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The Effect of Solvent Ratio and Extraction Time on Antioxidant Activity and Flavonoid Concentration of Kedawung Leaf (*Parkia Biglobosa*) Through Microwave-Assisted Extraction

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Abstract

Kedawung (*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 are able to counteract free radicals that enter the body by donating electrons and binding them. Currently, the microwave-assisted extraction (MAE) method is widely used because the solute mass transfer from the sample matrix into the solvent is higher than the Soxhlet method. The following study aimed to know the effect of solvent ratio and extraction time on the extraction yield, flavonoid concentration, and antioxidant activity of kedawung leaf through microwave-assisted extraction. In this method, we used 40% ethanol to make the varied solute: solvent ratio such as 1:20, 1:30, 1:40, and 1:50. The extraction time used in this method was 4-7 minutes respectively. Microwave-assisted extraction has good performance to extract the active substance in Kedawung leaves. The highest yield 16.36%, total flavonoid content (57.32±2,2 mg QE/g extract), and DPPH scavenging activity (88.87±1.062%) was obtained in the extraction with a solids-solvent ratio of 1:40 g/mL, at an extraction time of 6 minutes. This method promises to take the active substance in a short time.

INTRODUCTION

Indonesia is well-known for plants with pharmacological effects or healing effects on various diseases (Sholikhah, 2016). Many plants contain antioxidant compounds such as phenolic compounds, flavonoids, and xanthones. These compounds can be used as a natural antioxidant (Panche et al., 2016). Antioxidants are able to counteract free radicals that enter the body by donating electrons and binding them (Halliwell, 2012). Antioxidants function as an antidote against free radicals in the body to prevent cell damage (Kurutas, 2016).

Kedawung (*Parkia biglobosa*) has various pharmacological activities such as antibacterial, antifungal, and antioxidant activity. The microwave-assisted extraction (MAE) method is widely used because it can increase the solute's mass transfer rate from the sample matrix into the solvent faster than that of the Soxhlet extraction method (Kataoka, 2019). Besides, the time required for extraction is relatively short, and the obtained yield is higher than other extraction methods, such as maceration, soxhlet extraction, and ultrasonic.

One of the factors that influence the extraction yield is the extraction time studied in research conducted by Shuncheng et al. (2013) on the extraction of corn's husk using 4, 5, and 6 minutes time variation. The study has revealed that the best yield was obtained from the 6 minutes extraction time. The following study aimed to know the effect of solvent ratio and extraction time on the extraction yield, flavonoid concentration, and antioxidant activity of kedawung leaf through microwave-assisted extraction.

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MATERIALS AND METHODS

Materials

The materials used in this research were kedawung leaf (*Parkia biglobosa*) from Semarang, Central Java. Other materials which used: methanol (Merck, 98% purity 1060092500), 1,1 Diphenyl 2-Picryl Hydrazyl/DPPH (Sigma-Aldrich, 90% purity, D9132), quercetin hydrate (Sigma-Aldrich, 95% purity, 337951), ethanol (Merck, 96% purity), $AlCl_3$ (Merck) and distilled water.

The tools which used in this study are microwave (Samsung), analytical balances (Sartorius), oven (Memmert), moisture analyser (Radwag MAC50), UV-Vis spectrophotometer (Shimadzu 2480), and rotary evaporator (Scilogex).

Methods

This experimental study evaluated the solute effect: solvent ratio and the extraction time on the extraction yield, the flavonoid concentration, and the antioxidant activity of kedawung leaf (*Parkia biglobosa*) extract obtained through microwave-assisted extraction.

Raw Material Preparation

This research used a random sampling method. The kedawung leaf sample was randomly collected and processed by using the microwave-assisted extraction method. Preparation of raw materials is carried out like the previous research method.

Microwave Assisted Extraction (MAE)

The raw materials were extracted by microwave-assisted extraction with a time variation of 4-7 minutes (fixed condition were ratio of solid to solvent 1:30 g/mL, temperature of 40°C, ethanol concentration of 40%v and power of 300 Watt) and variation of the ratio of solid to solvent 1:20, 1:30, 1:40 and 1:50 g/mL, at fixed time conditions (best results from variations in extraction time), temperature of 40°C, ethanol concentration of 40%v and power of 300Watt. The extracts were filtered, and stored at room temperature before being examined.

Analysis of Total Flavonoid Content (TFC)

The modified aluminum chloride colorimetric method was used (Buanasari et al., 2021), 0.5 mL of the standard solution was diluted and each extract was mixed separately with 1.5 mL of ethanol (95%), 0.1 mL of aluminum chloride (10%), 0.1 mL of potassium acetate (1M) and 2.8 mL of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 426 nm by spectrophotometer. The results are expressed as percent by weight of flavonoids.

DPPH Scavenging Activity (DPPH-SA)

Antioxidant activity testing was carried out using the DPPH method. 1.0 ml of the extract sample solution was added with 3.0 m of the newly prepared 0.1 mM DPPH solution. The absorbance was measured with a spectrophotometer (Shimadzu Japan) at a wavelength of 514 nm. The extract activity at DPPH is expressed as shown in Eq. (1).

$$DPPH\ Scavenging\ activity = x\ 100\% \qquad (1)$$

RESULTS AND DISCUSSION

The results obtained that leaves's moisture content were 4.70%. It met the requirement for the standard moisture content of simplicia (<10%) (Sulasmi et al., 2016). The characteristics of the sample are listed in Table 1.

Table 1. The characteristic of raw material.

Properties	Leaf
Shape	Powder
Color	Green
Smell	Specific
Taste	Bitter
Texture	Smooth
Moisture Content	4.70%

The phytochemical screening aimed to identify the bioactive compounds of the extract. The results are listed in Table 2.

The test results showed that kedawung leaf contained bioactive compounds of flavonoids, saponins, and tannins. The test tubes that were carried out included alkaloids, flavonoids, saponins, and tannins. One indicator of the reaction in the test tube was a color change. The test results showed that kedawung (*Parkia biglobosa*) contained bioactive compounds of flavonoids, saponins, and tannins.

Table 2. The screening of Kedawung extract.

Bioactive Compound	Reagent	Result	Standard
Alkaloid	Dragendroff	-	Red or orange sediment
	Mayer	-	Sediment
	Wagner	-	Brown Sediment
Flavonoid	HCl + $MgSO_4$	+	Colored
Saponin	+ Aquadest + 2N HCl → Unremoved Foam	+	Foamed
Tannin	10% $FeCl_3$	+	Black, blue or green color

The Effect of Extraction Time and The Solid to Solvent Ratio on The Yield of Kedawung Leaf Extract

The effect of extraction time on percent yield is presented in Figure 1. The longer the extraction time increases the yield value to 6 minutes, after that it decreases.

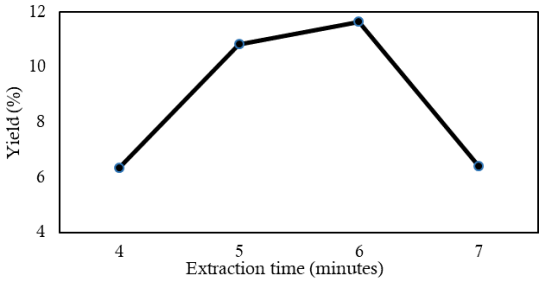

Figure 1. The effect of extraction time on the yield of kedawung extract.

Figure 1 shows that the amount of extract obtained is proportionate with the time needed during the extraction process. It means that the more time added, the higher the amount of section which is then obtained. The extraction time used in this research was 4-7 minutes. The highest extract yield is obtained from the extraction process using 6 minutes of extraction time, 11.66%. The 6 minute of the extraction time seems to be optimum. It means, after the 7 minute, the rise of the time extraction doesn't influence the amount of extract which is then obtained because all the solute particles have gotten good contact with all the solvent molecules (Mandal et al., 2007; Chen et al., 2007; Wang et al., 2010).

To determine the effect of the ratio of solvent solids on the percent yield, an extraction time of 6 minutes was used from the previous experiment. The amount of solvent was an essential factor that should be taken into account during the extraction process to ensure that all solute particle was in contact with the solvent molecules (Zhang et al., 2018).

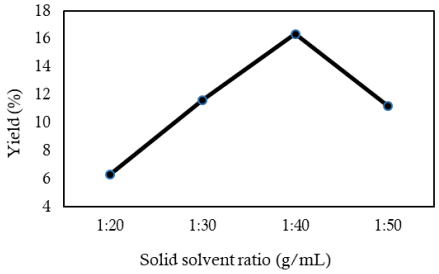

Figure 2. The effect of solid to solvent ratio on the yield of kedawung extract.

Figure 2 showed that the increase in the solvent amount is proportionate with the extract obtained during the extraction process. It means the more solvent added, the higher the obtained extract. The solute : solvent ratios used were 1:20, 1:30, 1:40 and 1:50, and the extract yields obtained were 6.3; 11.3; 16.36; and 11.2% respectively. The highest extract yield is obtained from the extraction process using a 1:40 ratio, 16.36%. It's also showed that the extraction yield decreases by 11.2% on the 1:50 ratio. The 1:40 ratio seems to be the optimum solute: solvent ratio. It means, after that concentration, the rise of the solvent doesn't influence the amount of extract, which is then obtained because all the solute particles have gotten good contact with all the solvent molecules. The higher solute: solvent ratio will increase the extract obtained in the general conventional extraction process. However, the higher solute: solvent ratio in microwave-assisted extraction will decrease the extract yield (Zhang et al., 2018). The heat radiation emitted by the tool cannot reach all solute particles with higher solvent amounts. Therefore, the extraction process doesn't function well (Kamaludin et al., 2014).

The Effect of Extraction Time and The Solid to Solvent Ratio on The Total Flavonoid Concentration (TFC) of Kedawung Leaf Extract

The effect of extraction time on TFC is presented in Figure 3. The longer the extraction

time increases the TPC value. At six minutes it gives the highest TFC value. This is similar to the yield gain.

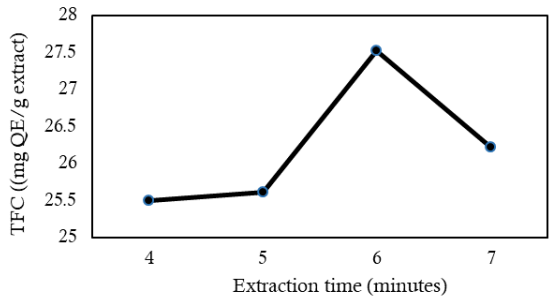

Figure 3. The effect of time extraction on the total flavonoid concentration.

Figure 3 showed that the highest total flavonoid concentration is obtained from the 6 minute of extraction time, the optimum extraction time. According to research conducted by Miollogo et al. (2009), kedawung leaf (*Parkia biglobosa*) has higher flavonoid content than its bark. It is in line with this research, in which the concentration of total flavonoid obtained was about 27.52 mg QE/g extract on the leaf.

Analysis results revealed that the solute: solvent ratio had different impacts on the total flavonoid content. Flavonoid has some unsubstituted hydroxy groups. Some polar molecules such as ethanol, methanol, ethyl acetate, or combination can be used for flavonoid extraction (Hidayati et al., 2019).

Figure 4 shows that increasing the solute:solvent ratio can increase the amount of flavonoids to a solid solvent ratio of 1:40 g/mL, then increasing the ratio further decreases the TFC value. The largest total flavonoid concentration at a ratio of 1:40 was 57.32 ± 2.2 mg QE/g extract. The heat exposure that occurred during the extraction process caused the degradation of the compound.

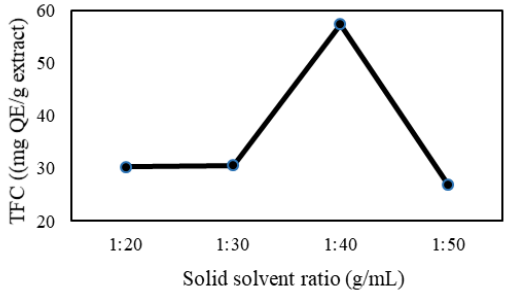

Figure 4. The effect of solid to solvent ratio on TFC of kedawung leaf extract.

Therefore the concentration found was lacking. According to the research conducted by Kusnadi *et al.* (2017), which used microwave-assisted extraction using a 1:15 ratio, the 1:15 ratio gave a lower concentration than that of the 1:10 ratio. This is in line with this study, where the total flavonoid concentration decreased after reaching the maximum ratio of 1:40.

The Effect of Extraction Time and The Solid to Solvent Ratio on The Antioxidant Activity of Kedawung Leaf

The longer the time increases the TFC value and also increases the antioxidant activity of the extract. This shows that the greater the TFC, the greater the antioxidant activity.

Figure 5 shows that the extraction time of 6 minutes resulted in an extract with the highest antioxidant activity of 86.40%. This is in accordance with the flavonoid content obtained at the extraction time of 6 minutes giving the largest flavonoid content.

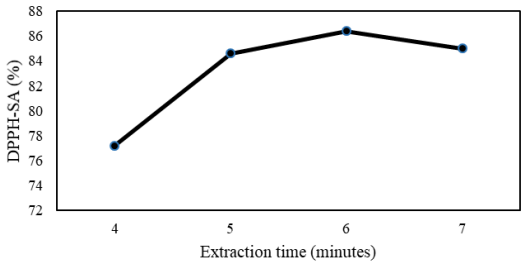

Figure 5. The effect of extraction time on the antioxidant activity of kedawung leaf.

When the DPPH was added to the kedawung (*Parkia biglobosa*) sample, the sample color changed from purple to yellow. The decrease of color intensity was measured at the wavelength of 515.4 nm, in which the maximum absorbance was given. The reduction of the absorbance showed the extract's potency to catch free radicals.

Figure 6 shows that the solute: solvent ratio increase can influence the flavonoid amount and thus the antioxidant activity. The total flavonoid concentration found in the leaf was 88.87 ± 1.06 % . However, the total flavonoid concentration had no significant influence on antioxidant activity because the bioactive compounds obtained had complex and various bioactive compounds with their antioxidant and prooxidant activity. For example, other than flavonoids, tannin can also function as an antioxidant that interferes with the assay of the

flavonoid's antioxidant activity (Komolafe et al., 2016).

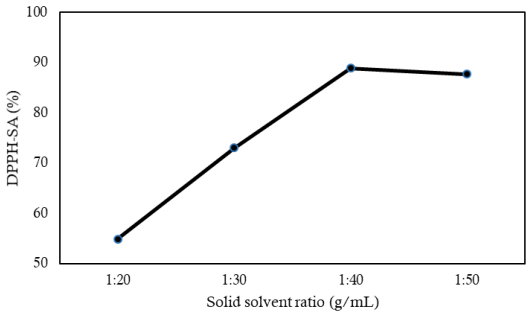

Figure 6. The effect of solid to solvent ratio on the antioxidant activity of kedawung leaf.

CONCLUSION

Microwave-assisted extraction has good performance to extract the active substance in Kedawung leaves. The highest yield 16.36%, total flavonoid content (57.32±2,2 mg QE/g extract), and DPPH scavenging activity (88.87±1.062%) was obtained in the extraction with a solids-solvent ratio of 1:40 g/mL, at an extraction time of 6 minutes. This method promises to take the active substance in a short time.

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