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Antibacterial Soap from Extract Spent Coffee Grounds using Microwave-assisted Extract Against *Staphylococcus aureus*

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ABSTRACT
This study investigates the application of Microwave-Assisted Extraction (MAE) to extract bioactive components from spent coffee grounds (SCG), enhancing the antibacterial properties of transparent soap. Spent coffee grounds, usually regarded as waste, include phenolics, alkaloids, flavonoids, and tannins which demonstrate potent antibacterial properties against Gram-positive bacteria such as *Staphylococcus aureus*. MAE effectively extracts these useful compounds using ethanol as a solvent, therefore improving the antibacterial power of the soap. Unlike the soap free of the extract, the inclusion of 3% spent coffee ground extract generated an inhibitory zone of 9.16 mm. The soap base is produced using the saponification process with palm oil and sodium hydroxide. The research additionally encompasses pH analysis, foam properties, and evaluations of client preferences. This research highlights the efficiency of MAE in upcycling coffee waste into a functional and sustainable product, offering enhanced antibacterial properties while contributing to waste reduction in personal care products.

Keyword: Antibacterial Soap, Microwave-Assisted Extraction (MAE), Spent Coffee Grounds (SCG) Extract.

ABSTRAK
Penelitian ini menyelidiki penerapan Microwave-Assisted Extraction (MAE) untuk mengekstraksi komponen bioaktif dari ampas kopi (SCG), yang dapat meningkatkan sifat antibakteri pada sabun transparan. Ampas kopi, yang biasanya dianggap sebagai limbah, mengandung fenol, alkaloid, flavonoid dan tannin yang memiliki sifat antibakteri yang kuat terhadap bakteri Gram-positif seperti *Staphylococcus aureus*. MAE secara efektif mengekstrak senyawa-senyawa berguna ini dengan menggunakan etanol sebagai pelarut, sehingga meningkatkan kekuatan antibakteri sabun. Berbeda dengan sabun yang tidak mengandung ekstrak, penambahan ekstrak ampas kopi bekas sebanyak 3% menghasilkan zona hambatan sebesar 9,16 mm. Basis sabun diproduksi melalui proses saponifikasi dengan minyak kelapa sawit dan natrium hidroksida. Penelitian ini juga mencakup analisis pH, sifat busa, dan evaluasi preferensi konsumen. Penelitian ini menyoroti efisiensi MAE dalam mengolah limbah kopi menjadi produk fungsional dan berkelanjutan, yang menawarkan sifat antibakteri yang lebih baik sekaligus berkontribusi pada pengurangan limbah dalam produk perawatan pribadi.

Kata Kunci: Ekstrak Ampas Kopi (SCG), Microwave-Assisted Extraction (MAE), Sabun Antibakteri.

1. Introduction

North Sumatra is a key region for Robusta coffee production in Indonesia, with plantations covering over 98,600 hectares and an anticipated 87,900 tons of production in 2023. Indonesia, the fourth-largest coffee producer globally, faces challenges in managing coffee waste, particularly spent coffee grounds (SCG) [1, 2]. Significant quantities of spent coffee grounds (SCG) are generated with each cup of coffee, containing bioactive compounds such as caffeine, tannins, and polyphenols that, if unmanaged, may pose environmental hazards. Conventional SCG disposal methods in North Sumatra can lead to trash accumulation, potentially harming the surrounding ecosystem [3, 4].

Despite these challenges, discarded spent coffee grounds are abundant in notable bioactive chemicals that have strong antioxidant and antibacterial effects [5, 6]. Antibacterial treatments effectively combat detrimental pathogens like *Staphylococcus aureus*, which can endure on contaminated surfaces and in filthy environments. The antibacterial characteristics of spent coffee grounds stem from their phenolic chemicals, which break bacterial cell structures and prevent cellular activity. Numerous investigations have shown that spent coffee ground extracts have antibacterial properties against pathogens like *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Enterococcus faecalis*. Spent coffee grounds exhibit antibacterial properties, notably against Gram-positive bacteria such as *Staphylococcus aureus* [7-10].These findings underscore the potential application of spent coffee grounds as a natural source of antibacterial agents.

Incorporating SCG into daily-use products, such as hand soaps, not only provides an eco-friendly solution but also leverages their antimicrobial properties for personal hygiene. The development of antibacterial hand soaps utilizing spent coffee grounds aligns with the growing demand for natural and sustainable personal care products. By harnessing the antimicrobial properties of coffee grounds, these soaps can effectively combat harmful pathogens, contributing to improved public health and environmental sustainability [8, 11, 12].

Many attempts have been performed to isolate bioactive compounds from spent coffee grounds (SCG); however, the circumstances of SGC result in a limited availability of residual bioactive chemicals. Consequently, a substantial quantity of solvent is necessitated, resulting in elevated expenses and prolonged extraction durations. An approach to diminish extraction time is to expedite the process by electromagnetic catalysis. Microwave-Assisted Extraction (MAE) is an efficient technique that use microwave radiation to rapidly produce heat, therefore expediting the extraction process [13]. In contrast to conventional techniques that necessitate prolonged extraction durations and significant solvent quantities, Microwave-Assisted Extraction (MAE) diminishes both solvent utilization and energy expenditure, rendering it a more efficient and environmentally sustainable method. Employing MAE accelerates the extraction process, increases sustainability, and minimizes solvent usage, therefore improving overall efficiency [14-16].

This study uses microwave-assisted extraction (MAE) to extract beneficial components from SCG for use in soap compositions, including hand soap [7, 17]. Unlike conventional soaps that often contain synthetic chemicals, the current trend favors herbal-based ingredients for sanitation. This shift aims to reduce irritation, enhance user compatibility, minimize allergies, and support environmental sustainability. The soap manufactured via saponification using sodium hydroxide and palm oil possesses antibacterial properties and is safe for use on the skin. MAE increases the concentration of bioactive compounds without harsh chemicals, resulting in a non-irritating, skin-friendly soap. Research shows that spent coffee grounds in personal care products can replace synthetic ingredients, improving skin hydration without causing irritation, making it ideal for both body and hand soap [18].

This study underscores the necessity of upcycling coffee grounds for trash management, sustainability, and personal care products. Bioactive compounds such as phenolics and caffeine can facilitate the circular economy and foster enduring sustainable solutions. Using microwave-assisted extraction (MAE), this study evaluates the antibacterial potential of spent coffee ground extracts against *Staphylococcus aureus* and examines the soap's irritability feature by analyzing its pH. The research also assesses the impact of varying quantities of spent coffee ground extracts in soap formulations, ensuring its suitability for skin use.

2. Material and Method

2.1 Equipments

Laboratory equipment used in this study included beaker glasses, Erlenmeyer flasks, thermometer, Microwave (Toshiba) 800 watt, rotary evaporator, portable hand mixer (Sonifer, SF-8055), vacuum rotary evaporator Buchi R-300 and FTIR spectrometer (Shimadzu)

2.2 Material

Spent coffee grounds were collected from a local coffee shop in Medan, North Sumatra Province, Indonesia. Palm oil by Sunco, olive oil by Dougo, distilled water by Merck, glycerin 99% (pa) by Smartlab, ethanol 96% (pa) by Merck, sodium chloride (pa) by Merck, Triethanolamine (TEA) by PETRONAS, stearic acid solid by Merck, sodium hydroxide solid from Merck, *Staphylococcus aureus.*

2.3 Preparation of Spent Coffee Grounds Extract

The spent coffee grounds were obtained from a local coffee shop in Medan, specifically the robusta spent coffee grounds (*Coffea canephora*). The samples were open-dried and oven-dried at 60°C for 3 hours to remove water content. Afterwards, 100 g of this dried sample were placed into a Erlenmeyer flask. Mix with 400 ml of ethanol and stir constantly. Then this flask was transferred into a microwave to carry out the MAE process; a microwave machine operating at 800 watts was utilized. To prevent the evaporation of the solvent, the flask was covered with a glass-based lid. The methods were slightly modified from Coelho, J.P., et al (2021), Microwave irradiation was applied for 12 cycles, with each cycle lasting 15 seconds. After one cycle, the flask was removed to measure the temperature and maintain the extraction condition < 60°C [19]. The operation of extracting SCG is illustrated in Figure 1. The MAE extraction procedure for the sample was repeated until a sufficient volume was obtained for evaporation. The resulting filtrate was then concentrated using a rotary evaporator set at 65°C and 80 rpm, yielding a concentrated spent coffee ground extract. This extract was transferred to a sterile container and stored in a desiccator until a stable weight was reached, ensuring the consistency of the extract's concentration prior to its use in the soap formulation.

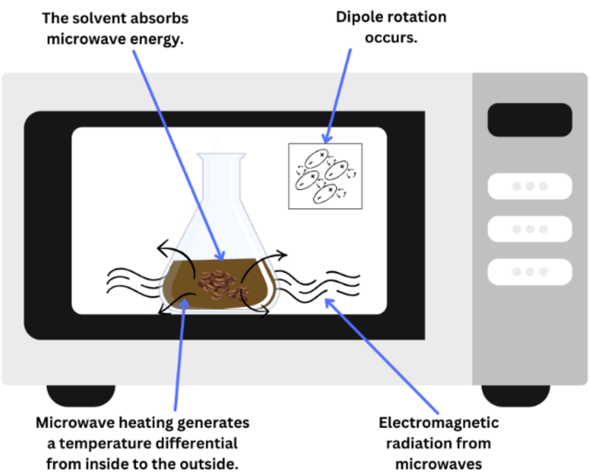

Figure 1. Cartoon illustration of the Microwave-Assisted Extraction (MAE) process for spent coffee ground extract

2.4 Soap Making Process

The soap-making procedure follows a previous study with slight modifications [20]. Initially, 60 ml of coconut oil was mixed with 70 ml of 60% sodium hydroxide and stirred continuously until the mixture became homogeneous. Then, 21 ml of stearic acid, 0.6 ml of 2 sodium chloride, 45 g of sugar, 45 ml of 70% ethanol, 40 g of glycerin, 30 ml of distilled water, and 3 ml of triethanolamine (TEA) were added into the first mixture. The mixture was stirred until fully blended. Next, varying amounts of SCG extract were added: 1.5 ml for F1, 3.0 ml for F2, and 4.5 ml for F3. The control formulation (F0) contained no extract. The mixture was stirred thoroughly and then poured into a mold to dry in open air.

2.5 Phytochemical Analysis of Spent Coffee Grounds Extract

Phytochemical analysis of the SCG extract was conducted using a modified Harborne method. The extract was mixed with 2 mg of magnesium powder and 3 mL of 2 M HCl. The presence of flavonoids was confirmed by the appearance of a stable orange, pink, or red coloration that persisted for at least three minutes. Alkaloids were detected by dissolving 4 mg of SCG material in ethanol from the purified extract, followed by filtration. The resulting filtrate was distributed into four test tubes. Tube 1 was treated with Mayer's reagent, tube 2 with Bouchardat's reagent, and tube 3 with Dragendorff's reagent, with five drops added to each. The formation of yellowish-white, brown, orange, or reddish-brown precipitates indicated the presence of alkaloids. Terpenoids were identified by the appearance of red, orange, or purple. Saponins were identified by the formation of a stable foam. SCG extract were identified by dissolving 1 mg of the purified extract in methanol. The resulting solution was mixed with 10 mL of distilled water and shaken vigorously. The presence of saponins was indicated by the formation of a stable foam that persisted for up to 10 minutes. Tannins compounds in the SCG extract were identified by dissolving the purified extract in methanol, followed by the addition of three drops of 5% $FeCl_3$ solution to the filtrate. The formation of a black colloidal precipitate indicated the presence of tannins. The results revealed the existence of several chemicals in the SCG extract.

2.6 Soap Testing Parameters

The testing procedure for transparent soap involved using a 5 g sample of soap diluted with 100 ml of distilled water, which was then homogenized and measured for foam stability. The pH level was measured by dissolving 1 g of soap in 10 ml of distilled water, and the moisture content was evaluated using gravimetric methods. A soap sample weighing 3-5 g was subjected to drying in an oven at 105°C until a consistent weight was gained. The hedonic test was performed to assess the color, shape, and fragrance of the soap formulation.

2.7 Antibacterial Efficacy Evaluation of Soap Utilizing Spent Coffee Ground Extract

The antibacterial test conducted was an inhibition zone test. The bacterium used in the study was *S.Aureus*. The antibacterial efficacy test was performed via the well-diffusion method with solid soap samples infused with different amounts of SCG extract. Three wells were established in each petri dish, into which the test samples were introduced. The dishes were incubated for 24 hours at 37°C. Following incubation, the inhibition zones surrounding the wells were studied and measured with a ruler, depending on the presence of clear areas around the wells. This signifies the lack of bacterial activity in the regions treated with the test samples.

3. Result and Discussion

3.1 Exctration

The spent coffee ground extraction process utilized ethanol. Ethanol was selected as the solvent in this procedure because of its versatility, enabling it to interact with diverse chemical constituents in plants, including non-polar, semi-polar, and polar molecules. Furthermore, ethanol has been demonstrated to be the most effective solvent for the extraction of phenolic and flavonoid components taking into account the active phenolic and flavonoid content present in SCG. The filtrate was subsequently evaporated to yield a concentrated extract from the SCG. Coelho et al. (2021) achieved a 6.98% yield using MAE with ethanol–water mixtures [21]. In our study, Microwave-assisted extraction (MAE) produced 71.5 grams of concentrated dark brown extract from 1000 grams of spent coffee grounds, resulting in a 7.15% w/w yield.

3.2 Extract Spent Coffee Ground Characterization

The phytochemical screening test of the Spent coffee grounds extract, as indicated in Table 1, showed good results for alkaloids, indicated by the development of a brown color in the extract. The phenol test confirmed positive results, indicated by the appearance of a dark green color in the extract. The flavonoid test produced positive results, shown by the development of an orange color in the extraction of the SCG.

Table 1. Identification of secondary metabolite compounds results

No	Secondary Metabolites	Indicator	Description	Result
1.	Alkaloid	Dragendorff.	Brown precipitate	+
		Mayer	Cream presipitate	+
		Bouchardart	Yellow precipitate	+
2.	Flavonoid	Magnesium + HCl	Orange	+
3.	Phenolic	$FeCl_3$ 10%	Dark green	+
4.	Saponin	Aquadest	Foam	+

Description: (+) = indicates presence of compoun, (-) = indicates absence of compound

This approach is a widely accepted method for identifying phytochemical constituents. Previous studies have shown that the ethanol-water mixture can be used to extract total polyphenols, chlorogenic acid, and caffeine from SCG [21]. In this study, saponins were identified by vigorous shaking of the extract, resulting in the formation of stable foam ranging from 1 cm to 10 cm in height, which remained intact for up to 10 minutes. The foam's persistence following the addition of a drop of 2 N hydrochloric acid further confirmed the presence of saponins. The formation of foam as a positive result in this test was attributed to the glycoside components in saponins, which undergo hydrolysis into glucose and other compounds that generate stable foam. Identifying the phytochemical constituents in plant extracts is essential for understanding their biological and pharmacological activities [22].

The Fourier transform infrared (FTIR) analysis was utilized to identify spent coffee grounds characteristic functional groups and spectra, as illustrated in Figure 2. The FTIR spectroscopy analysis indicated that the broadband at 3360 cm^{-1} is associated with the stretching of the O-H group, resulting from inter- and intra-molecular hydrogen bonding in polymeric substances such as alcohols, phenols, and carboxylic acids, as shown in pectin, cellulose, and lignin. The broad frequency range of O–H stretching vibrations suggests the presence of both free hydroxyl groups and hydrogen-bonded O–H groups typically found in carboxylic acids. The absorption band at 2925 cm^{-1} corresponds to the symmetric or asymmetric stretching of C–H bonds in aliphatic acids. Meanwhile, the peak observed at 1725 cm^{-1} is attributed to carboxyl functional groups, possibly originating from xanthine-based compounds such as caffeine [23].

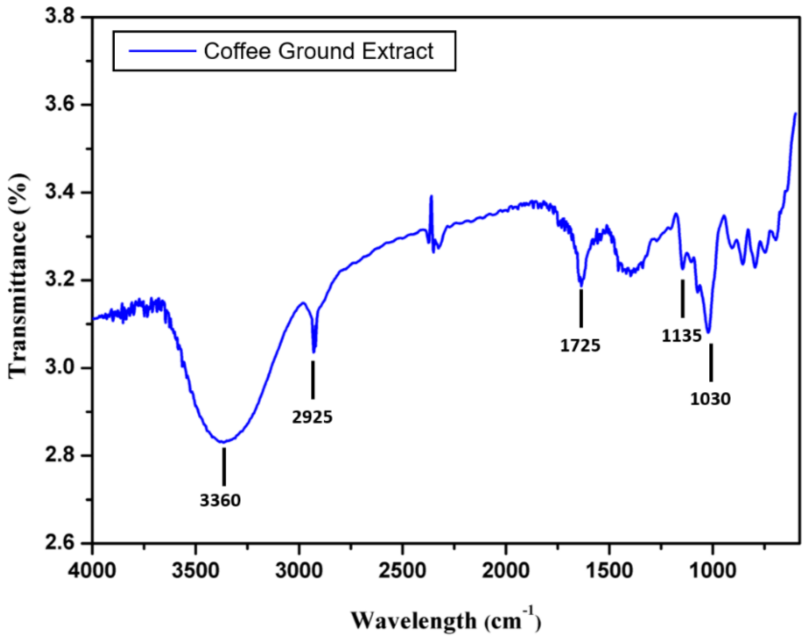

Figure 2. FTIR Spectrum of Spent Coffee Grounds Extract

The UV-Vis spectra for the SCG extract (formulations F1, F2, and F3) and the blank water sample are illustrated in Figure 3. The spectra for F1, F2, and F3 exhibit distinct peaks near 280 nm and within the range of 320–350 nm, signaling the presence of aromatic compounds and conjugated systems, such as phenolics and melanoidins.

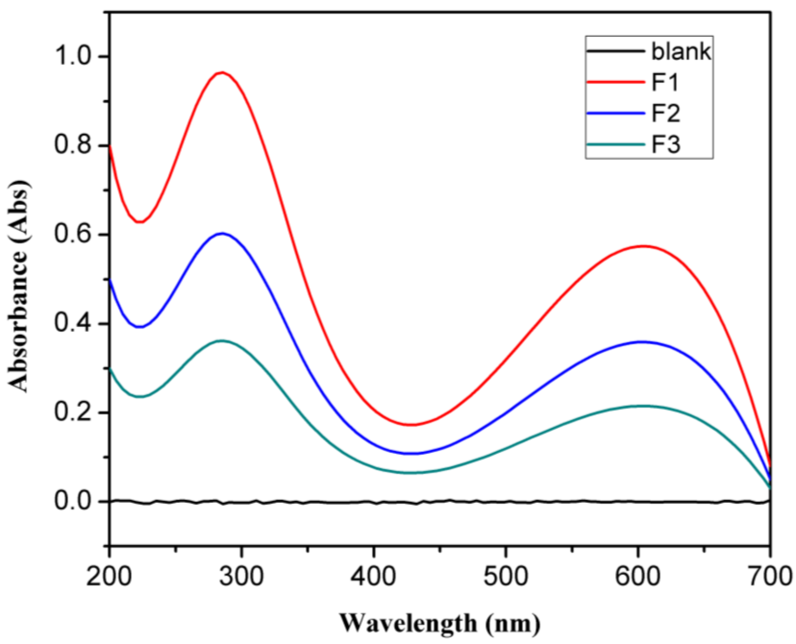

Figure 3. UV Vis Spectrum of Coffee Grounds Extract

As the concentration of SCG extract increases, the absorbance intensity also rises, as shown by the progression from F1 (blue line) to F2 (green line) and F3 (red line). This trend highlights a direct relationship between the concentration of bioactive compounds and UV-Vis absorbance. The peak at 280 nm is primarily attributed to phenolic compounds like chlorogenic acid and caffeic acid, while the broader peak within 320–350 nm reflects the presence of melanoidins, which are formed during coffee roasting [24]. These spectra demonstrate the gradual increase in the concentration of bioactive compounds across the formulations, which are vital for the soap's antibacterial and antioxidant properties. The UV-Vis analysis confirms the successful incorporation of spent coffee ground extract into the formulations, emphasizing its potential to enhance the functionality of personal care products, such as soap antibacterial.

3.3 Hedonic Evaluation of Spent Coffee Ground Extract Soap

The hedonic test results from the research encompassed participants' preferences regarding color, fragrance, and texture. A modest amount of SCG extract did not significantly impact the aroma, while it substantially affected the color and textural parameters.

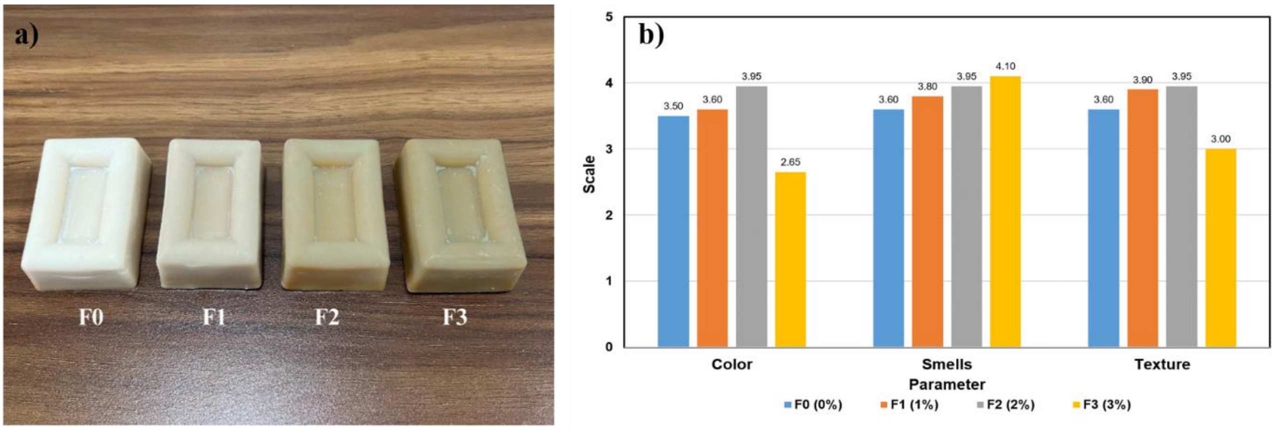

Figure 4. a) Appearance of Soap with Spent Coffee Ground Extract, b) Effect of Spent Coffee Ground Extract Concentration on Hedonic Test Parameters

The color variation in solid soap is determined by the different amounts of SCG extract utilized. Figure 4a. showed the appearance of the soap with varying number of extracts spent SCG. A study of panelists indicated a preference for the color in formulation F2. An increase in extract concentration yields a deeper color. The color derives from substances in the spent coffee grounds, including tannins. Throughout the drying process, tannins oxidize, which imparts a brown coloration.

One of the most important aspects of evaluating a product's quality, especially coffee-scented soap, is its aroma. Aroma affects the entire customer experience in addition to the product's attractiveness. No treatment substantially influenced the panelist's preferences for the soap scent, according to the hedonic analysis displayed in Figure 4b. This indicates that the panelists acceptance of each therapy was relatively uniform.

The panelists showed a preference for the texture of the soap, with evaluation scores ranging from 3.00 to 3.95. Formula F3 received the lowest average score of 3.00, while F1 and F2 obtained the highest average scores. However, analysis of variance indicated no significant differences in texture preferences among the various formulations. This similarity is likely due to the comparable textures produced by each treatment. Factors such as the ingredients used, storage duration, and moisture content influence the soap's texture. Additives like sugar and sodium chloride play a role in modifying texture, sodium chloride accelerates solidification, while increasing amounts of sucrose make the soap harder. This is attributed to sucrose's water-binding properties, which reduce the soap's moisture content, resulting in a denser and firmer texture.

3.4 Test of pH

A solution's acid or base level is indicated by its pH, or degree of acidity or alkalinity. According to Mezalin, [25] soap should have a pH of 9.0 to 10.8. The hydrolysis process of coffee-based soap is usually the cause of its high pH levels. Overly high pH soap can enhance the absorption of soap into the skin, which can cause dryness and irritation like sores, itching, or peeling.

Figure 5. illustrates the results of the pH measurement. The soap's average pH ranged from 9.52 to 10.10, with the F0 formulation having the highest average at 10.10 and the F3 formulation having the lowest at 9.52.

Variance analysis revealed no significant changes in the average pH values among the treatments. This suggests that including robusta spent coffee ground extract (1–3%) has not dramatically diminished the soap's pH, despite Garcia's, 2023 reporting that the pH of the SCG extract is roughly 5-6 [26].

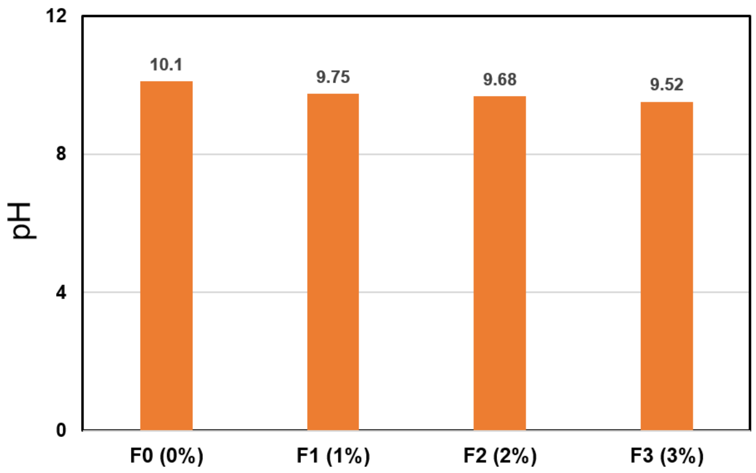

Figure 5. The Effect of Spent Coffee Ground Extract Concentration on Soap pH

3.5 Antibacterial tes Against S.aureus

The solid soap made from spent coffee grounds extract demonstrates the ability to inhibit bacterial growth, as evidenced by the formation of an inhibition zone. This study observes that as the concentration of spent coffee grounds extract increases, the inhibition zone also becomes larger. The results of the antibacterial activity tests, presented in Table 3, indicate that the diameter of the inhibition zones ranges from 6.34 mm to 9.20 mm. The highest average diameter of the inhibition zone was measured in formulations F2 and F3, each reaching 8,84 and 9,16 mm, indicating greater effectiveness. In contrast, formulation F0 produced the lowest inhibition zone at 6.36 mm, suggesting that without the addition of SCG extract, the antibacterial activity is significantly reduced. This finding highlights the potential SCG extract as an antibacterial agent that can be utilized in soap products.

Table 3. The effect of spent coffee grounds extract concentration on the antibacterial activity of soap

No	Treatment	Antimicrobial test (mm)		
		U1	U2	U_m
1.	F0	6.34	6.38	6.36
2.	F1	8.05	8.09	8.07
3.	F2	8.82	8.86	8.84
4.	F3	9.12	9.20	9.16

The inhibition zone formed during soap production can be attributed to the antibacterial or antimicrobial compounds present in the materials and extracts used. One ingredient that notably influences the inhibition zone is SCG extract. This extract, obtained through ethanol extraction, has proven effective in inhibiting the growth of methicillin-resistant *Staphylococcus aureus* strains [7]. The chemical constituents of SCG extract, including caffeine, phenolic compounds, and tannins [27], contribute to its antimicrobial properties [28].

4. Conclusion

This study effectively illustrated the efficacy of Microwave-Assisted Extraction (MAE) in isolating bioactive components from spent coffee grounds (SCG), these were later utilized in the development of antibacterial soap. The research emphasized the efficacy of MAE in enhancing the extraction process, delivering it more rapid and sustainable than traditional approaches. The antibacterial soap, containing different concentrations of SCG extract, demonstrated notable antibacterial efficacy against *Staphylococcus aureus*, with the 3% SCG extract formulation producing the most substantial inhibition zone of 9.16 mm. The inhibition zones against bacteria increase from 6.34 mm to 9.20 mm with rising extract concentrations.

Formulations containing 3% and 4.5% SCG extract demonstrated the highest effectiveness, with inhibition zones measuring 8.84 mm and 9.16 mm, respectively, while the formulation without the extract displayed a significantly lower zone of 6.36 mm. This study emphasizes the sustainable possibility of utilizing wasted coffee grounds (SCG) from coffee-producing areas to generate eco-friendly, antibacterial soaps. The soap compositions containing SCG extract were safe for dermal application, exhibiting acceptable sensory characteristics and robust antibacterial efficacy. This method not only mitigates waste but also fosters a circular economy. Future investigations may examine further bioactive chemicals derived from SCG and evaluate their prolonged environmental and economic effects.

5. Acknowledgements

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6. Conflict of Interest

Authors declare no conflicts of interest.

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