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The Comparison of Total Phenolic in The Extract of Brucea javanica L. Merr Using Maceration and Sonication Extraction Methods

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ABSTRACT

Brucea javanica L. Merr Fruit Seed is the traditional medicines in Indonesia that has acted as an antidiabetic, antioxidant, and anti-inflammatory. Phenolic compounds play a role in acting as a drug. The phenolic content in Brucea javanica extract had been shown to reduce inflammation symptoms and release of inflammatory mediators. In addition, total phenolic affects its antidiabetic activity as an alpha-glucosidase enzyme inhibitor. The amount of phenolic can be influenced by the extraction method used. The aim of this study was to compare the total phenolic content of wali fruit seed extract using maceration and sonication extraction methods. The advantage of maceration was the simplest and most economical method, but it took a long extraction time. Sonication had a short extraction time, but its ultrasonic energy was able to affect the active compound by the formation of free radicals. The method of this research is the extraction process using 96% ethanol as a solvent at room temperature(25°C) by maceration for 24 hours and sonication for 15 minutes. Screening of phenolic compounds with $FeCl_3$ reagent and determination of total phenolics by the Folin-Ciocalteau method. The results obtained were the total phenolic value with the maceration method was 61,1927±0,1560 mg GAE/g extract, while the total phenolic value with the sonication method was 67,9854±0,0968 mg GAE/g extract. This value was significantly different ($p<0.05$) statistically. Thus, it was found that the total phenolic by the sonication method was greater than the maceration method.

***KEYWORDS**: wali seeds, maceration, sonication, phenolic*

INTRODUCTION

Brucea javanica L. Merr is a medicinal plant. This plant is used as a traditional medicine in China for antidiabetic and antimalarial (Tang & Eisenbrand, 2013). *Brucea javanica* is scattered in several countries such as India, Sri Lanka, Vietnam, China, Indonesia, the Malay Peninsula to Northern Australia. In Indonesia, one of which is in the Sesaot Village area, West Lombok, West Nusa Tenggara, the seeds of the *Brucea javanica* fruit are empirically also used as an antidiabetic (Hamdin & Peni, 2017). Based on Simamora's research (2019), *Brucea javanica* seed extract has antidiabetic activity by inhibiting the alpha-glucosidase enzyme which plays a role in the breakdown of glucose. In addition, in vivo *Brucea javanica* extract reduces fasting blood sugar levels in diabetic rats (Manuaba et al., 2020). The *Brucea javanica* fruit seed extract has anti-inflammatory activity by inhibiting erythema and preventing the release of inflammatory mediators in mice (Amini et al., 2020; Yang et al., 2013). In the research of Risnadewi et al. (2019), *Brucea javanica*

fruit seed extract has an antioxidant activity which is classified as a strong antioxidant.

This *Brucea javanica* fruit seed extract contains secondary metabolites such as alkaloids, terpenoids, phenolics, and flavonoid (Amini et al., 2020; Muliasari et al., 2019). The content of phenolic compounds plays a role in antioxidant, antidiabetic, and anti-inflammatory activity (Ablat et al., 2017; Bouriche et al., 2016). Phenolics are compounds with a hydroxyl group (OH–) attached to an aromatic ring (Yunianti et al., 2020). The total phenolic content in the seeds of the *Brucea javanica* fruit is 36.9 – 98.5 mg GAE (gallic acid equivalent) per gram of extract (Hasni et al., 2017; Risnadewi et al., 2019). Based on Ablat et al. (2014), *Brucea javanica* fruit seeds have a total phenolic of 169.03 TAE (tannic acid equivalent) per gram of sample. In this study, only gallic acid was used as the standard because it was often used in several studies. It will make it easier for compared the result of this research.

The content of a compound can be influenced by several factors, one of which is the extraction method. Extraction is the process of separating the desired metabolite from the mixture (Azwanida, 2015). Based on Sari et al. (2018) different extraction methods such as maceration and sonication on *Eucheuma cottonii* extract resulted in different total phenolics.

Based on this, The total phenol content of Brucea javanica seed extract was compared by maceration and sonication methods. Maceration is one of the simplest and most economical conventional methods in both the process and the tools used. Sonication is an extraction method developed in the presence of ultrasonic waves so that it requires a shorter and more efficient time (Zhang et al., 2018). Both of these methods are suitable for thermolabile compounds with the maceration method representing the conventional extraction method while the sonication method represents the modern method. Thus, this study showed that the extraction method between maceration or sonication resulted in the highest total phenol content of *Brucea javanica* fruit seeds.

MATERIAL AND METHODS

Chemicals and Instrument

Brucea javanica seed taken in Sesaot (West Lombok, NTB), ethanol 96% (technical grade, Smartlab©), aquabidest (Waterone©), gallic acid standard p.a (Merck©) , Na_2CO_3 7,5 % (Merck©), Folin-ciocalteau reagent p.a (Merck©), $FeCl_3$ reagent (Merck©). Analytical balance (Kern©), Technical balance, sonicator, *rotary evaporator* (Heidolph©), Vortex (Labnet©), glass container, spectrophotometer UV-Vis (Specord®200 plus), beaker glass 50 mL, beaker glass 100 mL, volumetric glass 10 mL, volumetric glass 50 mL, micropipette, drop pipet, watch glass, stirring rod, mortar, pestle, filter cloth, filter paper.

Preparation sample

The purple to black *Brucea javanica* fruit was found in Sesaot Village, West Lombok, West Nusa Tenggara with coordinates of 8.5432983"S and 116.2410308"E. The *Brucea javanica* fruit that is sorted is wet and then washed until the flesh is gone and the seeds are taken. The seeds are then washed and dried. The dry seed shell is opened and the yellowish-white seed fill are taken. The fill of the seeds obtained are then made in powder form.

Extraction

Maceration

The *Brucea javanica* fruit seed powder was macerated using 96% ethanol as a solvent with a ratio of 2:5 (%w/v). The maceration process was carried out for 24 hours at room temperature and re-maceration was carried out 2 times (Risnadewi et al., 2019). The result of the maceration process was filtered using a filter cloth and then filter paper. The filtrate was evaporated with a rotary evaporator at 40^{o}C to remove the solvent.

Sonication

The seed powder of Brucea javanica was sonicated using 96% ethanol as a solvent. The ratio of powder and solvent was 2:5 (%w/v). Sonication was carried out at room temperature for 15 minutes. The results sonication were filtered using a filter cloth, then filtered by filter paper. Re-sonication was carried out 2 times. The result of all sonication processes which had been filtered was collected. The filtrate was evaporated with a rotary evaporator at 40^{o}C.

Phenolic Compound Screening

The thick extract was weighed as much as 50 mg and put into a test tube, the thick extract was added with 3 drops of $FeCl_3$ and the color change was observed. If there is a strong change in color to green, blue, red, purple, or black, the extract is identified as containing phenolic compounds (Shah & Seth, 2010).

Determination of Total Phenolic Content

Preparation of 500 ppm Gallic Acid Standard Solution

The method of this process had been modified by Alfian & Susanti (2012). Gallic acid was weighed as much as 50 mg. Diluted with aquabidest to 100 mL to produce a standard solution of gallic acid with a concentration of 500 ppm.

Determination of Operating Time

Determination of operating time according to Alfian & Susanti (2012) with modification. 300 µL of standard solution with a concentration of 30 ppm (µg/mL) were taken. 1,5 mL of Folin–Ciocalteu reagent (1:10) was added which had been diluted with aquabidest, then shaken and allowed to stand for 8 minutes. 1.2 mL of 7,5% Na_2CO_3 was added and shaken until homogeneous. The absorbance was measured in 0 – 90 minutes at a wavelength of 760 nm using a UV–Vis spectrophotometer.

Determination of Wavelength Maximum

Determination of wavelength maximum according to Alfian & Susanti (2012) with modification. 300 µL of standard solution with a concentration of 30 ppm (µg/mL) were taken and added 1,5 mL of Folin–Ciocalteu reagent (1:10) which has been diluted with aquabidest. It was homogenized and allowed to stand for 8 minutes. 1.2 mL of 7,5% Na_2CO_3 was added and allowed to stand at room temperature at operating time, then the absorbance was measured at a wavelength of 500–800 nm.

Preparation of Calibration Curve

A total of 5 variations of standard curve concentration were made from standard gallic acid solution by taking 0.4, 0.6, 0.8, 1, and 1.2 mL of standard solution and dissolving in 10 mL of aquabidest. 300 µL of each concentration of a standard solution with concentrations of 20, 30, 40, 50, and 60 ppm (µg/mL) was taken and put into a test tube. Each tube was added with 1.5 mL of Folin–Ciocalteu reagent which had been diluted with aquabidest (1:10) and homogenized. After being allowed to stand for 8 minutes, 1.2 mL of 7.5% Na_2CO_3 was added, then homogenized and allowed to stand for operating time at room temperature. The absorbance of each concentration was measured at the maximum wavelength. A calibration curve was made between the gallic acid concentration (ppm) and the obtained absorbance. Making this calibration curve through linear regression with Microsoft Excel software.

Measurement of Phenolic Content in Sample

This process was also carried out on 25 mg of ethanol extract of *Brucea javanica* seeds dissolved in 10 mL of aquabidest. As much as 300 µL of the sample with a concentration of 2500 ppm was taken. 1.5 mL of Folin–Ciocalteu reagent which had been diluted with aquabidest (1:10) was added and homogenized. After that let stands for 8 minutes. Added 1.2 mL of 7.5% Na_2CO_3 and homogenized then allowed to stand for operating time. The absorbance of the sample was measured using a UV–Vis spectrophotometer at the maximum wavelength. The phenol concentration of the extract was calculated with the absorbance obtained. Total phenolic was expressed as gallic acid equivalent per gram of *Brucea javanica* seed extract which was obtained through the following formula (Hapsari et al., 2018) :

$$TPC = \frac{X \; x \; V \; x \; Fp}{BS}$$

TPC = Total Phenolic Content (mg GAE/g)

X = Concentration (ppm)

V = Volume of extract solution (mL)

Fp = Dissolution factor

BS = Weight of sample (g)

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RESULTS AND DISCUSSION

Maceration and sonication are cold methods which means that there is no heating in the extraction process so they can be used

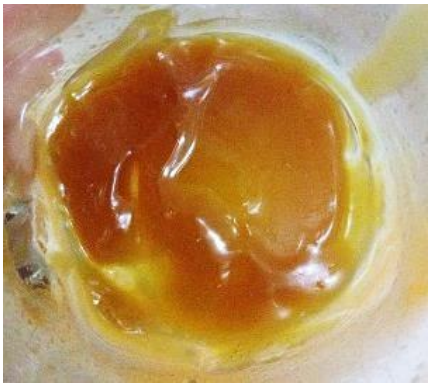


Figure 1. Viscous extract of *Brucea javanica* seed

on thermolabile compounds. According to Khoddami et al. (2013), phenolic compounds are unstable bioactive to heat or thermolabile. Therefore, this methods is suitable for taking the desired active component in the form of phenolic. Based on research by Risnadewi et al. (2019), proved that the extraction of *Brucea javanica* fruit seeds using the cold method in the form of maceration resulted in an extract with a higher phenolic content than the hot method in the form of soxhletation.

In this extraction process, 96% ethanol solvent was used. The use of this solvent is because 96% ethanol can attract the desired compound in the form of phenolics (Amini et al., 2020). This solvent is a universal solvent that can attract polar and non-polar compounds and has high extrasibility (Noviyanti, 2016).

The extract produced from both method is a thick, brownish-yellow extract. This extract has a characteristic odor of *Brucea javanica* seeds. The viscous extract can be seen in Figure 1.

Phenolics are compounds that have an aromatic ring with 1 or more hydroxyl groups (OH) attached to the aromatic ring. Phenolic compounds with more than 1 hydroxyl group are called polyphenolic compounds. In its pure state, it will be a colorless solid but if it is oxidized it will be dark in color (Yunianti et al., 2020). Qualitative analysis was carried out using a reagent in the form of $FeCl_3$. The principle of this method is the formation of complex compounds from the presence of bonds between O atoms in phenolics and Fe^{3+}ions in $FeCl_3$. The O atom as a basic ligand provides a free electron donor to the central atom of the Fe^{3+} ion so that a colored complex is formed (Ergina, 2014). This reaction can be seen in Figure 2.

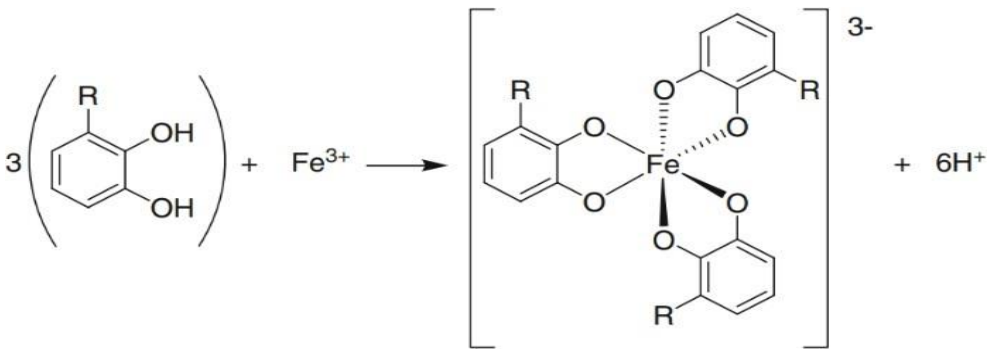


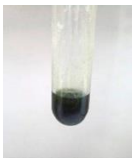
Figure 2. The reaction of complex compound between polyphenol and Ion $FeCl_3$ formation (Perron & Brumaghim, 2009)

Based on the experiment, it was found that in both extracts there was a color change from yellow to blue-black. This blue-black color indicates the presence of phenolic compounds in the sample (Shah & Seth, 2010). Thus, the extract using the maceration extraction method and the sonication method contained phenolic compounds. This color change can be observed in Table 1.

The method of determining the amount of total phenolic was used the Folin-Ciocalteau reagent. The principle of this method is the reduction of Folin-Ciocalteau reagent by phenolic compounds. The hydroxy group in phenolic reduces phosphomolybdate-phosphotungstate in the reagent to form a blue molybdenum-tungsten complex compound. This reaction requires alkaline conditions for the dissociation of protons in phenolics to become phenolic ions so that they can reduce reagents (Alfian & Susanti, 2012). Phenolic compounds are reacted with the Folin-Ciocalteau reagent in an acidic environment derived from the pH of the Folin-Ciocalteau itself. Therefore, Na_2CO_3 was added to make the pH becomes alkaline. This method has the advantages of being simple, fast, and accurate (Sánchez-Rangel et al., 2015). The darker the blue color produced, the more phenolic ions that form complexes compound indicating high the phenolic content (Tahir et al., 2017).

In this method, gallic acid is used as a standard. Using of gallic acid as a standard because it is a stable and natural simple phenolic acid (Ahmad et al., 2015). In performing the analysis by spectrophotometry, it is necessary to determine the operating time and maximum wavelength for measurement of the penolic content. Determination of the operating time to get the time that produced a stable

Table 1. The Result of Phenolic Screening

Extraction method	Before adding reagent	After adding reagent	Conclusion
Maceration			(+)
Sonication			(+)

absorbance and the maximum wavelength to get the wavelength with the maximum absorbance so that good results are obtained (Gandjar & Rohman, 2007). Based on the determination of the operating time and maximum wavelength, the measurements were carried out with 85 minutes as an operating time at a wavelength of 751 nm.

Determination of this standard curve is done by using variations in concentrations of 20, 30, 40, 50, and 60 ppm. According to Puspitasari & Prayogo (2017), good absorbance is in the range of 0.2-0.8. Based on the research, the absorbance results of all the concentration variations entered the range. The absorbance of standard concentration variations can be observed in Table 2. The calibration standard curve has an R-value of 0.997. A good R-value is close to 1. Furthermore, the equation on this standard curve used for the measurement of the total phenolic concentration in the sample. The resulting equation is Y = 0.011X + 0.088.

Based on the data of the total phenolic extract shown in Table 3, it was found that the extraction process by sonication was higher than maceration. The results of this sonication have a total phenolic value of 11.1% greater than the results of maceration. These results are following previous studies on *Solanum betaceaum* extract which produced higher total phenolics with the sonication method compared to the maceration method (Puspawati et al., 2019). Total phenolic content in plants is expressed in mg GAE/g. GAE (gallic acid equivalent) shows the equivalence of mg gallic acid in 1 gram of extract (Sari, 2017). This is related to the use of gallic acid as a standard for determining the total phenolic content.

Table 2. The absorbance of Gallic acid standard solution

Concentration (ppm)	Absorbance
20	0.3146
30	0.4289
40	0.5258
50	0.6373
60	0.7683

The total phenolic content of this *Brucea javanica* extract was included in the total phenolic range of previous studies, namely 36.9 – 98.5 mg GAE/g extract. Based on research by Risnadewi, et al., 2020, with a total phenolic content of 49.46 mg GAE/g extract, it showed strong antioxidant activity. The value obtained in this study from the maceration method was 23.7% greater and from the sonication method was 37.4% greater than the previous study. This antioxidant activity is related to an anti-inflammatory activity that can reduce the incidence of erythema in mice by considering UVB as an inducer. It leads to a reduction of new inflammatory mediators (Amini, et al, 2020). This total phenolic is also related to the antidiabetic activity of the extract of

Table 3. Total Phenolic Content of Extract Wali Seed by Maceration and Sonication Method

Method	Mean ± DS	Sig. (2-tailed)
Maceration	61.1927 ± 0,1560	0,004
Sonication	67,9854 ± 0,0968	

Brucea javanica. The higher the total phenolic in the extract, the higher the inhibition of the alpha-glucosidase enzyme (Simamora et al., 2019).

The maceration method is a simple extraction method using simple diffusion so that solvents from outside the cell will enter the cell. The solvent dissolves secondary metabolites in the cell and then returns to the outside of the cell with dissolved secondary metabolites (Susianti & Riyani, 2015). In the sonication method, there are ultrasonic waves that cause cavitation and implosion in cells. Cavitation and implosion cause cell wall damage. This damage to the cell wall makes it easier for solvents to enter and take up active compounds from plants (Ranjha et al., 2021). Based on this, it is known that the cavitation that occurs in the sonication process accelerates and increases the dissolution process in the cell. Based on Sari et al. (2018), the highest total phenolic content was obtained with the sonication method for 15 minutes compared to the microwave method for 15 minutes and maceration for 48 hours.

The results of the measurement of total phenolic by t-test showed that was a significantly difference between the results of the maceration and sonication extraction. It evidenced by the results of a significance value of less than 0.05. Before the t-test was carried out, the data on the determination of total phenolic in the *Brucea javanica* seed extract was homogeneous and normally distributed by normality test using *SPSS v.25*. This difference indicates that the extraction method affects the secondary metabolites produced in the sample.

CONCLUSION

Based on the research that has been done, it was found that the total phenolic by sonication method was greater than the maceration method. The total phenolic value of the sonication method was 67,9854±0,0968 mg GAE/g extract while the maceration method was 61,1927±0,15607 mg GAE/g extract and there was a statistically significant difference.

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