Journal of Biotechnology and Natural Science
Vol. 5, No. 1, June 2025, pp. 40-47
ISSN 2808-2508

The Effect of Extraction Method on Total Flavonoid Content of *Hedyotis corymbosa* L.

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ARTICLE INFO

Article history
Received: May 6th 2025
Revised: May 16th 2025
Accepted: May 23th 2025

Keywords
Hedyotis corymbosa L.
Maceration
Soxhletation
Total Flavonoid content
UAE

ABSTRACT

Hedyotis corymbosa L. (*H. corymbosa*) is an Indonesian herbal plant with many health benefits. This activity comes from secondary metabolite compounds, one of which is flavonoids. These compounds can be obtained through an extraction process, where the extraction method is one of the factors that can affect the levels of compounds. This study aims to determine the effect of conventional extraction methods: maceration and soxhletation with non-conventional methods, Ultrasound Assisted Extraction (UAE) on the total flavonoid content of *H. corymbose*. The sample was extracted using 70% ethanol solvent (1:10 w/v) with the maceration, soxhletation, and UAE methods. The total flavonoid content of the *H. corymbosa* extract was measured using UV-Vis spectrophotometry. The data obtained were then analyzed statistically using SPSS One-Way ANOVA, followed by Post Hoc Tukey with a 95% confidence level. The soxhletation method has the highest flavonoid content at 72.255±1.334 mg QE/g, followed by UAE at 69.118±1.782 mg QE/g, and maceration at 43.725±0.679 mg QE/g. Statistical analysis confirmed that the extraction method significantly influences total flavonoid content. While both soxhletation and UAE methods produced similarly high flavonoid contents, UAE offers a substantial advantage in efficiency due to its shorter extraction time, making it a promising alternative to conventional soxhletation.

How to Cite: Ulvia, R., Nurhasanah, D., Camelia, A. & Widianingrum, L. (2025). The Effect of Extraction Method on Total Flavonoid Contents of *Hedyotis corymbosa* L. *Journal of Biotechnology and Natural Science*, 5(1):40-47.

1. Introduction

H. corymbosa is one of the Indonesian herbal plants that is empirically used by the community to treat hepatitis, cancer, appendicitis, broken bones, and infections (Mukmilah et al., 2012). Based on previous studies, it also shows that *H. corymbosa* extract has pharmacological activity, including anticancer, antibacterial, antioxidant, analgesic, and hepatoprotective (Rahman et al., 2012). This activity is certainly inseparable from the secondary metabolite compounds contained in *H. corymbosa*, one of which is flavonoids. *H. corymbosa* extract is known to contain a total flavonoid compound of 2.52 mg QE/mg sample (Wahyuni et al., 2018). Flavonoid compounds are known for their beneficial effects on health. This is because flavonoids have anti-inflammatory, antioxidant, anticancer properties, and various other biological activities (Khoirunnisa & Sumiwi, 2019).
The extraction process can obtain flavonoid compounds. The selection of extraction methods 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). Previous studies show that differences in extraction methods affect the levels of total flavonoids and their antioxidant activity (Fadlilaturrahmah et al., 2020).

Extraction methods consist of conventional methods and non-conventional methods. Conventional extraction methods include maceration and soxhletation. Maceration involves soaking powdered simplicia in a specific solvent at room temperature, protected from light, and does not require heating (Putri et al., 2024). In contrast, soxhletation uses a solvent under continuous and repeated heating, allowing for efficient compound extraction (Riniati et al., 2019). Meanwhile, the Ultrasound-Assisted Extraction (UAE) method is a non-conventional or modern technique that employs high-frequency ultrasonic waves to create intensive agitation, enhancing the efficiency of the extraction process (Sari et al., 2024). This study aims to determine the effect of conventional extraction methods, namely maceration and soxhletation with non-conventional UAE method, on the total flavonoid content of *H. corymbosa*.

2. Methods

2.1. Ingredients

The materials used in this study were *H. corymbosa* obtained from Sidorejo, Harjobinangun, Pakem, Sleman, Special Region of Yogyakarta. $AlCl_3$, CH_3COOK, aquades, ethanol, 70% ethanol, Quercetin standard.

2.2. Plant determination and sample preparation

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

2.3. Sample extraction

2.3.1. Maceration method

Simplicia powder was extracted with 70% ethanol solvent (1:10 w/v). The sample was put into a maceration vessel, and solvent was added and left for 72 hours while stirring occasionally at the same time. The maceration vessel was placed in a dark environment to prevent direct sunlight exposure. After 72 hours, the maceration results were filtered to obtain a clear filtrate or extract, and re-maceration was carried out in the same method. After being filtered, each filtrate was combined and evaporated at 50℃ using a water bath until a thick extract was obtained.

2.3.2. Soxhletation method

The powdered simplicia is wrapped in filter paper and put into a Soxhlet thimble. Ethanol solvent is put into a round-bottom flask. The ratio between the amount of sample and solvent is 1:10 w/v. The heater is then turned on and the soxhletation process is carried out until the filtrate on the siphon arm becomes clear. The filtrate is then evaporated using a water bath at a temperature of 50ºC until a thick extract is obtained.

2.3.3 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. After being filtered, each filtrate was combined and evaporated with a water bath at a temperature of 50 ℃ until a thick extract was obtained. Each thick extract from maceration, soxhletation and UAE was weighed, and its yield value was calculated using the formula:

$$\% \text{ Yield} = \frac{\text{Extract weight (g)}}{\text{Sample powder weight (g)}} \times 100\% \qquad (1)$$

2.4. Total Flavonoid Content Test

2.4.1. Preparation of 10 % $AlCl_3$ solution

5 g $AlCl_3$ dissolved in 50 mL of distilled water in a 50 mL measuring flask.

2.4.2. Preparation of CH_3COOK 5%

981.4 mg of CH_3COOK was dissolved in distilled water in a 10 mL measuring flask to the line limit.

2.4.3. Preparation of standard quercetin solution

The quercetin standard was weighed as much as 10 mg dissolved with ethanol in a 100 mL measuring flask until a concentration of 100 ppm was obtained. The standard solution was made into a series of concentrations of 40, 50, 60, 70, and 80 ppm in a 10 mL measuring flask.

2.4.4. Determination of the maximum wavelength of quercetin

Standard quercetin 60 ppm, as much as 500 µL, was reacted with 500 µL ethanol, 100 µL CH_3COOK 5%, 100 µL $AlCl_3$ 10%, and 2.8 mL of distilled water. The solution was read using UV-Vis spectrophotometry at a wavelength of 200-700 nm. The wavelength obtained was 427 nm.

2.4.5. Determination of quercetin operating time

Quercetin standard 60 ppm 500 µL was added with 500 µL of ethanol, 100 µL of CH_3COOK 5%, 100 µL of $AlCl_3$ 10%, and 2.8 mL of distilled water. The absorbance of the solution was measured at a maximum wavelength of 427 nm for 60 minutes with an interval of 1 minute. The measurement results were obtained at operating time in the 26th minute.

2.4.6. Preparation of quercetin standard curve

The standard curve of quercetin standard used a concentration series of 40, 50, 60, 70, and 80 ppm. A total of 500 µL of each concentration series solution was reacted with 500 µL of ethanol, 100 µL of CH_3COOK 5%, 100 µL of $AlCl_3$ 10%, and 2.8 mL of distilled water. The solution was incubated for 26 minutes, and the absorbance was read at a wavelength of 427 nm.

2.4.7. Preparation of test solution

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

2.4.8. Determination of total flavonoid content

The *H. 42orymbose* extract test solution was reacted with ethanol, 100 µL CH_3COOK 5%, 100 µL $AlCl_3$ 10%, and 2.8 mL of distilled water. The test solution was incubated for 26 minutes, and the absorbance was read at a maximum wavelength of 427 nm.

2.5. Data Analysis

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

$$TCF = \frac{C \times V \times fp}{g} \qquad (2)$$

Where:
TFC = Total flavonoid content (mgQE/g)
C = Flavonoid concentration (x value)
V = Extract volume (mL)
Fp = Dilution factor
g = Sample weight (g)

The data obtained were analyzed statistically using the *Statistical Package for Social Sciences* (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 and *Post Hoc Tukey* with a 95% confidence level.

3. Results and Discussion

3.1. Plant determination and sample preparation

H. corymbosa was obtained from Sidorejo, Harjobinangun, Pakem, Sleman, Special Region of Yogyakarta and has been determined with a determination certificate number 220/Lab.Bio/B/V/2024. The purpose of the determination is to determine the truth of the plants to be studied and avoid errors in collecting materials and avoid the possibility of mixing the plants to be studied with other plants (Klau & Hesturini, 2021). The determination results show that the plants used are true *Hedyotis corymbosa* L.

H. corymbosa herb was harvested and wet sorting to eliminate dirt and foreign materials from the plant materials before washing, by discarding unwanted parts. This step ensures that the resulting simplicia are clean and appropriate for further use or processing (Wahyuni et al., 2017). The sample was dried using an oven at a temperature of 50 ℃ aims to maintain the secondary metabolite compounds of flavonoids so that they are not damaged because excessive heating can cause flavonoids to degrade (Sharma et al., 2015). Temperatures above 60℃ can lead to the degradation of flavonoid compounds, reducing their stability and efficacy (Kautsari et al., 2021).

The dried samples were ground using a grinder to expand the particle size and sieved with a 40 mesh sieve to obtain a uniform particle size.

3.2. Sample extraction

The extraction of *H. corymbosa* was performed using 70% ethanol, a polar solvent containing multiple hydroxyl (–OH) groups, enhancing its ability to dissolve polar flavonoid compounds more effectively than other solvents. (Nurhasanah et al., 2024). The obtained thick extract was observed organoleptically, and its yield value was calculated (Table 1). The extract has a thick texture, *H. corymbosa's* characteristic odor, and brown color. The results of the yield value calculation show that the maceration extraction method produces the highest yield value, followed by the soxhletation method, which is a conventional extraction method, and the lowest yield value is produced by the UAE extraction method, which is a non-conventional extraction method. The calculation of the yield value serves to determine the amount of secondary metabolites extracted by the solvent (Sari et al., 2024).

Table 1. Yield and organoleptic values of *H. corymbosa* extract

Extraction Method	Yield Value (%)	Organoleptic
Maceration	9.2	Color: Brown Odor: Characteristic Texture: Thick
Soxhletation	3.6	Color: Brown Odor: Characteristic Texture: Thick
UAE	1	Color: Brown Odor: Characteristic Texture: Thick

One of the factors that causes the difference in yield values is the difference in extraction time. Extraction for a long time will get a high yield value and increase solvent penetration in the sample. (Yulianti, 2014)The extraction process with the maceration method lasts for 72 hours compared to the soxhletation method, which is 4-6 hours to achieve 5-6 circulations and UAE for 30 minutes. The yield value obtained from various extraction methods has not met the requirements, where a good yield value is not less than 10%. This is influenced by differences in extraction methods, extraction temperature, type of material used, extraction time, and the comparison of solvents with the sample. Temperature and extraction time result in optimal contact time between the solvent and the sample, the penetration process between the solvent that enters the raw material is better, and causes more compounds to diffuse out of the cell (Prasetyo & Vifta, 2022).

3.3. Total flavonoid content of *H. corymbosa*

Determination of flavonoid content by the Colorimetric $AlCl_3$ method, with the principle on the formation of a stable complex between $AlCl_3$ and flavonoids, specifically by binding to the keto group at carbon position 4 and the hydroxyl group at carbon position 3 or 5 of the flavone and flavonol structures. To quantify total flavonoid content, quercetin was used as the standard comparison compound. Quercetin is a flavonol that possesses the required functional groups for complex formation, a keto group at position 4, and hydroxyl groups at positions 3 and 5 (Figure 1) (Azizah et al., 2014). The addition of $AlCl_3$ results in the formation of a colored complex with these hydroxy-ketone groups, which can be detected and measured in the visible wavelength range using UV-Vis spectrophotometry.

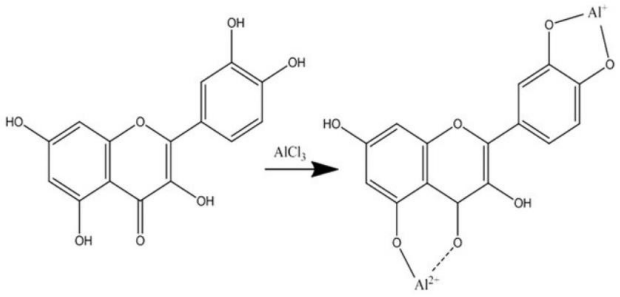

Figure 1. Complex formation of quercetin-$AlCl_3$ (Azizah et al., 2014)

The total flavonoid content was measured using the UV-Vis spectrophotometry method. This technique is based on the principle that when monochromatic light passes through a compound, part of the light is absorbed, while the rest may be reflected or transmitted. The amount of light absorbed by the sample is detected and recorded as an absorbance value, which correlates with the concentration of flavonoids present. This method offers several advantages: it is simple to perform, sensitive enough to detect very low concentrations, and provides rapid and accurate results. (Yulia et al., 2021).

The process of determining the total flavonoid content begins with measuring the maximum wavelength of the quercetin standard. The purpose of determining the maximum wavelength is to identify the wavelength at which the quercetin and $AlCl_3$ complex exhibits its highest absorbance. This step is crucial in spectrophotometric analysis, as measuring at the maximum wavelength ensures the greatest sensitivity, meaning that even small changes in concentration produce significant changes in absorbance. As a result, repeated measurements or replications will yield more consistent and accurate results, helping to minimize potential errors in the analysis (Suharyanto & Prima, 2020). The measurement results obtained the maximum wavelength of quercetin, which is 427 nm. The results are the same as those of previous studies, namely 427 nm for the maximum wavelength of quercetin (Aprilia et al., 2022).

The subsequent step involves identifying the operating time, which is the optimal duration needed before measuring a compound to ensure that its absorbance is at its most stable point. This is determined by monitoring changes in absorbance over time. Establishing the correct operating time is essential to reduce measurement errors, especially in this study where the absorbance being measured comes from a complex formed between quercetin and $AlCl_3$. Since the formation of this complex requires a certain amount of time to reach reaction stability, taking measurements too early may result in incomplete complex formation and inaccurate data (Suharyanto & Prima, 2020). The operating time obtained is 26 minutes.

Total flavonoid content was calculated using a linear regression equation y=0.0068x-0.029 obtained from the standard curve of quercetin. Sample absorbance 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 Flavonoid Content* (TFC) calculation formula. The results of the calculation of total flavonoid content can be seen in Table 2.

Table 2. Total Flavonoid Content

Extraction Method	Total Flavonoids (mg QE/g)±SD
Maceration	43.76±0.68
Soxhletation	72,26±1,33
UAE	69,12±1,79

The maceration method produced a total flavonoid content of 43.725 ± 0.679 mg QE/g, while the soxhletation method yielded the highest amount at 72.255 ± 1.334 mg QE/g, followed by the UAE method with 69.118 ± 1.782 mg QE/g. These results indicate that the extraction method influences the total flavonoid content in *H. corymbosa* extract. Among the three, the soxhletation method produced the highest total flavonoid content. This method works on the principle of repeated solvent reflux and filtration under heat, allowing for more efficient extraction while using a relatively small volume of solvent (Riniati et al., 2019). One critical factor affecting the efficiency

of extraction is temperature (Yuliantari et al., 2017). The high temperatures in the soxhletation process can break down plant cell walls, thereby enhancing the solvent's ability to extract secondary metabolites such as flavonoids. As a result, the soxhletation method enables a more complete extraction of flavonoid compounds from *H. corymbosa*.

After the soxhletation method, the UAE method produced the second highest total flavonoid content in *H. corymbose* extract. UAE is considered a non-conventional or modern extraction technique that improves upon the conventional maceration method by utilizing high-frequency ultrasonic waves, typically around 20,000 Hz, applied at a controlled temperature (Utami et al., 2020). The ultrasound waves create acoustic cavitation, the rapid formation, growth, and collapse of microscopic bubbles in the solvent, which generates localized high pressure and temperature. These mechanical effects lead to the rupture of plant cell walls, thereby facilitating the release of intracellular compounds such as flavonoids into the solvent (Sari et al., 2024). This process significantly enhances mass transfer between the plant material and the solvent, resulting in a more efficient and faster extraction compared to conventional methods such as maceration. In contrast, the maceration method simply involves soaking the powdered plant material (simplicia) in a suitable solvent at room temperature, with only occasional stirring. Since maceration does not involve mechanical cell disruption or heating, the release of bioactive compounds is generally slower and less complete, leading to lower flavonoid content.

The difference in extraction methods can also be related to the yield value with total flavonoid content. The yield of the extract obtained from the maceration method was 9.2% with a total flavonoid content of 43,725 ± 0.679 mg QE/g, while the soxhletation method was 3.6% and a total flavonoid content of 72,255 ± 1,334 mg QE/g and UAE produced a yield of 1% and a total flavonoid content of 69,118 ± 1,782 mg QE/g. High yield does not always mean high flavonoid content because extraction yield refers to the total amount of material extracted from the plant, including not just flavonoids but also other compounds such as sugars, proteins, chlorophyll, and other secondary metabolites (Utami et al., 2020). In contrast, flavonoid content specifically refers to the concentration or amount of flavonoid compounds within that extract. So, an extraction method might give a high total yield (lots of extract mass), but the extract might be diluted with non-flavonoid substances, leading to a lower percentage of flavonoids. On the other hand, a method with a lower total yield might selectively extract more flavonoids and fewer unwanted compounds, resulting in a higher flavonoid concentration per gram of extract.

Based on the statistical analysis performed using SPSS software, a One-Way ANOVA test was conducted to determine whether there were significant differences in the total flavonoid content of *H. corymbose* extracts produced by different extraction methods. The results of the ANOVA test showed a significant difference ($p < 0.05$), indicating that the type of extraction method used had a statistically significant effect on the total flavonoid yield. To further explore which specific groups differed from each other, a Post Hoc Tukey test was applied. This follow-up test is used to compare the mean values between each pair of extraction methods and assess whether the differences are statistically significant. The analysis revealed that there was no significant difference ($p > 0.05$) in total flavonoid content between the soxhletation method and the Ultrasound-Assisted Extraction (UAE) method, meaning that both methods produced comparable contents of flavonoids. However, both soxhletation and UAE methods showed a significant difference ($p < 0.05$) when compared to the maceration method, which has the lowest flavonoid content.

These findings suggest that both soxhletation and UAE are more effective than maceration in extracting flavonoids from *H. corymbosa*. While soxhletation is a conventional extraction method that relies on prolonged heating and repeated solvent cycling, UAE represents a modern and more efficient technique that utilizes ultrasonic waves to enhance cell wall disruption and compound release. An important practical advantage of the UAE method is its significantly shorter extraction time. In this study, UAE required only 30 minutes to complete the extraction process, compared to 5 hours for the soxhletation method. This major reduction in extraction time not only improves energy efficiency but also offers potential cost savings and scalability for industrial applications. Therefore, despite being a non-conventional method, UAE is a promising alternative to soxhletation, providing similar yields with more efficient operation.

4. Conclusion

The difference in the extraction method significantly influences the total flavonoid content in *H. corymbosa* extracts. Among the methods tested, soxhletation has the highest flavonoid content (72.255 ± 1.334 mg QE/g), followed closely by UAE (69.118 ± 1.782 mg QE/g), while maceration resulted in the lowest flavonoid content (43.725 ± 0.679 mg QE/g). Statistical analysis confirmed that soxhletation and UAE were both significantly more effective than maceration, although not significantly different from each other. UAE offers a major advantage in terms of time efficiency, making it a more practical and energy-efficient alternative. Further research is needed to evaluate the bioactivities of *H. corymbosa* extracts obtained by different extraction methods.

ACKNOWLEDGEMENT

We sincerely thank Universitas Jenderal Achmad Yani Yogyakarta for funding this research through its 2024 Internal Grant (SPK/219/LPPMUNJAYA/IV/2024).

REFERENCES

Aprilia, A.Y., Agustiani, N. & Salasanti, C.D. (2022). *Penentuan kadar flavonoid total dan aktivitas antipiretik ekstrak etanol buah kapulaga (Amomum compactum Soland. Ex Maton)*.

Azizah, D.N., Kumolowati, E. & Faramayuda, F. (2014). Penetapan kadar flavonoid metode $AlCl_3$ pada ekstrak metanol kulit buah kakao (*Theobroma cacao* L.). *Kartika : Jurnal Ilmiah Farmasi*, 2(2):Article 2. https://doi.org/10.26874/kjif.v2i2.14.

Fadlilaturrahmah, F., Wathan, N., Firdaus, A.R., & Arishandi, S. (2020). Pengaruh metode ekstraksi terhadap aktivitas antioksidan dan kadar flavonoid daun kareho (*Callicarpa longifolia* Lam). *Pharma Xplore : Jurnal Sains Dan Ilmu Farmasi*, 5(1):Article 1. https://doi.org/10.36805/jpx.v5i1.977.

Kautsari, S.N., Humaedi, A., Wijayanti, D.R. & Safaat, M. (2021). Kadar total fenol dan flavonoid ekstrak temu kunci (*Boesenbergia pandurata*) melalui metode ekstraksi microwave. *ALCHEMY Jurnal Penelitian Kimia*, 17(1):Article 1. https://doi.org/10.20961/alchemy.17.1.46497.96-104.

Khoirunnisa, I. & Sumiwi, S.A. (2019). Peran flavonoid pada berbagai aktivitas farmakologi. *Farmaka*, 17(2):131–142. https://doi.org/10.24198/jf.v17i2.21922.

Klau, M.H.C. & Hesturini, R.J. (2021). Pengaruh Pemberian ekstrak etanol daun dandang gendis (*Clinacanthus nutans* (Burm F) Lindau) terhadap daya analgetik dan gambaran makroskopis lambung mencit. *Jurnal Farmasi & Sains Indonesia*, 4(1):Article 1. https://doi.org/10.52216/jfsi.v4i1.59.

Marwati, M., Nur, S., Khairi, N. & Nursamsiar, N. (2022). Pengaruh metode ekstraksi terhadap aktivitas antioksidan ekstrak daun karamunting (*Rhodomyrtus tomentosa* (Aiton) Hassk) dengan metode DPPH. *Jurnal Ilmiah Farmasi Farmasyifa*, 5(2):Article 2. https://doi.org/10.29313/jiff.v5i2.9053.

Mukmilah, L., Udin, Z. & Lisnawati, E. (2012). Uji aktivitas antibakteri dari ekstrak rumput mutiara (*Hedyotis Corymbosa* (L.) Lamk). *Jurnal Kimia Valensi*, 2:107771. https://doi.org/10.15408/jkv.v2i5.297.

Nurhasanah, D., Ulvia, R. & Junita, F. (2024). The effect of ethanol concentration variations on the total phenolic and flavonoid levels of *Bauhinia purpurea* L. leaf extract. *Journal of Biotechnology and Natural Science*, 4(2):Article 2. https://doi.org/10.12928/jbns.v4i2.12060.

Prasetyo, D.A. & Vifta, R.L. (2022). Effect of extraction method on total flavonoid level of red ginger extract (*Zingiber Officinale* Var Rubrum). *Journal of Holistics and Health Sciences (JHHS)*, 4(1):Article 1. https://doi.org/10.35473/jhhs.v4i1.143.

Putri, C.E.E., Wulandari, D.M., Hasyim, U.H., Hasyim, D.I. & Ramadhan, M.S. (2024). Optimasi waktu maserasi pada ekstraksi daun pegagan (*Centella asiatica*) terhadap uji aktivitas antioksidan. *Prosiding Semnastek*. https://jurnal.umj.ac.id/index.php/semnastek/article/view/22497.

Rahman, S., Putra, B., Kosman, R. & Mustika, R. (2012). Uji aktivitas Imunoglobulin M (IgM) ekstrak etanol herba rumput mutiara (*Hedyotis corymbosa*) pada mencit (*Mus musculus*) jantan dengan metode hemaglutinasi. *As-Syifaa Jurnal Farmasi*, 4(2):Article 2. https://doi.org/10.56711/jifa.v4i2.79.

Riniati, R., Sularasa, A. & Febrianto, A.D. (2019). Ekstraksi kembang sepatu (*Hibiscus rosa-sinensis* L.) menggunakan pelarut metanol dengan metode sokletasi untuk indikator titrasi asam basa. *Indonesian Journal of Chemical Analysis (IJCA)*, 2(01):Article 01. https://doi.org/10.20885/ijca.vol2.iss1.art5.

Sari, I.P., Ulvia, R. & Pratama, N.P. (2024). Pengaruh metode ekstraksi terhadap kadar flavonoid total daun jeruk nipis (*Citrus aurantifolia*). *Jurnal Ilmiah Farmasi Attamru (JIFA)*, 5(2):100–113. https://doi.org/10.31102/attamru.2024.5.2.100-113.

Sharma, K., Ko, E.Y., Assefa, A.D., Ha, S., Nile, S.H., Lee, E.T. & Park, S.W. (2015). Temperature-dependent studies on the total phenolics, flavonoids, antioxidant activities, and sugar content in six onion varieties. *Journal of Food and Drug Analysis*, 23(2):243–252. https://doi.org/10.1016/j.jfda.2014.10.005.

Suharyanto, S. & Prima, D.A.N. (2020). Penetapan kadar flavonoid total pada juice daun ubi jalar ungu (*Ipomoea batatas* L.) yang berpotensi sebagai hepatoprotektor dengan metode spektrofotometri UV-Vis. *Cendekia Journal of Pharmacy*, 4(2):Article 2. https://doi.org/10.31596/cjp.v4i2.89.

Utami, N.F., Sutanto, S., Nurdayanty, S.M. & Suhendar, U. (2020). Pengaruh berbagai metode ekstraksi pada penentuan kadar flavonoid ekstrak etanol daun iler (*Plectranthus scutellarioides*). *Fitofarmaka: Jurnal Ilmiah Farmasi*, 10(1):Article 1. https://doi.org/10.33751/jf.v10i1.2069.

Wahyuni, R., Guswandi, G., & Rivai, H. (2017). Pengaruh cara pengeringan dengan oven, kering angin dan cahaya matahari langsung terhadap mutu simplisia herba sambiloto. *Jurnal Farmasi Higea*, 6(2):Article 2. https://doi.org/10.52689/higea.v6i2.104.

Wahyuni, W.T., Pitria, L.K.D. & Rahmat, A. (2018). Analisis kadar flavonoid dan antioksidan ekstrak daun kenikir (*Cosmos caudatus*), rumput mutiara (*Oldenlandia corymbosa*), dan sirsak (*Annona muricata*) dengan teknik spektrometri. *Analit: Analytical and Environmental Chemistry*. https://doi.org/10.23960/aec.v3i1.2018.p.

Yulia, M., Ningtyas, K.R. & Suhandy, D. (2021). Penggunaan UV-Vis spektroskopi dan kemometrika untuk uji keaslian kopi codot lampung. *Jurnal Ilmu Pertanian Indonesia*, 26(4):Article 4. https://doi.org/10.18343/jipi.26.4.479.

Yuliantari, N.W.A. (2017). Pengaruh suhu dan waktu ekstraksi terhadap kandungan flavonoid dan aktivitas antioksidan daun sirsak (*Annona muricata* L.) menggunakan ultrasonik. *Media Ilmiah Teknologi Pangan*, 4(1).

Yulianti, D. (2014). Pengaruh lama ekstraksi dan konsentrasi pelarut etanol terhadap sifat fisika-kimia ekstrak daun stevia (*Stevia Rebaudiana* Bertoni M.) dengan metode microwave assisted extraction (MAE). *Jurnal Bioproses Komoditas Tropis*, 2(1):35–41.