Study on Antioxidant Activity of *Anthemis nobilis* Extract in Liquid

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Abstract. The chamomile *Anthemis nobilis* dried flowers was selected as experimental material, and the active ingredients of *Anthemis nobilis* were extracted with water to determine the ability to remove DPPH and study the antioxidant capacity of *Anthemis nobilis* aqueous extracts. Single factor variable method, orthogonal experimental method and response surface method were used to select the optimum time, temperature and material/liquid ratio of chamomile. It achieves the maximum DPPH clearance. The experimental results showed that the time was 140 min. Temperature is set to 30℃; when the ratio of feed to liquid was set at 1:20, the DPPH clearance rate of the effective components in *Anthemis nobilis* water immersion solution reached a maximum of 96.79%.

1. Introduction
Chamomile is a plant belonging to the Asteraceae family, and its origin is in Europe. Studies have shown that many diseases are related to excess free radicals[1]. There are many antioxidant substances in nature, such as bioflavonoids, vitamin E, vitamin C, plant active alkalis, carotenes, xanthones, coenzyme Q10, tea polyphenols. The function evaluation of chamomile adopts in vitro evaluation methods, such as the oil resistance evaluation of antioxidant capacity[2], the determination of the ability to remove DPPH, the determination of the reducing ability, and the LOX enzyme inhibition test. The research on the antioxidant effect of the active ingredients in *Anthemis nobilis* extract has provided effective value for the commercial use of *Anthemis nobilis* tea [3]. The single factor test of *Anthemis nobilis* extract to scavenge DPPH free radicals; the orthogonal test of *Anthemis nobilis* extract to scavenge[4] DPPH free radicals; the response surface test of *Anthemis nobilis* extract to scavenge DPPH free radicals. The best time, best material-to-liquid ratio and best temperature combination were found through the three-factor experiment, and then the maximum scavenging rate of the *Anthemis nobilis* extract on DPPH free radicals was obtained[5].

2. Materials and Methods

2.1. Test material
The dried *Anthemis nobilis* flowers. Test reagent: DPPH (Hydrazyl, 2, 2-diphenyl-1-(2, 4, 6-trinitrophenyl) - (9Cl)): absolute ethanol (analytical purity). DPPH is a stable free radical, dark purple...
prism crystal. Because DPPH radical has a strong absorption peak in the center of 300-500nm, after adding the test sample, the solution appears dark purple, and becomes colorless or light yellow after neutralization with the solution. Using this feature, you can intuitively detect the progress of the reaction. By recording the change in absorbance at 520 nm in DPPH, you can get the number of initial free radicals[6]. The free radical scavenger is present, it will gradually disappear due to its pairing with a single electron, and the color of the reagent will become lighter. The degree of discoloration of the scavenger is quantitatively related to the number of electrons received, and a spectrophotometer can be used for rapid quantitative analysis. Using DPPH this property, the anti-oxidation test of the extract of *Anthemis nobilis* flowers was carried out.

2.2. Test method
The water immersion extraction method can directly use the raw liquid produced by the water immersion extraction method in the experiment. Compared with other methods, other methods are simple and efficient, easy to operate, and can achieve the purpose well. Three test methods were used in this test: single factor test, orthogonal test and response surface method test. Combining Box-Behnken design on the basis of single factor experiment and orthogonal experiment, the experiment data is analyzed through Design Expert 10.

2.2.1. Single-factor test of DPPH free radical scavenging by *Anthemis nobilis* extract
Select two factors that significantly affect the extraction of raw liquid by water immersion method, and select 5 levels for each factor to test, namely temperature and time for single factor test, and investigate the effect of each factor on the proposed *Anthemis nobilis* extract. The anti-oxidant effect of the stock solution. The time is set to 60 min, 90 min, 120 min, 150 min, 180 min, the temperature is set to 10℃, 40℃, 70℃, and 90℃.

2.2.2. Orthogonal test of *Anthemis nobilis* extract to scavenge DPPH free radicals
On the basis of the single-factor test of the extract of *Anthemis nobilis* to scavenge DPPH free radicals, taking temperature, time and material-to-liquid ratio as the influencing factors, and taking the scavenging rate of DPPH free radicals as the inspection index, an orthogonal test of 3 factors and 5 levels was carried out. The factors and levels are shown in Table 1.

| Level | A. Temp / ℃ | B. Liquid ratio | C. Time / min |
|-------|-------------|-----------------|--------------|
| 1     | 10          | 1:10            | 60           |
| 2     | 25          | 1:20            | 90           |
| 3     | 40          | 1:30            | 120          |
| 4     | 55          | 1:40            | 150          |
| 5     | 70          | 1:50            | 180          |

2.2.3. The response surface test of the extract of *Anthemis nobilis* in scavenging DPPH free radicals
On the basis of the single-factor test for removing DPPH free radicals and the orthogonal test for removing DPPH free radicals by the extract, the response surface test method is selected, and 3 factors (time min, temperature ℃, material-to-liquid ratio), 3 levels, and 3 levels are taken into consideration for a total of 17 tests. Point surface analysis test, response surface analysis factors and level codes are shown in Table 2.

| Code | A: Time | B: Temperature | C: Material-liquid ratio |
|------|---------|----------------|-------------------------|
| -1   | 120     | 30             | 1: 15                   |
| 0    | 150     | 40             | 1: 20                   |
| 1    | 180     | 50             | 1: 25                   |
Configuration 0.1 mmol/L of DPPH ethanol solution, taking 0.0394g DPPH, with 100mL of absolute ethanol in a volumetric flask to volume dubbed the mother liquor, using 10mL of absolute ethanol in 100mL of volumetric flask set Rong, ready to use, keep away from light. Add 2mL of the test sample solution and 2mL of DPPH solution to the same test tube, shake well, and measure the absorbance after standing in the dark at room temperature for 30 min. At the same time, determine the absorbance. After mixing 2mL of DPPH solution and 2mL of absolute ethanol. And the absorbance value of 2mL test sample solution mixed with 2mL absolute ethanol.

3. Results and discussion

3.1. Single factor test results

3.1.1. The influence of different temperatures on the antioxidation of Anthemis nobilis extract liquid
By setting the time for 120min, the material-to-liquid ratio is 1:30, the temperature is 10℃, 40℃, 70℃, and 90℃. The influence of different temperatures on the anti-oxidation of the chamomile extract liquid is shown in Fig 1.

3.1.2. The effect of different time on the anti-oxidation of Anthemis nobilis extracts liquid
By setting the temperature to 40℃, the material-to-liquid ratio is 1:30, and the time is 60min, 90min, 120min, 150min, and 180min, the effect of different time on the anti-oxidation outside of the chamomile extract liquid is shown in Fig 2. The effects of time and temperature on the scavenging of DPPH free radicals in vitro by night immersion of chamomile were investigated by single factor experiments. It can be seen that the temperature is 30℃-50℃ and the time is 120 min-180 min under the conditions of single factor test. The liquid has a higher scavenging rate of DPPH free radicals.

3.1.3. Orthogonal test results
On the basis of single factor performance, orthogonal experiment was used to analyze the influence of different time, temperature and liquid-to-material ratio on the anti-oxidation of Anthemis nobilis extraction liquid. The orthogonal factor level and result analysis are shown in Table 3. The analysis of variance of the orthogonal test results is shown in Table 4.
Table 3. Variances analysis of orthogonal experiments results

|            | Sum of square | D. of freedom | Mean square | F-Value | P-Value |
|------------|---------------|---------------|-------------|---------|---------|
| Temp/℃     | 50.000        | 24            | 2.083       | 4.146   | 0.029   |
| Liquid ratio| 53.840        | 24            | 2.243       | 0.251   | 0.024   |
| Time/min    | 50.000        | 24            | 2.083       | 4.156   | 0.029   |
| Deviation   | 48.420        | 24            | 1.892       | -       | -       |
| Total       | 202.26        | 96            | -           | -       | -       |

The order of the degree of influence of various factors on the anti-oxidation outside the chamomile extraction liquid is liquid-to-material ratio>time>temperature. The best combination of anti-oxidant removal rate is A3 B2 C4, that is, the temperature is in 40℃, the ratio of liquid to material is 1:20, and the time is 150 min (in Table 4).

Table 4. Orthogonal test and analysis of results

| №: | Temp/℃ | Liquid ratio | Time/ min | Purge | №: | Temp/℃ | Liquid ratio | Time/ min | Purge |
|----|---------|--------------|-----------|-------|----|---------|--------------|-----------|-------|
| 1  | 10      | 1:40         | 60        | 0.8952| 17 | 55      | 1:20        | 90        | 0.9280|
| 2  | 10      | 1:50         | 90        | 0.8938| 18 | 55      | 1:30        | 120       | 0.9269|
| 3  | 10      | 1:10         | 120       | 0.9399| 19 | 55      | 1:40        | 150       | 0.9342|
| 4  | 10      | 1:20         | 150       | 0.9086| 20 | 55      | 1:50        | 180       | 0.9410|
| 5  | 10      | 1:30         | 180       | 0.9122| 21 | 70      | 1:20        | 60        | 0.9582|
| 6  | 25      | 1:50         | 90        | 0.9078| 22 | 70      | 1:30        | 90        | 0.9540|
| 7  | 25      | 1:10         | 90        | 0.9201| 23 | 70      | 1:40        | 120       | 0.8781|
| 8  | 25      | 1:20         | 120       | 0.9146| 24 | 70      | 1:50        | 150       | 0.9417|
| 9  | 25      | 1:30         | 150       | 0.8983| 25 | 70      | 1:10        | 180       | 0.8194|
| 10 | 25      | 1:40         | 180       | 0.9145| 26 | 4.5497 | 4.4769     | 4.5501    | 0.9278|
| 11 | 40      | 1:30         | 60        | 0.9202| 12 | 4.5553 | 4.6433     | 4.6097    | 0.9410|
| 12 | 40      | 1:40         | 90        | 0.9138| 13 | 4.6153 | 4.6116     | 4.5781    | 0.9410|
| 13 | 40      | 1:50         | 120       | 0.9186| 14 | 4.5988 | 4.5358     | 4.6115    | 0.9278|
| 14 | 40      | 1:10         | 150       | 0.9288| 15 | 4.5513 | 4.6028     | 4.5211    | 0.9410|
| 15 | 40      | 1:20         | 180       | 0.9339| 16 | 0.0131 | 0.0333     | 0.0181    | 0.9410|
| 16 | 55      | 1:10         | 60        | 0.8688|  |      |            |           | -     |

3.1.4. Response surface test results

Combining the Box-Behnken design on the basis of single factor experiment and orthogonal experiment, taking time, temperature and material-to-liquid ratio as influencing factors, taking DPPH radical scavenging rate as the response value, and designing a 3-factor 3-level response surface test. Response surfaces the test design and test results are shown in Table 5.

Table 5. Design and results of Box-Behnken experiments

| №: | Temp/℃ | Liquid ratio | Time/ min | DPPH purge | №: | Temp/℃ | Liquid ratio | Time/ min | DPPH purge |
|----|---------|--------------|-----------|------------|----|---------|--------------|-----------|------------|
| 1  | 150     | 40           | 1:20      | 0.9502     | 10 | 150     | 30           | 1:15      | 0.9231     |
| 2  | 150     | 40           | 1:20      | 0.9487     | 11 | 120     | 40           | 1:25      | 0.9196     |
| 3  | 180     | 50           | 1:20      | 0.9325     | 12 | 150     | 50           | 1:15      | 0.926      |
| 4  | 120     | 40           | 1:15      | 0.9117     | 13 | 150     | 40           | 1:20      | 0.9205     |
| 5  | 120     | 30           | 1:20      | 0.9248     | 14 | 150     | 40           | 1:20      | 0.9479     |
| 6  | 180     | 30           | 1:20      | 0.9237     | 15 | 150     | 40           | 1:20      | 0.9456     |
| 7  | 180     | 40           | 1:15      | 0.8737     | 16 | 180     | 40           | 1:25      | 0.9001     |
| 8  | 150     | 50           | 1:25      | 0.9005     | 17 | 120     | 50           | 1:20      | 0.9078     |
| 9  | 150     | 30           | 1:25      | 0.9261     |  |      |             |           |            |
3.1.5. The establishment of regression model and analysis of variance

Design Expert 10 to test software regression analysis of the experimental data in Table 3-3 regression and analysis of variance, analysis of variance in Table 6, and the resulting multivariate quadratic regression equation DPPH radical scavenging of:

\[ CDPPH = 0.94 - 4.237A - 3.862B - 1.475C + 6.450AB - 4.625AC + 7.125BC - 0.019A^2 - 1.365B^2 - 0.022C^2. \]

Table 6. Variance analysis of regression model

| Source      | sum of square | Degree of freedom | Mean square | F      | P value | Significance |
|-------------|---------------|-------------------|-------------|-------|---------|--------------|
| model       | 4.605         | 9                 | 5.117       | 1.91  | <0.0001 | -            |
| A-time      | 1.437         | 1                 | 1.437       | 0.54  | 0.4874  | -            |
| B-temp      | 1.194         | 1                 | 1.194       | 0.45  | 0.5255  | -            |
| C-L/material| 1.740         | 1                 | 1.470       | 0.065 | 0.8060  | -            |
| AB          | 1.664         | 1                 | 1.664       | 0.62  | 0.4561  | -            |
| AC          | 8.556         | 1                 | 8.556       | 0.32  | 0.5893  | -            |
| BC          | 2.031         | 1                 | 2.031       | 0.76  | 0.4125  | -            |
| A2          | 1.522         | 1                 | 1.522       | 5.69  | 0.0485  | *            |
| B2          | 7.845         | 1                 | 7.845       | 0.029 | 0.8689  | -            |
| C2          | 2.092         | 1                 | 2.092       | 7.82  | 0.0266  | *            |
| Residual    | 1.872         | 7                 | 2.675       | -     | -       | -            |
| Lack of fit | 1.252         | 3                 | 4.172       | 2.69  | 0.1815  | -            |
| Pure error  | 6.205         | 4                 | 1.551       | -     | -       | -            |
| sum         | 6.477         | 16                | -           | -     | -       | -            |

Note: "***" means the difference is extremely significant (P<0.01); "**" means the difference is significant (P<0.05)

It can be seen from Table 6 that the quadratic regression model established by the response surface method is extremely significant (P<0.0001), and the lack of fit term is not significant (P=0.1815>0.05), indicating that the obtained model has a good degree of fit, and the lack of fit term interferes with the equation. The degree is small, indicating that the regression model can predict the response value well. Among them, the quadratic terms A2 and C2 are significant (P<0.05). The order of the degree of influence of each factor on the anti-oxidation outside of the chamomile extraction liquid is liquid-to-material ratio (C)>time (A)>temperature (B).

The interaction of time, temperature, and material-to-liquid ratio three factors to prevent oxidation outside the Anthemis nobilis extract liquid the response surface and contour results of the impact are shown in Fig 3. The degree of curved surface of the response surface graph indicates the degree of influence on the scavenging rate of DPPH free radicals. The larger degree of curved surface indicates the greater the effect, and vice versa, the smaller; and the contour line feeds back the significant degree of the interaction between the factors, the contour line showing the interaction between the oval represents more significant two factors, it indicates the circular contour lines showing no significant interaction between the two factors.
Fig 3. Response surface and contour lines of the interaction of time, temperature, and material-to-liquid ratio on the removal of DPPH

The DPPH clearance rate changes with the performance of the solid-liquid ratio increased and then decline; time on DPPH clearance is increased and then decreased (in Fig. 3). Temperature Effect on DPPH clearance tends to be almost constant. The maximum contour of the liquid-to-material ratio and time interaction surface tends to be elliptical, and the interaction is obvious; the time-temperature interaction surface is relatively flat, and the contour tends to be elliptical, and the interaction is obvious to the liquid-to-material ratio and time; the liquid-to-material ratio interacts with temperature. The curved surface is gentle, the contour lines tend to be elliptical, and the interaction is obvious relative to the liquid-to-material ratio and time.

4. Conclusions
The main research content of this paper is the determination of antioxidant indexes outside the *Anthemis nobilis* extract liquid, and the conclusions are as follows: (1) In the single factor test of the *Anthemis nobilis* extract liquid against DPPH free radicals, the temperature is 30℃-50℃. When the time is 120 min -180 min, and the material-to-liquid ratio is 1:15-1:25, it has the greatest impact on the antioxidation of the chamomile extract liquid. (2) Based on the single-factor test, the orthogonal test of the three-factor five-level *Anthemis nobilis* extract against the DPPH free radical was carried out, and it was found that the temperature was 40℃, the liquid-to-battery ratio was 1:20, and the time was 150 min. The theoretical clearance rate obtained by this combination can be the maximum. (3) On the basis of orthogonal experiment, the response surface test of *Anthemis nobilis* extract to DPPH free radical was carried out. The response surface test further refined the optimal time, temperature, and material-to-liquid ratio. The test results show that when the time is 140 min, the temperature is set to 30℃, and the material-to-liquid ratio is set to 1:20; the chamomile extract has a maximum DPPH clearance rate of 96.79%.

In this paper, the anti-oxidation research on the extract of dried flowers of *Anthemis nobilis* is carried out. There are many methods for extracting the active ingredients of chamomile for anti-oxidation determination, such as water extraction[7], ultrasonic assisted extraction, alkaline water extraction and alcohol extraction[8]. Through the analysis of Design Expert 10 software, the optimal combination of time, temperature, and material-to-liquid ratio is more detailed. A verification test was carried out. The test results showed that when the time was 140 min, the temperature was set to 30℃, and the material-to-liquid ratio was set to 1:20, the DPPH radical scavenging rate of *Anthemis nobilis* extract reached the maximum value 96.79%.
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