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Microwave Extraction of Antioxidant Polysaccharides from *Plantago depressa* and Their Effects on SOD and GSH-Px in *Drosophila melanogaster* Model

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Abstract

A microwave extraction method was developed to isolate antioxidant polysaccharides from *Plantago depressa* (psyllium), and the structure, free radical-scavenging ability, as well as in vivo antioxidant activity of psyllium polysaccharides were analyzed. The optimal condition for microwave extraction was as follows: duration of microwave radiation of 35 min, extraction temperature of 80 °C, and ratio of liquid to solid of 80:1 (mL/g). The yield of psyllium polysaccharides by microwave extraction was significantly higher than that by heating extraction ($p < 0.05$). The volumes of *P. depressa* samples notably increased after microwave extraction, which implied that microwave radiation might loosen the structure of cells and tissues of psyllium leaves and facilitate the exudation of target polysaccharides from leaf samples. The structure of polysaccharides was analyzed by infrared spectroscopy. The effective concentrations of psyllium polysaccharides scavenging DPPH• and ABTS•⁺ radicals by 50% (EC₅₀) were 0.20 and 0.10 mg/mL, respectively. Moreover, *P. depressa* polysaccharides increased activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in *Drosophila melanogaster*. In conclusion, microwave extraction seems to be an efficient method to isolate bioactive polysaccharides from *P. depressa*, which were a potential source of natural antioxidants.

Keywords: microwave extraction; psyllium; polysaccharides; antioxidant activity; SOD and GSH-Px; fruit fly



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1. Introduction

Plantago depressa (psyllium) belongs to the genus *Plantago* of the family Plantaginaceae. It is a traditional medicinal plant mainly used as expectorant, diuretic, and toxicide in China for thousands of years [1]. The family Plantaginaceae consists of one genus and about 270 species [2]. Among them, *P. depressa* and *P. asiatica* are recognized as the official sources

of medicinal materials in the Chinese pharmacopoeia. *P. depressa* is widely distributed in East and Central Asia as well as Europe [3]. It exhibits antioxidant, anti-hyperglycemic, anti-aging, anti-bacterial, and anti-inflammatory activities [4]. Recent pharmacological studies showed that psyllium also possessed efficacy against cancer, pimples, as well as diseases of digestive and respiratory organs [5].

The primary pharmacologically active components of psyllium are phenylpropanoids, polysaccharides, flavonoids, alkaloids, and triterpenoids [6]. Among them, polysaccharides are one of the most valuable bioactive substances [7]. Psyllium polysaccharides have been found to exhibit protective effects against inflammation, atherosclerosis, hyperglycemia, and tumor [8,9]. It is well known that reactive oxygen species (ROS) molecules and antioxidants are usually in balance in vivo. And an imbalance may lead to a host of serious and fatal illnesses, such as diabetes and cancer [10]. Plant polysaccharides are considered to be the important source of natural antioxidants [11]. It is of practical usefulness to investigate in vitro and in vivo antioxidant ability of polysaccharides from *P. depressa*.

Generally, extraction of bioactive polysaccharides from psyllium is the first step for their exploration and utilization. The most common extraction methods of polysaccharides include water decoction and alcohol precipitation, alkali extraction, enzymatic extraction, and microwave extraction [12,13]. Among them, microwave extraction has a high yield, short duration, low cost, and low solvent consumption in many cases [14]. It is of importance to optimize microwave extraction of antioxidant polysaccharides from *P. depressa*. The objective of the present study is to separate antioxidant polysaccharides from *P. depressa* by microwave extraction, optimize extraction condition using single factor experiments and orthogonal test, analyze the structure of polysaccharides by infrared (IR) spectroscopy, evaluate their free radical-scavenging activities in vitro, and study their in vivo antioxidant activity using *Drosophila melanogaster* model for the first time.

2. Materials and Methods

2.1. Materials

P. depressa were purchased at local pharmacy in Xi'an city of China, and identified by one of the authors (H.Z.), and a voucher specimen was deposited at International Joint Research Center of Shaanxi Province for Food and Health Sciences (China). The dried leaves of *P. depressa* were ground into powders (40 meshes). Afterwards, the powders were stored at room temperature in dark sealed containers until needed. Wild type of *Drosophila melanogaster* was obtained from Health Science Center, Xi'an Jiaotong University, China.

Superoxide dismutase (SOD) assay kit and glutathione peroxidase (GSH-Px) assay kit (colorimetric method) were bought from Nanjing JCB Institute (Nanjing, China). 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2-azino-bis-3-ethyl-benzothiazoline-*p*-sulfonic acid (ABTS) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Glucose (purity $\geq 99\%$), ascorbic acid (purity $\geq 99\%$), alpha-naphthol (purity $\geq 98\%$), concentrated sulfuric acid, phenol, acetone, propionic acid, potassium bromide (KBr), and other reagents were bought from Tianjing TL Chemical Reagent Co. (Tianjin, China).

2.2. Apparatus

Microwave extraction of polysaccharides from *P. depressa* was performed using a microwave/ultrasound-assisted extraction apparatus (Type CW-2000, XT Instrument Co., Ltd., Shanghai, China). Polysaccharides were concentrated using a freeze dryer (Type LGJ-10C, Beijing SH Scientific Instrument Factory Co., Ltd., Beijing, China) and a rotary evaporator (Type R-210, Buchi Labortechnik AG, Uster, Switzerland) equipped with a vacuum pump and a heating bath. Free radical-scavenging capacity of polysaccharides was deter-

mined using a microplate spectrophotometer (Type Multiskan GO, Thermo Fisher Scientific, Waltham, MA, USA). The structure of polysaccharides was analyzed using a Fourier transform infrared (FTIR) spectrometer (Type Tensor 27, Burkert Optics, Billerica, MA, USA).

2.3. Extraction Method

2.3.1. Microwave Extraction

Microwave extraction of polysaccharides from *P. depressa* was carried out as previously described with some modifications [15]. In short, 3 g of leaf powders of *P. depressa* was mixed with 240 mL of distilled water in conical flask and was then extracted in microwave extraction system (focused microwave oven at the atmospheric pressure). Microwave power was set at 200 W, and particle size of psyllium samples was 40 meshes. To optimize extraction condition of polysaccharides from *P. depressa*, influences of three extraction variables (duration of microwave radiation, extraction temperature, and ratio of liquid to solid) on extraction yield were investigated. And duration of microwave radiation, extraction temperature, and ratio of liquid to solid ranged from 35 to 85 °C, from 5 to 60 min and from 60 to 240 mL/g, respectively (see below). After extraction, the mixture was centrifuged at 3500 rpm for 10 min, and the supernatant was passed through a 0.45 µm poly-tetrafluoroethylene (PTFE) filter. The filtrate was preserved as the crude extract of *P. depressa* polysaccharides. The crude extract was purified and then freeze-dried [14,16].

2.3.2. Heating Extraction

Heating extraction of polysaccharides from *P. depressa* was conducted as previously described with some modifications [15]. In brief, 3 g of leaf powders (40 meshes) of *P. depressa* was mixed with 240 mL of distilled water, and then the mixture was heated at 80 °C for 35 min. After extraction, it was spun at 3500 rpm for 10 min. The resulting supernatant was filtered, and the crude extract of polysaccharides was purified and lyophilized.

2.3.3. Determination of Sample Volume

Comparisons of volumes of leaf samples and extraction yields of polysaccharides were carried out between microwave extraction and heating extraction. The batch of *P. depressa* samples in this assay was different to those in single factor experiments and orthogonal test. The samples of *P. depressa* after microwave extraction and heating extraction were oven-dried at 50 ± 5 °C for 3 h. Likewise, the sample of *P. depressa* without extraction was also oven-dried. The volumes of 0.1 g of treated (after extraction) and untreated (without extraction) samples were determined, respectively.

2.4. Qualitative and Quantitative Analyses of Polysaccharides

2.4.1. Phenol-Sulfuric Acid Method

Phenol-sulfuric acid colorimetry developed in our laboratory was used to quantify polysaccharides [11]. The absorbance of polysaccharides samples was measured at 490 nm, and the calibration curve was constructed with standard glucose solutions.

2.4.2. Molisch's Test

The samples of polysaccharides were identified using Molisch's test [17]. Glucose was used as positive control.

2.4.3. IR Spectroscopy

The structure of polysaccharides was analyzed by IR spectroscopy described by Wu et al. [18]. In short, the sample of polysaccharides was mixed with KBr powder. The mixture was ground and then pressed into a slice (1 mm) for IR spectroscopy. Data were recorded in the range from 4000 to 400 cm^{−1} with a spectral resolution of 1 cm^{−1}.

2.5. Radical Scavenging Activity Assay

2.5.1. DPPH• Assay

DPPH• radical scavenging activity of polysaccharides was determined according to Rumpf et al. and Zhang et al. [19,20]. Briefly, 4 mL of polysaccharides solutions at different concentrations (0.04, 0.08, 0.12, 0.16, 0.20 mg/mL) was mixed with 2 mL of DPPH ethanol solution, respectively, and then incubated at 37 °C for 30 min. The absorbance was determined at 517 nm. Ascorbic acid was used as positive control. EC₅₀ was defined as the effective concentration of polysaccharides reducing free radicals by 50%, which was used to estimate in vitro antioxidant activity [13].

2.5.2. ABTS•⁺ Assay

ABTS•⁺ radical scavenging activity of polysaccharides was measured according to Shang et al. [21]. In brief, 250 µL of polysaccharide solutions at various concentrations (0.1, 0.3, 0.5, 0.7, 0.9 mg/mL) was mixed with 4.75 mL of diluted ABTS•⁺ solution, and then incubated in darkness at 30 °C for 6 min. Afterwards, the mixture was detected at 734 nm immediately. Ascorbic acid was used as positive control. EC₅₀ was calculated to evaluate in vitro antioxidant activity of polysaccharides [13].

2.6. *Drosophila melanogaster* Experiment

2.6.1. Pretreatment and Treatment of Fruit Fly

Drosophila melanogaster experiments were approved by the Advisory Ethics Committee of International Joint Research Center of Shaanxi Province for Food and Health Sciences (IJRC-A2022-002), and they were performed according to a previously reported protocol in our laboratory [22]. Fruit flies were raised in a light/dark cycle (12 h of light and 12 h of darkness) at 25 °C and 60% relative humidity. Fruit flies were divided into four groups: female polysaccharides group, male polysaccharides group, female blank group, and male blank group. Among them, fruit flies in female and male polysaccharides groups were fed with conventional standard mediums supplemented with polysaccharides from *P. depressa* (at a final concentration of 0.01 mg/mL), whereas fruit flies in female and male blank groups were fed with standard mediums in the absence of psyllium polysaccharides. Afterwards, fruit flies were frozen to death at −20 °C, which were subsequently homogenized in saline. The homogenates were centrifuged at 3000 rpm at 4 °C for 15 min, and the supernatants were collected for SOD and GSH-Px assays. The contents of proteins were determined by the Bradford method [22].

2.6.2. SOD Assay

Activity of SOD was determined using a commercial assay kit [23]. The absorbance was measured at 550 nm.

2.6.3. GSH-Px Assay

Activity of GSH-Px was evaluated by a commercial assay kit [23]. The absorbance was analyzed at 412 nm.

2.7. Statistical Analysis

Extraction condition of polysaccharides from *P. depressa* was optimized using single-factor experiments followed by a three-factor and three-level orthogonal test (Table 1). In the orthogonal test, microwave extraction was performed for nine combinations of three extraction variables taking into account the possible interactions among these factors (see below). The batch of *P. depressa* samples in the orthogonal test was different from that in single factor experiments. The variance of the orthogonal test was calculated using SPSS

12.0 software (SPSS Inc., Chicago, IL, USA). All experiments were conducted at least in duplicate. Statistical significance was defined as $p < 0.05$, and high significance was defined as $p < 0.01$.

Table 1. Levels and factors in orthogonal test of microwave extraction of polysaccharides from *P. depressa*.

Level	Factors		
	A Duration of Microwave Radiation (min)	B Extraction Temperature (°C)	C Ratio of Liquid to Solid (mL/g)
1	25	60	80
2	30	70	100
3	35	80	120

3. Results and Discussion

3.1. Optimization of Microwave Extraction Condition

3.1.1. Single Factor Experiments of Microwave Extraction

In order to optimize microwave extraction of polysaccharides from *P. depressa*, the effects of different parameters (duration of microwave radiation, extraction temperature, and ratio of liquid to solid) on extraction yield were investigated systematically. As shown in Figure 1A, extraction yield of polysaccharides significantly increased from 8.21% to 14.78% ($p < 0.05$) when the duration of microwave radiation was prolonged from 5 to 30 min. However, excessive microwave exposure did not result in a higher extraction yield in the range of 30 to 60 min. The possible reason is that the majority of polysaccharides were released from psyllium after 30 min of microwave radiation.

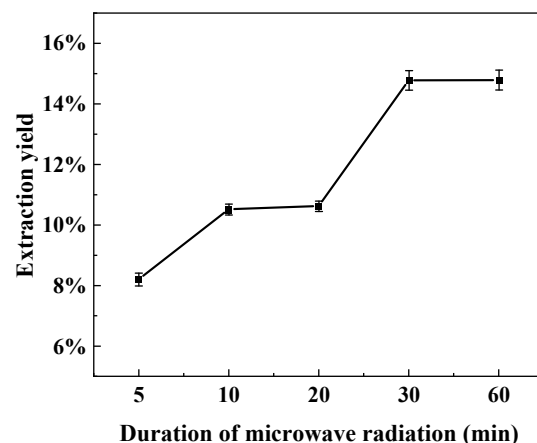
The influence of extraction temperature on extraction yield of polysaccharides is illustrated in Figure 1B. With the increase in extraction temperature from 35 °C to 75 °C, extraction yield increased significantly ($p < 0.05$). When extraction temperature exceeded 75 °C, the growth rate of extraction yield became slow ($p > 0.05$).

The ratio of liquid to solid is considered one of the most important factors in microwave extraction [14]. Figure 1C depicts the impact of ratio of liquid to solid on extraction yield. With the increase in ratio of liquid to solid, extraction yield first increased and then decreased rapidly. And the peak of extraction yield appeared at a ratio of liquid to solid of 120 mL/g.

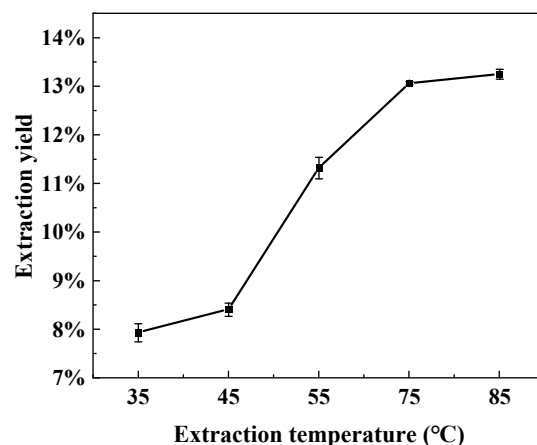
3.1.2. Orthogonal Test of Microwave Extraction

To further optimize extraction condition, a $L_9(3^3)$ orthogonal test was performed based upon the results of single factor experiments (Table 2). According to the results of range analysis, the influential order of three factors was ratio of liquid to solid (C) > extraction temperature (B) > duration of microwave radiation (A). According to variance analysis of the orthogonal test in Table 3, the ratio of liquid to solid significantly affected extraction yield ($p < 0.05$), while extraction temperature and duration of microwave radiation contributed slightly but non-significantly to extraction yield ($p > 0.05$). This tendency was in accordance with the result of range analysis (Table 2). On the basis of the orthogonal test, the optimal condition for microwave extraction was $A_3B_3C_1$, which included duration of microwave radiation of 35 min, extraction temperature of 80 °C, and ratio of liquid to solid of 80:1 (mL/g) (Table 2). However, the optimal condition ($A_3B_3C_1$) was not included in nine runs of the orthogonal test. Fortunately, the condition ($A_2B_3C_1$) in the sixth run was very similar to the optimal condition ($A_3B_3C_1$) (Table 2). Under the condition in the sixth run, extraction yield was 12.05% (Table 2), which was higher than those in other runs.

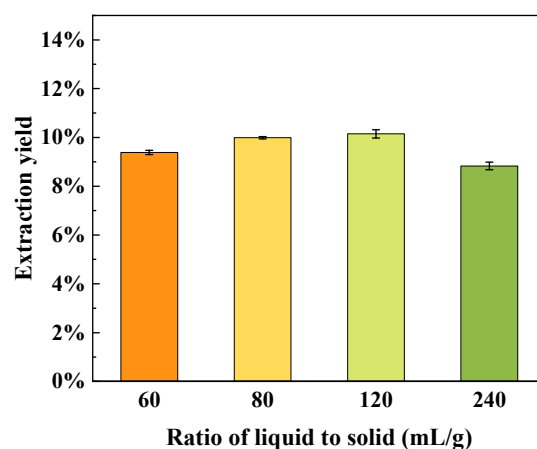
These results implied that the optimal condition obtained from the orthogonal test was a reasonable assumption. To validate the results of the orthogonal test, polysaccharides were extracted under the optimal condition ($A_3B_3C_1$). Finally, extraction yield reached 12.12% under the optimal condition, which was higher than those in all nine runs of the orthogonal test, demonstrating that $A_3B_3C_1$ was the optimal condition of microwave extraction of polysaccharides from *P. depressa*.



(A)



(B)



(C)

Figure 1. Effects of various parameters on extraction yield of polysaccharides from *P. depressa*. (A), duration of microwave radiation; (B), extraction temperature; and (C), ratio of liquid to solid.

Table 2. Orthogonal test design and results.

Run	Factor			Extraction Yield (%)
	(A) Duration of Microwave Radiation (min)	(B) Extraction Temperature (°C)	(C) Ratio of Liquid to Solid (mL/g)	
1	A ₁ (25)	B ₁ (60)	C ₁ (80)	10.50
2	A ₁ (25)	B ₂ (70)	C ₂ (100)	9.44
3	A ₁ (25)	B ₃ (80)	C ₃ (120)	9.68
4	A ₂ (30)	B ₁ (60)	C ₂ (100)	10.29
5	A ₂ (30)	B ₂ (70)	C ₃ (120)	9.49
6	A ₂ (30)	B ₃ (80)	C ₁ (80)	12.05
7	A ₃ (35)	B ₁ (60)	C ₃ (120)	9.30
8	A ₃ (35)	B ₂ (70)	C ₁ (80)	11.80
9	A ₃ (35)	B ₃ (80)	C ₂ (100)	10.96
T ₁	29.62	30.09	34.35	
T ₂	31.83	30.73	30.70	
T ₃	32.06	32.70	28.47	
k ₁	9.87	10.03	11.45	
k ₂	10.61	10.24	10.23	
k ₃	10.69	10.90	9.49	
Range *	0.81 ^b	0.87 ^b	1.96 ^a	

* Different superscript letters in this row mean significant differences ($p < 0.05$).

Table 3. Analysis of variance of orthogonal test.

Source of Variation	Quadratic Sum	Degree of Freedom	Mean Square	F-Value	p-Value
(A) Duration of microwave radiation	0.0001	2	0.0001	6.6695	$p > 0.05$
(B) Extraction temperature	0.0001	2	0.0001	6.7652	$p > 0.05$
(C) Ratio of liquid to solid	0.0006	2	0.0003	32.3174	$p < 0.05$
Error	0.0000	2	0.0000		
Sum	0.0009				

3.1.3. Comparison with Heating Extraction

To evaluate efficiency and repeatability of microwave extraction, extraction yields of polysaccharides were compared between microwave extraction and heating extraction. Each experiment was carried out in triplicate to calculate the relative standard deviation (RSD) of extraction yields. Notably, extraction yield by microwave extraction (10.01%) was higher than that by heating extraction (7.20%) ($p < 0.05$). In the presence of microwave radiation, extraction yield increased by 39.03%. RSD of extraction yields by microwave extraction (2.95%) was lower than that by heating extraction (3.49%), which confirmed relatively high repeatability of microwave extraction.

To provide clues on the mechanism underlying microwave extraction, the volumes of treated (after microwave extraction and heating extraction) and untreated (without extraction) samples were determined. *P. depressa* samples were divided into three groups: microwave extraction group, heating extraction group, and untreated control group. As seen in Figure 2, the volumes of psyllium samples in microwave extraction, heating extraction, and untreated control groups were 0.43, 0.40, and 0.25 mL, respectively. In comparison with the untreated control group, the volumes of *P. depressa* samples in microwave extraction and heating extraction groups significantly increased by 72% and 60% ($p < 0.01$). This result suggested that microwave radiation might facilitate swelling of leaf samples, loosen the structure of cells and tissues of leaves, increase specific surface area of leaf powders, promote the exudation of intracellular compounds from leaf samples, all of which might increase extraction yield of polysaccharides.

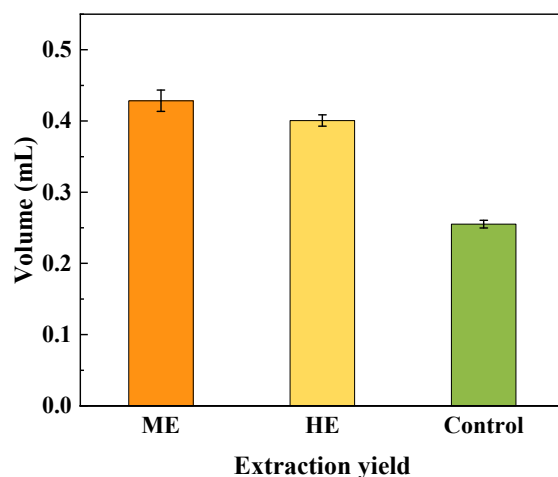


Figure 2. Influence of different extraction methods on volumes of leaf powders. ME, leaf samples after microwave extraction; HE, leaf samples after heating extraction; Control, leaf samples without extraction.

3.2. Molisch's Test and IR Spectroscopy

Under the treatment of H_2SO_4 , polysaccharides dehydrate into furfural and other derivatives. Furfural and its derivatives react with α -naphthol and form purple compounds [13]. In Molisch's test of *P. depressa* polysaccharides, purple was observed, indicating that the samples of polysaccharides obtained by microwave extraction belonged to carbohydrates.

As depicted in Figure 3, there were two strong absorption peaks at $3600\text{--}3000\text{ cm}^{-1}$, which might be attributed to the stretching vibration of O-H. The symmetric and asymmetric vibrations of C-H were responsible for the two absorption peaks at $2900\text{--}2800\text{ cm}^{-1}$. This suggested the presence of methyl, methylene, or methine in the molecules of the samples of *P. depressa* polysaccharides. The peak at 673 cm^{-1} indicated the presence of $-(\text{CH}_2)_n$ -chains. The peak at 873 cm^{-1} might be attributed to C-H scissor vibration, implying that there were β -glycosidic bonds in the samples. By contrast, the peak at 788 cm^{-1} suggested the presence of α -glycosidic bonds in the samples.



Figure 3. FTIR spectrum of polysaccharide samples from *P. depressa*. The abscissa and y-axis were absorbance and wavenumbers (cm^{-1}), respectively.

3.3. Antioxidant Capacities In Vitro

DPPH $\bullet$ and ABTS $\bullet^+$ assays have been frequently employed to evaluate in vitro antioxidant activity of plant extracts [22]. Fitting equations of dose–effect relationships between free radical scavenging capacities and mass concentrations of psyllium polysaccharides were listed in Table 4. With the increase in the concentration of polysaccharides from 0

to 0.33 mg/mL, DPPH• radical scavenging ability rose in a dose-dependent manner. At the concentration of 0.25 mg/mL, DPPH• radical scavenging activity exceeded 65%. EC₅₀ for DPPH• radicals was found to be 0.20 mg/mL. In the range of 0–0.16 mg/mL, the scavenging ability of polysaccharides on ABTS•⁺ radical was positively correlated with the concentration of polysaccharides. At the concentration of 0.16 mg/mL, the scavenging activity of polysaccharides exceeded 85%. EC₅₀ for ABTS•⁺ radicals was 0.10 mg/mL. These results conclusively revealed that polysaccharides from *P. depressa* obtained by microwave extraction possessed free radical scavenging activity.

Table 4. DPPH• and ABTS•⁺ radical scavenging activities of *P. depressa* polysaccharides.

Free Radical	Sample	Fitting Equation	R ²	EC ₅₀ /(mg/mL) *
DPPH•	Ascorbic acid	$y = 32.088 \ln(x) + 226.000$	0.9964	4.15×10^{-3b}
	<i>P. depressa</i> polysaccharides	$y = 265.994 x^{1.027}$	0.9437	0.20 ^a
ABTS• ⁺	Ascorbic acid	$y = 35.791 \ln(x) + 164.370$	0.9044	4.09×10^{-2b}
	<i>P. depressa</i> polysaccharides	$y = 683.321 x^{1.135}$	0.9908	0.10 ^a

* Different superscript letters in this column mean significant differences ($p < 0.05$).

3.4. Effects of Polysaccharides on SOD and GSH-Px in *Drosophila melanogaster*

Usually, SOD and GSH-Px are regarded as some of the most vital endogenous antioxidant enzymes against oxygen radicals [23]. Remarkably, *P. depressa* polysaccharides have a substantial impact on SOD and GSH-Px activities in *Drosophila melanogaster* model (Table 5). For GSH-Px assay, enzyme activity in male polysaccharides group was significantly higher than that in male blank group ($p < 0.05$) (Table 5). And GSH-Px activity in the female polysaccharide group was slightly higher than that in female blank group ($p > 0.05$). For SOD assay, enzyme activities in polysaccharide groups (both sexes of fruit flies) were significantly higher than those in blank groups ($p < 0.05$) (Table 5). Likewise, activities of SOD and catalase (CAT) in *Drosophila melanogaster* fed with mediums in the presence of *Lycium barbarum* polysaccharides (LBPs) were notably higher than those fed with standard mediums in the absence of LBPs ($p < 0.05$) [24]. Intriguingly, sex differences of SOD and GSH-Px activities were observed when *Drosophila melanogaster* was fed with *P. depressa* polysaccharides (Table 5). SOD activity in male and female *Drosophila melanogaster* fed with psyllium polysaccharides increases by 33.91% and 24.87%, respectively. And GSH-Px activity in male and female fruit flies increases by 54.15% and 2.84%, respectively. Similar trends in activities of antioxidant enzymes (e.g., SOD) in male and female fruit flies were found in case of *Lycium barbarum* polysaccharides [24] as well as *Bletilla striata* polysaccharides [25]. According to the results of in vivo and in vitro experiments in Tables 4 and 5, polysaccharides from *P. depressa* obtained by microwave extraction increased SOD and GSH-Px activities in *Drosophila melanogaster*. It can be concluded that polysaccharides from *P. depressa* were a potential source of antioxidants. Psyllium was listed as one of high-grade herbs in *Shennong's Classic of Materia Medica* (ca. 140–87 BC), the oldest book of traditional Chinese medicine in existence. According to *Shennong's Classic of Materia Medica*, psyllium could build up the physique (“*qing shen*” in Chinese), prolong lifespan, and delay aging (“*nai lao*” in Chinese). In addition, some herbalist in Guangxi Zhuang Autonomous Region of China treated diabetes with psyllium [26]. Since oxidant stress is extensively accepted to be implicated in hyperglycemia and aging [10,22,23,27], curative effects of *P. depressa* on diabetes and age-related diseases may be partly mediated by their antioxidant capacity. Given the pharmaceutical importance of *P. depressa* polysaccharides especially their relatively high bioactivities against oxidative stress (Tables 4 and 5), there is considerable interest in research and development of natural antioxidants and potential chemopreventive and anti-diabetic agents derived from psyllium polysaccharides. In the future, further investi-

gations will be required to validate the effectiveness of *P. depressa* polysaccharides against health problems related to ROS such as diabetes.

Table 5. Effects of *P. depressa* polysaccharides on SOD and GSH-Px activity (Mean $\pm$ SD) in *Drosophila melanogaster*.

Enzyme Activity (U/mg Protein)	Male		Female	
	Polysaccharides Group	Blank Group	Polysaccharides Group	Blank Group
SOD *	108.30 $\pm$ 6.04 ^a	80.87 $\pm$ 18.48 ^b	93.20 $\pm$ 7.08 ^a	74.64 $\pm$ 1.41 ^b
GSH-Px *	348.84 $\pm$ 22.71 ^a	226.30 $\pm$ 12.18 ^b	191.33 $\pm$ 15.87 ^a	186.05 $\pm$ 8.25 ^a

* Different superscript letters in this row mean significant differences ($p < 0.05$).

4. Conclusions

In the present study, a protocol of microwave extraction was developed for separation of antioxidant polysaccharides from *P. depressa*, the structure of polysaccharides was characterized, and in vitro as well as in vivo antioxidant activities of polysaccharides were analyzed. The optimal condition for microwave extraction was as follows: duration of microwave radiation of 35 min, extraction temperature of 80 °C, and ratio of liquid to solid of 80:1 (mL/g). Extraction yield of psyllium polysaccharides by microwave extraction was significantly higher than that by conventional heating extraction. The volumes of *P. depressa* samples significantly increased after microwave extraction, which implied that microwave radiation might loosen the structure of cells and tissues of psyllium leaves, facilitate the exudation of target polysaccharides from leaf samples, and accordingly increase extraction yield of polysaccharides. Polysaccharides extracted from *P. depressa* possessed DPPH• and ABTS^{•+} radical scavenging activities. Moreover, they increased SOD and GSH-Px activities in *Drosophila melanogaster*. These findings indicated that microwave extraction seems to be a relatively ideal technique for isolating antioxidant polysaccharides from *P. depressa*, which might be a potential source of antioxidants for prevention and treatment of some ROS-related diseases such as diabetes.

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