Comparison of microwave assisted extraction (MAE) with variations of power and infusion extraction method on antibacterial activity of rosetta calyx extract (*Hibiscus sabdariffa*)

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**Abstract.** This study aims to determine the effect of Microwave Assisted Extraction (MAE) extraction and infusion on total phenolic and antibacterial activity of rosetta calyx. Extraction using Infusion and Micro Assisted Extraction methods with variations in power of 10, 30 and 50. Followed by measurement of total phenolic content and its ability to inhibit test bacteria *Staphylococcus aureus* and *Escherichia coli*. The results of the evaluation revealed that the percentage of yield of rosetta calyx extract produced from the MEA method was 10, 30 and 50, respectively at 24.6%, 30.5% and 35.6% compared to the infusion method which was only 19.5%. The phenolic content of rosetta calyx extract from infusion method was 2.39%, while for MEA 10, 30 and 50 respectively were 2.72%, 2.53% and 2.18%. The results of extraction using infusion had inhibition zone diameters of 11.09 ± 0.96 mm against *E. coli* bacteria and 6.87 ± 0.05 mm against *S. aureus* which had the smallest resistance of all tests. While the results of extraction with the help of microwaves obtained the highest antibacterial activity at 30 power against *S. Aureus* at 12.23 ± 1.85 mm compared with all extract tests from the MAE method.

1. Introduction

Traditional medicine is an important part and is often underestimated in health services. In some countries, traditional or non-conventional medicine can be called complementary medicine. Traditional medicine has a long history of use in health care and disease prevention and treatment, especially for chronic diseases. The WHO 2014-2023 traditional treatment strategy was developed on the basis of the World Health Assembly's resolution of traditional medicine. The goal of this strategy is to support Member States in utilizing the potential contributions of traditional medicine, for health, and fitness. Promote safe and effective use of traditional medicines by regulating, researching and integrating traditional medicinal products [1].

One of the most popular plants lately is the Rosella (*Hibiscus sabdariffa* L.) plant which is often used for treatment and has been known to have extensive pharmacological activity. According to Purbowati (2014) states that rosettla calyx contain total phenolic, anthocyanin, vitamin C respectively are 19.45 ± 0.32; 13.51 ± 0.03; 20.47 ± 0.34 mg/g in the extraction method using 70% ethanol solvent [2].

In a previous study conducted by Wibowo et al. (2009) which states that the concentration of 25% v/v infusion of rosetta calyx has antimicrobial activity which is equivalent to the standard concentration of tetracycline HCl 18.62 µg/ml and Al Hashimi (2012) antibacterial activity of rosetta against *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* show different degrees of resistance [3], [4].
There are several factors that need to be considered in selecting extraction methods including time and cost efficiency and their effectiveness in producing targeted bioactive compounds. Conventional techniques such as infusion, sotkhet and reflux have disadvantages that the long extraction times, high handling and solvent costs. Lately, the extraction method of Microwave Assisted Extraction (MAE) has been widely used to extract of active compounds in natural ingredients [5]. Extraction using the MAE method utilizes microwave radiation to accelerate selective extraction through heating the solvent quickly and efficiently. The advantage of extraction using the MAE method is the reduced extraction time and the use of fewer chemical solutions [6].

Based on the description above, a study of the effect of microwave assisted extraction extraction methods on total phenolic contents and antibacterial activity of rosella calyx extract was carried out. To support this, an antibacterial activity of water extract was conducted from the microwave assisted extraction method against *Staphylococcus aureus* (gram positive) and *Escherichia coli* (gram negative) bacteria with various comparisons.

2. Materials and Methods

2.1 The Sample Preparation Process

Roselle calyx samples are sorted wet then the sample is washed using running water, then drying using a fruit dehydrator for 6-8 hours. The dried rosella calyxs are powdered using a blender then sifted using number 40.

2.2 Sample extraction

2.2.1 Microwave Assisted Extraction

Roselle powder was extracted using water solvents with a ratio of 1:10 (sample: solvent) with microwave variation power of 10, 30 and 50 for 15 minutes. The rosella extract is then filtered and concentrated with a freeze dryer until a dry extract is obtained.

2.2.2 Infusion

Rosella calyx extraction was carried out using the infusion method by placing 10 grams of sample into the infusion pan and then adding 100 ml of distilled water. The rosella that have been added to distilled water are heated for 15 minutes counting after the temperature in the pan reaches 900C, while stirring occasionally. The liquid extract of rosel is filtered and concentrated with a freeze dryer until a dry extract is obtained.

2.3 Determination of Total Phenolic

2.3.1 Determination of Maximum Wavelength

The raw gallic acid is weighed as much as 10 mg then dissolved with ethanol p.a in a 10 ml gourd and is sufficient to mark the limit. Measured the maximum wavelength of 656 nm, the maximum wavelength is determined.

2.3.2 Making a Raw Curve

Raw gallic acid is weighed as much as 10 mg then dissolved in ethanol p.a in a 10 ml gourd and the volume is sufficient to the mark limit. Furthermore, quantitative dilutions are made and if necessary gradually with ethanol pa 2, 4, 6, 8 and 10 bpj in a 10 ml measuring flask then add each with 2.5 ml of Folin Ciocalteu LP (7.5% in water), let stand for 8 minutes, 2 ml of 1% NaOH, and sufficed with
distilled water to the mark limit. After that homogenized and incubated for 60 minutes at room temperature.

2.3.3 Making Test Solution
Rosella extract was weighed as much as 10 mg then dissolved with ethanol p.a in a 10 ml gourd and the volume was sufficient to the mark limit. Then 0.5 ml of test each into a 5 ml measuring flask, added with 2.5 ml of Folin-Ciocalteau LP (7.5% in water), let stand for 8 minutes then added 2 ml of 1% NaOH. The concentrated test solution is diluted by taking 2 ml of each test solution in a 5 ml measuring flask. Adding with aqua pro injection to the mark limit.

2.3.4 Measurement with UV-Vis Spectrophotometer
Rosella extract was measured using a UV-Vis spectrophotometer at the maximum wavelength of gallic acid absorption. Blank measurements are carried out first, then each test solution is measured at its maximum absorption wavelength. Calculate phenol levels by comparing the concentration between the test solution and the comparison solution in% b / b.

2.4 Antibacterial test
2.4.1 Making of Mueller Hinton Agar (MHA) Medium
The MHA medium made by weighing 3.4 grams of medium was dissolved in 100 ml of distilled water, then dissolved (can be helped by heating). Then the medium was sterilized in an autoclave at 121°C for 15 minutes.

2.4.2 Making 0.5 Mc Farland solution
Mc Farland is made by making a solution of barium chloride anhydrous (BaCl2) 1% and anhydrous sulfuric acid solution (H2SO4) 1%. Then, the two solutions are made a mixture of solutions by taking 0.05 ml of 1% BaCl2 solution and 9.95 ml of 1% H2SO4 solution. The mixture is made in a sterile test tube which is covered with cotton and aluminum foil.

2.4.3 Preparation of Test Bacteria
One culture of bacteria that has been rejuvenated on MHA media is suspended into a sterile test tube containing sterile water until the turbidity is equivalent to a standard solution of 0.5 Mc Farland [9].

2.4.4 Antibacterial Activity Test
Antibacterial activity test was carried out by diffusion method to use paper diameter discs with 6 mm diameter with test bacteria (Escherichia coli and Staphylococcus aureus) with three repetitions. The paper disc was pressed using 20 μl micropipette with 5% concentration of rosella calyx extract and then placed on sterile MHA media which had been inoculated with test bacteria with spread method. Incubation was carried out at 37°C for 1 x 24 hours. Observations were made on the formation of inhibition zones around the paper disc.

3. Results and Discussions
3.1 Results of Rosella Calyx Extraction
Rosella calyx extraction was carried out by two methods consisting of the infusion method and Microwave Assisted Extraction (MAE) using 3 different power variations namely 10, 30 and 50. The solvents used for both methods were aquadest. The extract weight obtained from the infusion method was 1.95 g with a percent yield of 19.5%. While the extract weights for the MAE method using power 10, 30
and 50 were 2.46 g, 3.05 g and 3.56 g respectively with 24.6 percent, 30.5 percent and 35.6 percent rendemen. More can be seen in Table 1.

| Sample          | weight (g) |
|-----------------|------------|
|                 | Infusion   | MAE         |
|                 | P 10       | P 30        | P 50        |
| Dry simplicia   | 10         | 10          | 10          |
| Thick extract   | 1.95       | 2.46        | 3.05        | 3.56        |

Table 1. Results of extracting rosella

Based on table I, it can be seen that the extract produced is more by using the Microwave Assisted Extraction (MAE) method than the conventional method (infusa). This is due to the ability of microwave power that can break down the cell membrane structure so that active substances can escape from plant tissue and dissolve in the fluid of the dancer [7].

3.2 Measurement of Total Phenolic Levels and Antibacterial Activity

The results of data on measuring total phenolic levels of roSELLa calyx extract using the infusion and MAE methods can be seen in table 2 and figure 1.

Table 2. Results of the average measurement of total phenolic contents

| No. | Extraction Method | % total phenolic    |
|-----|-------------------|---------------------|
| 1.  | Infusa            | 2.39 ± 0.006        |
| 2.  | MAE power 10      | 2.72 ± 0.005        |
| 3.  | MAE power 30      | 2.53 ± 0.009        |
| 4.  | MAE power 50      | 2.18 ± 0.008        |

Figure 1. Results of total phenolic percentage

The measurement of total phenolic levels was determined using the Folin-Ciocalteau method by weighing as much as 10 mg extract and made 3 replications measured based on the standard ratio of gallic acid, phenolic content obtained from roSELLa calyx extract by infusion method was 2.39% while for MAE
method using power 10, 30 and 50 obtained total phenolic levels respectively 2.72%, 2.53% and 2.18%, this indicates that the lower power MAE produces a higher total phenolic level. Possible phenolic compounds are oxidized by heating. Based on Kurniasari, L., et al., 2008 on conventional heating processes that depend on the phenomenon of convection and conduction, most of the heat is lost to the environment. Whereas in the Microwave Assisted Extraction (MAE) process, the heat process occurs with specific targets and specific methods, so that no heat is lost to the environment, because the heating process takes place in a closed system, this causes MAE power 50 to have a lower phenolic level [8].

Antibacterial activity test was carried out in order to see the diameter of the inhibitory zone produced by rosetta calyx extract using different extraction methods namely infusa and MAE (power 10, 30 and 50).

| Bacteria | Infusion | Microwave asissted extraction | positive control |
|----------|----------|-------------------------------|-----------------
|          |          |                               |                 |
| S. aureus| 6.87±0.05| 10.34±3.50                    | 10.22±0.41      |
| E. coli  | 11.09±0.96| 9.77±0.42                    | 10.37±0.09      |
|          |          | 12.23±1.85                    | 11.78±2.53      |
|          |          | 11.17±0.50                    | 11.17±0.50      |

Based on the table above, it shows that the test of the antibacterial activity of the two extraction methods did not show a significant difference in inhibitory power. The results of extraction using infusion have inhibition of 11.09 ± 0.96 mm against E. coli bacteria and 6.87 ± 0.05 mm against S. aureus which has the smallest inhibition of all tests. Whereas the results of microwave assisted extraction obtained the highest antibacterial activity at power 30 against S. Aureus at 12.23 ± 1.85 compared to all extract tests of the MAE method.

4. Conclusions
Based on the results of research that has been done, it can be concluded that the phenolic content obtained from rosetta petal extract by the infusion method is 2.39% while for the MAE method using power 10, 30 and 50, the total phenolic levels are 2, respectively. 72%, 2.53% and 2.18%. This shows that the extraction method using microwave (Assisted Extraction) has a higher total phenolic level compared to the infusion method.

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