The antioxidant activity of Roselle (Hibiscus sabdariffa Linn) phenolic compounds in different variations microwave-assisted extraction time and power

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Abstract. The roselle flower bioactive compounds must be extracted out of the cell to facilitate their use. The use of microwave-assisted extraction methods is considered more effective and efficient, but improper use of microwave power and extraction time will actually cause damage to the bioactive compound. The focus of these study was to evaluate the effect of different microwave power and extraction time on chemical characteristics and antioxidant activity of roselle flower extract. The research design used in this study was randomized block design (RBD) trial with 2 (two) factors. The first factor is microwave power (100, 175, 250, 325 and 400 watts). Second one is extraction time (1, 3, 5, 7 and 9 minutes). The effect showed that the use of microwave power which give the best results is 325 watts with total anthocyanin 13.8591 mg/100g, vitamin C 110.88 mg/100g,. The best extraction time was 5 minutes with total anthocyanin 13.8381 mg/100g, vitamin C 121.44 mg/100g. The bioactive content is directly proportional to the antioxidant activity produced, namely IC50 of 0.515 μg/ml.

Keywords : Microwave Assisted Extraction (MAE; roselle; antioxidant

1. Introduction
Pollution of the environment in where we live and unhealthy lifestyles such as smoking, drinking alcoholic beverages and physical activity that can stimulate the free radicals cause damage in the body. Increasing the potential of free radicals causes the body must get additional antioxidants from the outside. However, synthetic antioxidant ingredients are not recommended for consumption because it can cause cancer effects in the long term. There are fears of the possibility of side effects from synthetic antioxidants causing natural antioxidants to become alternatives that are needed [5].

Roselle, one alternative plant that can be used as a source of natural antioxidants. Research conducted by [11] states that roselle extract contains a total anthocyanin bioactive compound of 14.80 ± 0.08 mg / g, with a total phenol of 23.77 ± 0.25 mg / g, vitamin C 10.74 ± 0.14 mg / g, IC5020.47 μL / mL, and microbial activity on Staphilococcus aureus 11.6 ± 0.3 and Escherichia coli 12.6 ± 0.6 mm. The content of these bioactive materials makes roselle has the ability as antioxidant and antibacterial agent.

The content of antioxidant compounds in roselle needs to be extracted out of the cell to facilitate its utilization. Several extraction techniques have been used to extract bioactive components. Extraction methods that have long been developed are conventional extraction methods such as maceration and soxhlet method. These techniques usually require more extraction time. so that it runs the risk of severe thermal degradation for most bioactive components such as antioxidants [7]. According to [1,12]. Conventional extraction methods
can spend 8, 16, 24 hours or more in an extraction process. In addition, conventional extraction techniques also require sufficient organic solvents and thermal energy to be considered less efficient. More innovative extraction methods are currently being developed such as ultrasound-assisted extraction, enzyme-assisted extraction and microwave-assisted extraction (MAE). Of the several methods, MAE is a method that is considered more environmentally friendly and effective. In addition, when compared with conventional methods, MAE has several advantages, namely requiring shorter extraction time and lower temperature compared to conventional extraction, thereby reducing the decrease in thermodabile components (Li et al., 2017). MAE is also suitable for extraction using water as a solvent because water has a dielectric coefficient of 78.3 so that it can absorb microwaves well. In addition, the advantage of water as a solvent for extraction is its abundance is large, non-toxic and will minimize the use of organic solvents [10,15,16].

The purpose of this study was to determine: 1) the effect of variations in microwave power on the chemical and physical characteristics of the extracts of roselle flower petals produced; 2) the effect of variations in the extraction duration of roselle calyx on chemical characteristics and physical extracts produced; 3) the interaction of microwave power variations and extraction time to the antioxidant activity of the extract produced.

2. Materials and Methods

This research was conducted in January-May 2018 in the Laboratory of Agricultural Technology, Study Program of Food Science and Technology, Department of Agricultural Technology, Faculty of Agriculture, Jenderal Soedirman University.

The materials used in this study were red roselle calyx (Hibiscus sabdariffa) by drying for 7 hours using a cabinet dryer obtained from Kediri and Bumi Herbal Dago (Bandung), aquadest, ethanol pa, acidic ethanol, 1% starch, iodine solution 0.01 N. The tools used in this study are microwave (electron), erlemeyer (pyrex), measuring cup (pyrex), digital scales (AND), analytical scales, blenders, sieves, UV-VIS spectrophotometers (Shimadzu), cuvettes, test tubes (pyrex), spatulas, pp plastic, filter paper, funnels, aluminum foil, fillers, 10 mL and 100 mL volumetric flasks, brown glass bottles, and measuring pipettes.

Extraction: The dried roselle calyces were ground for 1 minute using grinder and sieved in 60 mesh. The extraction with various concentrations of ethanol (50%, 60%, 70%, 80% and 90%), microwave power extraction (100, 175, 250, 325, and 400 W) and time of extraction (1, 3, 5, 7, 9, and 11 minutes) were conducted for each parameter using non factorial design. The extraction was done in the ratio dried roselle and solvent 1:10 w/v (wet weight), 10 g dried roselle powder, in 100 mL solvent. The slurry was radiated in microwave oven at regular intervals (one minute radiation and two minutes off) to keep the temperature not rise above the boiling point [16]. Roselle extract was filtered and concentrated with vacuum evaporator at 70°C, 44 cmHg and blowing with N₂ to ensure the solvent totally evaporate. The main response parameter that analyzed was total phenols.

Anthocyanin (Fuleki et al., 1968)

A total of 1 mL sample was added with 1 mL of acidic ethanol (95% ethanol +1.5 N HCl (85:15 v / v)) Stored overnight at 40 °C. Then diluted to 10 mL and centrifuged 5 minutes 1000 rpm. Measured absorbance at 535 nm.

Antioxidant activity (Al Hashimi, 2012)

0.6 mL of sample was dissolved in 0.12 mL of 98% ethanol and 2.88 mL of a 2.51% linoleic acid and 9 mL of a 40 mM phosphate buffer (pH 7.0) were added. The mixture was incubating at 40°C in a test tube in the dark for 3 days (72 hours). After incubation, a 0.1 mL
was taken from the mixture and diluted with 9.7 mL of 75% ethanol, followed by the addition of 0.1 mL of 30% ammonium thiocyanate. Precisely three minutes after adding the 0.1 mL of 20 mM Ferrous chloride in 3.5% hydrochloride acid, the absorbance of the red color was measured at 500 nm. The level of lipid peroxidation inhibition by each fraction was calculated from the absorbance ratio to that of a blank without any sample. A half lipid peroxidation inhibition expressed with IC$_{50}$.

The design used was a randomized block design (RCBD) with two factors. The factors used were microwave power (100, 175, 250, 325 and 400 watts) and extraction time (1, 3, 5, 7 and 9 minutes). The factors were arranged factorial so that 25 treatment combinations were obtained. Each treatment was repeated 2 times to obtain 50 experimental units. Total anthocyanin data and vitamin C of dried roselle flower extracts were analyzed using the F test (variance test) at a confidence level of 95% ($\alpha = 5\%$) with ANOVA and if there was a significant effect, then continued with the DMRT test (Duncan Multiple Range Test) at a 95% confidence level. Data on antioxidant activity were analyzed using linear regression.

3. Results and Discussion

Effect of variations in microwave power used on the total anthocyanin produced

The results showed that variations in microwave power (P) and extraction time (T) significantly affected the total content of anthocyanin.

Figure 1. Total anthocyanin in microwave power variations.

Figure 1 shows that extracted anthocyanin levels tend to increase from 100 watts of power and then reach the maximum point at 325 watts and decrease in subsequent microwave power variations. [2] state that when the microwave power increases, the extraction yield will increase. According to Chen et al (2008), the higher microwave power resulted the higher effect of microwave energy on biomolecules by ionic conduction and dipole rotation which results in power dissipated inside the solvent and plant material. This condition can generate molecular movement and heating. Linear to some extent before it begins to decrease or become insignificant. This is caused by the degradation or decomposition of heat-sensitive compounds (thermolabile). It is suspected that at 325 watts of power it is able to damage the cell walls of the roselle calyx so that the addition of power does not increase the amount of anthocyanin extracted, it will reduce the amount of anthocyanin compounds extracted because the higher exposure to microwaves can accelerate the rise in temperature which can damage the anthocyanin compounds. In addition, high exposure to microwaves causes reduced water as a solvent. The reduction of water as a solvent causes the bioactive compounds will not be maximally taken up in the solvent [4]. This is similar to the study of
[9] who reported that it requires 300 watts of power to produce Rosa-damascena flower extracts with the highest total anthocyanin.

Effect of variation of extraction time used on total anthocyanin produced

Figure 2 is a graph that shows the results of various analysis of the variation of extraction time variations on total anthocyanin.

Figure 2 shows that the total anthocyanin extract increased from the extraction time of 1 minute to 5 minutes. This is presumably because the longer the material is exposed to microwave radiation, it causes the rupture of the tissue of the material so that it will release the solute into the solvent [17]. This result also similar with P[15] that the yield of phenols is increasing until 5 minutes of extraction time. However, the use of time over 5 minutes will reduce the total anthocyanin produced. This is due to the longer extraction causes the resulting temperature to be higher, causing damage to anthocyanin compounds. In addition, the extraction time that exceeds the maximum time will cause the solution to become saturated and the anthocyanin decreases, so that the change in time will not give a real change in concentration [17].

[6] reported that it took 10 minutes to extract anthocyanin compounds from mangosteen peel waste. This could be due to [6] research using only 160 watts of power, allegedly the difference in power usage was also quite large. It will make a big difference in the extraction time. The results of research conducted by [9] showed that it only takes 5 minutes to extract the anthocyanin compound extract of Rosa-Damascena flower to the maximum with a power of 300 watts, the use of extraction time of more than 15 minutes will cause a decrease and a stable amount stable of the compound extracted anthocyanin.

The effect of variations in microwave power used on vitamin C produced

Figure 3 showed the results of the analysis of the influence of microwave power on vitamin C produced.
Figure 3 vitamin C content produced in microwave power of 100 watts produces the highest vitamin C when compared to other microwave power variations of 110, 88 mg / 100g. This can be caused because vitamin C has thermolabile characteristic especially in a state of dissolution so that the greater the power used, vitamin C will be more degraded due to rising temperatures. According to [18] the use of microwave power that exceeds 160 watts will cause the bioactive component to decrease especially vitamin C in the extraction of Clinachantus nutans plants. This is supported by the statement of [18] who extracted vitamin C from guava fruit by comparing the Nitrogen-protected microwave-assisted extraction (NPMAE) method to the usual MAE method, stated that in addition to rising temperatures and light factors, a decrease in vitamin C could also occur due to oxidation reactions due to extraction process that does not use nitrogen protection (closed system) so that the addition of power will accelerate the rate of oxidation. This is caused because vitamin C is very sensitive to changes in temperature, light, oxygen and pH.

Effect of variations in extraction time on vitamin C produced

Figure 4 is the result of the analysis of the effect of extraction time various on vitamin C produced, showing that the variation of extraction time produced the highest vitamin C content of 121.44 mg / 100 g using extraction variations for 1 minute.

[18] reported that extracting the bioactive component from the Clinachantus nutans plant using an optimal power of 160 watts requires a maximum extraction time of 60 seconds (1 minute). The use of extraction time that exceeds 1 minute will reduce the yield of extracted bioactive components. This is due to the longer extraction time will increase the microwave energy which causes high evaporation of the substrate so that vitamin C which dissolves easily in water will also evaporate. Different bioactive substances show different tendencies because the properties of substances are determined by their structure. The length of time of microwave radiation will damage the molecular structure of the substance thereby reducing the extraction yield.

Antioxidant Activity

Antioxidant activity expressed with IC$_{50}$ value. IC$_{50}$ is the concentration can inhibit 50% of free radicals. It means that the lower IC50 value, related to higher antioxidant activity. Figure 4 shows the results of data analysis of antioxidant activity using linear regression in each combination factors. These showed that the treatment of variations in power of 325 watts with 5 minutes extraction time has the highest antioxidant activity is along with the low IC$_{50}$ value, while the lowest antioxidant activity is the extraction result using 400 watts of power with 9 minutes extraction time.
Noted:
P1T1: 100 watts of power for 1 minute
P1T2: 100 watts of power for 3 minutes
P1T3: 100 watts of power for 5 minutes
P1T4: 100 watts of power for 7 minutes
P1T5: 100 watts of power for 9 minutes
P2T1: 175 watts of power for 1 minute
P2T2: 175 watts of power for 3 minutes
P2T3: 175 watts of power for 5 minutes
P2T4: 175 watts of power for 7 minutes
P2T5: 175 watts of power for 9 minutes
P3T1: 250 watts of power for 1 minute
P3T2: 250 watts of power for 3 minutes
P3T3: 250 watts of power for 5 minutes
P3T4: 250 watts of power for 7 minutes
P3T5: 250 watts of power for 9 minutes
P4T1: 325 watts of power for 1 minute
P4T2: 325 watts of power for 3 minutes
P4T3: 325 watts of power for 5 minutes
P4T4: 325 watts of power for 7 minutes
P4T5: 325 watts of power for 9 minutes
P5T1: 400 watts of power for 1 minute
P5T2: 400 watts of power for 3 minutes
P5T3: 400 watts of power for 5 minutes
P5T4: 400 watts of power for 7 minutes
P5T5: 400 watts of power for 9 minutes

Figure 5. Comparison of IC50 microwave power and extraction time of roselle calyx.

Figure 5 indicates that the antioxidant activity is higher when the antioxidant compounds contained in roselle calyx are extracted using a variety of microwave power and the appropriate extraction time. It is suspected that the antioxidant activity of each extract correlates with the content of vitamin C and anthocyanin. Vitamin C has a group free hydroxy which acts as a free radical scavenger and if it has a polyhydroxy group will increase antioxidant activity [13]. Anthocyanin compounds also greatly affect the antioxidant activity of extracts. This is supported by the statement of [3] which states that in several studies mentioned that the antioxidant activity of anthocyanins is 2-6 times greater than other common antioxidants such as ascorbic acid or better known as vitamin C. [8] reported in his research that pure extracts of the anthocyanin compound roselle have excellent antioxidant activity. Anthocyanin in the form of alcohol will reduce antioxidant activity while in the form of diglucoside will increase antioxidant activity. This is consistent with research conducted by [14] that high bioactive compounds such as total phenols, anthocyanins and vitamin C result in high antioxidant activity.
4. Conclusion
1. Variations in microwave power have a significant influence on the chemical and physical characteristics of roselle calyx extract. The best microwave power is at 325 watts of power, producing a total anthocyanin of 13.8591 mg / 100g, vitamin C 110.88 mg / 100g.
2. Variation of extraction time has a significant influence on the chemical and physical characteristics of roselle calyx extract. The best extraction time for 5 minutes produced a total anthocyanin of 13.8381 mg / 100g, vitamin C of 121.44 mg / 100g.
3. Variations in microwave power and extraction time affect the antioxidant activity of roselle calyx extract. The best antioxidant activity is found in the combination of microwave power of 325 watts with extraction time of 5 minutes, which produces an IC50 of 0.515 μg / ml.

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