Bangladesh J. Bot. **47**(1): 71-77, 2018 (March)

# MICROFLUIDIZATION-ASSISTED EXTRACTION OF POLYSACCHARIDE FROM *EUCOMMIA ULMOIDES* OLIV. LEAVES AND EVALUATION OF ITS ANTIOXIDANT ACTIVITY

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*Keywords*: Microfluidization-assisted extraction, *Eucommia ulmoides* leaves, Polysaccharide, Antioxidant activity

**Abstract**

Orthogonal test was used to optimize the process conditions of microfluidization-assisted extraction (MAE) of polysaccharide from *Eucommia ulmoides* Oliv. leaves. The effects of pressure, temperature and the time of extraction on the yields of polysaccharide and their antioxidative effect were investigated. Based on the single factor experiments, the optimal extraction conditions were as follows : pressure 200 MPa, extraction temperature of 60ºC and time extraction 60 min. Under these conditions, the polysaccharide yield was 1.86%. *E. ulmoides* leaves polysaccharide (EP) has a better scavenging effect on DPPH radicals and hydroxyl free radicals than ascorbic acid ($V_C$). MAE is a suitable method for extraction of polysaccharide from *E. ulmoides* leaves.

## Introduction

*Eucommia ulmoides* Oliv. (Xu *et al.* 2010), a traditional Chinese medicine, has been used for at least 2000 years. Recent studies have demonstrated that polysaccharide in *E. ulmoides* leaves polysaccharide (EP) exhibits various pharmacological benefits, such as anti-inflammatory, antioxidant, anti-bacterial and anti-fungal activities (Feng *et al*. 2016). Therefore, It is very useful to explore an effective extraction method for EP. Hot-water extraction (HWE) is a common method for extraction of polysaccharides extraction (Liu *et al.* 2016, Zhu *et al.* 2016). However, the HWE method has some disadvantages, such as higher temperature and longer extraction time but the efficiency of extraction is low. In recent years, microfluidization-assisted extraction (MAE) has been developed for the extraction of polysaccharides from plants (Huang e*t al.* 2012, Jing *et al*. 2016). MAE is not only cheap but also has a shorter extraction time and higher yield (Huang *et al*. 2013, Xia *et al*. 2012). So far, there is no report of using MAE in the extraction of EP and evaluation of its antioxidant activity. Thus, the objective of this work was to investigate the effect of extraction pressure, extraction temperature and extraction time on the yields of EP and subsequently to optimize the MAE conditions using orthogonal test methodology. Furthermore, scavenging effect of EP on DPPH radicals and hydroxyl free radicals had been studied.

## Materials and Methods

*Eucommia ulmoides* leaves (EL) were collected from experimental base located in countryside region of Zhengzhou, Henan province of China. The leaves were air-dried at 60ºC for 24 hrs and ground to powders through 80 meshes. Chemical reagents used in this study including 1,1-diphenyl-2-picrylhydrazyl (DPPH), D-glucose, salicylic acid, iron(II) sulfateheptahydrate and

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ethanol were obtained from Huafeng Chemical Reagent Co. (Zhengzhou, China). All the reagents were of analytical grade.

*Preparation of EP by MAE:* EL powder (10 g) was mixed with pure water in a 500 ml conical flask. Then, the mixture was homogenized at 20 MPa by a homogenizer (GJB30, Chaoli Co., Changzhou, China). Then it was treated with a high pressure microfluidhomogenizer (SCPH-10, SFPLTD, UK) at extraction pressure (0.1, 100, 150, 200 and 250 MPa, respectively) for twice. Then the mixture was kept at different temperature in water bath (30, 40, 50, 60 and 70ºC) for 20, 40, 60, 80 and 100 min. The mixture thus obtained was centrifuged at 5000 r/min for 20 min. The supernatant was concentrated to 30% of the original volume using a rotary evaporator (RE-52CS, Yanrong Co. China) under reduced pressure. Then ethanol was added until at 75%. Mixture was kept at 4ºC for 12 hrs. The extract was collected and was dried in a vacuum-freeze dryer (YB-FD-1, FS Co., China) to get EP.

*Preparation of Ep by HWE:* HWE of EP was used as the reference extraction method. Based on the results of preliminary experiments, the pre-treated sample was immersed in distilled water in a water bath for 6 hrs at 65ºC. After two replications, the solution also was centrifuged, concentrated, and dried as the same condition with MAE.

*Determination of extraction yield of EP:* Polysaccharides content was measured by phenol-sulfuric acid colorimetric method by using a UV-VIS spectrophotometer (Xinshiji T6, Puxi Co., Beijing, China).The yield of EP (%) was calculated using the following equation:

$$\text{Yield (\%)} = (\text{Weight of EP} / \text{weight of EL}) \times 100$$

*DPPH radical-scavenging activity:* The DPPH radical-scavenging activity of EP was measured by a previously described method (Zeng *et al*. 2016) with minor modifications. Briefly, the polysaccharides were mixed with 3 ml distilled water to prepare solution of series of concentration (0.5 - 2.5 mg/ml). Then these were mixed with 0.1 mmol/ml DPPH in methyl alcohol (1.0 ml). The mixture was kept at 25ºC in the dark for 30 min and the absorbance of the reaction mixture was measured at 517 nm using UV-VIS spectrophotometer. Ascorbic acid was used as a standard. The DPPH radical-scavenging activity was calculated according to the following equation:

$$\text{RSA (\%)} = \frac{1 - (A_1 - A_2)}{A_0} \times 100$$

where RSA is DPPH radical-scavenging activity, $A_0$ is a absorbance of only DPPH, $A_1$ is a absorbance of both sample and DPPH, $A_2$ is a absorbance of only sample.

*Hydroxyl radical-scavenging activity*: Hydroxyl radical-scavenging activity of EP was measured by a previously described method (Xie *et al*. 2016) by minor modifications. Briefly, the EP was added to 3 ml of distilled water to prepare a series of concentration (0.5 - 2.5mg/ml). Then they were mixed with 3.0 ml reaction liquid containing 1.0 ml $FeSO_4$ solution (10 mmol/l), 1.0 mL salicylic acid ethanol solution (2.0 mmol/l), and 1.0 ml $H_2O_2$ solution (5.0 mmol/l). The mixture was kept at 37ºC for 1 hr. The absorbance of the mixture was measured at 510 nm using a UV-VIS spectrophotometer. The hydroxyl radical scavenging activity was calculated according to the following equation:

$$\text{HSA (\%)} \frac{1 - (A_1 - A_2)}{A_0} \times 100$$

where HSA is hydroxyl radical-scavenging activity, $A_0$ is a absorbance of pure water, $A_1$ is a absorbance of sample and reaction solution, $A_2$ is a absorbance of sample solution.

*Statistical treatment of data*: All data are expressed as means ± standard deviation from triplicate samples. The significant differences among the treatments were determined using the p-value generated through t-test.

**Results and Discussion**

*Effect of extraction pressure on the yield of EP:* Extraction was carried out at different pressure at 65ºC for 60 min. The result is presented in Fig. 1.

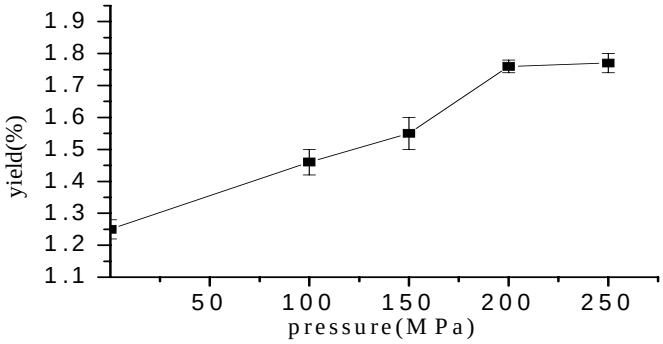


Fig. 1. Effect of extraction pressure on the yield of EP.

The yield of EP increased with the increase of the extraction pressure from 0.1 to 250 MPa and maximum yield reached at 1.76% at approximately 200 MPa. It can be explained that the high pressure and high-speed of jet make the EL cell wall ruptures. Polysaccharides were easy to release from broken cells. Extraction at 200MPa, almost all cell walls were fully broken and further increase in pressure will not increase significantly the yield. Therefore, the pressure of 200 MPa was chosen as the optimum pressure.

*Effect of extraction temperature on yield of EP:* Extraction was carried out at different temperature at 200 MPa for 60 min. The result is shown in Fig. 2.

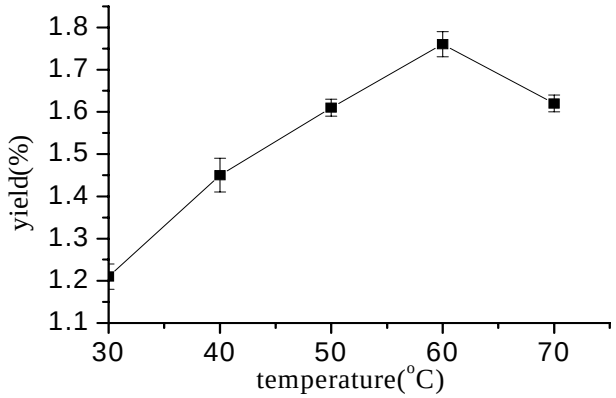


Fig. 2. Effect of extraction temperature on yield of EP.

The EP yield increased sharply from 1.21 to 1.76% (Fig. 2) when the temperature ranged from 30 to 60ºC. When the extraction temperature is higher than 60ºC, the yield of extraction will not increase. It can be explained that the extraction temperature until 60ºC can increase the polysaccharides diffusion coefficient, making an enhanced solubility of the polysaccharides in the extracting solvent. But when the extraction temperature is higher than 60ºC, it can lead to decomposition of the EP. Therefore, extraction at 60ºC was chosen as the optimum extraction temperature.

*Effect of extraction time on the yield of EP:* Extraction was carried out at different time and constant pressure of 200 MPa, and at 60ºC. The result is shown in Fig. 3.

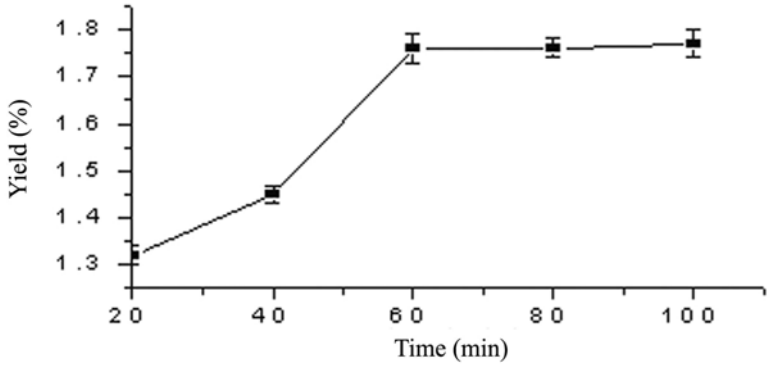


Fig. 3. Effect of extraction time on yield of EP.

The EP yield increased when the extraction time varied from 20 to 100 min and reached a maximum yield of 1.76% for approximately 60 min (Fig. 3). It can be explained that when time arrived at 60 min, the yield of polysaccharides started to maintain a dynamic equilibrium with increasing extraction time. Therefore, 60 min was chosen as the optimum extraction time for producing EP.

*Orthogonal test*: Based on the influence of the single level test, the yield of EP was selected as the main evaluating index. Extraction pressure (A), extraction temperature (B), extraction time (C) and blank (D) were chosen as main influencing factors. Results of orthogonal test are presented in Table 1.

**Table 1. Result of orthogonal test.**

| Sl. No. | (A) (MPa) | (B) (ºC) | (C) (min) | (D) | Yield (%) |
|---|---|---|---|---|---|
| 1 | 180 | 50 | 55 | 1 | 1.36 |
| 2 | 180 | 60 | 60 | 2 | 1.45 |
| 3 | 180 | 70 | 65 | 3 | 1.50 |
| 4 | 200 | 50 | 60 | 3 | 1.62 |
| 5 | 200 | 60 | 65 | 1 | 1.74 |
| 6 | 200 | 70 | 55 | 2 | 1.56 |
| 7 | 220 | 50 | 65 | 2 | 1.65 |
| 8 | 220 | 60 | 55 | 3 | 1.70 |
| 9 | 220 | 70 | 60 | 1 | 1.48 |
| $k_1$ | 1.437 | 1.543 | 1.540 | 1.527 | |
| $k_2$ | 1.640 | 1.630 | 1.517 | 1.553 | |
| $k_3$ | 1.610 | 1.513 | 1.630 | 1.607 | |
| R | 0.203 | 0.117 | 0.113 | 0.080 | |

According to the analysis of R, the optimum conditions were extraction pressure of 200 MPa, extraction temperature of 60ºC, extraction time for 60 min. At the best extraction parameters, the extraction was carried out with 1 kg material. Then the yield of EP was 1.86%. The variance analysis and F test were conducted. Both extraction temperature and extraction time did not significantly affect on the yield ($p > 0.05$), extraction pressure showed significantly effected on the yield ($p < 0.05$).

*Comparison of extraction yield with HWE*: Results on extraction yield of PE obtained by MAE and HWE methods are presented in Table 2. Remarkably, extraction yield of PE was higher than extraction yield of HWE. These results indicate that the application of MAE has a positive impact on extraction yield of PE. This is mainly attributed to the fact that high pressure and high-speed jet caused the EL cell wall to rupture and thus accelerate the extracting process so that the extraction of bioactive compounds obtained may be improved.

**Table 2. Comparison of the extraction yield with MAE and HWE.**

| Method | Yield (%) | Time (min) |
|---|---|---|
| MAE | 1.98 | 60 |
| HWE | 1.46 | 360 |

*DPPH radical scavenging activity:* Fig. 4 illustrates the scavenging activity of the EP samples on DPPH radicals. The scavenging rate increased from 42.2 to 78.6% as the concentration of EP increased from 0.5 mg/ml to 2.5 mg/ml. These results indicate that the EP exhibit significant scavenging ability, but the scavenging activity of EP was a little weaker than that of ascorbic acid.

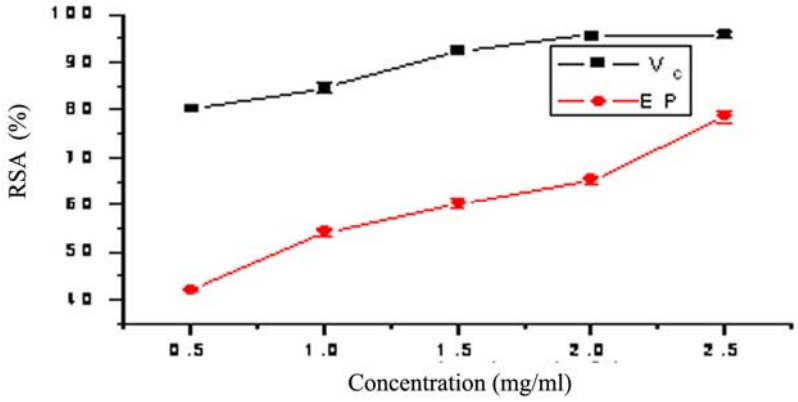


Fig. 4. DPPH radical scavenging activity of EP.

*Hydroxyl radical scavenging activity of EP*: Fig. 5 illustrates the hydroxyl radical scavenging activity of the EP samples. The scavenging rate increased from 12.8 to 58.8% as the concentration of EP increased from 0.5 to 2.5 mg/ml. These results indicate that the EP exhibit significant scavenging ability, but the hydroxyl radical scavenging activity of EP was weaker than that of ascorbic acid.

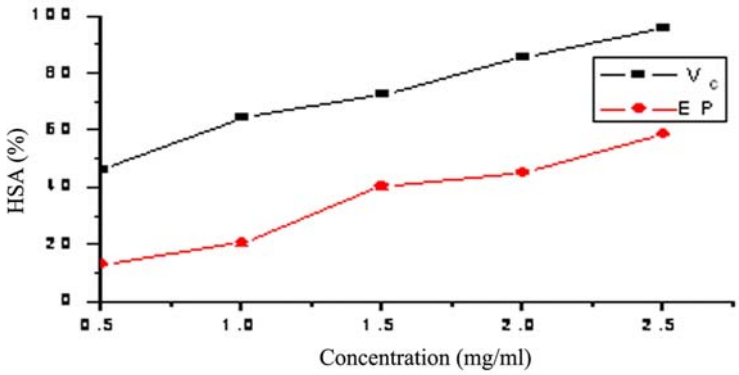


Fig. 5. Hydroxyl radical scavenging activity of EP.

A novel and an efficient MAE technique was employed to extract polysaccharides from EL. The maximum yield of 1.86% was obtained at extraction conditions of pressure 200 MPa, extraction time of 60 min, and extraction temperature of 60ºC. In comparison with HWE, MAE offered a higher extraction yield in a shorter time. Furthermore, the results of antioxidant activity assay demonstrated that EP extracted by MAE displayed a notable radical scavenging ability against DPPH and hydroxyl radicals, indicating that EP can be used as a natural antioxidants in functional food.

**Acknowledgements**

This research was financially supported by the National Key Research and Development Program of China ( 2017YFD060130205) and Henan province science and technology innovation talents(174200510002).

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*(Manuscript received on 24 April, 2017; revised on 17 July, 2017)*