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Extraction of Phenolic Compounds from Petai Leaves (*Parkia speciosa* Hassk.) using Microwave and Ultrasound Assisted Methods

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Abstract

The antioxidant has an activity to neutralize free radical compound that the body needs to avoid damage cells and tissues. Phenolic is one of the compounds that have an antioxidant activity. The influences of ultrasonic-assisted extraction (UAE) and microwave-assisted extraction (MAE) conditions on phenolic compounds of *Parkia speciosa* Hassk. leaves were investigated. The effects of temperature (40°C, 50°C, 60°C and 70°C), time (10, 30 and 50 minutes) and material-solvent ratio (1:10, 1:13, 1:15) were evaluated based on the yield, total phenolic content (TPC) and antioxidant activity. The result showed that the highest yield (15.82%) was obtained at 1:15 (w/w) of material-solvent ratio, 50°C of temperature and 50 minutes of extraction time for MAE. The highest yield of UAE is 15.53% that sample was obtained at 1:13 (w/w) of material-solvent ratio, 60°C of optimal temperature and 30 minutes extraction time. The highest IC_{50} of UAE method extract was 52.55 ppm, while the extract obtained using MAE method was 50.44 ppm. UAE is more stable at higher temperatures. Time and solvent which was used more efficient than MAE. Extract of petai leaves (Parkia speciosa Hassk.) were very potential to be used as a source of natural antioxidants because they have IC_{50} values from 41.39 to 66.00 ppm. Its antioxidants capacity is ranged from strong to very strong capacity.

INTRODUCTION

Antioxidants are compounds that inhibit the oxidation of other molecules due to free radicals that can cause damage to the unsaturated fatty acid, cell wall, vein, DNA base and lipid structure. This oxidation process will lead to illness such as diabetes, cancer, liver disease and kidney failure (Parr & Bowell, 2000). Plants with antioxidant activity will have compounds that can prevent free radical for example polyphenol, bioflavon, vitamin C, vitamin E, carotenoid and catechin (Hernani & Mono, 2002). Mostly used of antioxidant component was phenolic derivatives (Tahir et al., 2003).

Phenolic compounds ability to catch free radicals is 100 times more effective compared vitamin C and 25 times more effective to vitamin E (Septianingrum et al.,2012). While, flavonoid has an ability to capture free radicals 25 times stronger than vitamin C and its potential is 50 times higher than vitamin E (Shi et al., 2003). Based on those research phenolic compound is potentially used as natural antioxidant.

Generally, antioxidant from plants and seaweed are extracted by soaking it at room temperature or using maceration method (Jamal, 2013). Maceration is a simple and low cost method, but the disadvantages of this method are it requires many solvents and longer extraction time. Antioxidant compound of kedawung (*Parkia javanica Lam.)* has been extracted using acetone for 24 hours gave a 6% yield of kedawung (Olabinri,

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2013), when it was extracted using ethanol 70% form 72 hours yield 15.13% (Niken, 2010). It shows that both method require long time for extraction process, hence extraction method with short time is needed.

One of the improvement in extraction method is ultrasound and microwave assisted extraction methods. Extraction method, ratio of mixture to solvent, extraction temperature and extraction time are affecting the extraction process of phenolic compound from plants (Kojic et al., 2011). Free radical and total phenolic compound obtained from microwave assisted extraction with extraction time of 1 – 3 minutes was almost the same as obtained by maceration method with extraction time of 15 hours (Hongyan et al., 2012). *Ainsliaea milleofolium* that was extracted using microwave for 20 minutes yield phenolic compound of 62%, meanwhile maceration method for 72 hours with the same plant only produce 35% yield of phenolic compound (Mathur, 2011).

Another potential extraction method to be improved in this research was ultrasound assisted extraction method. It is due to ultrasound assisted extraction has shorter extraction time compared to maceration extraction method. Larger contact area between solid and liquid also become one of advantages ultrasound method. This happen due to direct contact between particles and the ultrasound wave (Wiyarno, 2010). Huan et al. (2009) studied the antioxidant extraction from folium eucommiae using ultrasound assisted method for 72 hours and yield 17.6% of flavonoid. Meanwhile, same material was extracted using maceration method for 2.5 hours and only gave 12% of flavonoid yield. Extraction of antioxidant from red grape has been conducted by Morelli (2012), the results showed that ultrasound method have 70% higher ability to attach DPPH radical than maceration method. It can be concluded that ultrasound and microwave assisted extraction can reduce extraction time and the amount of solvent for extraction process.

Generally extraction of phenolic compound from petai leaves (*Parkia speciosa* Hassk.) is using maceration method. The disadvantages of maceration method were require longer extraction time, use so many solvents and also solvents ability is defective (Meloan, 1999). This research is going to study ultrasound and microwave assisted extraction method in phenolic compound extraction of *Parkia speciosa* Hassk. Variables used in this research were extraction time, extraction temperature and ratio between feed with solven. This study has never been conducted before. From the experimental results we can decide which on is the most effective extraction method to obtain the certain antioxidant compound.

MATERIALS AND METHODS

Materials

Petai leaves (*Parkia spesiosa* Hassk) were collected from semarang, Central Java. Chemicals used were: Folin Ciocalteu Reagent (Merck), Na_2CO_3 (Merck, 99% purity), Gallic acid (Merck, 99% purity), ascorbic acid (Sigma, 99% purity), DPPH (Sigma, 90% purity), ethanol (Merck, 96% purity), methanol (Merck) and aquadest.

Equipment

Equipment used in this research were electrical balance (Satorius), oven (Memmert), microwave (Samsung), digital ultrasonic cleaner (Branson 2510), Spectrophotometer (Optima SP 300 and SP 3000), glassware (Pyrex), filter paper, 100 mesh strainer and aluminium foil.

Raw Material Preparation

Petai leaves were cleaned and dried without sunlight for 5 – 10 days. The dried leaves were then finely milled and sieved sing 100 mesh strainer. Finally, the materials were dried once again to reduce water content of 10%.

Extraction Process

Phenolic compound extractions were carried out to compare the yield obtained from ultrasound and microwave assisted extraction method. Sample and solvent were put into beaker glass and cover it with aluminium foil. For microwave assisted extraction, the beaker glass was inserted inside microwave oven while beaker glass was placed inside digital ultrasonic cleaner equipped with temperature control for ultrasound assisted extraction method. Experiments were carried out to study the effect of extraction time (10, 30 and 50 min), temperature (40, 50, 60 and 70°C) and sample to solvent ratio (1:10, 1:13 and 1:15 w/w). The solvent used in this research was solvent, since it is safe for food and ethanol is the best solvent compared to methanol, n-hexane and acetone in tannin extraction of *Mimosa pudica* leaves (Martono et al., 2012). Ethanol also the best choice

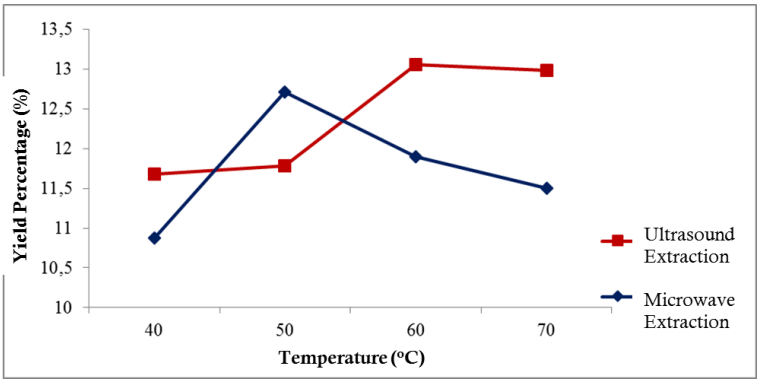

Figure 1. Effect of Extraction Temperature to the Extraction Yield

solvent in extraction of oleoresin and aldehyde from cinnamon (Jos et al, 2011).

Determination of Total Phenolic Contents

Total phenols were determined by using spectrophotometer with gallic acid as the standard solution based on Folin-ciocalteu method (Waterhouse, 1999). Total phenol values are expressed in terms of gallic acid equivalent (GA eq) which is measured using spectrophotometer (λ = 762 nm).

DPPH Radical Scavenging Activity

DPPH Radical –scavenging activity was determined according to the method reported by Benerje et al. (2005). DPPH stock was dissolved with 100 ppm of methanol solution. The mixture was diluted into 80 ppm of concentration. The absorbance was measured at 514 nm using spectrophotometer. 1 ml of the extract petai leaves sample with concentration of 100, 80, 60, 40 and 20 was mixed with 4 ml DPPH stock solution. This mixture was protected against the light. The IC_{50} of each extract was measured based on the concentration of antioxidant which reduces the free radical DPPH about 50%.

RESULTS AND DISCUSSIONS

This research was conducted to study the extraction method to obtain the antioxidant inside petai leaves *(Parkia speciosa* Hassk.) and find out its antioxidant activity. In the previous experiment, the average extraction yield obtained using maceration method was less than 10%.

The Influence of Extraction Temperature on Extraction Yield

Effect of extraction temperature was studied for both microwave and ultrasound assisted extraction method. The extraction time and material weight to solvent ratio were maintained at 10 minutes and 1:10. While Extraction temperature was varied by 40, 50, 60 and 70°C. Figure 1 shows the influence of extraction temperature to the yield for both method. It can be seen that ultrasound assisted extraction gave higher yield (highest yield obtained at 60°C) than microwave assisted extraction (highest yield obtained at 50°C).

In microwave assisted extraction, the yield is decreasing when the extraction temperature is higher than 50°C. At temperature of 60°C some antioxidants inside petai leaves extract were damaged due to high temperature. Due to this reason, the yield is decreasing at higher temperature. As stated by Liu et al. (2010) that at higher temperature, phenol compounds that act as antioxidant will be damaged. The damage was caused by several phenol compounds such as cinnamic acid and flavonols are easily degraded at temperature above 50°C (Liazid et al., 2007).

While in ultrasound assisted extraction, the increase of temperature has increase the yield. Higher temperature at ultrasound assisted extraction will increase the oxidants solubility (Amarnath, 2004). Previous research results conducted by Zou et al. (2014) showed that the optimum condition for phenolic compound extraction from Mango leaves was at 60°C and remain stable till 80°C.

The Influence of Extraction Time on Extraction Yield

The optimum temperature on the previous result was used for each extraction method. Material to solvent ratio was maintained at 1:10. Longer extraction time will increase extract amount, however it will become constant when equilibrium condition is achieved (Silva et al.,

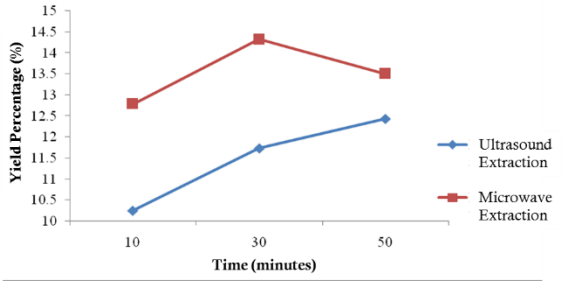

Figure 2. Effect of extraction time on the extraction yield

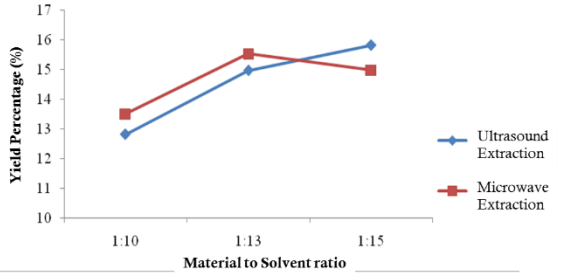

Figure 3. Effect of material to solvent ratio on the extraction yield

2007). Extraction times were varied by 10, 30 and 50 minutes. The results are shown in Figure 2.

Longer extraction time will increase the amount of extracted phenol. During extraction process, solute and solvent will have longer contact time. Phenolic compound will continuously dissolved into the solvent and stopped when solvent is saturated with the solute (Ghafoor et al., 2009). Fick's second law stated that at a certain time diffusion process will reach the equilibrium condition where at that time longer extraction time does not increase the extracted compound (Chew et al., 2011).

With the increasing of extraction time, the yield obtained from ultrasound assisted extraction is always higher than using microwave. Decrease of extraction yield in ultrasound assisted extraction was observed at extraction time of 30 minutes. Ultrasonic exposure at longer time and higher temperature might cause the damaged to the phenolic compound. In their research, Mario et al. (2010) mentioned that phenolic compound from plant can be extracted at temperature range of 35 – 120°C. Several phenolic compounds are sensitive to the temperature change, however each plant might have different phenolic compound characteristics.

The Influence of Material to Solvent Ratio on Extraction Yield

The material to solvent ratio in this experiments were varied by 1:10, 1:13 and 1:15. The experiments were conducted for both extraction method at each optimum extraction time and temperature based on the previous result. The influence of material to solvent ratio on extraction yield is presented in Figure 3.

Larger amount of solvent, the more compound diffuse into the solvent. Higher volume of the solvent will increase extraction yield. It is suspected that higher solvent volume leads to cell swelling. The increasing volume will increase the pressure of cell from the inside, it will make it stretch till finally break the cell and increase the molecules (Azmi et al., 2015). Extraction yield using microwave assisted method is increasing as the ratio increases. Meanwhile, extraction yield using ultrasound method is increasing with material: solvent ratio at 1:13 and decreasing at ratio of 1:15. At this ratio, the solvent already reached its saturation point.

Based on the optimization of extraction temperature, time and material to solvent ratio, it can be concluded that ultrasound assisted extraction is more optimum than microwave assisted extraction. Ultrasound assisted extraction is more stable at high temperature and has more efficient extraction time and solvent amount for higher extraction yield.

Total Phenolic Content Analysis

Total phenolic content in this experiment was determined using spectrophotometry. Total phenolic contents are expressed in terms of gallic acid equivalent per 100 gram dried extract weight (g GA eq/100 g). This is due to the unknown chemical structure of the phenolic compound contained in the extract. Standard curve for gallic acid is shown in Figure 4.

Total phenolic contents were obtained from extract samples at different process variable. It was chosen from the extract with highest yield at the best extraction temperature, time and material to solvent ratio. This measurement was intended to determine the average total phenolic content of petai leaves extract and compared it with the extraction yield. Total phenolic content analysis are shown in Table 1.

The increase of yield also followed by the increasing of total phenolic content. It shows that total phenolic content is affected by the yield percentage of phenolic compound. Therefore, selection of appropriate method which can give

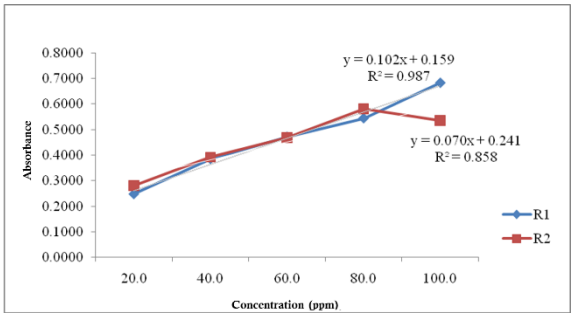

Figure 4. Gallic acid standard curve for Total Phenolic Content (TPC) determination.

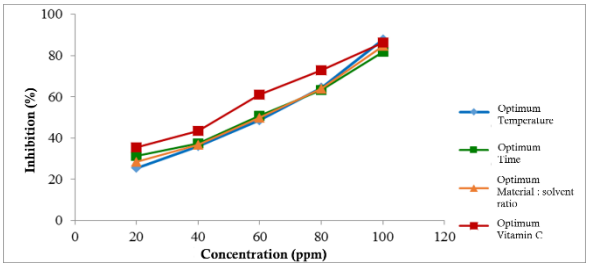

Figure 5. Correlation between concentrations of sample solution to the Vitamin C inhibition percentage.

Tabel 1. Total phenolic content (TPC) analysis

Sample	Microwave assisted extraction		Ultrasound assisted extraction	
	Yield (%)	TPC (mg GA eq/ g sample)	Yield (%)	TPC (mg GA eq/ g sample)
1	13.71	497.47	12.46	306.40
2	14.43	704.93	13.06	505.57
3	15.02	720.58	14.32	545.32

Notes : **1** (extract at optimum temperature), **2** (extract at optimum extraction time), **3** (extract at optimum material to solvent ratio)

Tabel 2. Antioxidant activity of petai leaves extract.

Sample	Antioxidant activity (IC_{50} = ppm)	
	Microwave assisted extraction	Ultrasound assisted extraction
1	66.00	57.46
2	43.92	55.22
3	41.39	44.98
Vitamin C	19.98	19.98

Notes : **1** (extract at optimum temperature), **2** (extract at optimum extraction time), **3** (extract at optimum material to solvent ratio)

high extract yield is needed to obtain large amounts of phenolic compound.

Antioxidant Activity Analysis

Antioxidant activity of petai leaves extracts were analyzed using DPPH method and expressed using IC_{50} value. Positive control used in this research was vitamin C. This test was carried out for both microwave and ultrasound assisted extraction method. Antioxidant activity for ultrasound assisted extraction results are presented in Figure 5.

Antioxidant activity is expressed as inhibition concentration (IC_{50}) value. IC_{50} from each extract was determined to obtain the absorbance intensity or about 50% of free radical DPPH scavenging. IC_{50} from vitamin C and petai leaf extract are shown in Table 2.

IC_{50} value was obtained from liner regression equation between concentration and inhibition percentage. The line was drawn from 50% inhibition line until it make an intersection with concentration axis. IC_{50} value illustrates the inhibition ability of sample and vitamin C solution. Vitamin C was used since it has good free radicals inhibition capabilities (Haryoto et al., 2017).

Extract with better antioxidant activity was found with lower value of IC_{50}. Specifically, sample that has IC_{50} less than 50 ppm is a very strong antioxidant, 50 – 100 ppm is a strong antioxidant, 100 – 150 ppm is a medium antioxidant, IC_{50} between 150 - 200 ppm is a weak antioxidant (Mardawati et al., 2008). As shown in Table 2, petai leaves have IC_{50} of 41.39 – 66.00 ppm showing that it is a strong – very strong antioxidant. Accordingly,

petai leaves (*Parkia speciosa* Hassk.) extract are potentially used as antioxidant source.

CONCLUSION

In this study, optimum yield by using microwave assisted extraction was 15.82% obtained at extraction temperature 50°C for 50 minutes extraction time with material to solvent ratio is 1:15. While ultrasound assisted extraction at temperature of 60°C, 30 minutes of extraction time and material to solvent ratio of 1:13 gave optimum yield of 15.53%. Average total phenolic content obtained from both method was 546 mg GA eq/g sample. Ultrasound assisted extraction shows more stability at higher temperature and also require more efficient extraction time and solvent amount. Petai leaves (*Parkia speciosa* Hassk.) extract are potentially used as antioxidant source since its IC_{50} value was 41.39-66.00 ppm indicating this extract has strong-very strong range antioxidant activity.

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