Study on Extraction of Polysaccharides from Flos Hibiscus and Its Antioxidation Activity

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Abstract. Polysaccharides were extracted from Flos Hibiscus by hot water extraction technique. Based on the results of single-factor experiments, the effects of extraction temperature, extraction time, mass ratio of solvent to material and extraction times on the extraction yield of polysaccharides were investigated by orthogonal experiment. The clearance effect of polysaccharides from Flos Hibiscus on hydroxyl free radical (·OH) and (O₂⁻) superoxide anion radicals was investigated. Design as follows: extraction temperature 80 °C, extraction time 3h, ratio of solvent to material 30:1ml/g. Under such conditions, the extraction yield of polysaccharide was 5.22%. The results also showed that the order of factor affecting the extraction yield of polysaccharide was extraction temperature > extraction time > ratio of solvent to material. The polysaccharides from Flos Hibiscus had strong scavenging activity against hydroxyl free radicals and superoxide radicals.

1. Introduction

Floss Hibiscus is the dried flower of Hibiscus Syracuse L... Hibiscus Syracuse L. is one of the most widespread garden shrubs in the world. Hibiscus Syracuse L. is a native of China [1]. There are nearly 300 species of hibiscus in the world. It is distributed in the global temperate and sub temperate regions, and is cultivated from the northeast to the south of China [2]. Floss Hibiscus is colorful, and it has a high yield and strong adaptability during the florescence in suitable cultivation environment. Floss Hibiscus is rich in protein, polysaccharide, anthocyanin, polyphenols and other functional ingredients with the effect of anti-bacterial and anti-oxidation. The edible custom of Floss Hibiscus has been preserved in southern Zhejiang, Southern Anhui and Southern Jiangxi. The cooking methods of Floss Hibiscus are various, such as cooked or Fried.

Polysaccharide is a kind of complex and bulky carbohydrate that is condensed and dehydrated by multiple monosaccharides. Polysaccharides can activate immune cells and enhance the immune function of the body. Polysaccharides also have no toxic effect on normal cells, which has developed into an immunotherapy. Polysaccharides are widely used in the food processing industry, which can improve food edible quality and processing characteristics. Polysaccharides also have a good effect on lipid peroxidation and can be used as emulsifier and stabilizer.

Floss Hibiscus is rich in polysaccharide. The researches of Floss Hibiscus are mainly concentrated in anthocyanin, polyphenols and total flavonoids [3-5]. The research and utilization of polysaccharides
from Floss Hibiscus are almost blank. Researches were made on the extraction process of polysaccharides from Floss Hibiscus in this paper, and the clearance effect of polysaccharides from Floss Hibiscus on hydroxyl free radical (·OH) and (O2-) superoxide anion radicals was investigated in order to provide relevant theoretical basis for the in-depth study and utilization of Hibiscus Syracuse L.

2. Materials and Methods

2.1. Materials and Instruments
Floss Hibiscus was picked from the dongoef road of Dezhou in June 2017, it was dried for 24 hours in 35~40 °C drying drum and pulverized.

The main experimental instruments include 5500 UV visible spectrophotometer (Shanghai Analytical Instrument Co., Ltd.), 601A digital super constant temperature water bath (Jinan Growing experimental instrument factory), Hei-Vap Value HL G3 rotary evaporator (German Heidolph company), DZF-6020 vacuum drying oven (Shanghai Boxing Industrial Company), MIKRO 22R high speed refrigerated centrifuge (German HETTICH company), AL204 electronic balance (Mettle Toledo instruments), etc.

2.2. Experimental Method

2.2.1. The extraction method of polysaccharides from Floss Hibiscus. The flowers were crushed into powders which were filtrated by 60 mesh screen. The solar extractor was used to degrease the powders. The powders were weighed and mixed with the deionized water as the extracting agent. The extraction conditions were prepared according to the different extraction conditions (extraction temperature, extraction time, mass ratio of solvent to material and extraction times). With rotating pan evaporation, the filtrate after extraction was merged and concentrated to a certain volume and mixed with ethanol for 24 hours. The centrifugation was precipitated and precipitated with anhydrous ethanol. 1/4 volume of chloroform and n-butane (v: v = 4:1) were added in order to remove most of the proteins, nucleic acids and other substances by violent oscillation 20 min with the separator funnel. The upper layer were centrifuged for 10min in 6000rpm/min. The operation was repeated until the middle layer of sediment had no denaturation protein.

2.2.2. The determination of polysaccharide from Floss Hibiscus content and calculation of extraction yield. The polysaccharide was determined by enthrone sulfuric acid method [6]. With glucose as the standard solution, glucose concentration (mg/ml) was the horizontal coordinate, and the absorbance value (A) was the standard curve of the ordinate. The regression equation was obtained as the following formula: A=2.162C+0.0098(R2=0.9995).

The polysaccharide content from Floss Hibiscus was calculated as the following formula (1):

\[
PC = \left( \frac{c \times N \times V}{M_1} \right) \times 100
\]  

PC: the polysaccharide content from Floss Hibiscus (%)
C: concentration of sample (mg/ml)
N: diluted multiples of sample (ml)
V: volume of sample (ml)
M1: the weight of crude polysaccharides (mg)

The extraction yield of polysaccharide from Floss Hibiscus was calculated as the following formula (2):

\[
F = \left( \frac{M_1}{M_0} \right) \times 100
\]  

2
F: the extraction yield of polysaccharide from Floss Hibiscus (%)
M1: the weight of crude polysaccharides (mg)
M0: the weight of Floss Hibiscus (mg)

2.2.3. Single factor test. (1) The effect of extraction temperature on the extraction yield of polysaccharide
2.0 g of the degreasing powders was precisely weighed in the triangle bottle and added with 50 ml deionized water at 60°C, 70°C, 80°C, 90°C, and 100°C respectively, extracted 1 h and leached 1 time. The filtrate is centrifuged after evaporation, concentration, cooling and removal of impurities. The polysaccharide soluble in 25 ml water in order to determine the content of polysaccharide and determine appropriate extraction temperature.

(2) The effect of extraction time on the extraction yield of polysaccharide
2.0 g of the degreasing powders was precisely weighed in the triangle bottle and added with 50 ml deionized water at 80°C, extracted 1 h, 2h, 3h, 4h, and 5h respectively, leached 1 time. The filtrate is centrifuged after evaporation, concentration, cooling and removal of impurities. The polysaccharide soluble in 25 ml water in order to determine the content of polysaccharide and determine appropriate extraction time.

(3) The effect of mass ratio of solvent to material on the extraction yield of polysaccharide
2.0 g of the degreasing powders was precisely weighed in the triangle bottle and added with 30 ml, 40 ml, 50 ml, 60 ml and 70 ml deionized water respectively, at 80°C, extracted 2 h and leached 1 time. The filtrate is centrifuged after evaporation, concentration, cooling and removal of impurities. The polysaccharide soluble in 25 ml water in order to determine the content of polysaccharide and determine appropriate mass ratio of solvent to material.

(4) The effect of extraction times on the extraction yield of polysaccharide
2.0 g of the degreasing powders was precisely weighed in the triangle bottle and added with 60 ml deionized water at 80°C, extracted 2 h, and leached 1 time and 2 time respectively. The filtrate is centrifuged after evaporation, concentration, cooling and removal of impurities. The polysaccharide soluble in 25 ml water in order to determine the content of polysaccharide and determine appropriate extraction times.

2.2.4. The optimization of the extraction process of polysaccharide from Floss Hibiscus. The effect of extraction temperature, extraction time, mass ratio of solvent to material, extraction times on t on the extraction yield of polysaccharide was studied by single factor test. In order to obtain the optimal extraction conditions, the extraction yield of polysaccharides was orthogonal with the factors of extraction temperature, extraction time and mass ratio of solvent to material. The factors and levels of orthogonal test were shown in Table 1.

Table 1. The factors and levels of orthogonal test

| Levels | Extraction temperature(A) | Extraction time(B) | Mass ratio of solvent to material(C) |
|--------|---------------------------|--------------------|-------------------------------------|
| 1      | 60                        | 2                  | 25:1                                |
| 2      | 70                        | 3                  | 30:1                                |
| 3      | 80                        | 4                  | 35:1                                |

2.2.5. The determination of the antioxidant properties of polysaccharide from Flos Hibiscus. (1) The determination of hydroxyl free radicals scavenging ability of polysaccharide from Flos Hibiscus
According to the Fenton reaction system [7], 2 ml 6mmol/L FeSO4 was mixed with 2 ml 6mmol/L salicylic acid-ethanol, 2 ml different concentration of polysaccharide from Flos Hibiscus respectively, and 2 ml 6mmol/L H2O2. The mixture reacted at 37°C for 1 h. Then the absorbance value was
measured at the wavelength of 510 nm. Standard curve of Vc standard solution was used to calculate the clearance rate of polysaccharide from Flos Hibiscus on hydroxyl free radicals.

The clearance rate of polysaccharide from Flos Hibiscus on hydroxyl free radicals was calculated as the following formula (3):

\[
CR = \left(1 - \frac{A_1 - A_2}{A_0}\right) \times 100
\]

CR: the clearance rate of polysaccharide on hydroxyl free radicals (%)
A0: the absorbance value of deionized water substituted for sample
A1: the absorbance value of sample
A2: the absorbance value of deionized water substituted for H2O2

(2) The determination of superoxide anion radicals scavenging ability of polysaccharide from Flos Hibiscus

According to pyrocatechol autoxidation [8], 3.5ml 0.05 mol/L Tris-HCL (pH 8.2) was mixed with 0.2ml 20mmol/L EDTA, 0.5ml different concentration of polysaccharide from Flos Hibiscus respectively. The mixture reacted at 25°C for 20 min, and mixed with the isothermal 0.5ml 10.0 mmol/L pyrocatechol. The mixture was immediately shaken well and reacted accurately for 4min. The reaction was terminated by 1ml 8 mol/L HCL. The equivalent volume of water was substituted for the pyrocatechol as a control group.

The clearance rate of polysaccharide from Flos Hibiscus on superoxide anion radicals was calculated as the following formula (4):

\[
CR = \left(1 - \frac{A_1}{A_0}\right) \times 100
\]

CR: the clearance rate of polysaccharide on hydroxyl free radicals (%)
A0: the absorbance value of deionized water substituted for sample
A1: the absorbance value of sample

3. Results and Analysis

3.1. Analysis of single factor test results

3.1.1. The effect of extraction temperature on the extraction yield of polysaccharide. Results in Figure 1 showed, the extraction yield of polysaccharide increased with the increase of temperature. The extraction yield was highest at 4.42% in 80°C, and fell slightly with the increase of temperature after more than 80°C. The reason may be related to the damage of the structure of polysaccharide due to high temperature. Therefore, the extracting temperature should be controlled at 80°C.

3.1.2. The effect of extraction time on the extraction yield of polysaccharide. Fig. 2 showed, the extraction yield of polysaccharide increased with the extension of extracting time. In 3h the extraction yield was highest at 4.58%, but there was no significant change in the extraction yield of polysaccharides after 3h. This indicated that the polysaccharides from Flos Hibiscus were almost completely dissolved.

3.1.3. The effect of mass ratio of solvent to material on the extraction yield of polysaccharide. The effect of mass ratio of solvent to material on the extraction yield of polysaccharide was shown in Fig. 3. The extraction yield of polysaccharide increased significantly when the mass ratio of solvent to material was increased from 15:1 to 30:1. When the mass ratio of solvent to material was 30:1, the extraction yield reached the highest point, 4.77%. When the mass ratio of solvent to material was increased to 35:1, the extraction yield of polysaccharide decreased. When the mass ratio of solvent to
material was 30:1, polysaccharide had reached saturation. The decrease of the extraction rate of polysaccharide may be related to the decrease of interaction between the material molecules. Similar phenomena had been reported in the research of polysaccharide extraction technology such as winter flower and chicken leg mushroom [9, 10]. Considering that the material solvent was larger than the general assembly, the work of concentration, precipitation, and centrifugation and so on would be larger than before, so the mass ratio of solvent to material was more suitable than 20:1-30:1.

3.1.4. The effect of extraction times on the extraction yield of polysaccharide. Fig. 2 showed, the polysaccharide was completely extracted after 2 extraction. Therefore, the number of suitable extraction was 2.
Fig. 3 The effect of mass ratio of solvent to material on the extraction yield of polysaccharide

Fig. 4 The effect of extraction times on the extraction yield of polysaccharide

Fig. 5 Hydroxyl free radicals scavenging ability of Vc and polysaccharide
Table 2. The results of orthogonal test

| Serial number | Extraction temperature (A) | Extraction time (B) | Mass ratio of solvent to material (C) | The extraction yield (%) |
|---------------|-----------------------------|---------------------|--------------------------------------|--------------------------|
| 1             | 1                           | 1                   | 1                                    | 2.98                     |
| 2             | 1                           | 2                   | 2                                    | 3.88                     |
| 3             | 1                           | 3                   | 3                                    | 4.18                     |
| 4             | 2                           | 1                   | 2                                    | 4.17                     |
| 5             | 2                           | 2                   | 3                                    | 4.53                     |
| 6             | 2                           | 3                   | 1                                    | 4.71                     |
| 7             | 3                           | 1                   | 3                                    | 4.44                     |
| 8             | 3                           | 2                   | 2                                    | 5.16                     |
| 9             | 3                           | 3                   | 1                                    | 5.11                     |
| x1            | 3.68                        | 3.86                | 4.13                                 |                          |
| x2            | 4.47                        | 4.38                | 4.54                                 |                          |
| x3            | 4.90                        | 4.81                | 4.38                                 |                          |
| R             | 1.22                        | 0.95                | 0.39                                 |                          |

3.2. The results of orthogonal test

According to the orthogonal test of L934, the results were shown in Table 2. The main relation of the factors affecting the extraction yield of polysaccharide was extraction temperature > extraction time > mass ratio of solvent to material. The optimal combination was A3B2C2. The extraction yield of polysaccharide from Flos Hibiscus was 5.22% after three parallel tests. The optimum extraction process was that extraction temperature was 80°C, extraction time was 3h and mass ratio of solvent to material was 30:1.
3.3. The determination results of antioxidant properties of polysaccharide from Flos Hibiscus

3.3.1. The determination results of hydroxyl free radicals scavenging ability of polysaccharide. Fig. 5 showed, the clearance rate of hydroxyl free radicals was increased with the increase of concentration of polysaccharide solution. There was a certain amount of relationship between them. When the concentration of polysaccharide solution reached 4 mg/ml, the clearance rate of •OH was 52.2%.

3.3.2. The determination results of superoxide anion radicals scavenging ability of polysaccharide. Fig. 6 showed that Vc and polysaccharide from Flos Hibiscus had a scavenging ability on O2-. The clearance rate increased obviously with the increase of polysaccharide. The scavenging ability of polysaccharide from Flos Hibiscus was lower than that of Vc. When the concentration of polysaccharide solution reached 2 mg/ml, the clearance rate of O2- is over 50%.

4. Conclusion

(1) Polysaccharide were extracted from Floss Hibiscus by hot water extraction technique. The optimal extraction process was that extraction temperature was 80°C, extraction time was 3h and mass ratio of solvent to material was 30:1 by single factor tests and orthogonal experiment. In that condition the extraction yield of polysaccharide from Floss Hibiscus was highest at 5.22%.

(2) The main relation of the factors affecting the extraction yield of polysaccharide from Floss Hibiscus was extraction temperature > extraction time > mass ratio of solvent to material.

(3) Polysaccharide from Floss Hibiscus had strong scavenging ability to remove •OH and O2-. The scavenging ability was positively correlated with the concentration of solution of polysaccharide.

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