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Comparative Antioxidant Activity of *Brucea javanica* (L) Merr Seed Extract Derived from Maceration and Soxhletation Method

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Abstract. Free radicals are an atom or molecule that has unpaired electrons which make them reactive and unstable that caused various damages to the cells. The natural antioxidants are very needed to inhibit free radicals. *Brucea javanica* (L) M known as "Wali", is one of the local plants that potential as a natural antioxidants due the content of phenolic compound. The aim of this study was to determine the antioxidant activity by using DPPH method and the total phenolic content in the ethanol extract of Wali seeds derived from maceration and soxhletation. The results of antioxidant activity measurements showed that ethanol extract of Wali seeds derived from maceration d a strong activity (IC_{50} of 64,703 ppm), while the extract from soxhletation had a moderate activity (IC_{50} of 121,739 ppm). The total phenolic compounds responsible to the antioxidant activity of ethanol extract of Wali seeds derived from maceration and soxhletation were 4.946,35 g EAG/100g and 3.830,72 mg EAG/100g, respectively. The ethanol extract of Wa eds derived from maceration has higher antioxidant activity and total phenolic content than the extract from soxhletation with a significant different ($p<0.05$).

INTRODUCTION

Free radicals are molecules that have unpaired electrons which cause them very reactive and unstable. Free radicals in the activated form can damage healthy cells such as proteins, lipids, carbohydrate and DNA, so these cells lose their function and structure [1]. The activity of free radicals could be inhibited by antioxidant by donating its one electron to free radicals, breaking the chain reaction (polymerization) and turning it into a more stable product [2]. Antioxidant can be obtained from natural and synthetic way. The antioxidant recently used mostly the synthetic antioxidants such as *Buthylated hydroxytoluene* (BHT), *Buthylated hydroxianisol* (BHA), and *Tert-butylhydroquinone* (TBHQ) which can effectively inhibit oxidation [3]. However, the usage of synthetic antioxidants were showed to have adverse effect on human due to their hepatotoxic and carsinogenesis. Therefore, the searched of natural antioxidants are very needed because it has lower side effect [4].

Wali plant (*Brucea javanica* (L) Merr) is one of many plants that potential as natural antioxidant due to the chemical content such as tannin, flavonoid, alkaloid, triterpenoid and quassinoid [4]. The part of Wali plant that mostly used is the leaves, while the bitter fruit and seeds are still rarely used [6,7]. In this study, we investigate the antioxidant activity of ethanol extract of Wali seed derived from maceration and soxhletation. The antioxidant activity of extracts from natural ingredients will give different result if we extracted it in different ways. For example, studies reported that pineapple peels extracted by maceration, soxhletation and reflu giving greater antioxidant activity in extracts from soxhletation followed by reflux and maceration results [8]. Hence, this study aims to evaluate the antioxidant activity of ethanol extracts Wali seeds derived from maceration and soxhletation by using DPPH as a free radicals inducing agent. Besides that, we also measured the total phenolic content that is commonly known act to inhibite free radicals.

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MATERIALS AND METHODS

Sampling of Wali (*Brucea javanica* (L) Merr)

The sampling of Wali fruits was conducted in Sesaot, Narmada, Lombok Barat (8°32'44"S 116°14'27'E). Determination of Wali seeds was done in Laboratory of Biology, University of Mataram, Indonesia. The Wali seeds that have been collected were washed with water to remove dirt and dried. The dried grayish seeds were split to get the contents which are yellowish and mash with blender.

Extraction of Wali (*Brucea javanica* (L) Merr) Seed

Samples were extracted according to Ablat et al. with modification [9]. Briefly, 300 g simplicia of Wali seeds were extracted with 96% ethanol (2:5) w/v by maceration and soxhletation. Extraction by maceration was done by immersing simplicia at room temperature for 3 x 24 hours with 2 changes of solvents. Extraction with soxhlet was done by wrapping the simplicia in filter paper and putting it in a soxhlet (thimble) tube. The extraction process with soxhlet was done at a temperature of 70°C until the solvent cycle droplets become clear. The extracts from maceration and soxhletation were concentrated using rotary evaporator at 40°C.

Phytochemical Screening

Phytochemical screening was conducted following the method as follows [10]:

Flavonoid Test

2 mL of extracts was added Mg powder and a few drops of HCl concentrated. Positive flavonoid were indicated by the formation of yellow color.

Tannin Test

2 mL of extracts was added drop by 1% $FeCl_3$. The presence of tannin is indicated by the formation of a dark green color.

Terpenoid Test

2 mL of extracts was added 1 mL of chloroform and a few drops of H_2SO_4 concentrated. The presence of terpenoids is indicated by the formation of purple.

Alkaloid Test

2 mL extracts plus a few drops of Dragendorf reagent. The presence of alkaloid is indicated by the formation of sediment.

Qualitative Test of Antioxidant Activity

Ten mg of ethanol extract of Wali seeds from maceration and soxhletation was dissolved using 1 mL 96% ethanol. The solution was spoted on the Thin Layer Chromatography (TLC) plate using capillary pipes. The TLC plates that have been doped were eluted with buthanol:glacial acetic acid:water (3:1:1), and subsequently sprayed with 0,1 mM DPPH solution. The presence of antioxidant activity was characterized by yellow patches with a purple background [11].

Determination of Free Radical Scavenging Activity (Diphenyl-picrylhydrazyl)

Ethanol extract derived from maceration and Soxhletation were measured for antioxidant activity using diphenyl-picrylhydrazyl (DPPH) radical scavenging activity [12,13]. The extract sample stock solution (1000 ppm) was eluted to concentrations of 20, 40, 60, 80 and 100 ppm with ethanol p.a. The reaction mixture containing 2 mL sample of various concentrations and 2 mL DPPH solution were incubated at room temperature in the dark condition for 40 minutes. After that, the absorption rate was measured at 516 nm wavelength and converted to the percentage of antioxidant activity using the following formula:

$$\% \text{ inhibition} = \frac{Blank\ absorbance - sample\ absorbance}{Blank\ absorbance} \text{ x } 100\ \%$$

An amount of 2 mL ethanol p.a and 2 mL DPPH were used as a blank. While ascorbic acid (AA) used as

positive control with various concentrations at 2, 4, 6, 8 and 10 ppm.

Determination of Total Phenolic Content

Wali seeds ethanol extract derived from maceration and soxhletation were measured for total phenolic content by using Folin-Ciocalteu reagent [14]. The sample stock solution (1000 ppm, 200 µL) were added by 0,4 mL of Folin-Ciocalteu and 4 mL of 7% Na_2CO_3 and then incubated at room temperature for 30 minutes. After that, the absorption rate was measured at 744 nm wavelength. An amount of 2 mL ethanol 96%, 0,4 mL Folin-Ciocalteu and 4 mL of 7% Na_2CO_3 were used as a blank. The total phenolic content was expressed as mg of gallic acid equivalent (EAG)/gr ethanol extract of Wali seeds by the following formula [15].

$$\text{Total Phenolic Content} = \frac{\text{Concentration of gallic acid x Volume of extract solution}}{\text{weight of extract}}$$

Statistical analysis

Data analysis was performed by using *SPSS* version 16.0 with a T test for total phenolic compound and *One Way ANOVA* followed by a *Post Hoc test* for antioxidant activity.

RESULTS AND DISCUSSION

Phytochemical Screening

Phytochemical screening showed that the ethanol extract of Wali seeds extracted both by maceration and soxhletation were positively contained flavonoid, tannins, terpenoids and alkaloids (Table 1). This result based on the color changes or sediment that occurs after the addition of the reagents. The content of flavonoids and tannins in the ethanol extract of Wali seeds can inhibit free radicals due to the hydroxyl group as a reducing agent (act as hydrogen donor) to free radicals [16].

TABLE 1. Phytochemichal Screening Result

Sample / Secondary metabolite	Maceration	Soxhletation	Result
Flavonoids	+	+	Yellow colour
Tanins	+	+	Blackish green colour
Terpenoids	+	+	Purple colour
Alkaloids	+	+	Orange sediment formed

Qualitative Antioxidant Activity

The ethanol extract of Wali seed screened by TLC method showed the positive activity as antioxidant. As shown in Figure 1, the sample producing yellowish bands on the purple background were considered as antioxidant activity [17].

FIGURE 1. Qualitative Test of Antioxidant Activity Ethanol Extract of Wali Seeds by Maceration (M), Soxhletation (S) and Ascorbic Acid (AA) The Yellowish Band Indicated Antioxidant Activity Comparing With Standard Ascorbic Acid.

Determination of Free Radical Scavenging Activity

The free radical scavenging activity of the ethanol extracts Wali seeds derived from maceration and soxhletation were determined following DPPH method. The principle of measuring antioxidant activity with the DPPH method is by measuring the intensity of purple color changes of the DPPH solution using a UV-Vis spectrophotometer. The presence of antioxidant compounds in the sample used causes the color change of DPPH solution from deep purple to pale yellow. This discoloration occurs due to the DPPH radical inhibition caused by the reaction of hydrogen atoms in the sample to form diphenyl-picrilhydrazine compounds [18]. As shown in Table 2 and Figure 2, the ethanol extract of Wali seeds by maceration showed higher free radical scavenging activity than the ethanol extract by soxhletation in all concentration used. The DPPH radical scavenging activities of the ethanol extract of Wali seeds were increased as the extract concentrations increased. This happens because the greater the concentration, the more hydrogen atoms reduce DPPH, so the absorbance of DPPH decreases and the% inhibition increases.

TABLE 2. Antioxidant Activities of Ethanol Extract of Wali Seeds derived from Maceration and Soxhletation.

Concentration (ppm)	% Inhibition	
	Maceration	Soxhletation
20	39.17±0.04	32.19±0.05
40	44.30±0.03	38.19±0.11
60	46.91±0.05	41.20±0.06
80	53.22±0.33	42.94±0.13
100	60.43±0.11	45.96±0.14

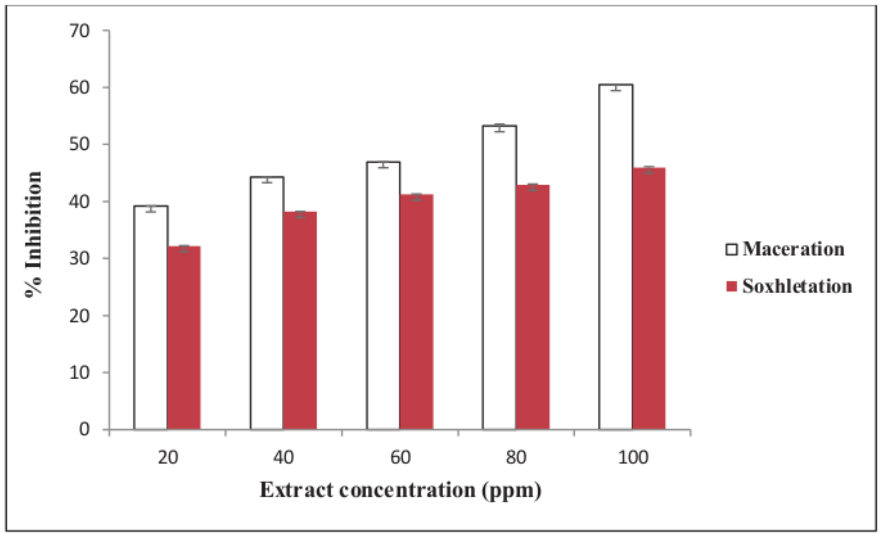

FIGURE 2. Antioxidant Activities of Ethanol Extract of Wali Seeds derived from Maceration and Soxhletation.

The intensity of antioxidant activity is repressed by the the value of IC_{50} that calculated from the linier regression equation of % inhibition curve. The IC_{50} value is defined as the extract concentration that can inhibit free radicals by 50% [19]. Antioxidant activity test showed that the ethanol extract of Wali seeds derived from maceration had IC_{50} values of 64,708 ppm, higher than the IC_{50} value of the extract derived from soxhletation (IC_{50} values of 121,739 ppm). Based on that result, ethanol extract of Wali seeds from maceration categorized to have a strong antioxidant activity because the IC_{50} values in range of 50-100 ppm. Whereas ethanol extract of Wali seeds from soxhletation has moderate antioxidant activity with IC_{50} in the range of 101-150 ppm [20]. Statistical analysis showed that the antioxidant activity of the two extracts were significantly different ($p < 0.05$).

The compound used as a comparison in this study was ascorbic acid or vitamin C. Ascorbic acid was chosen because it is a secondary antioxidant that has a free hydroxyl group that acts as a free radical scavenger and can prevent chain reactions [13]. In this study, the IC_{50} value of ascorbic acid was 5.888 ppm. This showed that ascorbic acid has a very strong activity in inhibiting DPPH radicals.

Determination of Total Phenolic Content

Based on Table 3 and Figure 3, the total phenolic content of ethanol extract of Wali seeds derived from maceration and soxhletation were 4.946,5 ± 139,195 mg GAE/100g sample and 3.830,7 ± 176,705 mg GAE/100g sample, respectively. This means in the 100 grams of simplicia extracted by maceration equivalent to 4.946,5 mg gallic acid and while the ethanol extract by soxhletation equivalent to 3.830,7 mg gallic acid. Statistical test showed that the total phenolic content in the two extract were significantly different ($p<0.05$).

TABLE 3. Total Phenolic Content of Ethanol Extract of Wali Seeds Derived from Maceration and Soxhletation

Concentration	Total Phenolic Content (GAE/100g sample)	
	Maceration	soxhletation
1000 ppm	4.946,5±139,195	3.830,7±176,705

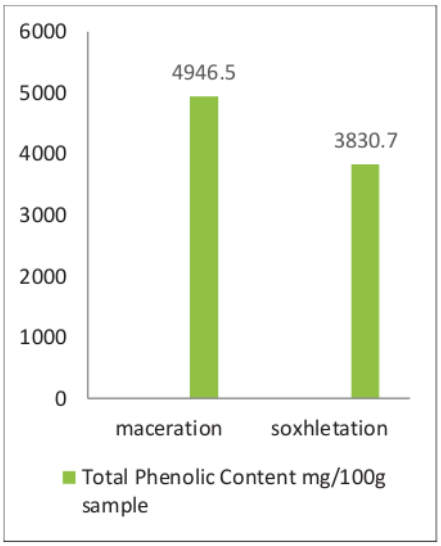

FIGURE 3. Total Phenolic Content of Ethanol Extract of Wali Seeds Derived from Maceration and Soxhletation

The differences in the total phenolic content in ethanol extract of Wali seeds was due to the differences in the extraction methods. Flavonoids and phenolic are very sensitive and unstable which can be degraded by temperature [21]. So, total phenolic content in ethanol extract by soxhletation is less due to the flavonoid and phenolic content in the ethanol extract that has been damage because of the high temperature and long periode of heating [22].The high temperature can damage and disturb the stability of fragments in plant, resulting in the reduced flavonoid and phenolic compounds. The IC_{50}value of the extract from maceration was higher because the total phenolic content in maceration extracts is higher than the extracts of soxhletation. According to Sivaci and Duman [23], the higher the total phenolic content, the higher the antioxidant activity.

Phenolic compounds including flavonoid and tannin were the most potential antioxidants containing in the extracts because they rich of hydroxyl groups. These hydroxyl groups act as hydrogen donor that stabilize the free radicals through the electron transfer mechanism so that the oxidation process is inhibited [24,25]. The higher the total phenolic content contained in an extract, the higher the hydroxyl group is, so the antioxidant activity is higher.

CONCLUSION

In the present work, ethanol extract of Wali seeds from maceration has higher antioxidant activity with IC_{50} 64,703 ppm (strong activity) compared to ethanol extract from soxhletation with IC_{50} 121,739 ppm (moderate activity).The bioactive compounds responsible for the antioxidant activity of the extracts were phenolic compounds, with the total phenolic content of 4.946,5 mg GAE/100 sample and 3.830,7 mg GAE/100 sample from maceration and soxhletation, respectively. Antioxidant activity and total phenolic content in ethanol extract of Wali seeds from maceration and soxhletation showed significant differences with p values < 0.05.

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