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Prediction of the antiglycation activity of polysaccharides from *Benincasa hispida* using a response surface methodology

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DPPH• Free Radical (PubChem CID: 2735032)
Methanol (PubChem CID: 887)
Na_2HPO_4
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ABSTRACT

Benincasa hispida is a popular vegetable in China. Our previous experiments suggested that polysaccharides isolated from *B. hispida* fruits (PBH) have antiglycation effect and DPPH• free radical scavenging activity. Ultrasonic treatments can be used to extract polysaccharides from *Benincasa hispida* (PBH). The aim of this study was to investigate the correlation between the ultrasonic treatment conditions and the antiglycation activity of PBH. A mathematical model was generated with an artificial neural network (ANN) toolbox from MATLAB to analyze the effects of ultrasonic treatment conditions on antiglycation activity. The response surface plots showed relationships between ultrasonic extraction conditions and bioactivity. The R^2 value of the model was 0.9919, which suggested good fitness of the neural network. The application of genetic algorithms showed that the optimal ultrasonic extraction conditions resulted in the highest antiglycation activity for PBH. These were 150 W, 46 °C, and 35 min. These conditions produced a predicted antiglycation activity of 41.2%; the actual activity was 40.9% under optimal conditions. This is very close to the predicted value. The experimental data indicated that the PBH possessed both antiglycation and antioxidant activities. The maximum actual value of antiglycation was 101.7% that of the positive control, and the PBH inhibited the DPPH• free radicals with an EC_{50} value of 0.98 mg/mL. This is 66.2% that of ascorbic acid. These results explained the observations that *B. hispida* can decrease glucose levels in diabetic patients. The experimental results also showed that the ANN could be used for optimization and prediction.

1. Introduction

Medical and nutritional research has long emphasized the health benefits of eating vegetables, fruits and other plant-based foods to reduce the risk of diseases such as Type II diabetes, cardiovascular conditions, cancer, and obesity (Rekhy & McConchie, 2014). Phytochemicals – the functional non-nutrient compound in fruits, vegetables, and other plants – has been suggested to be responsible for their bioactivity in reducing the risk of major chronic diseases.

Benincasa hispida (Thunb.) Cogn. is a member of the Cucurbitaceae family and is an important vegetable in China. It is widely cultivated in East and South Asia (Zaini, Anwar, Hamind, & Saari, 2011). The fruit of *B. hispida* is named "Donggua" in Chinese, and it is a rich source of nutrients such as polysaccharides, proteins, vitamins, and minerals (Zaini et al., 2011). Many chemical components extracted from *B. hispida* fruit have various biological activities and pharmacological functions. For example, flavanoids, saponins, and organic acids are effective agents to reduce diuretic swelling, and aqueous extracts can lower blood glucose (Bimakr et al., 2012; Jayasree et al., 2011). Thus, *B. hispida* fruits are a medicinal and

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edible plant and are a standard component of the Asian diet (Zaini et al., 2011).

Polysaccharides are important functional ingredients in many medicines and foods (Jin, Huang, Zhao, & Shang, 2013). Because of their unique biological activities, many recent studies have investigated these macromolecules (Wijesinghe & Jeon, 2012). Our previous experiments have suggested that polysaccharides isolated from *B. hispida* fruits (PBH) could probably expand the clinical applications of PBH.

Non-enzymatic glycation of proteins was confirmed to play a significant role in normal aging and the development of chronic diabetic complications (Yang, Zhao, & Jiang, 2009). Protein glycation, oxidation, and nitration are the most important non-enzymatic protein modifications involved in the formation of endogenous protein aggregates (Ott et al., 2014). Non-enzymatic glycation of proteins by reduction with carbohydrates and further oxidation, rearrangements, and eliminations would result in the formation of advanced glycated end-products (AGEs) (Maritim, Sanders, & Watkins, 2003). This might further alter peptide structure, function, and stability. There have been many reports of the accumulation of AGEs under pathophysiological conditions such as diabetes. Therefore, identifying effective inhibitors of AGEs formation will have a meaningful impact on human health (Yamagishi et al., 2012).

Peyroux and Sternberg (2006) reported that trapping reactive dicarbonyl species and inhibiting their oxidation can prevent the formation of AGEs. The polysaccharides isolated from *B. hispida* are suggested to have antiglycation activity, and this may explain the effects of PBH in treating diabetic patients—an application of PBH in traditional Chinese medicine that has already been used for hundreds of years.

PBH is usually extracted by juicing or boiling. However, these methods reduce the efficiency of polysaccharides. Ultrasonic treatment has been employed to extract natural polysaccharides for decades (Hromadkova & Ebringerova, 2003) and has been shown to yield higher efficiency in polysaccharide extraction. The efficiency of ultrasonic treatment varies depending on ultrasonic power, temperature, and time. Each of these variables can affect the polysaccharide structure and enhance its bioactivity (Chen et al., 2012). Both antiglycation and free radical scavenging activities are linked to the molecular mass and structure of the polysaccharide (Yang et al., 2009).

In folk medicine, people believe that eating *B. hispida* fruits can reduce the blood glucose of diabetic patients. This lead researchers to study this food more intensely. Prediction of the antiglycation activity of polysaccharides isolated from the fruits of *B. hispida* has not yet been described using artificial neural networks (the response surface method). Therefore, our goal here was to identify correlations between ultrasonic conditions and antiglycation activity of PBH using a multilayer feed-forward neural network trained with an error back-propagation algorithm. A mathematical model was built between independent variables (ultrasonic power, temperature, and time) and the dependent variable (antiglycation activity). Genetic algorithms optimized the ultrasonic extraction conditions of the PBH preparation to obtain the highest antiglycation activity.

2. Materials and methods

2.1. Plant materials

Fresh *B. hispida* fruit was purchased from a farmer's market on Changzhou Island, Guangzhou city, Guangdong province, China. The material was identified by Professor C. Y. Yan, Guangdong Pharmaceutical University, China. The fruit was selected by judging that it was free of defects and bottle-green in color. The fruit was sliced and dried at 80 °C in an oven to a constant weight. The fruit was then cut into small pieces.

2.2. Chemicals

Aminoguanidine, sodium azide, bovine serum albumin (BSA), glucose, and DPPH• (2,2-diphenyl-1-picrylhydrazyl) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Phenol and sulfuric acid were purchased from Guangzhou Reagent Co. (Guangzhou, China). All other reagents used in this study were of analytical grade.

2.3. Extraction and quantification of PBH

Eight grams of *B. hispida* fruits were extracted with 80 mL distilled water under the selected ultrasonic power, temperature, and time with an ultrasonic cleaner (KH-400KDB, Hechuang Ultrasonic Equipment Co., Kunshan, China). The extract was vacuum filtrated and concentrated to 15 mL using a rotary evaporator under vacuum. Anhydrate ethanol was added until the ethanol concentration reached 75%, as measured by an alcoholmeter. The mixture was maintained for 24 h at 25 °C to precipitate polysaccharides. These were then lyophilized to yield a precipitate powder for further bioactivity assays. The amount of polysaccharides was determined using a phenol-sulfuric acid method with glucose as the standard control (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The extraction rate was calculated using the following equation:

$$Extractionrate(\%) = W_1/W_2 \times 100\%$$

where W_1 was the amount of polysaccharide, and W_2 was the weight of *B. hispida*.

2.4. Antiglycation activity assay

The antiglycation activity was measured according to our previous method with some modifications. Aminoguanidine was used as a positive control (Zhao, Yang, Yang, Jiang, & Zhang, 2007). A stock solution with 5% (w/v) BSA, 1 M glucose, and 0.1% (w/v) sodium azide in pH 7.4 phosphate buffer (Na_2HPO_4 and NaH_2PO_4) was prepared. The PBH was dissolved in the stock solution to a concentration of 0.5 mg/mL. The negative control was free of aminoguanidine and polysaccharides. The bacteria were removed by membrane filtration with a 0.22-μm filter. Solutions were stored in 15-mL centrifuge tubes and incubated at 37 °C for 4 weeks in the dark. The AGEs were determined using a fluorospectrophotometric method with an emission wavelength of 440 nm and an excitation wavelength of 370 nm. The percentage of antiglycation activity was calculated as following formula:

$$G\% = \frac{A_{control} - A_{sample}}{A_{control}} \times 100\%$$

where A_{sample} and $A_{control}$ are the fluorescence of the sample and negative control groups, respectively.

2.5. DPPH• free radical scavenging activity assay

The DPPH• free radical scavenging capacity of PBH was determined using a test described by Yan, Kong, and Ou (2012). Ascorbic acid was used as a positive control. A 1 mM solution of DPPH• in ethanol was prepared daily before ultraviolet (UV) measurements. Next, 100 μL of various concentrations of PBH or ascorbic acid in distilled water were added to 100 μL freshly prepared DPPH•, and the mixture was shaken mildly. The DPPH• free radical reduction was measured by determining the absorbance at 517 nm after incu-