Analysis of the Total Flavonoid Content in Northern and Southern Diverse Wormwood Herb and Different Concoction Products

Kaicheng Yang, Dong Chen, Jingwei Gao, Ming Fan, Yun Zhuang*

Jilin Agricultural Science and Technology University, Jilin 132109, China

*corresponding author.

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Abstract: Objective: To investigate the relationship between the concoction of northern and southern Diverse Wormwood Herb and its total flavonoid content of active ingredients. Methods: Spectrophotometric method. Results: The total flavonoid content in the southern Diverse Wormwood Herb and its concoction products were in the following order: vinegar sizzling > fried yellow > fried charcoal > boiling > cutting > wine steaming; while the total flavonoid content in the northern Diverse Wormwood Herb and its concoction products were in the following order: wine steaming > fried yellow > vinegar sizzling > fried charcoal > boiling > cutting. Conclusion: The flavonoid content of the raw and concoction products of Northern Diverse Wormwood Herbo was better than that of South Diverse Wormwood Herbo, and the flavonoid content of South Diverse Wormwood Herbo increased significantly after vinegar sizzling and fried yellow compared with the raw products. The flavonoid content of northern Diverse Wormwood Herb was significantly increased compared with the raw product after wine steaming and frying.

1. Introduction

Diverse Wormwood Herb is the dried above-ground part of Artemisia anomala S. Moore, family Asteraceae, or Siphonostegia chionensis Benth, family Xuan Shen [1]. In the Tang Materia Medica, it is recorded that Diverse Wormwood Herbo is a common herb used in Chinese medicine to invigorate blood and stop bleeding in clinical practice. Southern Diverse Wormwood Herb Qi Artemisia has the efficacy of healing wounds and stopping bleeding, breaking blood and opening the meridians, eliminating food stagnation, awakening the spleen and opening the stomach [2]. It is mainly produced in the south in Guangxi and Fujian. It has significant curative effects on inflammatory diseases such as acute jaundice, pharyngitis, chronic bronchitis, tonsillitis, and is particularly effective in the treatment of liver disease, as well as in the treatment of malaria, external use for conjunctiva, sores, eczema, and often for bleeding from traumatic injuries. NorthernLiu Yao Yin Xing Cao has no blood-breaking, anti-distension, has the effect of clearing heat and dampness, and also activates blood circulation and dispels blood stasis, and is mainly used for the treatment of jaundice, urinary disorders, painful bruises from falls, bloody dysentery and excessive leucorrhea [3]. In clinical application, in addition to the raw product, different concoctions are applied, for example, the Chinese Pharmacopoeia records that it is cut off and dried in the sun, in the Lei Gong Concoction Theory it is steamed in wine, in the Bo Ji of the Song Dynasty it is fried, and in the Pu Ji Fang it is said to be fried to treat cold pain in the abdomen, and in the Bai Wen the concoction method for it is boiled. Modern pharmacological studies have shown that the flavonoids present in large quantities in Diverse Wormwood Herb have anti-inflammatory, anti-platelet aggregation, painful bouts, and hepatoprotective effects. In order to explore the significance of the concoction of Northern and South Diverse Wormwood Herb, we selected the active ingredient flavonoids in Northern and South Diverse Wormwood Herb as indicators to determine the changes in the content of Northern and South Diverse Wormwood Herb and different concoction products.

2. Apparatus, drugs and reagents

752 UV-visible spectrophotometer (Shanghai Aoppler Instruments Co., Ltd.). The control rutin

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was purchased from China Hefei Bomei Biological Company. The rest of reagents were Analytical reagent.

The raw product Diverse Wormwood Herb was identified by Prof. Ma Yao of the Institute of Traditional Chinese Medicine as the dried above-ground parts of Artemisia chrysanthemi (South) and Yin Xing Cao (Northern) of the family Asteraceae.

3. Determination of the total flavonoid content in diverse wormwood herb and different concoction products

3.1 Concoction

1) Cutting. After the sample was dried, it was cut into 10-15mm pieces.
2) Charcoal frying. Heat the cleaned sample with martial fire until its surface color turns into burnt black and a slight smoke comes out, take it out and let it cool.
3) Boiling. The net selected samples are soaked and boiled until there is no dry heart inside and removed, and then cooled.
4) Vinegar sizzling. Add the net selected samples with appropriate amount of rice vinegar, smother and moisten, wait until the vinegar is absorbed by the herbs, fry dry with civil fire, remove and cool.
5) Steaming with wine. Add the selected samples to the appropriate amount of yellow wine, smother and moisten until the yellow wine is absorbed, place the samples in a steamer, steam for 30min, remove and cool.
6) Frying. Heat the selected samples over a moderate fire until the color is deepened, remove, and let cool.

3.2 Sample solution preparation

Take about 2g of each of the raw Diverse Wormwood Herb and each of the concoction products, weigh them precisely and wrap them with filter paper. Put them in the Soxhlet extractor and refluxed with 80% ethanol for 3h to extract all the flavonoids. Wash with a small amount of 80% ethanol for several times, and incorporate into the extraction solution, then quantitatively transfer into a 100ml volumetric flask, add 80% ethanol to the scale, shake well, that is, the sample solution.

3.3 Determination of total flavonoid content

3.3.1 Preparation of standard curve

0.0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ml of rutin 80% ethanol control solution (0.104 mg-ml-1) was taken into a 200 mL volumetric flask, and 5 mL of each extract was put into a 50 mL volumetric flask for fixing [4]. Then 2 mL of each was placed in a 25 mL volumetric flask, 1.5 mL of 5% sodium nitrite solution was added and shaken well for 6 min, then 1.5 mL of 10% aluminum nitrate solution was added and shaken well for 6 min, and then 10 mL of 1 mol/L NaOH solution was added and shaken well, fixed, shaken well, and placed for 15-20 min, and the absorbance was measured at a wavelength of 510 nm .It can be seen in Figure 1 that a linear regression of concentration on absorbance gave y=0.1102x-0.1004. R2=0.996.

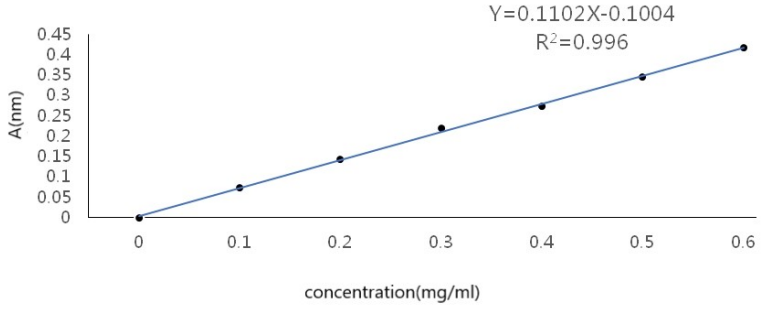

Figure 1 Standard Curve

3.3.2 Determination of content

The absorbance was measured according to the method under standard curve preparation, and the total flavonoid concentration in each dilution was derived from the regression equation and then the percentage content was calculated. The results are shown in Table 1.

3.3.2.1 Determination of the total flavonoid content of southern Diverse Wormwood Herb and its concoction products

Table 1 Total flavonoid content of South Diverse Wormwood Herb and its concoction

Artemisia annua	A(510nm)	Flavonoid mass (mg)	Percentage content (%)
Chopped	0.047	66.88	3.344
Fried with charcoal	0.050	68.24	3.412
Boiled	0.048	67.33	3.367
Sizzling with vinegar	0.064	74.74	3.737
Steamed in wine	0.046	66.58	3.329
Stir-fried yellow	0.054	69.90	3.495

As shown in Table 1, the total flavonoid contents of southern Diverse Wormwood Herb were in the order of vinegar sizzling > fried yellow > fried charcoal > boiled > cut > wine steaming after different concoction methods.

Table 2 Multiple comparisons of the total flavonoid content of South Diverse Wormwood Herb

Concoction method	Mean	F(0.05)	F(0.01)
Chopped	66.88	c	C
Fried with charcoal	68.24	c	C
Boiled	67.33	c	C
Sizzling with vinegar	74.74	a	A
Steamed in wine	66.58	c	C
Stir-fried yellow	69.90	b	B

From Table 2, it can be seen that the flavonoid content of South Diverse Wormwood Herb is higher after vinegar sizzling and fried yellow, and there is a significant difference between the raw product and other concoction methods.

3.3.2.2 Determination of total flavonoid content in Northern Diverse Wormwood Herb and concoction products

Table 3 Total flavonoid content of northern Diverse Wormwood Herb and concoction products

Siphonostegia chinensis benth	A(510nm)	Flavonoid mass (mg)	Percentage content (%)
Chopped	0.057	71.42	3.571
Fried with charcoal	0.060	72.93	3.647
Boiled	0.059	72.17	3.609
Sizzling with vinegar	0.061	73.08	3.654
Steamed in wine	0.072	78.37	3.919
Stir-fried yellow	0.066	75.35	3.768

Table 3 shows that the total flavonoid content of Northern Diverse Wormwood Herb and each concoction is in the following order: steamed in wine > fried in yellow > sizzled in vinegar > fried

in charcoal > boiled > cut, and the total flavonoid content of Northern Diverse Wormwood Herb is higher after steamed in wine and fried in yellow.

Table 4 Analysis of variance of total flavonoid content in different concoctions of Northern Diverse Wormwood Herb

Concoction method	Average	F(0.05)	F(0.01)
Chopped	71.42	c	C
Fried with charcoal	72.93	c	C
Boiled	72.17	c	C
Sizzling with vinegar	73.08	c	C
Steamed in wine	78.37	a	A
Stir-fried yellow	75.35	b	B

From Table 4, it can be seen that the flavonoid content of Northern Diverse Wormwood Herb Siphonostegia chinensis Benth is higher after wine steaming and fried yellow, and there is a significant difference between the raw product and other concoctions.

3.3.3 Precision examination

Table 5 Precision determination

Frequency	1	2	3	4	5	6	Average	RSD(%)
A(nm)	0.051	0.052	0.048	0.047	0.046	0.052	0.049	0.27

The absorbance values were measured at 510 nm by taking the extracts of the cut sections of southern Diverse Wormwood Herb, and the results are shown in Table 5. The relative standard deviation of the experiment was 0.27%, which indicated that the precision of the instrument used in the experiment was good and the experimental data had high confidence.

3.3.4 Stability study

The absorbance at 510 nm was measured at 10 min, 20 min, 30 min, 40 min, 50 min and 60 min, and the results were shown in Table 6.

Table 6 Stability experiments

Time（min）	10	20	30	40	50	60	Average	RSD(%)
A(nm)	0.046	0.048	0.048	0.050	0.045	0.050	0.048	0.21

3.*3.5 Repeatability examination*

After repeating the experiment three times, the average value of flavonoid content after each concoction method and RSD value were obtained, and the results were shown in Table 7, which indicated that the method was well reproducible.

Table 7 Repeatability experiments of Southern Diverse Wormwood Herb

Artemisia chinensis	Mean value of flavonoid content	RSD(%)
Chopped	66.88	1.35%
Fried with charcoal	68.24	0.66%
Boiled	67.33	1.17%
Sizzling with vinegar	74.74	0.93%
Steamed in wine	66.58	2.19%
Stir-fried yellow	69.90	1.99%

4. Summary and discussion

In this experiment, the flavonoid content of the raw and most of the concoction products of Northern Diverse Wormwood Herb was better than that of South Diverse Wormwood Herb. We speculate that because of the inferior efficacy of the southern Diverse Wormwood Herbo, the southern Diverse Wormwood Herbo was not included in the pharmacopoeia.

Now the pharmacopoeia recorded in the Diverse Wormwood Herb concoction method is only cut and dried, and on the experimental results and data analysis, the Northern Diverse Wormwood Herb after wine steaming, fried yellow and other methods of concoction, its extracted total flavonoid content is significantly higher than the raw product, into the medicine using wine steaming and other concoction methods, can better play the medicinal effect.

The highest flavonoid content of Artemisia annua South after vinegar sizzling, if you want to treat liver-related diseases, you can use the vinegar sizzling method when adding the medicine, and vinegar enters the liver meridian. Some studies have shown that flavonoids in liver can affect the activity of drug metabolizing enzymes to some extent, and reasonable application will have better therapeutic effect.

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