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The Effect of Extraction Method on Total Phenolic Contents of *Hedyotis corymbosa* (L.) Lam.

Rengganis Ulvia[1], Devika Nurhasanah[1], Azhar Camelia[2], Lucia Widianingrum[2]

[1]*Department of Pharmacy, Faculty of Health, Universitas Jenderal Achmad Yani Yogyakarta, Yogyakarta, Indonesia*
[2]*Undergraduate Program, Department of Pharmacy, Faculty of Health, Universitas Jenderal Achmad Yani Yogyakarta, Yogyakarta, Indonesia*

* *Corresponding author* Email: rengganisulvia@gmail.com

ABSTRACT

Background: *Hedyotis corymbosa* (L.) Lam. is an Indonesian herbal plant that has benefits as an anticancer, antibacterial, and antioxidant due to the content of secondary metabolite compounds, one of which is phenolic. This activity is influenced by the levels of active compounds contained in it. The levels of active compounds such as phenolics in an extract can be influenced by the extraction method.

Objective: To determine the effect of extraction methods on the total phenolic content of *H. corymbosa*

Research methods: *H. corymbosa* was extracted using maceration, soxhletation, and Ultrasound-Assisted Extraction (UAE) methods with 70% ethanol solvent (1:10 w/v). Each extract obtained was tested for total phenolic content using the UV-Vis Spectrophotometry method. The total phenolic content data obtained were then analyzed statistically using Statistical Product and Service Solutions with the One-Way ANOVA and Posy Hoc Tukey tests with a confidence level of 95%.

Results: The total phenolic content of *H. corymbosa* extract using the maceration extraction method was 53.585 ± 0.910 mg GAE/g, the soxhletation method was 67.827 ± 1.105 mg GAE/g, and the UAE method was 59.615 ± 1.421 mg GAE/g. Based on statistical analysis using the SPPS One-Way Anova test, there was a significant difference ($p<0.05$) in total phenolic content between extraction methods.

Conclusion: Differences in extraction methods significantly affect the total phenolic content of *H. corymbosa* extract. The highest total phenolic content was produced using the soxhletation extraction method, followed by the UAE method and the maceration method.

Keywords (bold): *Hedyotis corymbosa* (L.) Lam.; Extraction Method; Total Phenolic Content; the UV-Vis Spectrophotometry

INTRODUCTION

Hedyotis corymbosa (L.) Lam. or often called pearl grass is an Indonesian herbal plant that has health benefits. The plant has been used to treat infectious diseases, broken bones, appendicitis, cancer, and hepatitis (Mukmilah *et al*., 2012). *H. corymbosa* extract has been scientifically proven to have hepatoprotective, antibacterial, antioxidant, analgesic, and anticancer activities (Rahman *et al*., 2012). *H. corymbosa* extract contains secondary metabolite compounds including phenolics, quinones, flavonoids, tannins, coumarins, terpenoids, essential oils, and alkaloids (Das and Bharali., 2020). One of the compounds that plays an active role in pharmacological activity is phenolic compounds. Based on previous studies, *H. corymbosa* extract has been scientifically proven to have hepatoprotective, antibacterial, antioxidant, analgesic, and anticancer activities. *H. corymbosa* extracted using the soxhletation method has antioxidant activity with an IC_{50} value of 58.26 µg/ml, where this activity shows a strong correlation with the total phenolic content (Das and Bharali., 2020).

Phenolic compounds can be obtained by the extraction process. Extraction methods consist of conventional and non-conventional methods. Conventional or traditional methods include maceration and soxhletation and non-conventional or modern methods include Ultrasound Assisted Extraction (UAE). The choice of extraction method plays a major role in the levels of compounds and biological activity of an extract. The higher the levels of active compounds, the better the activity (Marwati *et al*., 2022). Research conducted by Ramayani *et al* (2021) showed that differences in extraction methods affect the levels of total phenolics and flavonoids and the antioxidant activity of noni leaves (Ramayani *et al*., 2021). Therefore, this study aims to determine the effect of conventional extraction methods, namely maceration, and soxhletation, with non-conventional methods, namely Ultrasound-Assisted Extraction (UAE) on the total phenolic content of *H. corymbosa* extract.

RESEARCH METHODS

Tools and Materials

The tools used in this study were a 40 mesh sieve, maceration vessel, porcelain cup, grinder (Fomac), micropipette (Ohaus), water bath, soxhlet apparatus (Pyrex), sonicator (GT-Sonic), UV-Vis spectrophotometer (Thermo Scientific Genesys 10S), analytical balance (Ohaus PAJ1003), and glassware (Iwaki). The materials used in this study included *H. corymbosa* obtained from Sidorejo, Harjobinangun, Pakem, Sleman, Special Region of Yogyakarta, Gallic acid (Sigma), Aquades, Folin-Ciocalteau (Sigma), Ethanol p.a, Ethanol 70%.

Research Procedure

1. Plant Determination and Sample Preparation

H. corymbosa was obtained from Harjobinangun, Pakem, Sleman, Special Region of Yogyakarta, and determined in the Biology Learning Laboratory, Faculty of Applied Science and Technology, Ahmad Dahlan University. The samples used in this study were all parts of *H. corymbosa* plant. A total of 1 kg of *H. corymbosa* was harvested, then wet sorted and washed to remove dirt. The sample was dried using an oven at a temperature of 50℃ for 72 hours, ground using a grinder, and sieved with a 40 mesh sieve.

2. Sample Extraction

2.1 Maceration Method

H. corymbosa simplicia powder was extracted with 70% ethanol solvent (1:10 w/v) for 72 hours. The sample was put into a maceration vessel and solvent was added while stirring occasionally at the same hour in a dark environment to prevent exposure to direct sunlight. After 72 hours, the maceration results were filtered to obtain filtrate, and re-maceration was carried out in the same way. After being filtered, each filtrate was combined and evaporated using a water bath at a temperature of 50℃ until a thick extract was obtained (Ulvia *et al*., 2024).

2.2 Soxhletation Method

The simplicial powder was wrapped in filter paper and put into a soxhlet lead. Ethanol solvent was put into a round bottom flask with a ratio of sample to solvent of 1:10 w/v. The heater at a temperature of 70℃ was then turned on and the soxhletation process was carried out until the filtrate on the siphon arm turned clear. The filtrate was then evaporated using a water bath at 50ºC to obtain a thick extract (Sirumpea *et al*., 2021).

2.3 UAE Method

H. corymbosa simplicia powder was added with ethanol solvent (1:10 w/v). The homogeneous mixture was extracted with a sonicator for 30 minutes at a temperature of 27℃. The extraction results were filtered to obtain the filtrate and re-extracted with the same method. After being filtered, each filtrate was combined and evaporated with a water bath at a temperature of 50℃ until a thick extract was obtained (Ulvia *et al*., 2024).

Each thick extract from maceration, soxhletation, and UAE was weighed and its yield value was calculated using the formula:

$$\%\text{Yield} = \frac{Extract\ weight\ (g)}{Simplicia\ weight\ (g)} \text{ x } 100\%$$

3. Total Phenolic Content Test

3.1 Preparation of 20% Sodium Carbonate Solution

5 g of Sodium Carbonate powder and dissolve it with distilled water in a 25 mL measuring flask.

3.2 Preparation of Standard Gallic Acid Solution

10 mg of gallic acid standard dissolved with ethanol p.a in a 10 mL measuring flask until a concentration of 1000 ppm was obtained. The standard solution was made in a series of concentrations of 300, 400, 500, 600, 700, and 800 ppm in a 10 mL measuring flask (Alim *et al*., 2022).

3.3 Determination of the Maximum Wavelength of Gallic Acid

A standard of 600 ppm gallic acid 100 µL was reacted with 7.9 mL of distilled water, 500 µL of Folin-Ciocalteau was left for 8 minutes, and 1.5 mL of 20% sodium carbonate was added. The solution was read using UV-Vis spectrophotometry at a wavelength of 200-800 nm (Alim *et al*., 2022). The wavelength obtained was 776 nm.

3.4 Determination of the Operating Time of Gallic Acid

A standard of 600 ppm gallic acid as much as 100 µL was reacted with 7.9 mL of distilled water, 500 µL of Folin-Ciocalteau was left for 8 minutes, and 1.5 mL of 20% sodium carbonate was added. The absorbance of the solution was measured at a maximum wavelength of 776 nm for 2 hours with a time interval of 1 minute (Alim *et al*., 2022). The measurement results obtained an operating time of 1 hour 45 minutes.

3.5 Preparation of Gallic Acid Standard Curve

The standard curve of gallic acid used a concentration series of 300, 400, 500, 600, 700, and 800 ppm. A total of 100 µL of each concentration series solution was reacted with 7.9 mL of distilled water, 500 µL of Folin-Ciocalteau was left for 8 minutes, and 1.5 mL of 20% sodium carbonate was added. The solution was incubated for 1 hour 45 minutes and the absorbance was read at a wavelength of 776 nm (Alim *et al*., 2022)

3.6 Preparation of test solutions

For each extract of *H. corymbosa* from maceration, soxhletation, and UAE as much as 10 mg was dissolved in 10 mL of ethanol p.a until a concentration of 1000 ppm was obtained.

3.7 Determination of total phenolic content

The *H. corymbosa* extract test solution was reacted with 100 µL with 7.9 mL of distilled water, 500 µL of Folin-Ciocalteau was left for 8 minutes, and 1.5 mL of 20% sodium

carbonate was added. The test solution was incubated for 1 hour 45 minutes and the absorbance was read at a maximum wavelength of 776 nm (Alim *et al*., 2022).

The absorbance of the obtained sample is entered into the linear regression equation of the standard curve of gallic acid y = bx + a to obtain the phenolic concentration (x value). The total phenolic content is determined by the formula below:

$$\text{TPC} = \frac{C \,.\, V \,.\, fp}{g}$$

Information:

TPC = Total phenolic content (mg QE / gram)

C = Phenolic concentration (x value)

V = Volume of extract used (mL)

Fp = Dilution factor

G = Weight of sample used (g)

The data obtained were analyzed statistically using the Statistical Package for Social (SPSS) Software. Normality test with Shapiro-Wilk and homogeneity with Levene Statistic ($p > 0.05$). Normally distributed and homogeneous data were continued with a one-way ANOVA test with a 95% confidence level and post hoc Tukey.

RESULTS AND DISCUSSION

Extraction is performed to isolate secondary metabolites from their mixtures using suitable solvents. The choice of extraction method depends on the characteristics of the material and the specific compound being separated. In this study, several extraction techniques were utilized, including maceration, Soxhlet extraction, and ultrasound-assisted extraction (UAE). Variations in the extraction methods can significantly influence the concentration of the extracted metabolite compounds. This study aims to determine the effect of *H. corymbosa* extraction method on total phenolic content. *H. corymbosa* was obtained from Sidorejo, Harjobinangun, Pakem, Sleman, Special Region of Yogyakarta. The sample was determined with the aim of determining the truth of the plant in the Biology Learning Laboratory, Faculty of Applied Science and Technology, Ahmad Dahlan University. The determination results showed that the plant is *Hedyotis corymbosa* (L.) Lam.

The harvested *H. corymbosa* was wet sorted to remove dirt. The sample was then dried using an oven at a temperature of 50°C for 78 hours. The purpose of drying is to reduce the water content so that it can be stored for a long time and to facilitate the extraction process (Amin, 2023). The dried sample was ground with a grinder to reduce the particle size and

sieved with a 40 mesh sieve to obtain a simplicial powder with a uniform and fine size to facilitate the extraction of compounds during the extraction process (Setiawan *et al*., 2017).

H. corymbosa was extracted using three different extraction methods, namely the conventional maceration and soxhletation method and the non-conventional Ultrasound-Assisted Extraction (UAE) method using the same solvent, namely 70% ethanol. 70% ethanol solvent is a solvent that has been proven to be widely used to extract total phenolic compounds in plants (Alim *et al*., 2022). The thick extract obtained was observed organoleptically, the extract has a thick texture, distinctive odor, and brown color (Table 1). The yield value of the thick extract was also calculated to determine the amount of secondary metabolites extracted by the solvent (Ulvia *et al*., 2024).

TABLE 1. Yield Value and Organoleptic of *H. corymbosa* Extract

Extraction Method	Yield Value (%)	Organoleptic
Maceration	9,2	Color: Brown Odor: Specific for *H. corymbosa* Form: Viscous liquid Taste: Bitter
Soxhletation	3,6	Color: Brown Odor: Specific for *H. corymbosa* Form: Viscous liquid Taste: Bitter
UAE	1	Color: Brown Odor: Specific for *H. corymbosa* Form: Viscous liquid Taste: Bitter

The results of the calculation of the yield value in Table 1 show that the maceration extraction method produces the highest yield value, which is 9.2%, followed by the soxhletation method of 3.6% and UAE of 1%. The yield value is said to be good if it is more than 10% (Purwanti, A., 2022). The yield value produced from all extracts does not meet the requirements for good yield. The low yield value can be influenced by the weight of the

simplicia powder used, the more simplicia powder used, the higher the yield value produced (Yanti *et al*., 2024). The yield value created from each extraction method is different. Several of the factors that causes the difference in yield value is the length of extraction time. Extraction over a long time will get a high yield value and increase solvent penetration in the sample (Yulianti, 2014). The length of extraction time results in optimal contact time between the solvent and the sample, so that the penetration process between the solvent that enters the raw material is better and causes more compounds to diffuse out of the cell (Prasetyo and Vifta, 2022). In addition to extraction time, temperature can also affect the yield value. Temperature influences the permeability of the simplicial cell walls, making them weaker. The weaker the cell walls, the easier it is for the solvent to extract the active substances from the cells. As the temperature increases, the movement of active substance particles occurs more rapidly (Damanik *et al*., 2014).

The extraction process using the maceration method lasts for 72 hours compared to the soxhletation method, which takes 4-6 hours to achieve 5-6 circulations, and UAE for 30 minutes so that with this time difference, the maceration method produces the highest yield value compared to other methods. The maceration method involves stirring the mixture every 24 hours for three days, leading to a higher yield compared to the Soxhlet and ultrasonic-assisted extraction (UAE) methods. This increased yield is due to the more frequent contact between the sample and the solvent achieved through continuous stirring. The constant stirring creates greater pressure between the solvent and the cells in the pearl grass leaves, resulting in a more effective dissolution of organic compounds in the ethanol. After this process, each extract obtained was tested for total phenolic content using UV-Vis spectrophotometry. Each extract obtained is then tested for total phenolic content using the UV-Vis spectrophotometry method (Setyowati *et al*., 2014).

The UV-Vis spectrophotometry method has the principle that the incoming light source is polychromatic light which is passed through a monochromator so that it becomes monochromatic light which is then forwarded through the cell containing the sample. Some of the light will be absorbed by the cell and some will be forwarded to the photocell which functions to convert light energy into electrical energy. The electrical energy will provide a signal to the detector which will then be converted into an absorption value (absorbance) of the substance being analyzed (Miarti and Legasari, 2022). The advantages of this method are that the method is quite simple, can determine very small levels of substances, and the results obtained are quite fast and accurate (Yulia *et al*., 2021).

The process of determining the total phenolic content begins with measuring the maximum wavelength of the gallic acid standard. The maximum wavelength of gallic acid obtained is 776 nm. The next stage is to determine the operating time that will be used as the incubation time, the operating time obtained is 1 hour 45 minutes. Total phenolic content is calculated using a linear regression equation obtained from the standard curve of gallic acid y = 0.0011x-0.0231 as in Figure 1. The absorbance of the sample is distributed as the y value in the linear regression equation and the x value as the content. The x value is distributed into the Total Phenolic Content (TPC) calculation formula. The results of the calculation of total flavonoid content can be seen in Table 2.

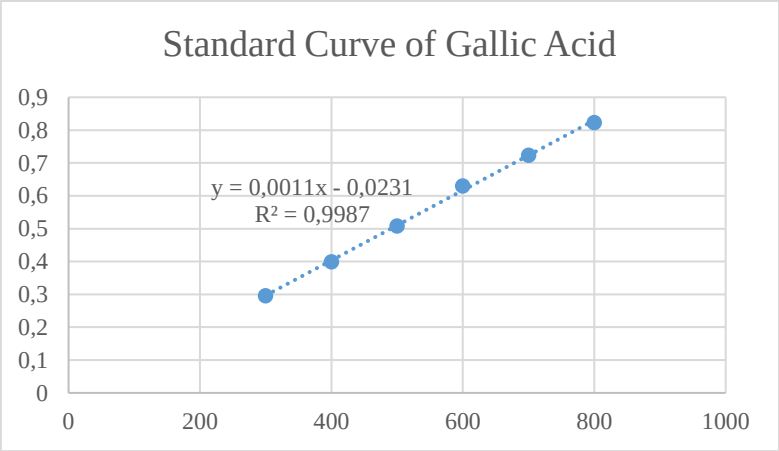

FIGURE 1. Standard Curve of Gallic Acid

TABLE 2. Total Phenolic Content of *H. corymbosa* Extract

Extraction Method	Total Phenolic Content (mg GAE/g) ± SD	CV (%)
Maceration	53,585 ± 0,910	1,7
Soxhletation	67,827 ± 1,105	1,6
UAE	59,615± 1,421	2,4

The maceration method produced a total phenolic content of 53.585 ± 0.910 mg GAE/g, the soxhletation method of 67.827 ± 1.105 mg GAE/g, and the UAE method of 59.615 ± 1.421 mg GAE/g. The CV value produced by all methods was good because it was less than 5% (Table 4). Based on the results of the total phenolic content test, showed that differences in extraction methods affected the total phenolic content of *H. corymbosa* extract. The soxhletation method produced the highest total phenolic content followed by

the UAE and maceration methods. The soxhletation method is an extraction method that involves heating with the principle of repeated filtration so that the results obtained are perfect and the solvent used is relatively small (Riniati *et al*., 2019). Several factors influence the extraction process, including temperature and extraction time. In the Soxhlet extraction method, high temperatures can break down plant cell walls, making it easier for the solvent to extract secondary metabolites from the plant cells. Additionally, an optimal extraction time is crucial; if the extraction time is too long, it can lead to hydrolysis of the extract, while a time that is too short may not extract all the active compounds from the material. Therefore, the Soxhlet method is more effective in extracting total phenolic compounds from *H. corymbosa* extract compared to the maceration and UAE methods (Riniati *et al*., 2019).

The UAE method has the highest total phenolic content of *H. corymbosa* extract after the soxhletation method. The UAE method is a modification of maceration that uses the assistance of high-frequency ultrasonic waves, namely 20 kHz (20000 Hz) at a controlled temperature (Utami *et al*., 2020). Ultrasonic waves can break down cell walls which will help release active compounds, compared to the maceration method which only involves soaking the simplicia in a solvent at room temperature with periodic stirring. So the UAE method produces higher total phenolic content compared to the maceration method. The maceration extraction method produces the smallest amount of phenolic content. This method involves soaking powdered Simplicia in a suitable solvent at room temperature (Nuraeni and Kodir., 2021). Since the maceration method does not involve heating or the use of ultrasonic waves, the extraction process does not occur optimally, resulting in a lower yield of compounds, including phenolics.

Based on statistical analysis using SPSS with a one-way ANOVA test, there is a significant difference ($p < 0.05$) from each extraction method on the total phenolic content of pearl grass extract. Then a further statistical test was carried out with Post Hoc Tukey, the results showed that the maceration method was significantly different from the soxhletation method and significantly different from UAE ($p < 0.05$).

The results of this study can serve as a valuable reference for the pharmaceutical industry to enhance product quality by increasing phenolic content. Extraction methods are crucial in the pharmaceutical field for obtaining optimal bioactive compounds, which can be utilized in various pharmaceutical and health products. As extraction technology advances, it continues to contribute to the development of more effective, safe, and beneficial drugs for human health.

CONCLUSION

The difference in extraction methods significantly affected the total phenolic content of *H. corymbosa* extract. The best total phenolic content of *H. corymbosa* extract was produced by the soxhlet extraction method of 67,827 ± 1,105 mg GAE/g followed by the UAE method of 59,615 ± 1,421 mg GAE/g and the maceration method of 53,585 ± 0,910 mg GAE/g.

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