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Research on the extraction condition of antioxidant active ingredients of bitter tea

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Abstract: *The experiments were conducted with Bittering tea of the Wintergreen family as the raw material to determine the best conditions for the extraction of functional substances from Bittering tea extracts by four factors and five levels of thought. Through the comprehensive analysis of the five indicators, the comprehensive optimal extraction conditions of bitter tea extract were 73℃, 6.31 g/L, 95.6 min, pH=7, at which the DPPH radical scavenging rate was 93.81%, the superoxide anion radical scavenging rate was 66.23%, the hydroxyl radical scavenging rate was 76.51%, the concentration of polyphenols in the extract was 185.30 mg/L, and the concentration of flavonoids was 74.99 mg/L.*

Keywords: *Bitter tea; Optimal extraction conditions; Free radical scavenging*

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I. Introduction

Bitter tea is a kind of substitute tea, which rich in polyphenols, flavonoids, saponins, amino acids and other active substances, with anti-inflammatory, antiseptic, antipruritic, weight loss, blood pressure and other pharmacological functions[1-3]. In the last 20 years, there have been more investigations into the cultivation, production, physical and chemical research and application of bitter tea, but the fine processing, deep processing, management and management of bitter tea, as well as the commercial industrialisation of talent technology is still relatively potential[4-5]. In order to deeply utilize the resources of big leaf bitter tea and enhance its economic value, the qualitative and quantitative analysis of the active substances of bitter tea and the investigation of the physiological capacity should be further determined, and the progress of the research on the development and utilization of the deep processing of big leaf bitter tea should be enhanced[6-8]. Now China's research on the nutritional organs of big-leaf bitter tea is still on the traditional exploration of young leaves, while the research and development of other parts of bitter tea (such as old leaves, bitter tea flowers, bitter tea seeds, seed husks, bitter tea bark, bitter tea roots, etc.) is rarely studied in the relevant literature, and there is a great demand for its development in medicinal uses and other aspects[9]. With the development of the economy and the people's standard of living having improved considerably compared to the past, the demand for excellent beverages has become greater and greater[10]. However, in general, most of the research on bittering tea in China is at the theoretical stage[11]. In this experiment, bittering tea processed from the leaves of bittering tea holly tree was used as raw material to optimise the extraction conditions of bittering by determining the polyphenol and flavonoid content and free radical scavenging ability (including scavenging of DPPH radicals, hydroxyl radicals and superoxide anions). The experiment was carried out to determine the optimum extraction conditions by testing the five indexes of DPPH radical scavenging rate, superoxide anion radical scavenging ability, hydroxyl radical scavenging ability and polyphenol and flavonoid contents of bittering tea extracts at different temperature, concentration, time and pH.

II. Experiment

2.1 Experimental reagents

Bitter tea (purchased from Zibo Tea Factory); distilled water; 0.2 mmol/L ethanol solution of DPPH radical; 0.05 mol/L Tris-HCl, pH 8.2; 2.5 mmol/L o-triphenol solution; 12 mol/L concentrated hydrochloric acid; 6 mmol/L ferrous sulfate; 6 mmol/L hydrogen peroxide; 6 mmol/L salicylic acid solution; 0.1 mol/L Folin-Ciocalteu reagent; saturated sodium carbonate solution; 0.1 mol/L ethanol (30%) solution of aluminium trichloride; pH=3 and pH=4 sulphuric acid solutions; pH=10 and pH=11 sodium hydroxide solutions; pH=1 hydrochloric acid solution; anhydrous ethanol; buffer solution configuration method.

2.2 Apparatus

DL-5-B centrifuge (Shanghai Anting Scientific Instrument Factory); UV-1700 ultraviolet spectrophotometer (Shimadzu, Japan); AUY-220 electronic analytical balance (Shimadzu Testing and Measuring Division); DT2000 electronic analytical balance (Changshu Yio Instruments Co., Ltd.); WMZK-72 electric thermostatic water bath (Shanghai Yuejin Medical ZD type stainless steel distilled water apparatus (Shanghai Guangdi Instruments Co., Ltd.); SHZ-D (Ⅲ) circulating water vacuum pump (Gongyi Yuhua Instruments Co., Ltd.); RRHP-100 type universal high-speed pulverizer (O'Klafbauer Co., Ltd.)

2.3 Assay indicators and methods

2.3.1 Determination of DPPH radical scavenging ability

The 3 mL of DPPH radical in ethanol (0.2 mmol/L) was added to 3 mL of the extract, and the reaction was carried out at room temperature for 30 min in the dark, centrifuged and the OD value was measured at 517 nm.

2.3.2 Determination of superoxide anion radical scavenging ability

An autoxidation method was used for the determination of o-triphenol. The autoxidation of o-benzenetriol occurs under alkaline conditions, resulting in the formation of coloured intermediates and superoxide anion radicals, which have a catalytic effect on the autoxidation. The reaction was terminated immediately with 10 mol/L HCL 0.1 ml (or two drops) in a water bath at 25℃ for 4 min, and the absorbance A1 value was measured at 325 nm. Distilled water was used as a blank instead of the sample and the clearance was calculated according to the following formula.

2.3.3 Determination of hydroxyl radical scavenging ability

Two extracts of 1 mL each and 1 mL of distilled water were taken, making a total of three portions (labelled a, b and c respectively). a and b were extracts and c was distilled water. Add 1 mL of $FeSO_4$ at a concentration of 6 mmol/L and 1 mL of H2O2 at a concentration of 6 mmol/L respectively, mix and leave for 10 min. a add 1 mL of salicylic acid at a concentration of 6 mmol/L, mix well and leave for 30 min, measure the absorbance at a wavelength of 510 nm and record as C1. b add 1 mL of distilled water, mix well and leave for 30 min. After 30 min, the absorbance was measured at a wavelength of 510 nm and recorded as C2; c, 1 mL of salicylic acid at a concentration of 6 mmol/L was added, mixed well and left to stand for 30 min, and the absorbance was measured at a wavelength of 510 nm and recorded as C3.

Hydroxyl radical scavenging rate = [1-(C1-C2)/C3] x 100%

2.3.4 Method for the determination of polyphenol content

The polyphenol content was determined using the Folin-Ciocalteu method with distilled water for zeroing. Mix 1 mL of sample solution with 2.0 mL of Folin-Ciocalteu reagent, let stand for 5 min, add 4.0 mL of saturated Na_2CO_3 solution, react for 1 h at 30℃ in a water bath, cool, and measure the absorbance at 747 nm. The polyphenol content of the sample was expressed as CHA (chlorogenic acid) equivalents.

2.3.5 Determination of flavonoid content

Flavonoid content was determined by the $AlCl_3$ method with 30% ethanol zeroing. 3.0 mL of sample solution was mixed with 3 mL of 0.1 mol/L AlCl3 ethanol solution (30% ethanol) and the reaction was carried out at room temperature for 10 min, and the absorbance at 430 nm was measured. The flavonoid content of the samples was expressed as rutin equivalents.

III. Results and Discussion

3.1 Multi-factor experiments

From the single-factor experiment, it was found that the optimum temperature for the extraction of antioxidant active substances and their antioxidant properties of bitter tea was around 60℃, the optimum concentration was around 6 g/L, the optimum time was around 90 min and the optimum pH=7. The four-factor five-level orthogonal experiment was established as shown in the following table 1.

Table 1 Experimental protocol

Factors	Temperature（℃）	Concentration（g/L）	Time（min）	pH
-2	50	2	30	5
-1	60	4	60	6
0	70	6	90	7
1	80	8	120	8
2	90	10	150	9

3.2 DPPH radical scavenging rate

After recording the DPPH radical scavenging rate data from the 30 sets of experiments, the data were entered into the Design-Expert software to obtain the following different response surfaces.

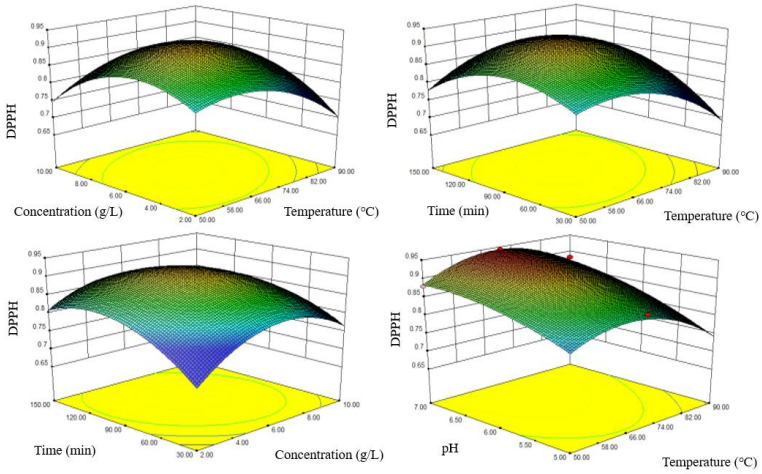

Figure 1. Response surface of DPPH radical scavenging rate data

Summary of this subsection: it can be concluded that the optimum temperature for DPPH radical scavenging by bitter tea was 69°C, the optimum concentration was 5.99 g/L, the optimum time was 90.3 min, the optimum pH was 7 and the maximum scavenging rate was 94.03%.

3.3 Superoxide anion radical scavenging rate

After recording the superoxide anion scavenging rate data from the 30 sets of experiments, the data was entered into Design-Expert software to obtain the following different response surfaces.

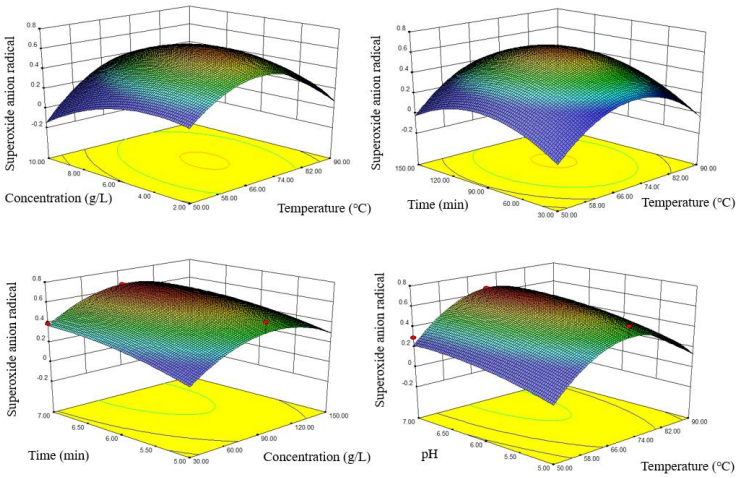

Figure 2. Superoxide anion radical scavenging data response surface

Summary of this subsection: it can be concluded that the optimum temperature for superoxide anion radical scavenging by bitter tea was 72℃, the optimum concentration was 5.76 g/L, the optimum time was 94.1 min, the optimum pH was 6.84 and the maximum scavenging rate of superoxide anion was 66.51%.

3.4 Hydroxyl radical scavenging rates

The hydroxyl radical scavenging rate data from the 30 sets of experiments were entered into Design-Expert software to obtain the following different response surfaces.

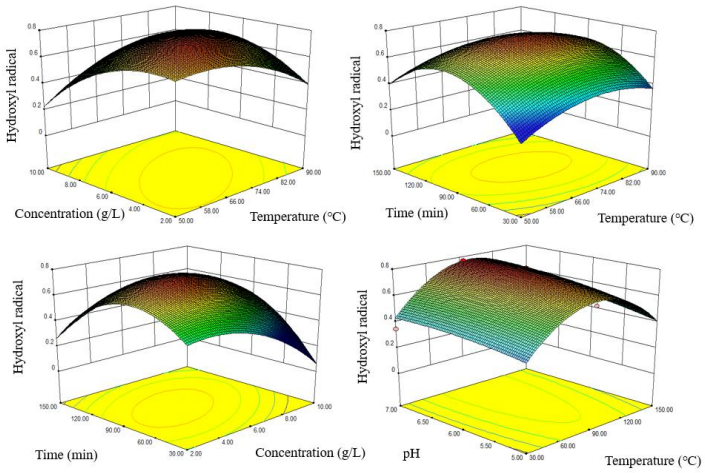

Figure 3. Response surface of hydroxyl radical scavenging rate data

Summary of this subsection: it can be concluded that the optimum temperature for hydroxyl radical scavenging by bitter tea was 77°C, the optimum concentration was 6.38 g/L, the optimum time was 96.7 min, the optimum pH was 7 and the maximum scavenging rate of hydroxyl radicals was 76.94%.

3.5 Determination of polyphenol content

The absorbance of 30 sets of measured polyphenol content was recorded and the data was entered into the Design-Expert software to obtain the following different response surfaces.

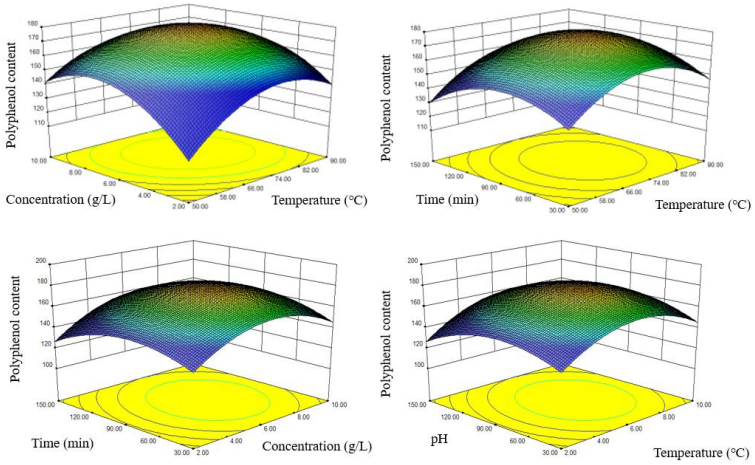

Figure 4. Response surface for polyphenol content data

Subsection Summary: it can be concluded that the optimum temperature for the extraction of polyphenols from bitter tea was 83oC, the optimum concentration was 6.75g/L, the optimum time was 98.8min, the optimum pH was 7 and the maximum concentration of polyphenols in the extract was 187.35mg/L.

3.6 Determination of flavonoid content

The absorbance of 30 sets of measured polyphenol content was recorded and the data was entered into the Design-Expert software to obtain the following different response surfaces.

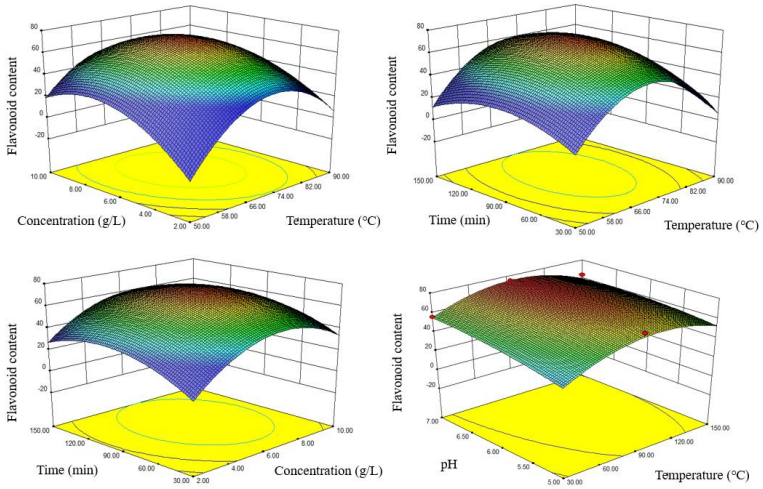

Figure 5. Flavonoid content data response surface

Summary of this subsection: According to Figure 6, it can be concluded that the optimum temperature for the extraction of flavonoids from bitter tea was 71 °C, the optimum concentration was 6.53 g/L, the optimum time was 98.1 min, the optimum pH = 7 and the maximum concentration of flavonoids in the extract was 75.66 mg/L.

3.7 Comprehensive analysis

The following table was obtained by comprehensive analysis of the five assay indicators, thus expecting to obtain the optimum temperature, concentration, time and pH, through the Design-Expert software.

Table 2 Summary of experimental results

	Tempe-ra ture	Concen-tra tion	Time	pH	Corresponding values
DPPHFree radical scavenging rate	69	5.99	90.3	7.00	94.03%
Superoxide anion radical scavenging rate	72	5.76	94.1	6.84	66.51%
Hydroxyl radical scavenging rate	77	6.38	96.7	7.00	76.94%
Polyphenol Extraction	83	6.75	98.8	7.00	187.35mg/L
Flavonoid Extraction	71	6.53	98.1	7.00	75.66mg/L
General	73	6.31	95.6	7.00	

IV. Conclusion

This experiment was carried out to optimize the extraction conditions of bittering by determining the polyphenol and flavonoid contents, free radical scavenging ability (including scavenging of DPPH radicals, hydroxyl radicals and superoxide anions) of bittering tea processed from the leaves of wintergreen tree. The experiment was mainly conducted by the idea of four factors and five levels. Through the comprehensive analysis of the five indicators, the optimum temperature of bittering tea extract was 73oC, the optimum concentration was 6.31g/L, the optimum time was 95.64min, and the optimum pH was 7. At this time, the scavenging rate of DPPH radicals was 93.81%, the scavenging rate of superoxide anion radicals was 66.23%, the scavenging rate of hydroxyl radicals was 76.51%, and the extract The concentration of polyphenols in the extract was 185.30 mg/L and the concentration of flavonoids was 74.99 mg/L. This experiment finally obtained the optimal conditions for the extraction of bitter tea, determined the antioxidant capacity of bitter tea of the family Wintergreenaceae and achieved the purpose of the experiment.

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