The Effect Of Microwave AID Extraction Temperature Of Kedawung Leaves (Parkia biglobosa) On Antioxidant Activity And Flavonoid Leavels

Ferika Indrasari1*, Buanasari1
1Department of Pharmacy, STIFERA, Semarang, Indonesia

*Corresponding author : ferikaindrasari89@gmail.com

Abstract Parkia biglobosa contains various ingredients such as alkaloids, saponins, tannins, flavonoids, and terpenoids. The flavonoid content in kedawung is thought to have an antioxidant effect. Antioxidants have the ability to provide electrons, bind and have a free radical chain reaction. Currently, the microwave-assisted extraction (MAE) method is widely used because extraction using MAE can increase the mass transfer rate of solutes from the sample matrix into the solvent compared to the Soxhlet method. One of the factors affecting microwave-assisted extraction is temperature because a higher temperature will increase the solubility of the solute in the solvent. The temperature is limited by the boiling point of the solvent used. This study aims to determine the effect of temperature on the solvent in order to get the best extraction results. This method uses temperature variations to determine the highest levels of flavonoids from microwave-assisted extraction of leaves and bark of kedawung, the temperature used is 30°C, 40°C, 50°C, 60°C with ethanol solvent at a wavelength of 300 W. The results of this study say that there is an effect of temperature on the extraction results. microwave-assisted to the yield of kedawung leaf extract (Parkia biglobosa) at the best temperature of 40°C. Kedawung leaves (parkia biglobosa) have high levels of flavonoids so they have antioxidant activity.

1. Introduction

Indonesian is well known for plants that have pharmacological effects or cure towards various diseases. (Agestia, 2009). Many plants contain antioxidant compounds such as phenolic compounds, flavonoids and xantones. This compound can be used as a natural antioxidant (Efendi, 2007). Antioxidants have the ability to provide electrons, bind and have a free radical chain reaction. (Halliwell, 2012). Antioxidants function as an antidote to free radicals in the body so they can prevent from the cell damage (Syafiiuddin, 2015).

Kedawung (Parkia biglobosa) has various pharmacological activities such as antibacterial, antifungal and antioxidant activity. Currently, the microwave-assisted extraction (MAE) method is widely used because it can increase the mass transfer rate of the solute from the sample matrix into the solvent compared to the Soxhlet extraction method (Mandal et al, 2007). In addition, the time required for extraction is relatively short, and the obtained rendement is high compared to other extraction methods, such as maceration, soxhlet extraction, and ultrasonic.

A study suggested that temperature might increase the rendement from 168,464,05% to 183,511,80%. By increasing the temperature from 40 oC to 60 oC, the antioxidant activity decreased. It was because of the decomposition phenomena during the increase of the heat. Hence, the temperature of 40 oC would be used as a standard temperature for the futher measure.
This study aimed to evaluate the effect of temperature of microwave aid extraction on the antioxidant activity of parkia biglobosa’s leaf extract and its concentration.

2. Methods

a. tools and materials
The materials used were simplicia of kedawung leaves (Parkia biglobosa), 10% AlCl₃, 5% acetic acid, 2,2-Diphenyl-1-PicryHydrazyl / DPPH, and 40% technical ethanol, methanol p.a, Kuarcetin, distilled water. The tools used were blender, knife, electric scale (Ohaus), oven, microwave, spectrophotometer (UV mini-1240), beaker glass (pyrex), Whatman paper, 100 mesh and aluminum foil, Blender (Miyako), Erlenmeyer (Pyrex), stirring rod, porcelain cup, test tube (Pyrex), dropper pipette, volume pipette, measuring pipette, micropipette, measuring flask, moisture content (MC).

b. Research design
This was an experimental study in which we evaluated the effect of temperature of microwave-assisted extraction on the yield obtained as well as its antioxidant activity.

c. Extraction process.
The extraction was carried out using the microwave assisted extraction (MAE) method. The leaves and bark of kedawung (Parkia biglobosa) were extracted by using 40% ethanol with the ratio of material: solvent 1:20. Time required for extraction was 6 minutes, with a power of 300 W. The solution was then irradiated in a microwave in the temperature of 30 °C, 40 °C, 50 °C, 60 °C. The yield of the extract was then calculated. The extraction was left to the room temperature.

d. Analysis of total flavonoid levels
1. maximum wavelength measurement
1 ml of quercetin solution of 100 ppm was taken and added with 1 ml of 10% AlCl₃ and 8 ml of 5% CH₃COOH. The absorbance of the solution was then measured by using UV-Vis spectrophotometer at the wavelength of 350-450 nm.

2. operating time measurement.
1 ml of quercetin solution of 100 ppm was taken and added with 1 ml of 10% AlCl₃ and 8 ml of 5% acetic acid. The absorbance of the solution was then measured at the obtained wavelength in the intervals of 2 minutes until a stable absorbance was obtained.

3. determination of the quercetin standard curve
A standard solution of quercetin with the concentration of 60, 80, 100, 120, 140 ppm was then made. As much as 1 ml of each solution was then taken and added with 1 ml of 10% AlCl₃ and 8 ml of 5% acetic acid. Let stand for 16 minutes. The absorbance of the solution was then measured at the maximum wavelength.

4. Total Flavonoid measurement.
The 1000 ppm extract solution was taken as much as 1 ml, added with 1 ml of 10% AlCl₃ and 8 ml of 5% acetic acid, let stand for 16 minutes. The absorbance readings were carried out at the maximum wavelength.

e. Antioxidant activity measurement using DPPH
1. Maximum Wavelength measurement
1 ml of 0.1 mM DPPH blank solution in p.a methanol was added to the p.a methanol to bring the volume to the mark on the 5 ml volumetric flask. Let stand for 30 minutes in a dark place. Solution’s absorbance was then measured using UV-Vis spectrophotometer at the wavelength of 450-550 nm (Molyneux, 2004).

2. Operating Time measurement
1 ml of 0.1 mM DPPH solution was added with a standard solution of 6 ppm quercetin to the limit mark on a 5 ml volumetric flask. The solution was then read at a maximum wavelength of 516 nm with a time interval of 2 minutes (Maulina, 2014).

3. determination of the quercetin standard curve
A standard solution of quercetin with the concentration of 0.5, 1, 2, 4, 8 ppm was then made. As much as 1 ml of the standard solution of 0.4 mM was added with the standard solution of quercetin to bring
to the mark on the 5 ml volumetric flask, then left in a dark place for 26 minutes. The absorbance was then read at a maximum wavelength of 516 nm (Nihlati et al., 2008).

d. Determination of IC50 Value

1000 ppm extract solution was made in a concentration of 10, 20, 30, 40, 50 ppm. A total of 1 ml of 0.4 mM DPPH solution was added to each sample solution to bring the volume to the mark on the flask. The solution was allowed to stand in a dark place for 26 minutes. The solution was then read at a maximum wavelength of 516 nm.

3. Results and Discussions

From the results, it was obtained that the moisture content of leaves and the stem was of 4.70% and 9.026% respectively. It met the requirement for the standard moisture content of simplicia (<10%). (Kumalaningsih, 2006). The characteristics of sample were listed on the table 1:

| Characteristic of Kedaung Simplicia | Daun | Kulit Batang |
|------------------------------------|------|-------------|
| Bentuk                             | Serbuk | Serbuk     |
| Warna                              | Hijau  | Coklat      |
| Bau                                | Bau khas | Tidak berbau |
| Rasa                               | Pahit  | Pahit       |
| Tekstur                            | Halus  | Halus       |
| Kadar Air                          | 4,70 % | 9,026%      |

The fitochemical screening aimed to identified the bioactive compounds of the extract. The results are listed in the table 2:

| Uji Fitokimia | Pereaksi | Hasil | Standar |
|---------------|----------|-------|---------|
| Dragendorff   | -        | Endapan merah atau jingga |
| Mayer         | -        | Endapan |
| Wagner        | -        | Endapan coklat |
| Flavonoid     | HCl + MgSO4 | + | Warna |
| Saponin       | + aquadest + HCl 2N busa | + | Terbentuk |
| Tannin        | Larutan besi (III) klorida 10% | + | Warna biru hitam atau hijau |

The test results showed that kedawung contained bioactive compounds of flavonoids, saponins, and tannins. The test tubes that were carried out included the tests for alkaloids, flavonoids, saponins and tannins. One indicator of the reaction in the test tube was a change in color. The test results showed that kedawung contained bioactive compounds of flavonoids, saponins, and tannins.
Based on the graphic 1, it showed that the temperatures influenced the amount of extract produced during the extraction using microwave. The temperature used were 30 °C, 40 °C, 50 °C, 60 °C. Based on the yield produced, it could be said that the largest yield was produced at a temperature of 40 °C. That temperature affects total flavonoid levels, where the highest levels of total flavonoids in leaf extract and bark of kedawung are found at 40 °C (leaves 33.78 + 5.64 mg QE / g extract, bark 15.66 + 2.77 mg QE / g extract), while at a temperature of 30 °C (leaf 22.48 + 5.64 mg QE / g extract, bark 9.67 + 2.77 mg QE / g extract) at 50 °C (leaf 25.71 mg + 5.64 QE / g extract, bark 11.18 + 2.77 mg QE / g extract), and at a temperature of 60 °C (leaf 21.23 + 5.64 mg QE / g extract, bark 9.93 + 2.77 mg QE / g extract). This is because phenolic compounds and antioxidant activity are not heat resistant so that when extracted at high temperatures these compounds will be damaged or even lost (Widya et al., 2010).

The increase in temperature affects the rendement percentage between the leaf extract and the bark of kedawung at a temperature of 30 °C (73% leaves, 64.33% stem bark). At the temperature of 40 °C is found the highest rendement percentage (85.80% leaves, 85% stem bark). At the temperature of 50 °C is found the % rendement of leaves (85.20%) and stem bark (84.60%). At the temperature of 60 °C is found the % rendement of leaves (71.60%) and bark (82.86%). Antioxidant activity is influenced by the number of flavonoid compounds present in the extract of leaves and bark of kedawung, the more flavonoid compounds, the more antioxidant activity will increase (Kemit ddk, 2015).
4. Conclusions

There is an effect of temperature on microwave-assisted extraction results on the yield of kedawung leaf extract (Parkia biglobosa). The maximum level of total flavonoid of kedawung leaf extract is obtained at the temperature of 40 oC (33.78 ± 5.64 mg QE / g extract), the maximum antioxidant activity of kedawung leaf extract (Parkia biglobosa) is found at the temperature of 40 oC (85.8).

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