Optimization of ultrasound extraction of Wolfberry Flavonoids and its antioxidant activities

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Abstract. The solidtosolvent ratio, ethanol concentration, extraction temperature and extraction time were investigated for extracting total flavonoids from wolfberry. The optimal conditions were found to be extracted at the ratio of material to solvent 1:20 (g/mL), 60 ℃ for 40 min with 70% (v/v) ethanol. The D101 macroporous resin was chosen to purify the flavonoids from the crude extracts, the ORAC value of purified flavonoids was enhanced 83.38% than that of the crude one.

Introduction

Wolfberry(*Lycium barbarum*) is one of the bestknown traditional Chinese herbal medicine. It has been widely used as healthy food for thousands of years in China[1]. The main active constituents of Wolfberry include polysaccharides, flavonoids and trace elements. Many researches showed that wolfberry fruits and its extracts possess pharmacological and physiological effects, e.g., brighten eyes, nourish the liver, nourish Yin and strengthen kidney, antioxidant, antifatigue, antiaging, lowering bloodglucose and serum lipids, immunity enhancement and anticancer[2].

Lots of previously published literatures about wolfberry were focus on polysaccharides, however, the papers about flavonoids were rare. Hence, the present work investigates the optimal parameters for extracting flavonoids from Wolfberry. Moreover, the crudes extracts were purified by macroporous resin and its antioxidant activities in vitro were measured by oxygen radical absorbance capacity (ORAC) assay.

Materials and methods

Materials

The Wolfberry were kindly provided by Ningxia Tang Ming Pharmaceutcal Co., Ltd., the herbs were ground with a DFY1000C omnipotent disintegrator (Wenling Dade machinery Co., Ltd., China) to get a relatively homogenous drug powder and then sieved through 100mesh screen. The Wolfberry powder was stored in a desiccator.

The standard of Rutin (>98%) was purchased from Bei Na Chuang Lian Biotechnology Institute of Beijing. All other chemical agents were analytical grade.

Extraction methods

Ultrasoundassisted extraction (UAE) experiments were carried out in a DCTZ2000 multipurpose thermostatic ultrasonic extraction machine (Beijing Hongxianglong Biotechnology Co., Ltd., China). Briefly, about 50 g Wolfberry powder was extracted by using ethanol in a designed material to solvent ratio (1:5-1:30 g/mL), ethanol concentration(50-100%, v/v), extraction temperature(30-80℃) and sonication time(10-60 min). After extraction, the mixtures were centrifuged in a centrifuge (L550, Xiangyi Instrument Co. Ltd.,

Changsha, China) at 2811×g for 10min, and the supernatants were collected and put into a volumetric flask, adjusted to appropriate volume with corresponding solvent.

Purification of wolfberry flavonoids by D101 macroporous resin

As the good performance on purification of natural products, the macroporous adsorption resins are widely used in the recovery or separation of flavones and polyphenols. On the basis of preliminary experiments, the D101 macroporous resin was chosen for separating flavonoids from wolfberry extracts.

The crude flavonoids was extracted under the optimal conditions, after centrifuging, the supernatants were collected, and the remaining ethanol was removed by rotary evaporation, the protein was removed by Sevage reagent (nbutyl alcohol:chloroform=1:5, v/v), the polysaccharides were eliminated by 95% ethanol. After the removal of protein and polysaccharides, the rest of extract was concentrated by reducing pressure and dried by vacuum freeze, finally, wolfberry flavonoids is obtained and stored in a 80℃ freezer for further use.

Exactly 25 g of the pretreated D101 macroporous resin was added into a 1000 mL triangular flask, then 500 mL crude extracts of wolfberry was added. The flask was shaken on an oscillator (160 r/min) at 25 ℃ for 24 h to reach the adsorption equilibrium. After the adsorption equilibrium had been reached, the resins were washed twice using 500 ml distilled water and then desorbed by 500 mL 95% (v/v) ethanol solution in a flask, which was shaken on an immersion oscillator (130 r/min) at 25 ℃for 24 h.

Determination of total flavonoid content

The total flavonoid content was determined by using the method reported by WANG [3] with minor modification. Briefly, 1 mL sample solution was put into a 25 mL volumetric flask and mix with 1 mL 5% $NaNO_2$, after6minstanding, added 1 mL 10% $Al(NO_3)_3$ and stand for 6 min, then 10 mL 4% NaOH was added, at last, the mixture was adjusted to 25 mL with 70% ethanol, stir and let stand for 30 minutes. The absorbance was measured at 510 nm by ultravioletvisible spectrophotometer (TU1810, Beijing Purkinje General Instrument Co., Ltd.), and the 70% ethanol was employed as a blank control. Rutin was adopted as a standard, the regression equation was as flowed, $y = 0.0798x+0.0003$ ($R^2= 0.9989$), and the total flavonoid contents of extracts were expressed as rutin equivalents. All measures were performed in triplicate.

Determination of oxygen radical absorbance capacity

The radicalscavenging capacity was determinedby the ORAC assayusing fluorescein(FL)as the fluorescent indicator in according to Dávalos [4]. Briefly, the reaction was carried out at 37℃ in 75mM phosphate buffer (pH 7.40). 20μLof antioxidant (trolox or extracts) and 20μLof10nM FLwere putin the well of the black 96well microplates, and then the microplate was incubated at 37°C for 15min before 160 μL AAPH solutions (0.10M) was addedrapidly to each well as peroxyl generator to trigger reaction. The microplate reader from BioTek Instruments was programmed to record the fluorescence reading atexcitation of 485nm and emission of 535nm at 1min interval for 60minusing software Gen 5^{TM}. And phosphate buffer was used as blank control, and the standard trolox at different concentrations (0, 6.25, 12.50, 25, 50 and 100μM)were used to construct a calibrator. All the tests were performedin triplicate.

The regression equation between the net AUC and the antioxidant concentration was calculated. Theregression was $y =93221x+8124810$ ($R^2=0.986$).The final ORAC values for extracts were expressed as trolox equivalent (TE).

Results and discussion

Effects of material to solvent ratio

Generally, the yield of extracts will improveas the increase of solvent volume. Extractions were carried out at different material to solvent ratio (1:5-1:30, g/mL) while the other parameterswere set as follows: at 50℃

with 60% ethanolfor 20 min. The effects of different material to solvent ratioon flavonoids yieldwas shown in Fig.1, the flavonoids yield enhanced with the increase of material to solvent ratio. Before 1:20g/mL,the yield of flavonoids increase rapidly, however, it was become very slowly as the ratio was abovethat. Considered the concentrate and to lowerthe costs, the value of 1:20 g/mL was employed as the optimal ratio of material to solvent for the experiments.

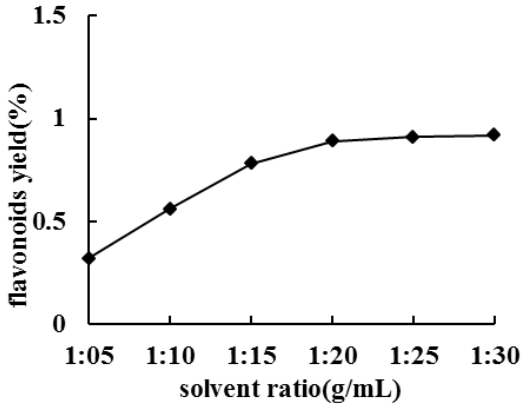

Fig.1 Effects of material to solvent ratio on flavonoids yield.

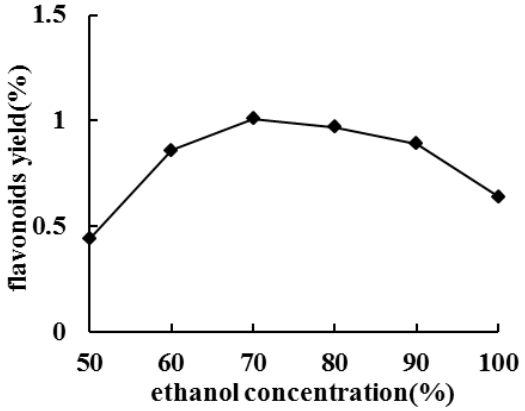

Fig.2 Effects of ethanol concentration on flavonoids yield.

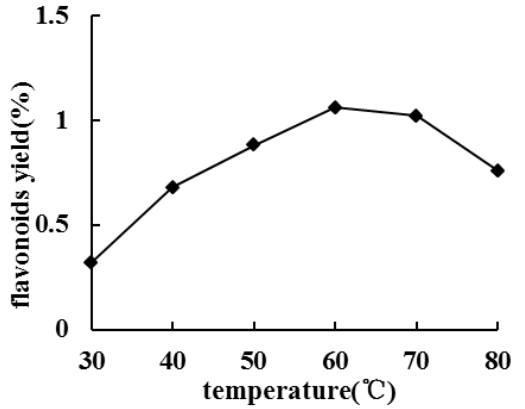

Fig.3 Effects of temperature on flavonoids yield.

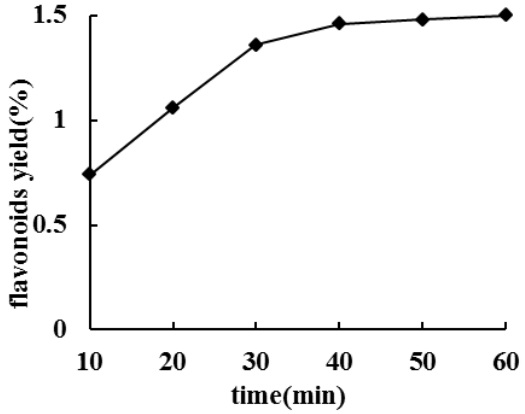

Fig.4 Effects of time on flavonoids yield.

Effects of ethanol concentration

50 g herbs powder was placed into the extracted pool for extracting with 1000 mL ethanol at 50 ℃for 20 min. The concentration of ethanol varied from 50% to 100%. As seen in Fig.2, the ethanol concentration could great influence the flavonoids yield, it increased with the increase of ethanol concentration when the ethanol volume percentage in the solvent was lower than 70% (v/v), but the yields were decreased when the cthanol concentration was higher than 70%. Thus, the 70% was considered as the best ethanol concentration for this process.

Effects of extraction temperature

The effects of different temperature (30-80 ℃) on the yield of flavonoids were carried out with 1000 mL 70% ethanol for 20 min.As shown in Fig.3, the yield of flavonoids enhancedwith the increase of temperature from 30 to 60 ℃, however, the yields were decreased when the temperature was higher than60℃. The results indicatedthat increasetemperature properlymay improve the flavonoids yield as higher temperature may break down the plant cell wallsmore thoroughly, but on the other hand, higher temperature may resolvetheflavonoids, leading todecreasethe recovery. According to the results, 60℃was chosen for the experiments.

Effects of extraction time

Use In general, extraction time play a vital role in enhancing the extraction efficiency of phytochemicals. The yield of wolfberry flavonoids extracted over different time from 10 to 60 min was seen in Fig.4, when the other factors were designed as follows: ratio of material to solvent 1:20, ethanol concentration 70%, ultrasonic temperature 60 ℃. The results indicated that the yield of flavonoids increased greatly with the duration of extraction time from 10 to 40 min; however, the yield enhanced slowly when the action time longer than 40 min. Consider to reduce energy consumption and save time, 40 min was chosen as the optimal time for the trials.

The ORAC value of wolfberry flavones

The wolfberry flavonoids were extracted by above process (ratio of material to solvent 1:20, ethanol concentration 70%, ultrasonic temperature 60 ℃, extraction time 40 min), and then purified by D101 macroporous resin. The antioxidant activities of crude extracts and the purified flavonoids were measured by ORAC assay. The results were shown in Table 1, the ORAC value of purified flavonoids was nearly twice as that of the crude extracts, reached to 70.60 μmol TE/L. The results indicated that the 60% ethanol extracts from wolfberry possess good antioxidant capacity; moreover, the activity was enhanced sharply by using D101 marcoporous resin for purification.

Table 1. The ORAC value of flavonoids

Fractions	Concentration (mg/mL)	ORAC value (μmol TE/L)
Crude extracts	5	38.45
Purified flavonoids	5	70.60

Conclusion

The ultrasoundassistant extraction process was developed for extracting flavonoids from wolfberry. The optimal parameters were found as solvent ratio 1:20 (g/mL) with 70% (v/v) ethanol at 60℃ for 40 min. The yield of flavonoids reached 1.48% under these conditions, and the D101 macroporous resin was proven as an effective and efficient purification technique, which could greatly improve the antioxidant activity.

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