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Development of ultrasonic-assisted extraction of antioxidant compounds from Petai (*Parkia speciosa Hassk.*) leaves

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Abstract. Research on Petai (*Parkia speciosa Hassk.*) suggests it has an antihypertension, antidiabetic, analgesic, and antiulcer effects. In the present study, an ultrasonic-assisted extraction method was developed for the effective extraction of active compound from petai leaves. Some parameters such as ethanol concentration (0, 20, 40, 60, 70, 80, 100 %v), solid-to-liquid ratio (1:5; 1:10; 1:15; 1:20; 1:25; 1:30; 1:35; 1:40; 1:50 g/mL), extraction time (15, 20, 25, 30, 35, 40, 50 minutes) and extraction temperature (40, 45, 50, 55, 60, 65, 70°C) were studied and evaluated base on extract yield and 1,1-diphenyl-2-picry hydrazyl (DPPH) scavenging activity. The result showed that the highest extract yield was obtained at 40% ethanol concentration, 1:30 (%w/v) of solid-to-liquid ratio, 30 minutes and 65°C of temperature with DPPH scavenging activity 92.53 ± 0.87% and extract yield 21.25 ± 2.38%. The result obtained is helpful for the utilization of Petai leaves, and also indicated that ultrasonic-assisted extraction is a very recommended method for the extraction of active compounds from plant material.

1. Introduction

Parkia speciosa Hassk., known as petai leaves, are a Mimosaceae family that contains amino acids such as lysine, aspartine, glucine, alanine, valine and leucine [1] and carbohydrates content of 13g/100g [2]. Petai leaves are also rich in mineral content such as calcium, phosphorus, magnesium, manganese [2] and sulfur [3]. Petai leaves (*Parkia speciosa Hassk.*) can also be used for the treatment of hypertension, diabetes, and headaches [4,5,6]. Petai leaves can function as antiulcer [7], while petai seed leaves contain vitamin C which is quite a lot and phenolic compound which can perform an antioxidant. Petai leaves can be utilized as a natural source of antioxidant [8].

Extracts of petai plants have been widely performed both for the skin of the fruit [9,10,11], the leaf part [7,12], and seed [13]. Common extractions to take antioxidants from petai plants are soaking extraction at room temperature or maceration. The maceration extraction is straightforward and economical, but the disadvantage is that it requires a lot of solvents and a long time. Previous studies have compared between microwave assisted and ultrasonic-assisted extractions to extract antioxidant compounds in petai leaves, and ultrasonic-assisted extraction provides an alternative method that can be developed to take the active compound of petai leaves [12].

Another advantage of ultrasonic-assisted extraction is surface contact between the solids and the wider liquid, due to the direct contact between particles and ultrasonic waves [14]. Extraction of the antioxidant compound from *Eucommiae folium* with ultrasound-assisted extraction resulted the flavor

content of flavonoids for 72 minutes reached 17.6% while the maceration with heating for 2.5 hours only reached 12% [15]. An antioxidant study of red grapes showed extrapolation using ultrasonic method had a DPPH radical binding ability of 70% higher than that of maceration [16].

Research on the extraction of ultrasonic assisted petai plants (*Parkia speciosa Hassk.*) by finding process conditions regarding solvent concentration, solids: solvents ratio, time, and temperature for optimal yield and scavenging activity have never been reviewed before.

2. Methods

Materials

The main ingredient of this research is petai leaves (*Parkia spesiosa* Hassk.) obtained from Semarang area, Central Java. Other materials which used: methanol reagents (Merck), DPPH (Sigma-Aldrich, 90% purity), ethanol (Merck, 96% purity), and aquadest.

Equipment

The tools which used in this study are electric scales (Sartorius), oven (Memmert), moisture analyzer (Radwag MAC50), digital ultrasonic cleaner (Branson 5210), UV-Vis spectrophotometer (Shimadzu 2480), rotary evaporator (Scilogex), Whatman filter paper, 100 mesh filter, vacuum pump and aluminum foil.

Raw Material Preparation

Petai leaves are uniformed with the size of 100 mesh and water content less than 10%w/w. Petai leaves (*Parkia speciosa Hassk.*) are cleaned and aerated without sun for 5-10 days. The dried leaves are smoothed with 100 mesh size followed by drying until the moisture content is less than 10%w/w.

Ultrasonic-assisted Extraction (UAE)

The extraction method is used ultrasonic-assisted extraction with ethanol solvent. The ultrasonic instrument used in digital ultrasonic cleaner (Branson 5210) with 200 W ultrasonic power and 40 kHz frequency, equipped with digital temperature and time control. Petai leaves powder (5.0 g) was weighed carefully in a glass tube, then added with solvent and homogenized. The tube was sealed and inserted into the water in digital ultrasonic cleaner that has been regulated process conditions. Extraction was performed to determine the yield of extract and scavenging activity produced by ultrasonic-assisted extraction with solvent concentration variables (0, 20, 40, 60, 70, 80, 100 percent of volume), solid (g): solvent (mL) ratio(1:5; 1:10; 1:15; 1:20; 1:25; 1:30; 1:35; 1:40; 1:50), time (15, 20, 25, 30, 35, 40, 50 minutes), and temperature (40, 45, 50, 55, 60, 65, 70°C). After extraction, sample was centrifuged and filtered using vacuum pump with Whatman. The solvent was separated from extract by rotary evaporator.

1,1 Diphenyl 2-Picryl Hydrazyl Scavenging Activity (DPPH-SA)

DPPH scavenging activity test was carried out by the method of Banerjee *et al*., 2005 [17]. The 1.0 mL sample extract solution was added 3.0 mL 0.1 mM DPPH solution. The absorbance was measured by spectrophotometer (Shimadzu Japan) at 514 nm. The extract activity on DPPH is expressed as:

$$\text{DPPH radical scavenging sctivity (\%)} = \frac{\text{A Blank - A sample}}{\text{A Blank}} \text{ X } 100\%$$

3. Result and Discussion

Effect of Ethanol Concentration on Extracts and DPPH-SA

Solvents that are often used for extraction include methanol, ethanol, and water. In this study used ethanol because it was safer for medicine and food than methanol. Water is also a safe and cheap solvent, but its effectiveness in extracting is lower than ethanol-water. The effect of ethanol concentration (0, 20, 40, 60, 70, 80, 100 percent volume) on yield extract and DPPH scavenging activity was studied with

fixed parameters: 1:10 solid-liquid ratio; time 20 minutes, temperature 40°C with frequency 40 Hz. The yield of extract is presented in Figure 1A. When ethanol concentration was increased from 0 to 40% extract yield increased to 10.04 ± 1.09%w. After concentration above 40%, yield tends to decrease even for 100% concentration was obtained yield 5.50 ± 0.53%w.
The results in this variable also tested its DPPH-SA and presented in Figure 2A. The graph profile shows the same characteristics as extract yield calculation. DPPH-SA increased from 0-40% ethanol concentration of 71.90±0.03 to 88.25±0.09% and decreased with increasing ethanol concentration above 40%. Zou *et al*. 2014 use mixture of water-ethanol to take the active compound on mango leaves and the best results with 40% ethanol. The results of this extraction variable also found an excellent ethanol-water mixture to take active substance content in petai leaves, and 40% ethanol gave the largest extract yield and DPPH-SA which will be used for further research [18].

Effect of Solid: Liquid Ratio against Extract Yield and DPPH-SA
The influence of solid-liquid ratio on extract yield and DPPH-SA was studied by variation (1:5; 1:10; 1:15; 1:20; 1:25; 1:30; 1:35; 1:40; 1:50 g/mL) and fixed parameters: ethanol concentration 40%, extraction time 20 minutes, temperature 40°C with frequency 40 Hz. The results are presented in Figure 1B (effect on extract yield). When the solid: liquid ratio is raised from 1:5 to 1:30,extract yield also increases, and after that, it tends to remain even down at 1:50 ratio. In general, the greater use of solvents increases extracted active substance [19]. But the use of too high solvents will be much wasted and ineffective, the lack of solvent leads to less optimum extraction performed [20]. So the selection of solvent amount is crucial.

The results of DPPH-SA measurements are shown in Figure 2B. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) compound is a stable free radical having nitrogen free radicals commonly used to measure antioxidant activity of extract. Free radicals are unpaired unstable electrons, and their molecules are highly reactive. DPPH radical scavenging activity by antioxidants occurs through provision of protons to radicals. Compounds capable of donating protons show strong radical scavenging activity. Some phenolic compounds in the extract release their hydrogen atom to bind 1,1-diphenyl-2-picrylhydrazyl and then convert to 1,1-diphenyl-2-picryl hydrazine (nonradical). Once stable, these compounds do not attack other molecules to form new radicals. An increase of 1,1-diphenyl-2-picryl hydrazine nonradical amount will be characterized by color change from purple to yellow. Figure 2B showed an increase of the solid: liquid ratio from 1:5 to 1:30 will increase DPPH-SA even though not significantly, and increasing of further solvent gave DPPH-SA value fixed and tended to decrease. The results of this study are in line with other UAE studies. Oancea *et al*. 2013 reported a not-so-great difference in the anthocyanin yield of a gold black sweet cherry at 10:1 - 20:1 (v/w) ratio for 20 minutes using UAE [21]. Petigny *et al*. 2013 reported an insignificant effect of solvent ratios at 4-10% on Boldo leaves yield [22]. Wardhani *et al*. 2013 reported the effect of *Eucheuma cottonii* seaweed on solid solvent ratio at 3:1-10:1 (v/w) ratio is also insignificant [23]. The highest DPPH-SA in solid: liquid ratio 1:30 equal to 88.19±0.92%. Solid: solventratio 1:30 gives the largest extract yield and DPPH-SA and will be used for further research. The results of this ratio are in line with research of Zou *et al*. 2014 to take active compounds through UAE [18].

Effect of Extraction Time on Extract Yield and DPPH-SA
The effect of extraction time was done with time variation (15, 20, 25, 30, 35, 40, 50 minutes), and as fixed parameter of 40% ethanol concentration, solid: liquid ratio 1:30 g/mL, 40°C with frequency 40 Hz. The results are presented in Figure 1C. Yield continues to increase from 15 to 30 minutes. Longer extraction times allow more contact time for cavitation bubbles broke sample cells, increasing extracted active substances [24]. Solid-fluid extraction is a mass transfer phenomenon which compounds in a solid matrix migrate into the solvent through diffusion and osmotic ultrasound-induced mechanisms. The ultrasound application in the form of solid-liquid extraction forms a cavitation bubble which disconnects the sample cell to solvent facilitate penetration into the cell. Maximum yield result is 92.85±0.61% at

30 minutes. Yield decreasedmore than 30 minutes. The results show that ultrasound can accelerate formation of equilibrium for active substance dissolution between sample wall and extraction solvent in short time. This effect is an advantage of UAE compared to conventional extraction methods [25]. Prolonging the extraction time no longer affects active compound amount of *Orthosiphon* which is significantly removed when the diffusion process reaches equilibrium [26]. In this study, the maximum is 30 minutes and will be used for further research.

DPPH-SA for time variation is shown in Figure 2C and showed an upsurge of activity, although not too large from 15-30 minutes after which the DPPH-SA decline. Extraction time extension after the equilibrium time causes phenolic active ingredient, exposed to light and oxygen for longer time. This exposure allows the phenolic compounds to become oxidized which in turn decreases the obtained DPPH-SA. Also, prolonging the extraction time may lead to the accumulation of compounds that may increase oxidation [27].

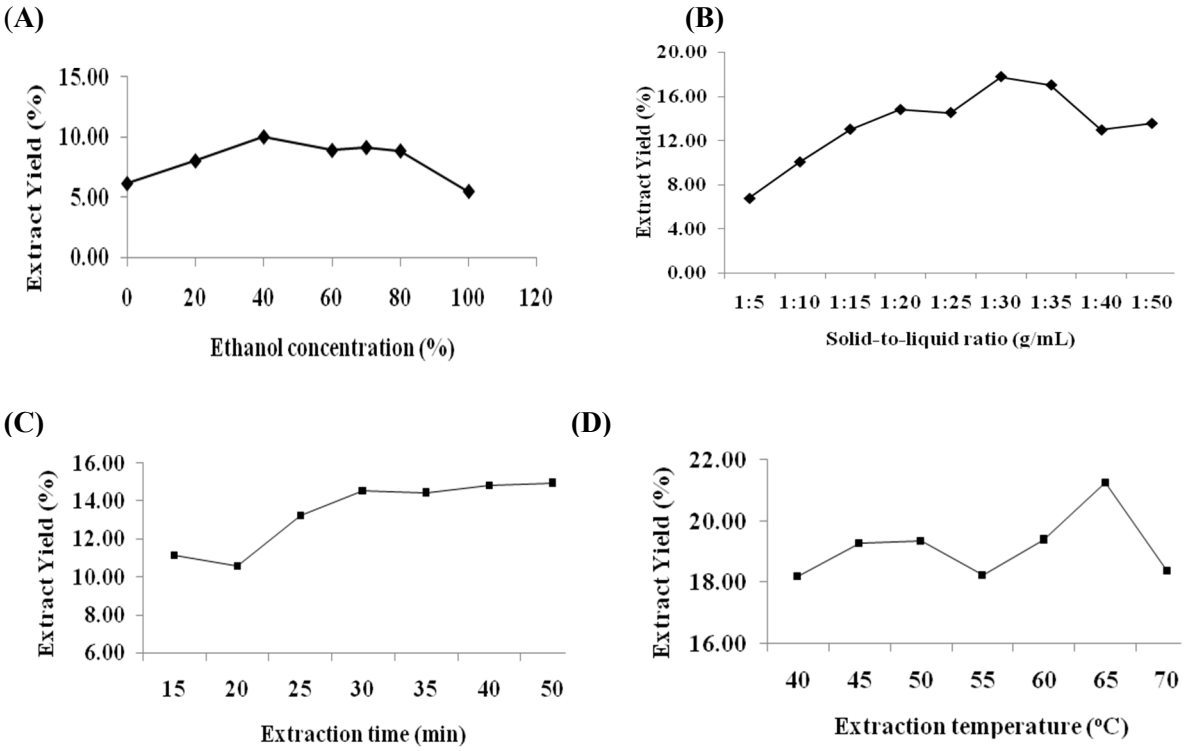

Figure 1. Effect of extraction parameters on extract yield. **(A)** Effect of ethanol concentration on extract yield; **(B)** Effect of solid-liquid ratio on extract yield; **(C)** Effect of time on extract yield; **(D)** Effect of temperature on extract yield.

Effect of Extraction Temperature on Extract Yield and DPPH-SA

The influence of extraction time was carried out with temperature variations (40, 45, 50, 55, 60, 65, 70°C), and as fixed parameter of 40% ethanol concentration, solid: liquid ratio of 1:30 g/mL, 30 minutes with frequency 40 Hz. The results are shown in Figure 1D for extract yield and 2D for DPPH-SA results. The best temperature in this study was found 65°C with yield of 21.25 ± 2.38% and DPPH-SA of 92.53 ± 0.87%.Heo et al. 2005 found 70°C as the best extraction temperature of various seaweed antioxidant compounds [28]. Wardhani *et al*. 2013 found the best temperature for extraction of phenolic compounds at 55°C with methanol solvent [23]. Zou *et al*. 2014 found an optimum temperature of 60°C to extract mango leaves with 40% ethanol solvent [18].

The best extraction temperature difference shows that ultrasound application reduces phenolic extraction optimum temperature compared with conventional extraction. In this process, the heat gradually propagates from solvent to solid. Meanwhile, direct contact between solid and ultrasonic waves in the UAE allows reaching optimum temperatures that do not damage extracts compared to conventional extractions. Ultrasound assists mechanical effects with compression cycles and continuous evacuation that allow greater solvent absorption into sample matrix, increasing contact surface area between solid and liquid phases, so that the solute diffuses fast from solid phase to solvent [29].Phenolic compounds are unstable at higher temperatures. Thus high extraction temperatures may increase extract impurity due to joint extraction of other undesirable compounds [30]. However, several previous studies have shown that ultrasound induction can maintain extracts with a high-temperature range between 60-80°C.

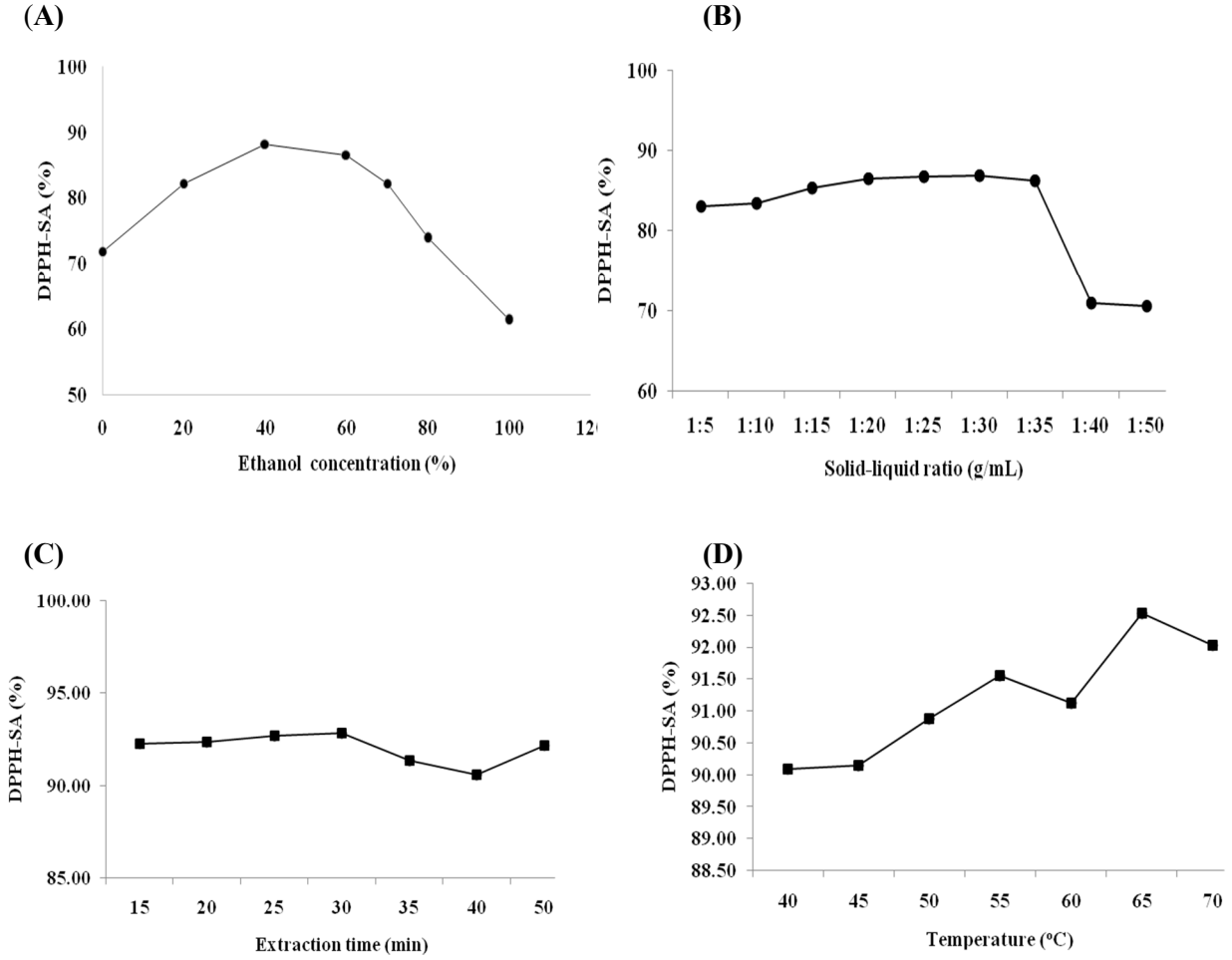

Figure 2.Effects of extraction parameters on DPPH-SA. (A) Effect of ethanol concentration on DPPH-SA; (B) Effect of solid: liquid ration on DPPH-SA; (C) Effect of time on DPPH-SA; (D) Effect of temperature on DPPH-SA.

4. Conclusion

In this study, an ultrasonic-assisted extraction method has been developed for extraction of Petai leaves active compound (*Parkia speciosa Hassk.*). Ultrasonic waves are tools that can efficiently improve extraction results. The results showed that ethanol concentration, solid-liquid ratio, and extraction time affected extraction result in extract yield and DPPH-SA value. The best-operating conditions are 40% ethanol, 1:30 solid-liquid ratio, and 30 minute extraction time at 65°C with 40Hz ultrasound frequency. The highest yield value reached 21.25±2.38% and DPPH-SA of 92.53±0.87%.

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