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Flavonoid Analysis of Moringa Leaf Extract and Its Utilization in Antibacterial Transparent Soap Formulation

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ABSTRACT

Staphylococcus aureus bacteria are normal flora on the skin, but if the number exceeds the normal limit, these bacteria can cause infection. One effort to prevent infection is to maintain skin cleanliness by bathing using soap containing antibacterial ingredients such as flavonoid compounds found in Moringa leaves. This study aims to analyse flavonoid compounds in Moringa leaves and formulate them into transparent solid soap preparations. Soap formulations were carried out with variations in the concentration of Moringa leaves ethanol extract, namely 2.5%, 3.5%, and 4.5%. Flavonoid analysis was carried out qualitatively using Mg reagent and quantitatively using UV-Vis spectrophotometry method with $AlCl_3$ reagent. The physical properties of the soap preparations tested included organoleptic, pH, foam height, foam stability and transparency. Antibacterial activity testing was carried out using the well diffusion method. Moringa leaves ethanol extract has a total flavonoid content of 19.5479 $\pm$ 0.0657 (% w/w EQ). The higher the concentration of Moringa leaves ethanol extract in the formula, the stronger the antibacterial activity produced. The 4.5% Moringa ethanol extract formulation showed the highest antibacterial activity while maintaining acceptable physical properties for transparent soap. These findings suggest its potential use in natural antibacterial soap products. The 4.5% Moringa ethanol extract formulation showed the highest antibacterial activity while maintaining acceptable physical properties for transparent soap. These findings suggest its potential use in natural antibacterial soap products.

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ABSTRAK

Bakteri Staphylococcus aureus merupakan flora normal pada kulit, namun apabila jumlahnya melebihi batas normal, bakteri ini dapat menyebabkan infeksi. Salah satu upaya pencegahan infeksi adalah menjaga kebersihan kulit dengan mandi menggunakan sabun yang mengandung bahan antibakteri seperti senyawa flavonoid yang terdapat pada daun kelor. Penelitian ini bertujuan untuk menganalisis senyawa flavonoid dalam daun kelor dan memformulasikannya menjadi sediaan sabun padat transparan. Formulasi sabun dilakukan dengan variasi konsentrasi ekstrak etanol daun kelor yaitu 2,5%, 3,5%, dan 4,5%. Analisis flavonoid dilakukan secara kualitatif menggunakan reagen Mg dan secara kuantitatif menggunakan metode spektrofotometri UV-Vis dengan reagen $AlCl_3$. Sifat fisik sediaan sabun yang diuji meliputi organoleptik, pH, tinggi busa, stabilitas busa dan transparansi. Pengujian aktivitas antibakteri dilakukan dengan metode difusi sumuran. Ekstrak etanol daun kelor memiliki total kandungan flavonoid sebesar 19,5479 + 0,0657 (% w/w EQ). Semakin tinggi konsentrasi ekstrak etanol daun kelor dalam formula, maka semakin kuat aktivitas antibakteri yang dihasilkan. Ekstrak etanol daun kelor dengan konsentrasi 4,5% dalam formula sabun padat transparan memiliki kategori aktivitas antibakteri yang kuat dan memenuhi persyaratan fisik sediaan sabun padat transparan. Formulasi ekstrak etanol Moringa 4,5% menunjukkan aktivitas antibakteri tertinggi dan memenuhi persyaratan sifat sediaan sabun padat transparan. Hasil ini menunjukkan potensi penggunaannya dalam produk sabun antibakteri alami.

Kata Kunci:
Daun Kelor; Flavonoid; Sabun Padat Transparan; Antibakteri; Kosmetika Alami

1. Introduction

The presence of microorganisms around us can cause infections such as skin infections. Skin infections can be caused by various microorganisms, one of which is bacteria. Bacteria are actually normal microorganisms in the skin, but if the number of bacteria in the skin exceeds normal limits, it can cause infection. One of the bacteria that can infect human skin is *Staphylococcus aureus*. *Staphylococcus aureus* bacteria can be found on several objects such as keyboards, wristbands, escalator handrails, and door handles.[1],[2],[3],[4]. Efforts to prevent bacterial infections can be made by paying attention to body hygiene, one of which is by using cleansing products. One of the most commonly used cleansing products is soap.

Bath soap is one of the skin care cosmetic products in solid, soft/liquid, and foaming form that functions as a cleaning agent. The effectiveness of bath soap in killing germs can be increased by adding antibacterial ingredients to the bath soap preparation formula. This aligns with Setiawati et al. [5] who state that the main parameter in designing soap products is their antibacterial properties, which means that antibacterial ingredients can be added in formulating bath soaps. Antibacterial agents can be derived from natural sources [6]. The inclusion of natural ingredients in soap formulations is driven by the growing interest in natural cosmetics [7]. Based on the *Research Series of the Embassy of the Republic of Indonesia in Brussels* [8] currently, herbal cosmetic products are growing rapidly in the Global and European markets. The growth rate of the European market for herbal cosmetics has increased from 2013-2018 by 1.04%.

Natural antibacterial ingredients that can be used in bath soap formulations can be derived from the Moringa plant. Moringa leaves contain flavonoid compounds [9]. One of the flavonoid compounds in moringa leaves is quercetin. Quercetin has a strong potential in inhibiting bacteria [6]. Given the relationship between flavonoid compounds found in moringa leaves and their ability as antibacterials, this study aims to analyse flavonoid content in Moringa leaves extract and evaluate its incorporation in antibacterial transparent soap formulations.

2. Methods

Tools and Materials

The tools used in this research are glassware (Iwaki), *waterbath* (HH-6), glass funnel (Herma), analytical balance (OHAUS®), autoclave (YCQ. SG41), stainless wok, aluminium pan, spatula, filter paper, scoop, filter cloth, horn spoon, stirring rod, *aluminium foil*, autoclave (Ningbo kaipo), oven (*Drawell*), drop pipette, ruler, pH meter (PH-02 *auto calibrate*), tweezers, ose needle, *cork borer*, micropipette (DragonLAB), soap mould, petri dish (HERMA), *magnetic stirrer*, cotton swab, *dehydrator* (GETRA®), 20 mesh sieve, caliper, Bunsen lamp, UV Vis spectrophotometer (Genesys 150).

The ingredients used in the manufacture of transparent soap are ethanol extract of *moringa* leaves (*Moringa oleifera* Lam.), 96% ethanol, stearic acid, VCO, glycerin, NaOH (Merck), sugar (sucrose), NaCl (Merck), *cocomide* DEA, distilled water. Materials for flavonoid analysis test were magnesium powder, concentrated HCl (Merck), $AlCl_3$ 10% (Sigma Aldrich), quercetin (Sigma Aldrich)(.) Materials for antibacterial test were *Nutrient Agar* (Merck), NaCl 0.9%, *Staphylococcus aureus* bacteria (ATCC) and antiseptic soap.

Flavonoid Extraction and Analysis

Moringa leaves were extracted using the maceration method with 96% ethanol distillation solution. Flavonoid analysis was carried out qualitatively and quantitatively. Qualitative analysis used concentrated HCl reagent and magnesium powder. Quantitative analysis was performed using UV Vis spectrophotometric method with 10 % $AlCl_3$ reagent. The standard curve used for quantitative analysis of flavonoids used quercetin standard.

Transparent Solid Soap Formulation

The formulation of transparent solid soap containing Moringa leaves ethanol extract was adapted from previous research [10]. The soap preparation began by melting stearic acid, followed by the gradual addition of other ingredients including virgin coconut oil (VCO), glycerin, NaOH solution (30%), ethanol (96%), and sucrose. All components were mixed at a controlled temperature of 70–80 °C with continuous stirring to produce a homogeneous soap base. The detailed composition of each formula, including the concentration and function of each ingredient, is presented in **Table 1**.

Once a uniform base was achieved, Moringa leaves ethanol extract was added at concentrations of 2.5%, 3.5%, and 4.5%, corresponding to Formula 1, 2, and 3, respectively. The addition was performed carefully to maintain transparency and ensure even distribution of the active compounds. After thorough mixing, the soap mixture was poured into molds and allowed to cure at room temperature. This curing process was necessary to complete saponification and stabilize the final physical form of the soap before further evaluation.

Table 1. Transparent solid soap formula of moringa leaves ethanol extract

Material	Formula 1	Formula 2	Formula 3	Function
leaves extract	2,5	3,5	4,5	Substance
VCO	30	30	30	Fatty acid base
acid	10,5	10,5	10,5	Soap hardener
NaCl	0,3	0,3	0,3	Forms the core of the soap and accelerates the formation of solids
30% NaOH	30,45	30,45	30,45	Alkali (Base)
96% Ethanol	22,5	22,5	22,5	Solvents and Transparency
Sucrose	22,5	22,5	22,5	Transparency
Glycerin	19,5	19,5	19,5	Humectants
Cocomide DEA	1,5	1,5	1,5	Surfactants and foam formers
Distilled water	150	150	150	Solvent

Evaluation of Physical Properties

After the curing period, the physical properties of the transparent solid soap formulations were evaluated to assess their quality and acceptability. The parameters assessed included organoleptic characteristics, pH, foam height, foam stability, and transparency.

The organoleptic evaluation was performed using sensory observation of the soap's appearance, smell, and color. This test was conducted visually and olfactorily to ensure that each formulation exhibited a consistent blackish-green color typical of Moringa extract, a pleasant herbal scent, and a solid, well-formed structure without surface defects [11].

The pH test was carried out by dissolving 1 gram of the solid soap into 10 mL of distilled water, and the pH value was measured using a calibrated digital pH meter. This test aimed to ensure that the soap's pH fell within the acceptable range of 8 to 11 for solid soap, as defined by national standards, to ensure user safety and compatibility with skin [11].

The foam height test involved placing 1 gram of the soap sample into a test tube containing 10 mL of distilled water. The tube was then shaken by turning it upside down for 1 minute. The resulting foam was measured using a ruler to determine its height in millimeters. This test assessed the soap's ability to produce sufficient lather [11].

The foam stability test was performed immediately after the foam height measurement. After the foam was left undisturbed for 5 minutes, its height was re-measured. This test indicated how long the foam could maintain its structure, which reflects the product's perceived effectiveness and user satisfaction during washing [12].

The transparency test was done through visual inspection under normal lighting. The soap was evaluated for clarity and the absence of cloudiness or suspended particles. Transparent appearance was considered an indicator of a well-formulated product with proper homogenization and solvent compatibility [10].

Antibacterial Analysis of Transparent Solid Soap

Antibacterial activity testing was performed aseptically using the pitting diffusion method, with *nutrient agar* media. The diameter of the zone of inhibition was measured after the test sample inserted in the test medium containing a suspension of *Staphylococcus aureus* bacteria was incubated at 37 ^{o}C for 24 hours.

Data Analysis

The data obtained from antibacterial activity testing were analyzed statistically using One-Way ANOVA to determine the effect of different concentrations of Moringa leaf ethanol extract in the transparent soap formulations against *Staphylococcus aureus*. When a significant difference was found ($p < 0.05$), a Post-Hoc test was conducted to identify which specific groups showed statistically significant differences in the diameter of the inhibition zones [13]. All analyses were performed using SPSS version 25.

3. Results and Discussion

Extraction and Analysis of Moringa Leaves Flavonoids

Extraction is the withdrawal of phytochemical compounds from plant cells using an aqueous solution. One of the compounds targeted as antibacterial in Moringa leaves is flavonoids, so the maceration method chosen is maceration. According to Wulan *et al.* [9], flavonoids in moringa leaves are extracted using the maceration method with 96% ethanol distillation solution.

Qualitative analysis of flavonoids is carried out using Mg powder reagents and concentrated HCl. The reagents used in this test can reduce the structure of flavonoids, causing the formation of yellow or orange complexes [14]. The results of the qualitative analysis showed that the ethanol extract of Moringa leaves contained flavonoid compounds as indicated by the formation of an orange-colored solution after the test sample was reacted with concentrated HCl and Mg powder.

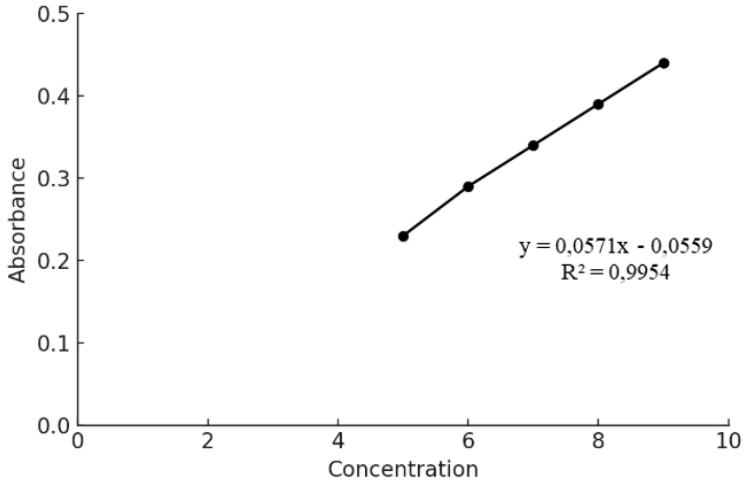


Figure 1: Standardised curve of quercetin

The quantitative analysis aimed to determine the total flavonoid content present in the ethanol extract of Moringa leaves. This determination was carried out using a UV-Vis spectrophotometric method with quercetin as the standard, and the resulting calibration curve is presented in **Figure 1.** Based on the analysis, the total flavonoid content was found to be 19.5479 ± 0.0657% (w/w EQ), as shown in **Table 2**. This value indicates a relatively high flavonoid concentration, demonstrating the potential of Moringa leaves as a natural source of bioactive compounds with antibacterial properties.

Table 2. Total flavonoid content of ethanol extract of moringa leaves

Replication	Absorbance	Radar (% w/w EQ)
1	0,489	19,5551
2	0,495	19,6097
3	0,498	19,4789
	Average	**19,5479 + 0,0657**

Compared to the study conducted by Fatmawati *et al.* [15] , which reported a total flavonoid content of 16.69 ± 0.74% (w/w EQ), the result obtained in this study is slightly higher. The variation in flavonoid levels may be attributed to several influencing factors, including the geographical origin of the plant, the time of harvest, post-harvest handling, and differences in extraction techniques, particularly in maceration duration and solvent polarity.

Importantly, the flavonoid content measured in this study exceeds the minimum requirement specified by the Indonesian Herbal Pharmacopoeia, which mandates a minimum of 6.30% calculated as quercetin [14]. These findings further support the rationale for utilizing Moringa leaf extract in functional formulations, such as antibacterial transparent soap, where flavonoids serve as key active ingredients.

Formulation of Moringa Leaves Ethanol Extract Transparent Solid Soap

The formulation of transparent solid soap containing Moringa leaves ethanol extract was carried out using the hot process method, which allows for faster curing. In this process, solid soap is produced by saponifying fatty acids with a base (NaOH). The primary source of fatty acids in this formulation was virgin coconut oil (VCO), known for its high content of lauric and myristic acids, which contribute to the soap's foaming properties [16][17].

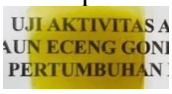
Formula 1 Formula 2 Formula 3

Figure 2. Transparent solid soap preparation of moringa leaves ethanol extract

Figure 2 illustrates the resulting soap bars from the three formulations prepared in this study, containing varying concentrations of Moringa leaves ethanol extract: 2.5%, 3.5%, and 4.5% for Formula 1, Formula 2, and Formula 3, respectively**.** Each formulation yielded a solid, transparent soap bar with a consistent shape and surface appearance, suitable for further evaluation of physical and antibacterial properties.

The soap that had passed the *curing* period was then tested for physical properties of the preparation which included organoleptic test, homogeneity test, pH test, foam height test, foam stability test and transparency test. The test results are listed in **Table 3**.

Table 3. Test results for physical properties of transparent solid soap preparations

Test Parameters	Formula 1	Formula 2	Formula 3	Requirements
Organoleptic	Transparent solid, blackish-green in colour, with a characteristic moringa odour	Transparent solid, blackish-green in colour, with a characteristic moringa odour	Transparent solid, blackish-green in colour, with a characteristic moringa odour	Solid, transparent, odour as per extract
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous
pH	9,31 ± 0,036	9,16 ± 0,031	9,20 ± 0,030	8-11
Foam Height (mm)	102,6 ± 0,557	103,3 ± 1,528	104,0 ± 1,000	13-220
Foam Stability (%)	68,90 ± 1,015	67,46 ± 0,681	68,33 ± 0,833	60-70
Transparency	Transparent 	Transparent 	Transparent 	Transparent

The resulting pH value is in the range of 9.16 to 9.31, this result is by the research of Aris et al which gave the results of a transparent solid soap preparation of moringa leaves ethanol extract having a pH of 9 [11]. The resulting pH value meets the requirements of SNI (3532-2016) the pH of soap which is in the pH range of 8-11.

The foam height produced was in the range of 102.6 to 104 mm, with foam stability values of 67.46 to 68.90%. The resulting foam stability value is almost the same as the research of Aris *et al*. [11] which gave results of 63.7 to 69.2%.

Antibacterial Analysis

The results of the antibacterial activity test are listed in **Table 4**. Soap formula 1 provides strong category antibacterial activity, while soap formulas 2 and 3 provide very strong antibacterial activity. When compared to the positive control (antibacterial soap on the market), the three soap formulas provide better antibacterial activity. The test results of the soap base (negative control) also provide better antibacterial activity compared to the positive control, this is in line with research conducted by Turangan *et al*. [12]. The inhibition zone in the soap base formulation can be caused by the use of *Virgin Coconut Oil* (VCO) in the soap base. VCO contains lauric acid ranging from 50-

70% which has antibacterial properties [12]. The antibacterial mechanism of action of lauric acid is by damaging the peptide chains that make up peptidoglycan, so that the bacterial cell wall becomes weak and undergoes lysis, this makes the bacteria unable to withstand external influences and soon dies [18]. In addition, the use of NaCl in the formulation of transparent solid soap preparations can have an antibacterial effect. Chloride ions in NaCl can interfere with the work of bacterial enzymes, causing protein denaturation and causing bacterial death [19]. The use of the active ingredient moringa leaves ethanol extract can increase antibacterial activity as seen from the diameter of the inhibition zone. The higher the concentration of moringa leaves ethanol extract formulated in transparent solid soap preparations, the greater the diameter of the inhibition zone formed. These results are in line with research conducted by Aris *et al*. [11], the increase in the concentration of moringa leaves juice in the formulation of solid soap preparations can increase its effectiveness as an antibacterial.

Table 4. Results of measuring the diameter of the bacterial inhibition zone

	Replication				Antibacterial potential
Formula	**R1 (mm)**	**R2 (mm)**	**R3 (mm)**	**Mean ± SD**	
Formula 1	17,3	17,6	17	17,30 ± 0,300	Strong
Formula 2	21,53	21,86	21,9	21,76 ± 0,203	Very strong
Formula 3	25,8	25,4	25,2	25,47 ± 0,306	Very strong
Control (-)	12,25	12,30	12,45	12,33 ± 0,104	Strong
Control (+)	5,65	5,45	5,30	5,47 ± 0,176	Medium

The inhibition zone data obtained were then statistically analyzed using the One-Way ANOVA test to see the effect of variations in the concentration of moringa leaves ethanol extract formulated in the form of transparent solid soap preparations against *Staphylococcus aureus* bacteria. The data requirements can be tested statistically using the One-Way ANOVA test, namely the data must meet the requirements of the normality test and homogeneity test showing a p-value> 0.05 [13]. The results of the normality test and homogeneity test on the research data showed a p-value> 0.05, which means that the data can be analyzed using the One-Way ANOVA test. The results of the One-Way ANOVA test can be seen in Table 5.

Table 5. One-Way ANOVA test results

	Sum of Squares	**f**	**Mean Square**	**f**	**Sig.**
Between Groups	743.501	4	185.875	3490.832	.000
Within Goups	.532	0	.053		
Total	744.033	4			

Based on **Table 5**, it can be seen that the significance value obtained is .000, meaning that there is an effect of variation in the concentration of moringa leaves ethanol extract formulated in the form of transparent solid soap preparations on the antibacterial activity of *Staphylococcus aureus* with a significance value of <0.05. Post-Hoc test is a test conducted to see whether or not there is a significant difference between each solid soap formulation of moringa leaves ethanol extract against *Staphylococcus aureus* bacteria. From this test, all significant values resulting from comparisons between groups were obtained, namely .000, where this value indicates that variations in the concentration of

moringa leaves ethanol extract make a significant difference to the inhibition zone produced. An increase in Moringa extract concentration was associated with enhanced antibacterial activity, as reflected by a wider inhibition zone, particularly at the 4.5% formulation. This suggests a dose-dependent response, likely due to the higher availability of bioactive flavonoid compounds at elevated concentrations. Flavonoid compounds can destroy bacterial cell membranes by changing the membrane fluidity of hydrophilic and hydrophobic regions of the bacterial cytoplasmic membrane [20].

In addition, the mechanism of flavonoids as an antibacterial is by inhibiting the synthesis of nucleic acids and inhibiting the release of toxins from bacteria [21]. Flavonoid levels correlate with antibacterial ability or activity [22]. In this study, the total flavonoid value of moringa leaves ethanol extract was 19.5479± 0.0657 (w/w EQ), the extract obtained if formulated into a transparent solid soap dosage form of 4.5% provides very strong antibacterial effectiveness.

This study has several limitations that should be acknowledged. First, the antibacterial activity was only tested against a single bacterial strain, limiting the generalizability of the results to broader microbial spectra. Second, no stability tests were conducted to evaluate the long-term physical and chemical properties of the soap formulations under various storage conditions. Third, the study did not assess the potential for skin irritation or user acceptability, which are critical parameters for topical cosmetic products. Finally, the analysis focused solely on flavonoid content, while other bioactive compounds that may contribute to antibacterial effects, such as alkaloids or saponins, were not evaluated. Future research should address these aspects to validate the safety, efficacy, and market readiness of the formulated soap.

4. Conclusion

The ethanol extract of *Moringa oleifera* leaves was found to contain a total flavonoid content of 19.5479 ± 0.0657% (b/b). At a concentration of 4.5% in a transparent solid soap formulation, the extract demonstrated very strong antibacterial activity and met the essential physical quality standards of transparent soap preparations. These findings suggest the potential application of *M. oleifera* extract as a natural antibacterial agent in cosmetic formulations. Further studies are recommended to evaluate long-term stability, safety, and broader antimicrobial activity to support product development.

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Conflicts of Interest:

The authors declare no conflict of interest regarding the publication of this article.

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