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Optimization of the extraction of polysaccharides from tobacco waste and their biological activities

Yanqiu Jing[a], Yuzhen Gao[b], Weifeng Wang[c], Yuyuan Cheng[d], Ping Lu[e], Cong Ma[f,*], Yuehua Zhang[e]

[a] Henan Agricultural University, National Tobacco Cultivation and Physiology and Biochemistry Research Centre, Key Laboratory for Tobacco Cultivation of Tobacco Industry, Zhengzhou 450002, China
[b] Tobacco Industry Co., Ltd., Technology Research and Development Center in Gansu province, Lanzhou 730050, China
[c] Guangxi Zhuang Autonomous Region Provincial Branch of CNTC, Nanning 530022, China
[d] Nanyang Branch of Henan National Tobacco Corporation, Nanyang 473000, China
[e] Technology Center of Chinese Tobacco Industrial Company in Henan, Zhengzhou 450000, China
[f] Henan Provincial Branch of CNTC, Zhengzhou 450052, China

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ABSTRACT

A response surface methodology was used to optimize the parameters for extracting the polysaccharides from tobacco waste (TWPs) using hot water. The extraction process, carried out under the following optimized parameters: an extraction temperature of 90 °C, a ratio of water to raw material of 54, and an extraction time of 115 min, allowed an experimental yield of 28.32 ± 1.78%. The chemical composition analysis showed that TWPs were composed of mannose, rhamnose, glucuronic acid, galacturonic acid, glucose, galactose and arabinose with the following molecular ratio: 1.00:2.69:1.29:2.29:5.23:6.90:3.92. The molecular weights of its four major fractions were 0.558, 1.015, 16.286, and 151.194 kDa. Bioactivity experiments showed that TWPs not only decreased the reactive oxygen species level in salt-stressed tomato seedlings, but also possessed significant antioxidant activities *in vitro*. Antioxidant activity *in vivo* further showed that TWPs could significantly increase the activities of antioxidant enzymes including superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT), and decrease the level of malondialodehyde (MDA). In addition, according to the acute toxicity test, TWPs did not cause behavioral changes or any death of mice. This study provides an effective method to utilize tobacco waste resources.

1. Introduction

Tobacco (*Nicotiana tabacum* L.) is one of the most widely planted economic crops. China is the world's largest tobacco producer and consumer, and contributes about 40% of the world's total tobacco production [1]. Tobacco cultivation and cigarette manufacture industries produced more than 200 million tons of tobacco waste materials annually. The waste includes the stems, leaf veins and roots of tobacco plants, as well as low-grade and defective tobacco leaves. They have a strong smell and cause serious environmental contamination [2]. According to an incomplete statistical analysis in 2008, more than 366, 200 tons of tobacco stems were disposed as rubbish worldwide. This represents an enormous waste of a natural plant resource and results in serious environmental pollution [3]. Therefore, the reutilization of this industrial waste and its exploitation as potential biomass material are important.

Polysaccharides are a primary class of bio-macromolecules, undoubtedly as important as proteins, nucleic acids, and lipids. They play a variety of vital roles in physiological processes, including structural support, cell recognition, information transfer, and immune defense [4]. In recent years, there has been a growing interest in polysaccharides obtained from higher plants exhibiting diverse chemical structures and biological activities. The polysaccharides extracted from higher plants are widely used in the food, pharmaceutical, and many other industries [5]. It is well known that a large amount of polysaccharides exists in tobacco waste, including stems, leaves, and roots. Thus, the efficient extraction of polysaccharides from tobacco waste resources is of great significance for the utilization of this resource. To the best of our knowledge, there is no information available about polysaccharides

* Corresponding author.
E-mail address: macong9666@163.com (C. Ma).

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from tobacco waste. Aqueous extraction is the most commonly used method to extract plant polysaccharides, and the extraction process is usually optimized via a mathematical analysis. The response surface methodology (RSM) is useful for investigating the relation between several variables. This methodology has been widely used to optimize polysaccharide extraction processes [6–8]. In the current study, the objective was to optimize the conditions (the extraction temperature, the ratio of water to raw material, and the extraction time) to be used to extract polysaccharides from tobacco waste using RSM. The antioxidant activities of the extracted polysaccharides, including the scavenging activity against DPPH radicals, hydroxyl radicals, superoxide radicals as well as the reducing power and total antioxidant activity were assayed *in vitro*. Furthermore, the chemical characteristics of the extracted polysaccharides including the monosaccharide composition and the molecular weight, and the *in vivo* antioxidant activities were also determined.

2. Materials and methods

2.1. Experimental materials and chemicals

1,1-Diphenyl-2-picryl-hydrazyl (DPPH), 2′,7′-dichlorofluorescin diacetate (DCFH-DA), ascorbic acid, and standard monosaccharides were obtained from Sigma-Aldrich (St. Louis, MO, USA). Dextran T-series standards were from the National Institutes for Drugs and Biological Products (Beijing, China). Assay kits for superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT) and malondialdehyde (MDA) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The male Kunming mice were purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). All other reagents were of analytical grade. Other reagents used in this study were of the highest quality available from commercial vendors.

2.2. Preparation of polysaccharides

Dried tobacco waste was ground in a rotary mill and sieved (10 mesh) to obtain a fine powder. The fat-soluble materials were removed by extractions using 95% (v/v) ethanol during a reflux of 2 h at 90 °C for three times. After the insoluble residue was dried in an oven at 50 °C, the pretreated sample was extracted by the distilled water in a designed extraction temperature (80, 90 and 100 °C), extraction time (60, 90 and 120 min) and the ratio of water to raw material (40, 50 and 60). The water extraction solutions were separated from insoluble residue by centrifugation (4000 rpm for 10 min). The supernatant was concentrated using a rotary evaporator at 60 °C under vacuum. The extract was then precipitated by adding 95% (v/v) ethanol to a final concentration of 80% and incubad at 4 °C for 24 h. The precipitated polysaccharides were collected by centrifugation (4000 rpm/min for 10 min). After washing three times with absolute ethanol, the polysaccharides extracted from tobacco waste (TWPs) were obtained using lyophilization. The polysaccharide content was measured following the phenol-sulfuric method. The TWP yield (%) resulting from the extractions was calculated following the equation:

$$\text{TWP yield}(\%) = [(\text{the polysaccharide content of extraction, g})/\text{the weight of the pretreated samples, g})] \times 100$$

Table 1
Box-Behnken experimental design (BBD) with the independent variables.

Run	Extraction temperature (A/°C)	Ratio of water to raw material (B)	Extraction time (C/min)	TWP yield (%)
1	80	40	90	24.78
2	100	40	90	23.25
3	80	60	90	26.22
4	100	60	90	26.44
5	80	50	60	25.67
6	100	50	60	24.82
7	80	50	120	26.90
8	100	50	120	26.65
9	90	40	60	24.10
10	90	60	60	26.27
11	90	40	120	25.97
12	90	60	120	28.35
13	90	50	90	27.75
14	90	50	90	27.92
15	90	50	90	28.72
16	90	50	90	28.14
17	90	50	90	27.97

2.3. Single factor experimental design

The effects of the extraction temperature, the ratio of water to raw material and the extraction time were first studied by a single-factor experiment by changing only one of the mentioned parameter while the other factors were kept constant in each experiment. The effect of each factor was evaluated by determining the TWP yield. One-way analysis of variance (ANOVA) was used to compare the means of different groups in the experiment.

2.4. Experimental design and statistical analysis

After determining the preliminary coverage of the independent variables through the single-factor test, a 17-run Box-Behnken design (BBD) with three independent variables (A, the extraction temperature; B, the ratio of water to raw material; C, the extraction time) at three levels was employed to optimize the TWP extraction process. As shown in Table 1, these three factors designated as *A,B and C*, were coded as +1, 0 and −1 for high, intermediate, and low values, respectively. For the statistical calculations, the three test variables were coded according to the following equation:

$$X_i = \frac{x_i - x_0}{\Delta x} i = 1, 2, 3$$

where X_i is the coded value of the independent variable, x_i is the actual value, x_0 is the value of x_i at the center point, and Δx is the step change. To predict the optimized conditions, the relation between the independent variables and the response (TWP yield) was fitted by a second-order polynomial equation.

$$Y = A_0 + \sum_{i=1}^{3} A_i X_i + \sum_{i=1}^{3} A_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} A_{ij} X_{ij}$$

where Y is the dependent variable (TWP yield), A_0 is an intercept, and A_i, A_{ii}, and A_{ij} are the coefficients that were estimated by the model. X_i and X_j are the levels of the independent variables that represent the linear, quadratic and cross-product effects of the X_1, X_2, and X_3 factors on the response, respectively. The model evaluated the effect of each independent variable on the response. The experimental design was analyzed and the predicted data were calculated using the Design-Expert software (v.8.0.6.1 trail, Stat-East, Inc, Minneapolis, USA) in order to estimate the response of the independent variables. The designated variables and the experimental values of the responses are given in Table 1.