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The effect of extraction methods on total flavonoid levels and antinflammatory activity of seagrass (*enhalus acoroides*) leaf extract on rabbits

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Abstract

Seagrass (*Enhalus acoroides*) has been identified as a promising candidate for natural anti-inflammatory agents due to its content of secondary metabolites, including flavonoids, steroids, and alkaloids. The extraction method employed can significantly influence the total flavonoid content. This study aimed to quantify the total flavonoid content derived from different extraction methods and assess the anti-inflammatory activity of these extracts in rabbits, using varying concentrations of 1%, 25%, 50%, 75%, and 100%. The extraction techniques utilized in this study were infusion and Ultrasonic-Assisted Extraction (UAE). The resulting extracts underwent phytochemical screening, and total flavonoid content was quantified using the colorimetric method. The extract with the highest flavonoid concentration was subjected to an anti-inflammatory activity test on rabbits with induced burn wounds. Data were analyzed using SPSS, encompassing normality tests, homogeneity tests, and non-parametric analyses, including the Kruskal-Wallis and Mann-Whitney tests, to determine significant differences in anti-inflammatory activity across the various extract concentrations. The UAE method, using ethyl acetate as a solvent, yielded the highest flavonoid content at 68.836±10.44 mgQE/g. The anti-inflammatory activity of the extracts was evident through a reduction in burn wound size from day one to day fourteen. The *Enhalus acoroides* leaf extract, obtained via the UAE method with ethyl acetate and containing flavonoids, alkaloids, and steroids, exhibits significant anti-inflammatory activity in rabbits (*Oryctolagus cuniculus*). There was no statistically significant difference ($p > 0.05$) in the reduction of wound diameter between the 50%, 75%, and 100% extract concentrations and the positive control (Bioplacenton®) over the fourteen-day treatment period.

Keywords

Antiinflammatory, Infundation, Total flavonoid levels, Ultrasound assisted extraction, Enhalus acroides

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Introduction

In Indonesia, the prevalence of burn injuries reaches 0.7%, with the majority occurring in males (1.04%) compared to females (1.02%) [1]. First-degree burn symptoms are characterized by limited damage to the epidermis, dry and reddened skin without blistering, and pain due to irritation of sensory nerve endings. These symptoms are indicative of inflammation, including erythema and irritation. The healing time for first-degree burns is typically around 14-21 days [2]- [3]. Anti-inflammatory treatment is essential for managing burn injuries, as it helps to reduce inflammation, alleviate pain, and promote the healing process. The study by [4] demonstrated that several medicinal plants have been proven to exhibit anti-inflammatory therapeutic effects with minimal or no side effects. The anti-inflammatory activity of these plants has been shown to be effective in treating inflammatory conditions through traditional medicine. Therefore, the development of anti-inflammatory agents from natural sources, such as seagrass (*Enhalus acoroides*), is being explored as a potential treatment.

Seagrass (*Enhalus acoroides*) is a marine plant that contains secondary metabolites with potential anti-inflammatory properties. Phytochemical screening of seagrass for its secondary metabolites has been conducted, as reported by [5], indicating that *Enhalus acoroides* leaves contain alkaloids, flavonoids, triterpenoids, steroids, tannins, and saponins. In this study, only the leaves of the seagrass were utilized. According to [6], the compounds responsible for anti-inflammatory activity are flavonoids, alkaloids, and steroids. The mechanism by which flavonoids exert their anti-inflammatory effect involves the inhibition of cyclooxygenase, lipoxygenase, and the production of arachidonic acid. Steroids, in the body, inhibit phospholipase A2, an enzyme responsible for releasing arachidonic acid, which is subsequently metabolized by cyclooxygenase and lipoxygenase enzymes, leading to the release of inflammatory mediators.

In the study by [7], the total flavonoid content of *Enhalus acoroides* leaves was reported to be 3.5697%. Several factors can influence the total flavonoid content, including temperature, heating duration, and the volume of solvent used. According to [8], increased temperature and prolonged heating can damage plant fragments that are heat-sensitive, potentially compromising the stability of compounds within the plant. Additionally, the more solvent used, the greater the amount of flavonoid compounds extracted. Therefore, optimizing the extraction time is essential to determine the point at which the extract yields the highest percentage of flavonoid content. Based on the aforementioned background, the author aims to investigate the total flavonoid content obtained through different extraction methods, as well as the anti-inflammatory activity of the extracts on rabbits at concentrations of 1%, 25%, 50%, 75%, and 100%.

Method

Preparation of test animals

Male New Zealand rabbits (Oryctolagus cuniculus), approximately 2.5 months old and weighing between 1.5 to 2 kg, were utilized as test animals. A total of six rabbits were acclimatized over a period of 7 days to adjust to the environmental conditions, during which general observations were conducted to monitor the animals' adaptation to their surroundings [9].

Administration of seagrass leaf extract to test animals

The fur on the dorsal region of the rabbit was shaved to expose an area of approximately 6 cm^2 (2x3 cm), which was previously measured with a ruler and marked with a marker. Prior to inflicting the injury, the rabbit was anesthetized, and the shaved area was subjected to injury using a coin heated over a Bunsen burner for 5 minutes. The heated coin was then applied to the skin for 5 seconds to induce a first-degree burn, characterized by localized damage to the epidermis, dry skin, erythema, the absence of blisters, and pain due to sensory nerve irritation. Subsequently, the burn area was moistened with a towel or cloth dampened with cold water for several seconds. Each rabbit was assigned to four control groups on its back. The test animals were treated with a dosage of 2 x 1 per day, applied topically with sterile gauze. The diameter of the burn wounds was monitored, and the area of the wounds was measured in square centimeters over a period of 14 days [9]-[3].

Data analysis

Data analysis was processed using SPSS Statistics Version 25 software, which analyzes normally distributed data using the Shapiro-Wilk normality test and tests for homogeneity using Levene's Test. Subsequently, One-Way ANOVA was conducted to examine differences in inflammation among the rabbits. If the resulting data were found to be non-normally distributed and non-homogeneous, non-parametric analysis was continued using the Kruskal-Wallis test, followed by the Mann-Whitney U test for further analysis.

Research ethics

The research ethics for this study have been assessed and approved by the Medical/Health Research Bioethics Committee of the Faculty of Medicine, Sultan Agung Islamic University, Semarang, under reference number 284/VII/2024/Bioethics Committee.

Results and Discussion

Percentage yield and moisture content of seagrass leaf extract

According to [10], the yield percentage is essential for determining the amount of extract produced during extraction. The difference in the initial sample weight does not proportionally affect the yield percentage and total flavonoid content (Table 1 and 2).

This implies that even if a large initial sample is used, it does not necessarily result in a high yield percentage and total flavonoid content [11]. In a study conducted by [12], the yields of ethanol extracts at concentrations of 30%, 50%, 70%, and 96% were 2.60%, 1.88%, 1.88%, and 1.92%, respectively. The total flavonoid content in each ethanol extract at 30%, 50%, 70%, and 96% was found to be 12.3, 8.9, 18.0, and 44.7 mg equivalent quercetin/g of extract. These results indicate that the yield weight does not determine the total flavonoid content obtained. The results of the moisture content extraction met the range for the moisture content of extracts, which is 5-10%. Excessive moisture content can facilitate microbial growth and reduce the stability of the extract [13].

Table 1. Results of percentage yield of seagrass leaf extract (*Enhalus acoroide*)

Methods and Solvents Used	Initial Weight of Sample (kg)	Weight of Extract (gram)	% Yield
Infusion with Aquades Solvent	3	14.48	0.48
UAE with Ethyl Acetate Solvent	7	20.56	0.29
UAE with n-Hexane Solvent	4	9.55	0.23

Table 2. Results of the moisture content percentage of seagrass leaf extract (*Enhalus acoroide*)

Methods and Solvents Used	Moisture Content (%)	Average Moisture Content (%)
Infusion with Aquades Solvent	5.94	5.77 ± 0.34
	5.98	
	5.38	
UAE with Ethyl Acetate Solvent	5.37	5.62 ± 0.28
	5.57	
	5.92	
UAE with N-Hexane Solvent	5.94	5.54 ± 0.49
	5.69	
	4.99	

Phytochemical screening of seagrass leaf extract

Table 3. Results of phytochemical screening of seagrass leaf extract

Type of Test	Reagent	Standard Color	Results		
			Infusion	UAE Ethyl Acetate	UAE N-Hexane
Alkaloid	Concentrated H_2SO_4 + Mayer	Presence of precipitate	Positive	Positive	Negative
Flavonoid	Mg Powder + Concentrated HCl	Formation of red, yellow, or orange alcoholic amine layer	Positive	Positive	Positive
Steroid and Triterpenoid	Glacial CH_3COOH + H_2SO_4	Steroid: Blue or green Triterpenoid: Red or purple	Negative	Positive	Positive
Saponin	-	Presence of foam	Positive	Negative	Negative
Tanin	FeCl3	Greenish black	Positive	Negative	Negative

Based on the results of phytochemical compound identification (Table 3), the seagrass extract obtained through the infusion method using aquadest as a solvent tested positive for alkaloids, flavonoids, saponins, and tannins. This finding aligns with the research by [14], which reported that extraction of seagrass (*Enhalus acoroides*) using polar liquid phases positively indicated the presence of alkaloids, flavonoids, saponins,

and tannins. The seagrass extract obtained through the UAE method using ethyl acetate as a solvent tested positive for alkaloids, flavonoids, and steroids. These results are consistent with the findings of [15], which also indicated that extraction of seagrass (*Enhalus acoroides*) with ethyl acetate positively contained alkaloids, flavonoids, and steroids. The seagrass extract obtained through the UAE method using n-hexane as a solvent tested positive for flavonoids, steroids, and triterpenoids. This result is consistent with the research by Nurafni and Nur [14], which indicated that extraction of seagrass (*Enhalus acoroides*) with n-hexane positively contained flavonoids and steroids.

Determination of total flavonoid content in seagrass leaf extract determination of quercetin standard curve

Table 4. Results of linear regression equation

Concentration	Absorbance
20 ppm	0.1824
30 ppm	0.2358
40 ppm	0.2988
50 ppm	0.3732
60 ppm	0.4384
70 ppm	0.5054

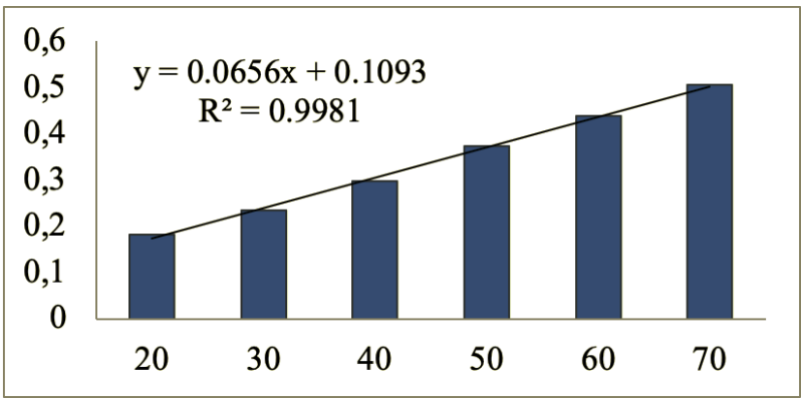

Figure 1. Standard curve graph

The absorbance results for quercetin demonstrate a linear relationship with concentration, indicating that higher concentrations on the quercetin standard curve correspond to greater absorbance values (Table 4). The linear regression equation obtained is y= 0.0066x + 0.0436, with a correlation coefficient (r) of 0.9981 (Figure 1). A correlation coefficient (r) value approaching 1 signifies a very strong correlation between concentration and its absorptive capacity [16][17][18].

Total flavonoid content determination in seagrass extract

The results of the total flavonoid content calculation were analyzed, revealing data that were not normally distributed and not homogeneous, with a significant value of $p < 0.05$, as shown in the following Table 5. Statistical tests were conducted to determine the differences among the groups. Normality testing was performed to assess whether the data followed a normal distribution, revealing that the data was neither normal nor homogeneous, with a significant value of $p < 0.05$. Subsequently, a non-parametric test,

specifically the Kruskal-Wallis test, was employed to examine significant statistical differences. The results indicated $p > 0.05$, suggesting that there were no significant differences among the groups.

Table 5. Results of total flavonoid content test in seagrass leaf extract

Sample	Replicate	Absorbance	Total Flavonoid Content (MgQE/G)	Average Total Flavonoid Content (MgQE/G)
Infusion	1	0.3403	4.495	4.339±0.28
	2	0.3411	4.507	
	3	0.3086	4.015	
UAE Ethyl Acetate	1	0.4194	56.93	68.836±10.44
	2	0.5483	76.46	
	3	0.5262	73.12	
UAE n-Hexane	1	0.3404	4.496	6.61±2.1
	2	0.6191	8.719	
	3	0.4802	6.615	

The infusion method with distilled water compared to the UAE method using ethyl acetate showed a significant outcome with $p < 0.05$, indicating a meaningful difference in total flavonoid content. Conversely, the comparison between the infusion method with distilled water and the UAE method using n-hexane yielded a non-significant result with $p > 0.05$, suggesting no meaningful difference in total flavonoid content. Additionally, the Mann-Whitney test comparing the ultrasonic extraction method using ethyl acetate to the ultrasonic extraction method using n-hexane resulted in a significant finding with $p < 0.05$, indicating a meaningful difference in total flavonoid content.

The highest flavonoid content was obtained from the UAE method using ethyl acetate. The solvent used in extraction can influence the flavonoid content in the extract. Ethyl acetate is a semi-polar solvent with the ability to extract both polar and nonpolar flavonoid compounds. Several free flavonoids are present in plants, such as flavones, flavonones, and flavonols, which are readily soluble in semi-polar solvents. Some types of flavonoids can be dissolved in nonpolar solvents, such as polymethoxyaglycone and isoflavones. Glycosides of these flavonoids have been removed, allowing them to be soluble only in nonpolar solvents. Extracts obtained through the infusion method using distilled water exhibited the lowest flavonoid content, likely due to the polar nature of distilled water, which results in fewer dissolved compounds compared to slightly polar and nonpolar solvents [17]. In addition to solvent factors, the extraction method can also affect the flavonoid content in the extract. In the study by [19], the infusion method yielded a rutin content of 20.38 ± 0.6, while the UAE method yielded a rutin content of 24.49 ± 0.41. The highest rutin content was found using the UAE method, as UAE is effective in extracting rutin compounds.

Anti-inflammatory activity test of seagrass leaf extract in rabbits

The anti-inflammatory activity test was conducted on the backs of rabbits injured with a heated coin. Observations and administration of the extract were performed at

concentrations of 1%, 25%, 50%, 75%, and 100% over a period of 14 days (Table 6 and Figure 2).

Table 6. Burn wound area on the back of rabbits (cm^2) for each control of seagrass leaf extract

Day	K-	K+	K1%	K25%	K50%	K75%	K100%
1	3.27	3.08	3.26	2.85	3.30	2.98	2.87
2	3.27	2.30	3.26	2.85	2.46	2.73	2.73
3	3.27	2.13	3.25	2.85	2.23	2.31	2.11
4	3.27	2.08	3.24	2.84	2.17	2.25	1.84
5	3.26	1.82	3.24	2.83	2.05	2.11	1.62
6	3.25	1.86	3.22	2.82	1.94	2.09	1.74
7	3.25	1.43	3.22	2.82	1.14	0.99	1.10
8	3.25	1.13	3.21	2.81	0.94	0.94	0.95
9	3.25	0.95	3.19	2.81	0.86	0.78	0.80
10	3.23	0.80	3.18	2.81	0.75	0.63	0.69
11	3.21	0.67	3.18	2.79	0.63	0.55	0.53
12	3.21	0.54	3.15	2.78	0.52	0.44	0.38
13	3.21	0.49	3.15	2.78	0.42	0.34	0.27
14	3.21	0.39	3.14	2.78	0.38	0.23	0.23

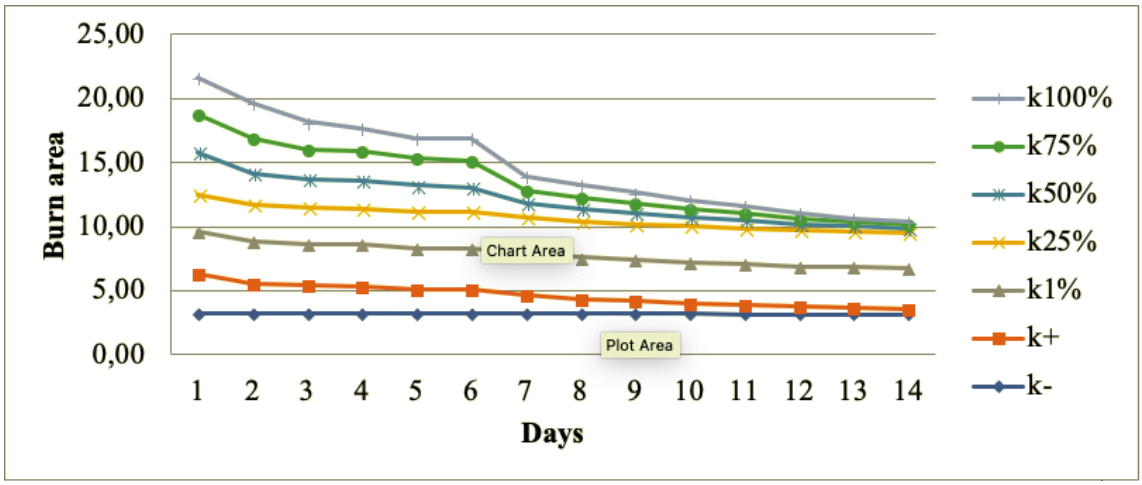

Figure 2. Decrease in burn area in rabbits

Table 7. Negative control compared to positive control

Mann-Whitney Test	Comparison	p-value
K-	K+	0.000
	K1%	0.016
	K25%	0.000
	K50%	0.000
	K75%	0.000
	K100%	0.000

The results of the Mann Whitney test on the data regarding the diameter of the rabbit wounds in the negative control compared to the positive control and the concentrations of 1%, 25%, 50%, 75%, and 100% showed significant results ($p < 0.05$), indicating that there are meaningful differences among the variables concerning anti-inflammatory activity (Table 7).

The results of the Mann-Whitney test comparing the positive control with the 1% extract and the 25% extract showed significant results ($p < 0.05$), indicating a meaningful difference in anti-inflammatory activity (Table 8). However, when comparing the

positive control with the 50%, 75%, and 100% extract concentrations, the results were not significant ($p > 0.05$), indicating no meaningful difference in anti-inflammatory activity. The results of the Mann-Whitney test for the 1% extract concentration compared to the 25%, 50%, 75%, and 100% extract concentrations showed significant results with $p < 0.05$, indicating a meaningful difference in anti-inflammatory activity (Table 9). The results of the Mann-Whitney test comparing the 25% extract concentration with the 50%, 75%, and 100% extract concentrations showed significant results with $p < 0.05$, indicating a meaningful difference in anti-inflammatory activity (Table 10). The results of the Mann-Whitney test for the 50% extract concentration compared to the 75% and 100% extract concentrations showed $p > 0.05$, indicating that there is no significant difference in anti-inflammatory activity (Table 11). The results of the Mann-Whitney test for the 75% extract concentration compared to the 100% extract concentration showed no significance, with $p > 0.05$, indicating that there is no significant difference in anti-inflammatory activity (Table 12).

Table 8. Comparing the positive control with extract concentrations of 1%, 25%, 50%, 75%, and 100%.

Mann-Whitney Test	Comparison	p-value
K+	K1%	0.000
	K25%	0.000
	K50%	1.000
	K75%	0.708
	K100%	0.181

Table 9. 1% Extract compared to 25%, 50%, 75%, and 100% extract concentrations

Mann-Whitney Test	Comparison	p-value
K1%	K25%	0.000
	K50%	0.000
	K75%	0.000
	K100%	0.000

Table 10. 25% Extract concentration compared to the 50%, 75%, and 100% extract concentrations

Mann-Whitney Test	Comparison	p-value
K25%	K50%	0.000
	K75%	0.000
	K100%	0.000

Table 11. Comparing the 50% extract concentration with the 75% and 100%

Mann-Whitney Test	Comparison	p-value
K50%	K75%	0.647
	K100%	0.227

Table 12. 75% extract concentration compared to 100% extract concentration

Mann-Whitney Test	Comparison	p-value
K75%	K100%	0.164

The negative control exhibited no inflammatory activity similar to the positive control and extract concentrations of 1%, 25%, 50%, 75%, and 100%, because the negative control, which is Na CMC, did not produce anti-inflammatory effects. This result aligns with literature stating that Na-CMC, as a base and solvent for extracting, does not have activity to treat inflammation, and the control group did not experience a reduction in

inflammation. Na-CMC was chosen as a negative control due to its low toxicity and better dispersion in water compared to other suspensions [20].

In the data on the diameter of rabbit wounds, the comparison of the 1% extract concentration with the 25%, 50%, 75%, and 100% extract concentrations yielded significant results ($p < 0.05$), indicating a meaningful difference among the variables regarding anti-inflammatory activity. This result is consistent with the data for the 25% extract concentration compared to the 50%, 75%, and 100% extract concentrations, which also showed significant results ($p < 0.05$), indicating a meaningful difference among the variables regarding anti-inflammatory activity. The analysis shows that there is no anti-inflammatory activity at the same level in the 1% and 25% extract concentrations compared to the 50%, 75%, and 100% extract concentrations. This is in line with Almaidah (2018) statement that a higher concentration of extract will provide greater anti-inflammatory activity.

In the data on the diameter of rabbit wounds, the comparison of the 50% extract concentration with the 75% and 100% extract concentrations, as well as the comparison of the 75% extract concentration with the 100% extract concentration, yielded significant results ($p > 0.05$), indicating that there is no meaningful difference among the variables regarding anti-inflammatory activity. From this analysis, the 50%, 75%, and 100% extract concentrations exhibit the same anti-inflammatory activity, effectively reducing the diameter of burn wounds in rabbits.

According to [22], alkaloids possess antibacterial and anti-inflammatory effects and help constrict blood vessels (vasoconstriction) at the onset of a wound, aiming to reduce bleeding that occurs during the hemostasis, inflammation, and proliferation phases. According to [23], flavonoids have pharmacological activity as anti-inflammatories through several pathways, including the inhibition of cyclooxygenase (COX) and lipoxygenase enzyme activities, inhibition of leukocyte accumulation, inhibition of neutrophil degranulation, and inhibition of histamine release. Flavonoids also exhibit astringent properties, enhancing wound healing by reducing wound size and accelerating recovery. Steroid compounds can play a role in improving the wound healing process due to their anti-inflammatory effects, which reduce swelling during the inflammatory phase. According to [24], Bioplacenton contains 0.5% neomycin sulfate and 100 mg of placenta extract to help prevent infection and inflammation. Neomycin sulfate, which belongs to the aminoglycoside antibiotic family, binds to the 30S ribosomal subunit of bacteria, leading to misreading of t-RNA and inhibiting bacterial growth and reproduction. Bioplacenton not only contains active antibiotic ingredients but also provides comfort upon use, and the placenta extract aids in the regeneration of burned skin, restoring it to its original form. Placenta extract is commonly used as a topical treatment to accelerate the healing of burns, chronic wounds, and other skin injuries. The substances in placenta extract generally work by increasing transforming growth factor-beta (TGF-beta) in the early stages of wound healing and vascular endothelial growth factor (VEGF) in the later stages.

Conclusion

The extraction method influences the flavonoid content in the extract of seagrass leaves (*Enhalus acoroides*). In the infusion extraction method using distilled water, the flavonoid content was measured at 4.339 ± 0.28 mgQE/g; the ultrasonic extraction method using ethyl acetate yielded 68.836 ± 10.44 mgQE/g; and the ultrasonic extraction method using n-hexane resulted in 6.61 ± 2.1 mgQE/g. The extract of seagrass leaves (*Enhalus acoroides*) obtained through the UAE extraction method using ethyl acetate, which contained flavonoids, alkaloids, and steroids, demonstrated anti-inflammatory activity against rabbits (*Oryctolagus cuniculus*). This was evidenced by the lack of significant differences ($p > 0.05$) between the extracts at concentrations of 50%, 75%, 100%, and the positive control (Bioplacenton®) in reducing the diameter of wounds in rabbits over the treatment period from the first day to the fourteenth day.

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Conflict of Interest

The authors declare no conflict of interest.

References

[1] Sari Dina Permata, Sari Gigih Kenanga, and Saraswati Maulita, "Uji Aktivitas Gel Ekstrak Daun Kelor (Maringa Oelifera L.) dari Kabupaten Blora Terhadap Penyembuhan Luka Bakar PD Kelinci," *Joseph (Journal of Science and Pharmacy)*, vol. 2, pp. 28–41, 2022.

[2] W. R. B. Santosa and R. Anggraini, "Perbandingan Efektifitas Tumbukan Daun Cocor Bebek dan Rebusan Daun Sirih Terhadap Penyembuhan Luka Bakar Derajat II Pada Tikus Wistar Jantan," *Jurnal Insan Cendekia*, vol. 8, no. 1, pp. 74–79, 2021.

[3] G. R. W. Sanjaya, N. M. Linawati, I. G. K. N. Arijana, I. A. I. Wahyuniari, and I. G. N. S. Wiryawan, "Flavonoid dalam Penyembuhan Luka Bakar pada Kulit: Flavonoids in Healing Burns on the Skin," *Jurnal Sains dan Kesehatan*, vol. 5, no. 2, pp. 243–249, 2023.

[4] S. Hidayati, F. Oktavianti, D. A. Susanti, and Q. Aini, "Aktivitas Antiinflamasi In Vitro dan In Vivo Ekstrak Etanol Daun Mangga Arumanis (Mangifera indica L.): In Vitro and In Vivo Anti-Inflamatory Activities of Ethanol Extract Mangifera indica L. Leaves," *Jurnal Sains dan Kesehatan*, vol. 4, no. 5, pp. 488–494, 2022.

[5] E. R. Taminggu and T. Tahril, "Identifikasi Senyawa Metabolit Sekunder Pada Batang dan Daun Lamun (Seagrass) di Teluk Palu," *Media Eksakta*, vol. 18, no. 1, pp. 6–11, 2022, doi: 10.22487/me.v18i1.1016.

[6] M. Yusuf, I. Sari, and A. Wijaya, "Efek Antiinflamasi Ekstrak Etanol Daun Lamun (Enhalus acoroides) Terhadap Mencit (Mus musculus) Jantan Yang Diinduksi Karagen," *Jurnal Ilmiah Manuntung*, vol. 7, no. 2, pp. 165–174, 2021.

[7] I. D. Rahakbauw and T. Watuguly, "Analisis Senyawa Flavonoid Daun Lamun Enhalus Acoroides di Perairan Pantai Desa Waai Kabupaten Maluku Tengah," *BIOPENDIX: Jurnal Biologi, Pendidikan dan Terapan*, vol. 3, no. 1, pp. 53–62, 2016, doi: 10.30598/biopendixvol3issue1page53-62.

[8] F. Maryam, Y. P. Utami, S. Mus, and R. Rohana, "Perbandingan Beberapa Metode Ekstraksi Ekstrak Etanol Daun Sawo Duren (Chrysophyllum cainito L.) Terhadap Kadar Flavanoid Total Menggunakan Metode Spektrofotometri UV-VIS," *Jurnal Mandala Pharmacon Indonesia*, vol. 9, no. 1, pp. 132–138, 2023, doi: 10.35311/jmpi.v9i1.336.

[9] E. Yolandari, S., & Mustiqawati, "Uji Efek Ekstrak Etanol Daun Jambu Biji (Psidium Guajava L) Sebagai

Antiinflamasi Pada Kelinci (Oryctolagus Cuniculus)," *JURNAL Promotif Preventif*, vol. 5, no. 1, pp. 117–129, 2022.

[10] S. R. Lamadjido, U. Umrah, and J. Jamaluddin, "Formulasi dan Analisis Nilai Gizi Bakso Kotak dari Jamur Tiram Putih (Pleurotus Ostreatus)," *Jurnal Farmasi Galenika (Galenika Journal of Pharmacy) (e-Journal)*, vol. 5, no. 2, pp. 166–174, 2019, doi: 10.22487/j24428744.2019.v5.i2.13149.

[11] N. Pawarti, M. Iqbal, D. A. Ramdini, and C. Yuliyanda, "Pengaruh Metode Ekstraksi Terhadap Persen Rendemen dan Kadar Fenolik Ekstrak Tanaman yang Berpotensi sebagai Antioksidan The Effect of Extraction Methods on Percent Yield and Phenolic Content of Plant Extracts Potentially as Antioxidants," *Jurnal Medula*, vol. 13, no. 4, pp. 590–593, 2023.

[12] E. Pujiastuti and D. El'Zeba, "Perbandingan Kadar Flavonoid Total Ekstrak Etanol 70% Dan 96% Kulit Buah Naga Merah Hylocereus Polyrhizus) dengan Spektrofotometri," *Cendekia Journal of Pharmacy*, vol. 5, no. 1, pp. 28–43, 2021, doi: 10.31596/cjp.v5i1.131.

[13] R. R. Kamilah, K. S. Rahmasari, W. Wirasti, and V. A. Nur, "The Effect of Solvent Extraction in Making Natural Sweetener from Lemba Fruit (Curculigo latifolia) on Total Sugar Content," *The 16th University Research Colloqium*, pp. 714–722, 2022.

[14] Nurafni and R. M. Nur, "Identifikasi Senyawa Bioaktif Jenis-Jenis Lamun Di Perairan Pulau Morotai," *Seminar Nasional Pendidikan Biologi Kepulauan Aula Banau*, vol. 18, no. 20, pp. 26–32, 2018.

[15] Hasan, Suryadi, and Djufri, "Uji Aktivitas Antidiabetes Ekstrak Etil Asetat Daun Lamun (Enhalus acoroides) Pada Mencit (Mus musculus)," *Journal Syifa Sciences and Clinical Research (JSSCR)*, vol. 4, no. 1, pp. 293–305, 2022.

[16] N. R. K. Nisaa and V. H. , Abd. Malik, "Analisis Kadar Total Flavonoid Ekstrak Etanol Kulit Pisang Cavendish (Musa paradisiaca var. Sapientum) Menggunakan Metode Spektrofotometri Uv-Vis," *Jurnal Sains dan Kesehatan*, vol. 3, no. 4, pp. 586–592, 2021.

[17] N. K. L. Puspa Yani, K. Nastiti, and N. Noval, "Pengaruh Perbedaan Jenis Pelarut Terhadap Kadar Flavonoid Total Ekstrak Daun Sirsak (Annona muricata L.)," *Jurnal Surya Medika*, vol. 9, no. 1, pp. 34–44, 2023, doi: 10.33084/jsm.v9i1.5131.

[18] E. F. Yanti and N. Purwanti, "Penetapan Kadar Falvonoid Total Dan Uji Aktivitas Ekstrak Etanol Daun Makadamia (Macadamia integrifolia) Dengan Metode DPPH," *Journal of Islamic Pharmacy*, vol. 7, no. 2, pp. 100–103, 2023, doi: 10.18860/jip.v7i2.17522.

[19] A. Chahyadi and Elfahmi, "The influence of extraction methods on rutin yield of cassava leaves (Manihot esculenta Crantz)," *Saudi Pharmaceutical Journal*, vol. 28, no. 11, pp. 1466–1473, 2020, doi: 10.1016/j.jsps.2020.09.012.

[20] D. Y. Wiratma, K. Manurung, S. Supartiningsih, and M. F. Telaumbanua, "Uji Aktivitas Ekstrak Etanol Bawang Putih (Alliumsativum L.)Yang Dihitamkan Sebagai Anti Inflamasi yang Diinduksi Oleh Karagenan Terhadap Mus musculus," *Jurnal Farmanesia*, vol. 4, no. 2, pp. 116–121, 2017, doi: 10.51544/jf.v4i2.2712.

[21] M. F. Almaidah, "Uji Aktivitas Anti Inflamasi Ekstrak Etanol Daun Buni (Antidesma Bunius L. Spreng) Terhadap Tikus Putih (Rattus Norvegicus)," *Pakistan Research Journal of Management Sciences*, vol. 7, no. 5, pp. 1–2, 2018.

[22] T. Carolina, D. Fitri Nugraha, and U. H. Fetriyah, "Activity Test Of Chloroform Extract Sangkareho (Callicarpa Longifolia Lam.) Leaf On Wound Healing In Male Wistar Rats," *Jurnal Surya Medika*, vol. 7, no. 2, pp. 166–173, 2022.

[23] S. Lallo, B. Hardianti, H. Umar, W. Trisurani, A. Wahyuni, and M. Latifah, "Aktivitas Anti Inflamasi dan Penyembuhan Luka dari Ekstrak Kulit Batang Murbei (Morus alba L.)," *Jurnal Farmasi Galenika (Galenika Journal of Pharmacy) (e-Journal)*, vol. 6, no. 1, pp. 26–36, 2020, doi: 10.22487/j24428744.2020.v6.i1.14661.

[24] N. Sari, M. Latief, P. S. Farmasi, P. S. Kimia, and U. Jambi, "Uji Aktivitas Ekstrak Etanol Daun Sungkai (Peronema Canescens Jack) Terhadap Penyembuhan Luka Bakar Pada Kelinci Jantan (Oryctolagus cuniculus)," *Indonesian Journal of Pharma Science*, vol. 4, no. 1, p. 11, 2022.