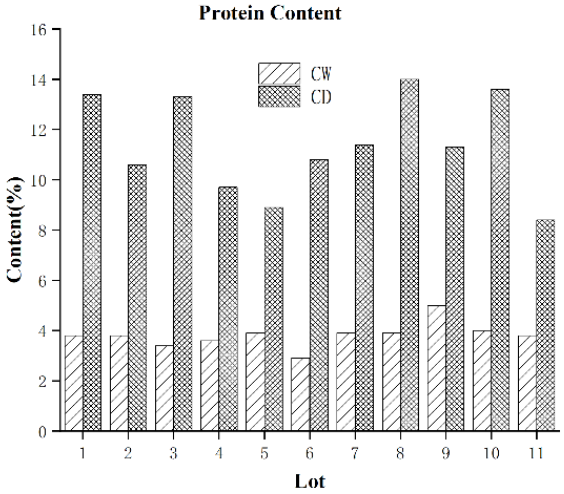

Figure 8. Different cultivars of protein content.

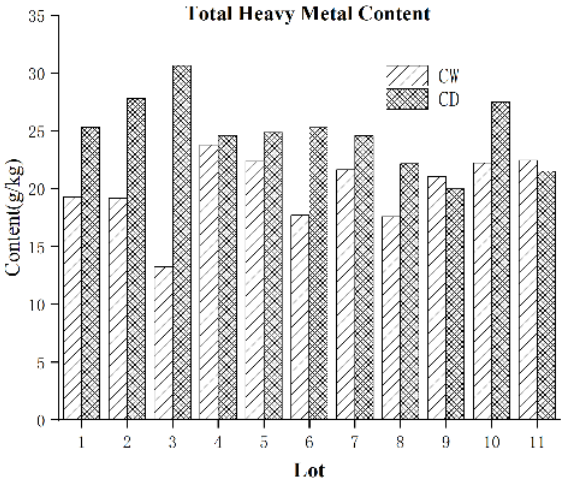

Figure 9. Different cultivars of total heavy metal content.

3.2. Analysis of antioxidant activity results of CW and CD

The DPPH free radical scavenging ability, ABTS free radical scavenging activity and superoxide radical scavenging activity index ORAC of the collected CW and CD samples were detected. The results were analyzed as follows: The comparative analysis of the content differences of biological activity indexes between CW and CD is shown in **Figures 10–12**. It can be seen from the diagram that the DPPH free radical scavenging activity, ABTS free radical scavenging activity and ORAC superoxide free radical scavenging activity of CW are higher than those of CD. The average values of DPPH free radical scavenging activity, ABTS free radical scavenging activity and ORAC superoxide free radical scavenging activity of 11 batches of CW were 26.72%, 49.55% and 65, 895.34 μmol TE/L, respectively. The average values of DPPH free radical scavenging activity, ABTS free radical scavenging activity and ORAC superoxide radical scavenging activity of 11 batches of CD samples were 7.48%, 7.57% and 14, 579.72 μmol TE/L, respectively. The three indexes of antioxidant activity were analyzed by *t* test, and the *p* values of the difference analysis of each index were < 0.01, indicating that there was a significant difference in antioxidant activity between CW and CD. It is believed that the difference in antioxidant activity between CW and CD is due to the difference in active substances between the two species.

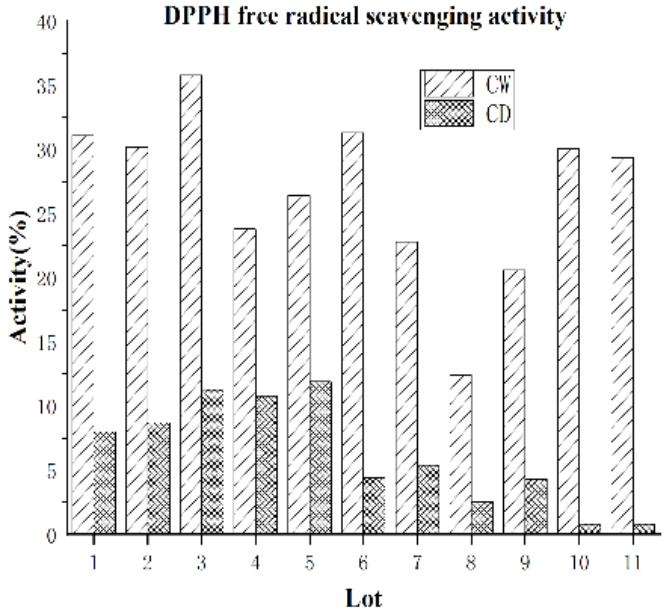

Figure 10. Different cultivars of DPPH scavenging activity.

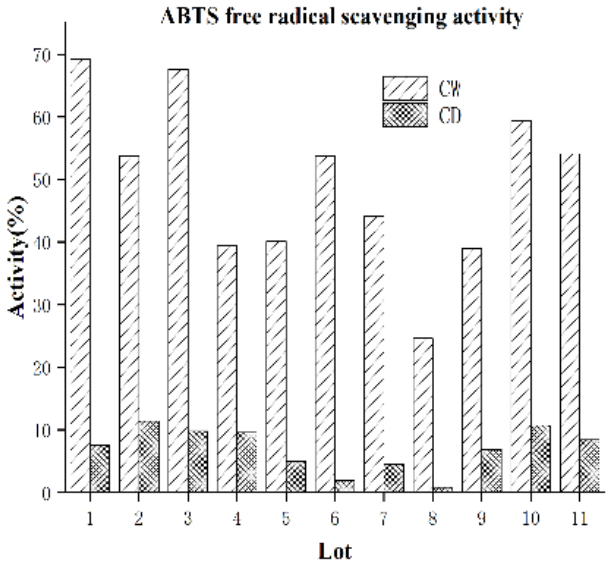

Figure 11. Different cultivars of ABTS scavenging activity.

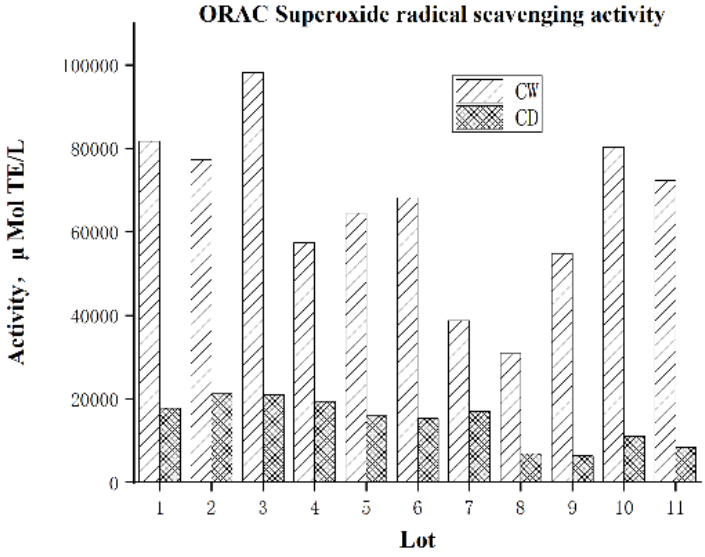

Figure 12. Different cultivars of ORAC scavenging activity.

3.3. Correlation analysis

Pearson correlation analysis was used to analyze the correlation between the quality indexes of Cistanches herba in different producing areas [28,29], and the results are shown in **Table 4**. From the table, it can be seen that the total polyphenol content was significantly positively correlated with the total flavonoid content, total triterpenoid content and phenylethanoid glycoside content ($P < 0.01$), and the total polyphenol content was significantly negatively correlated with the proanthocyanidin content, total amino acid content, protein content and total heavy metal content ($P < 0.01$). The content of total flavonoids was positively correlated with the content of total triterpenoids and phenylethanoid glycosides ($P < 0.01$), and negatively correlated with the content of proanthocyanidins, total amino acids, protein content and total heavy metals ($P < 0.01$). The content of total triterpenes was significantly

positively correlated with the content of phenylethanoid glycosides ($P < 0.01$). The content of proanthocyanidins was significantly positively correlated with the content of amino acids, proteins and heavy metals ($P < 0.01$), and negatively correlated with the content of phenylethanoid glycosides ($P < 0.01$). The content of phenylethanoid glycosides was significantly positively correlated with amino acids, protein and total heavy metals ($P < 0.01$), and the content of amino acids was significantly positively correlated with protein content and total heavy metals ($P < 0.01$). Protein content was significantly positively correlated with heavy metal content ($P < 0.01$).The superoxide radical scavenging activity index ORAC, ABTS radical scavenging activity and DPPH radical scavenging activity were significantly positively correlated with the contents of total polyphenols, total flavonoids, total triterpenoids, total polysaccharides and phenylethanoid glycosides ($P < 0.01$), and were significantly negatively correlated with the contents of proanthocyanidins, amino acids, proteins and heavy metals ($P < 0.01$), indicating that the antioxidant capacity of Cistanches herba was closely related to the contents of total polyphenols, total flavonoids, total triterpenoids, total polysaccharides, phenylethanoid glycosides, proanthocyanidins, amino acids, proteins and heavy metals.

Table 4. The results of correlation analysis between the indicators.

Index	Total polyphenol	Total flavonoids	Total triterpenoids	Total polysaccharides	Proanthocyadin	Phenylethanol glycosides	Amino acid	Protein	Total heavy metal	ORAC	ABTS	DPPH
Total polyphenol	1											
Total flavonoids	0.998**	1										
Total triterpenoids	0.711**	0.727**	1									
Total polysaccharides	0.521	0.533	0.377	1								
Proanthocyadin	−0.617**	−0.61**	−0.299	−0.278	1							
Phenylethanol glycosides	0.993**	0.994**	0.71**	0.528	−0.612**	1						
Amino acid	−0.736**	−0.724**	−0.573**	−0.306	0.666**	−0.737**	1					
Protein	−0.91**	−0.904**	−0.704**	−0.48	0.549**	−0.908**	0.845**	1				
Total heavy metal	−0.654**	−0.653**	−0.634**	−0.495	0.552**	−0.693**	0.711**	0.681**	1			
ORAC	0.966**	0.974**	0.731**	0.585**	−0.564**	0.968**	−0.653**	−0.858**	−0.617**	1		
ABTS	0.976**	0.975**	0.741**	0.54**	−0.623**	0.981**	−0.718**	−0.902**	−0.679**	0.959**	1	
DPPH	0.967**	0.97**	0.646**	0.537**	−0.582**	0.974**	−0.664**	−0.904**	−0.62**	0.965**	0.96**	1

Notes: The "**" indicates a significant correlation at the 0.01 level.

3.4. Cluster analysis

Based on the average value of the quality indexes of different samples of different varieties of Cistanches herba [30], the clustering analysis of Cistanches herba materials samples was carried out, and the results were shown in **Figure 13**. From **Figure 13**, it can be seen that when the distance is 12.5, Cistanches herba medicinal materials samples are well classified into two categories, indicating that there is a very obvious gap between different varieties of Cistanche, and the varieties of Cistanches herba medicinal materials can be roughly judged according to the test results.

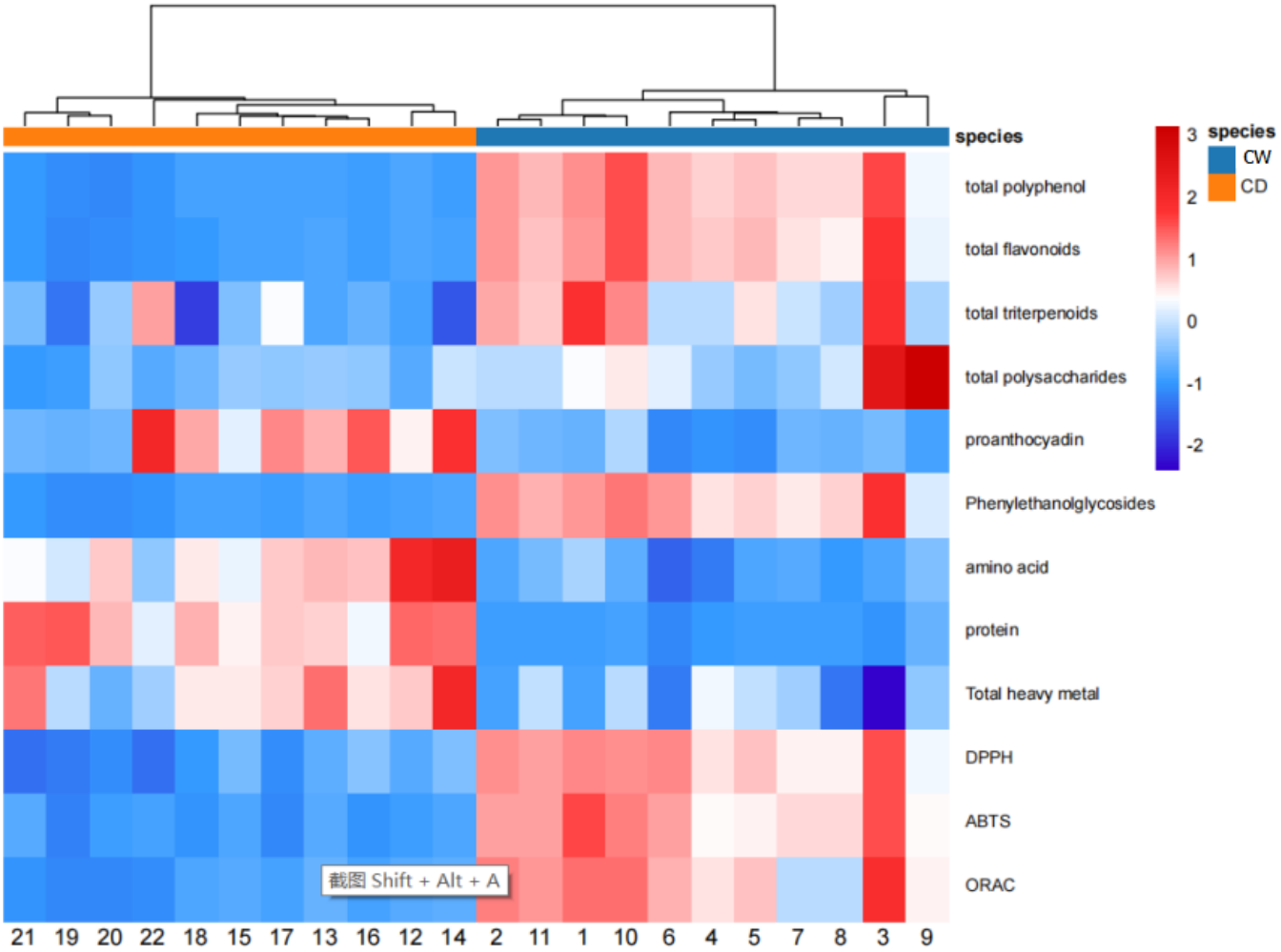

Figure 13. The result of HCA.

3.5. Principal component analysis and comprehensive quality evaluation

The principal component analysis of each index was carried out, and the principal components were extracted according to the eigenvalue and contribution rate of each principal component, which could improve the efficiency and reliability of the quality evaluation of Cistanches herba [31]. The variance contribution rate of each principal component is shown in **Table 5**. It can be seen from **Table 5** that the eigenvalues of the four principal components extracted are all > 0.5, and the

cumulative contribution rate of variance is 94.15%, indicating that the four principal components extracted can effectively represent most of the information of all indicators and comprehensively reflect the quality characteristics of Cistanches herba.

The principal component factor is rotated, and the load value can reflect the relationship between the principal component factor and the indicators. The results are shown in **Figure 14** and **Table 6**. It can be seen from **Figure 14** and **Table 6** that PC1 is mainly related to total polyphenols, total flavonoids, phenylethanoid glycosides and protein content. PC2 was mainly related to the content of total polysaccharides and proanthocyanidins, and PC3 was mainly related to the content of proanthocyanidins, total polysaccharides and total triterpenes. PC4 is mainly related to the content of total polyphenols, total flavonoids and total triterpenoids.

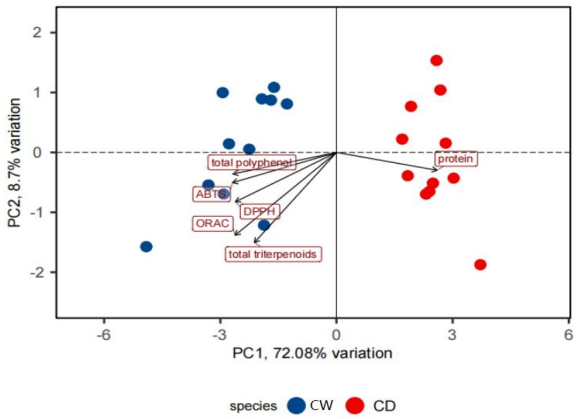

Figure 14. The diagram of PCA.

Table 5. Percentage of variance of principal components.

Principal Component	Eigenvalue	Variance contribution rate/%	Accumulative contribution rate/%
1	6.393	72.08	72.08
2	0.837	8.70	80.78
3	0.676	7.51	88.29
4	0.527	5.86	94.15

Table 6. Vector load coefficient of each principal component factor.

Variable	1	2	3	4
Total olyphenol	0.961	0.035	0.079	0.241
Total flavonoids	0.960	0.059	0.082	0.238
Total triterpenoids	0.768	0.238	0.427	−0.243
Total polysaccharides	0.576	0.614	−0.520	−0.004
Proanthocyadin	−0.682	0.529	0.408	−0.069
Phenylethanol glycosides	0.965	0.043	0.067	0.199
Amino acid	−0.843	0.343	−0.022	0.186
Protein	−0.939	0.015	−0.134	−0.065
Total heavy metal	−0.799	0.006	0.141	0.52

The antioxidant capacity of the medicinal material needs to rely on the active substances contained in it. In order to explore the antioxidant capacity of the medicinal material, the partial least squares method was used to explore the antioxidant capacity of the medicinal material and the content of the active substances contained in the medicinal material [32]. The relationship between the antioxidant capacity and the content of active substances in Cistanches herba is as follows:

DPPH free radical scavenging activity = 0.693 × X1 + 0.281 × X2 − 0.033 × X3 − 2.314 × X4 + 0.626 × X5 − 0.372 × X6 − 0.866 × X7 − 0.118 × X8 + 4.61 × X9 + 9.128;

ABTS free radical scavenging activity = 1.267 × X1 + 0.503 × X2 + 0.187 × X3 + 3.169 × X4 + 0.994 × X5 − 17.035 × X6 − 0.817 × X7 − 0.097 × X8 + 4.404 × X9 + 1.489;

Superoxide radical scavenging activity index ORAC = 1803.445 × X1 + 706.334 × X2 + 1253.24 × X3 + 6081.999 × X4 + 1222.102 × X5 − 9588.69 × X6 − 719.575 × X7 + 819.644 × X8 + 6097.07 × X9 − 37255.

X1: Total polyphenols; X2: Total flavonoids; X3: Total polysaccharide; X4: Total triterpenes; X5: Phenylethanoid glycosides; X6: Proanthocyanidins; X7: Protein; X8: Heavy metals; X9: Amino acids.

4. Discussion

In this paper, the bioactive substances and antioxidant activities of CW and CD were compared and analyzed. The results showed that the quality and antioxidant activity of different varieties of Cistanches herba were significantly different. The contents of total polyphenols, total flavonoids, total triterpenes, total polysaccharides and phenylethanoid glycosides in CW were higher than those in CD. The contents of proanthocyanidins, amino acids, proteins and heavy metals in CD were higher than those in CW. The ABTS and DPPH free radical scavenging activity and superoxide radical scavenging activity index ORAC of CW were higher than those of CD. Cluster analysis could divide 22 batches of medicinal materials into 2 categories. Four principal components were extracted by principal component analysis, and the cumulative contribution rate of variance was 94.15%. The relationship equation between antioxidant activity and bioactive substances was constructed, which provided methods and means for the quality prediction and analysis of medicinal materials. The results of this study will provide basic data and reference for quality evaluation, product development and standardization of Cistanches herba in China.

Author contributions: Conceptualization, XL; methodology, QM and ZH; validation, HS; formal analysis, YH; investigation, JZ; data curation, YZ; writing—original draft preparation, XL; writing—review and editing, QM; visualization, QM; supervision, QL; project administration, QY. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest: The authors declare no conflict of interest.

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