



Formulation and Evaluation of Anti-Dandruff Hair Mask from Lime Peel Extract (*Citrus aurantifolia*): Antibacterial Activity, Stability, and Safety Assessment

Andini Putri Maharani^{1*}, Eriska Agustin², Dani Prasetyo³

Universitas Kader Bangsa Palembang, Indonesia

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Corresponding author*:

andinipm03@gmail.com

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Abstract: Dandruff is a common scalp condition caused by *Staphylococcus aureus*, leading to itching, irritation, and reduced self-confidence. Natural alternatives, such as lime peel (*Citrus aurantifolia*), which contains antibacterial compounds like flavonoids and saponins, show potential as an effective anti-dandruff agent. **Objective:** This study aimed to formulate a hair mask using lime peel extract as an anti-dandruff agent and evaluate its physical quality, safety, and antibacterial activity. **Methods:** A quantitative experimental approach was used, preparing three formulations with 5%, 10%, and 15% lime peel extract. Evaluations included organoleptic, homogeneity, pH, spreadability, stability, irritation, and antibacterial activity tests against *Staphylococcus aureus*. **Results:** Formulations F0, F1, and F2 remained stable for 28 days, while F3 showed phase separation from days 21 to 28. All formulations had pH values within the safe range (4.5–6.5). Spreadability was acceptable, and irritation tests revealed that only F3 caused irritation. F3 showed the largest inhibition zone in antibacterial tests, indicating the highest antibacterial efficacy. **Implications:** This study suggests that higher concentrations of lime peel extract enhance antibacterial efficacy but may compromise stability and cause irritation. Further optimization is needed to improve safety and stability while maintaining effectiveness. **Originality:** This research contributes original insights into the use of lime peel extract for anti-dandruff treatments, offering a natural alternative to synthetic agents. The study provides valuable data for developing safer and more effective cosmetic formulations.

Keywords: Lime peel extract, *Citrus aurantifolia*, anti-dandruff, hair mask, antibacterial activity, *Staphylococcus aureus*.

INTRODUCTION

Indonesia, as a tropical country with a hot and humid climate, faces significant challenges in hair and scalp care (Sunsilk, 2023). In particular, women who wear hijabs experience increased sweating, creating an ideal environment for the growth of microorganisms such as *Staphylococcus aureus*, which contribute to dandruff formation (Oki et al., 2024). Dandruff, along with its associated symptoms such as itching, irritation, and discomfort, significantly impacts the quality of life (Farida, 2020; Susanti & Nasikhah, 2022). Therefore, finding effective and safe solutions to address dandruff is crucial. Natural ingredients, such as lime peel (*Citrus aurantifolia*), offer a promising alternative for dandruff treatment (Sari & Asri, 2022). Lime peel, often considered waste, contains active

compounds like flavonoids, hesperidin, and nobiletin, which have antibacterial and antimicrobial properties (BAWEKES et al., 2023). In addition, lime peel contains essential oils, vitamin C, citric acid, saponins, and alkaloids, all of which may help reduce excess oil and combat dandruff by reducing microbial activity on the scalp (Batra's, 2023). Previous studies (Farida, 2020; Mulyani & Aulia, 2022), have shown that lime peel extract can act as an effective antibacterial agent.

Several studies have explored the antifungal properties of lime peel extract, specifically its effects on *Malassezia* sp., the primary agent responsible for dandruff. Hidayah demonstrated that a 25% lemon juice solution resulted in a 30% reduction in the growth of *Malassezia* sp (Hidayah, 2010). However, this result is significantly lower compared to ketoconazole, which achieved a 93% reduction in fungal growth. Sreekanth and Kaithavalappil found that lemon juice showed significant antifungal activity at concentrations above 0.1% w/v, indicating its potential as an anti-dandruff agent (Sreekanth & Kaithavalappil, 2016). Despite these findings, the antifungal activity of lime peel extract was found to be less potent than synthetic treatments like ketoconazole, which is widely used for its high efficacy in fungal inhibition (Hidayah, 2010). This highlights a limitation in the effectiveness of lime peel extract as a standalone treatment for dandruff.

The comparative efficacy of lime peel extract against established antifungal agents has also been investigated. While lime peel extract does exhibit some antifungal activity, studies show that it is less effective than synthetic antifungal treatments such as ketoconazole. Hidayah found that the minimum inhibitory concentration (MIC) of lemon juice is higher than that of ketoconazole, suggesting that significantly higher concentrations of lemon juice are required to achieve comparable effectiveness (Hidayah, 2010). Additionally, Bhattacharya and Ghosh highlighted the success of FERMENZA®, an alternative natural formulation, in outperforming both lime peel extract and traditional treatments against *Malassezia furfur* (Bhattacharya & Ghosh, 2025). These findings emphasize the need for further research into more effective natural alternatives to synthetic anti-dandruff agents.

While lime peel extract shows promise as an anti-dandruff agent, its limitations in terms of efficacy compared to synthetic treatments point to the need for continued innovation in this area. Bhattacharya and Ghosh found that natural alternatives like FERMENZA® exhibit superior antifungal efficacy, which could lead to better outcomes for individuals seeking natural treatments (Bhattacharya & Ghosh, 2025). This underscores

the ongoing need to explore more effective formulations and identify natural ingredients that can offer comparable or superior results to conventional treatments. Furthermore, many studies on lime peel extract have not yet explored its synergistic effects when combined with other natural ingredients, which could enhance its antifungal activity.

This study aims to formulate a hair mask preparation using lime peel extract (*Citrus aurantifolia*) as a natural alternative for hair care to address dandruff more safely and in an environmentally friendly manner. The study will evaluate the physicochemical characteristics and stability of the hair mask, as well as its antibacterial effectiveness against *Staphylococcus aureus*, a primary cause of dandruff. In response to the limitations identified in previous studies, such as the lower effectiveness of lime peel extract compared to synthetic agents like ketoconazole ([Hidayah, 2010](#)), this research seeks to develop a more effective hair mask formulation. The study will also explore the potential of combining lime peel extract with other natural ingredients to enhance its efficacy in treating dandruff. Furthermore, the research aims to contribute new insights into the development of natural hair care products with potential applications in the pharmaceutical and cosmetic fields, offering a safer, more effective, and environmentally friendly solution compared to synthetic chemical-based products.

This study hypothesizes that a hair mask formulated with lime peel extract (*Citrus aurantifolia*) will effectively reduce dandruff by inhibiting *Staphylococcus aureus*, a primary cause of dandruff. It is expected that increasing the concentration of lime peel extract will enhance its antibacterial efficacy, as demonstrated by a larger inhibition zone in antibacterial tests. However, this increased concentration may also affect the stability of the formulation, potentially causing phase separation at higher concentrations, as observed in the F3 formula. The research will evaluate the balance between enhanced antibacterial activity and the stability of the product, aiming to develop a natural, effective, and safe anti-dandruff solution that could serve as a viable alternative to synthetic treatments.

RESEARCH METHOD

This research is a quantitative experimental study aimed at testing the effectiveness of a hair mask formulation made from lime peel extract (*Citrus aurantifolia*) for treating dandruff. The research was conducted in May 2025 at the Pharmacy Laboratory of Universitas Kader Bangsa Palembang. The focus of this study is to evaluate the physicochemical properties, stability, and antibacterial effectiveness of the formulated hair

mask. Specifically, the study analyzes the effect of varying concentrations of lime peel extract (5%, 10%, and 15%) in the formulation.

The experimental design was chosen because it allows for controlled manipulation of the independent variable (lime peel extract concentration) to measure its effect on the dependent variable (dandruff treatment effectiveness). Quantitative methods are applied to ensure objective data collection, especially in evaluating the antibacterial activity against *Staphylococcus aureus*, one of the primary causes of dandruff, as well as testing the physical properties of the hair mask formulation. This design is ideal for assessing the potential efficacy and stability of a natural-based product compared to synthetic alternatives.

The primary data sources for this research include experimental results obtained from the formulation of the hair mask, phytochemical screening, and various laboratory tests. These tests involve evaluating the physicochemical characteristics of the hair mask, such as organoleptic tests, pH, viscosity, stability, homogeneity, irritation, and antibacterial activity. The secondary data, such as previous studies on lime peel extract's antibacterial properties, are also referenced to guide the formulation process and testing procedures ([Anindita et al., 2022](#); [Safitri et al., 2023](#)).

Data collection was carried out through a series of controlled laboratory experiments. The process began with preparing the lime peel extract using maceration and phytochemical screening to identify active compounds. The hair mask was formulated by blending the lime peel extract with other ingredients, and then subjected to tests for physical stability, pH, viscosity, and antibacterial activity. The antibacterial activity was assessed using the disk diffusion method against *Staphylococcus aureus*. All tests were performed in triplicate to ensure the reliability and accuracy of the data.

Data analysis involved both descriptive and inferential statistical methods. Descriptive statistics were used to summarize the results of the organoleptic evaluation, pH, viscosity, and other physical tests. The antibacterial effectiveness was evaluated by measuring the inhibition zones in the disk diffusion tests. The results were analyzed to determine correlations between the concentration of lime peel extract and the effectiveness of the hair mask. Statistical analysis, such as analysis of variance (ANOVA), was conducted to assess any significant differences between the different formulations (F0, F1, F2, and F3) in terms of their stability, antibacterial activity, and overall performance as an anti-dandruff product.

The following Table 1 shows the composition of the hair mask formulations used in the study:

Table 1. Hair Mask Formulation

Ingredient	Function	Concentration (%)
Lime Peel Extract	Active Ingredient	5
Stearic Acid	Emulsifier	15
Cetyl Alcohol	Emulsifier	0.6
Liquid Paraffin	Emollient	3
Triethanolamine (TEA)	Emulsifying Agent	2
Propylene Glycol	Humectant	10
Aquadest (Water)	Solvent	Add 100

Source: Handbook of Pharmaceutical Excipients

This approach ensures a comprehensive understanding of the potential of lime peel extract as an effective and stable ingredient for anti-dandruff hair mask formulations, and provides a foundation for further development of natural, safe alternatives in hair care products.

RESULT AND DISCUSSION

This study aims to evaluate the effectiveness of a hair mask formulated with lime peel extract (*Citrus aurantifolia*) as an anti-dandruff agent. The research was conducted at the Pharmacy Laboratory of Universitas Kader Bangsa Palembang. The research process began with the preparation of lime peel extract, followed by phytochemical screening to identify the active compounds, and the formulation of the hair mask with varying concentrations of lime peel extract. Subsequently, physicochemical evaluations were conducted on the hair mask formulation, including organoleptic tests, pH testing, spreadability testing, stability testing, homogeneity testing, irritation testing, and antibacterial testing.

Plant Determination

The plant determination was performed by the Herbarium of Institut Teknologi Bandung to ensure that the lime peel used in this study was indeed from the *Citrus aurantifolia* species.

Phytochemical Screening

Phytochemical screening was carried out to identify the natural chemical compounds in the lime peel extract that could function as antibacterial agents. The compounds tested included flavonoids, alkaloids, tannins, and saponins.

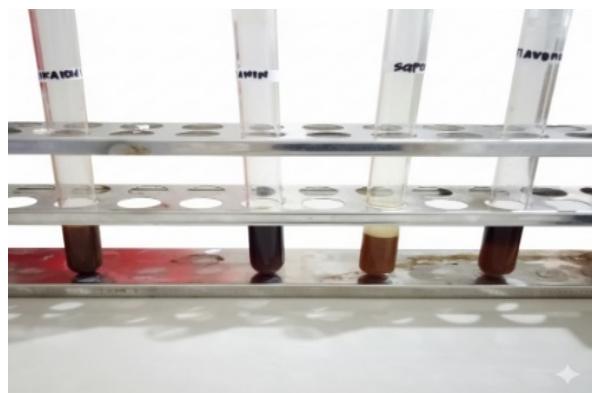


Figure 2. Phytochemical Screening

This figure represents the phytochemical screening results, showing the identification of active chemical compounds in the lime peel extract. The compounds tested include flavonoids, alkaloids, tannins, and saponins.

Organoleptic Test

The organoleptic test was conducted to evaluate the physical properties of the hair mask formulation. This test involved observing the appearance, color, scent, and texture of the hair mask across different formulations. The results showed consistency in texture, with slight variations in color and scent based on the concentration of lime peel extract added to each formulation.



Figure 3. Hair Mask Formulations F0, F1, F2, F3

Table 3. Organoleptic Test Results

Formula	Day	Color	Scent	Texture
F0	0	White	Odorless	Cream
F0	7	White	Odorless	Cream
F0	14	White	Odorless	Cream

Formula	Day	Color	Scent	Texture
F0	28	White	Odorless	Cream
F1	0	Yellow	Odorless	Cream
F1	7	Yellow	Odorless	Cream
F1	28	Yellow	Extract-like scent	Cream
F2	0	Brownish Green	Extract-like scent	Cream
F2	28	Brownish Green	Extract-like scent	Cream
F3	0	Dark Brown	Odorless	Cream
F3	28	Greenish Brown	Odorless	Cream

The results from the organoleptic test on all formulas (F0, F1, F2, and F3) showed that the texture of the formulations remained consistent, despite color differences caused by the addition of lime peel extract. The color of formula F2 (10% lime peel extract) and F3 (15% lime peel extract) tended to be darker compared to F0 and F1.

Stability testing revealed that all formulas, except F3, remained stable over a 28-day storage period at room temperature. Formula F3, which contains the highest concentration of lime peel extract, experienced phase separation from day 21 to day 28.

Homogeneity Test

The results from the homogeneity test, as shown in Table 4, indicate that all formulas (F0, F1, F2, and F3) remained homogeneous and did not show any phase separation.

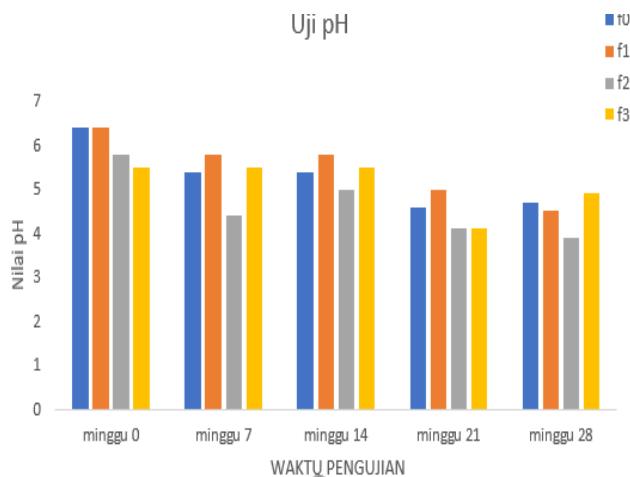
Table 4. Homogeneity Test Results

Formula	Day 0	Day 7	Day 14	Day 21	Day 28
F0	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
F1	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
F2	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
F3	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous

The test results show that all formulations maintained homogeneity throughout the 28-day storage period without any phase separation

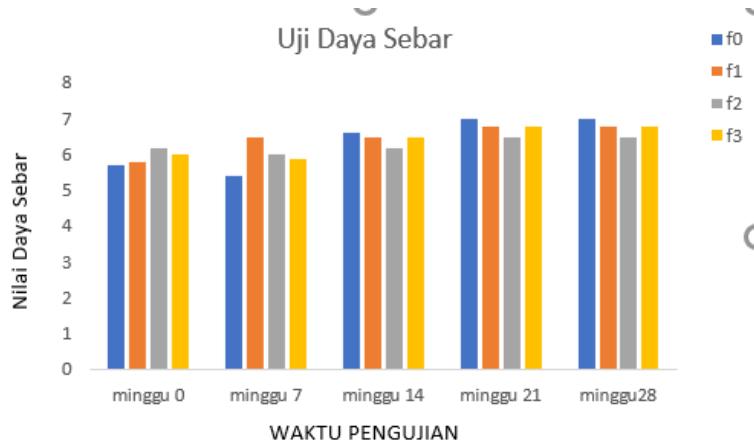
pH Test

The results of the pH test, as shown in the figure, indicate that the formula F0 (cream base without extract) has a stable pH range of 4.6–6.4. Formula F1 (5% lime peel extract) shows a pH range of 4.5–6.4, while F2 (10% extract) has a pH between 4.4–5.8, and F3 (15% extract) demonstrates a pH range between 4.1–5.5, all of which are within the acceptable range for scalp use.

**Figure 4.** pH Test Results

Spreadability Test

The results of the spreadability test, as shown in the figure, indicate that all formulations (F0, F1, F2, and F3) exhibited good spreadability, with spread distances ranging from 5.7 to 7.0 cm. Formula F2 demonstrated the best spreadability, with the lowest standard deviation (0.21), while F0 (cream base) exhibited the highest standard deviation (0.74), indicating greater variability in its spreadability.

**Figure 5.** Spreadability Test Results

Viscosity Test

The viscosity test results showed that all formulations (F0, F1, F2, and F3) fall within the viscosity range specified by the SNI standard for topical preparations, which is between 2,000 and 50,000 cPs (Purwaningsih et al., 2020). Formula F0 had the highest viscosity (17,000 cPs), followed by formula F1 (12,833 cPs), formula F2 (10,500 cPs), and formula F3 (8,000 cPs).

**Figure 6.** Viscosity Test Results

Irritation Test

The results of the irritation test, presented in Figure 7 and Table 5, show that formulas F0, F1, and F2 did not cause any irritation on rabbit skin. However, formula F3 (15% lime peel extract) caused irritation, with an erythema score of 2 on day 2 (48 hours) and a score of 3 on day 3 (72 hours), indicating severe irritation.

**Figure 7.** Irritation Test

The observations are shown in the figure above and are further clarified in the following table:

Table 5. Irritation Test Results

Formula	Erythema (24, 48, 72 hours)	Edema (24, 48, 72 hours)
F0	0, 0, 0	0, 0, 0
F1	0, 0, 0	0, 0, 0
F2	0, 0, 0	0, 0, 0
F3	0, 2, 3	0, 0, 0

Notes:

1. Erythema: 0 = none, 1 = very slight, 2 = moderate, 3 = moderate-severe, 4 = severe.
2. Edema: 0 = none, 1 = barely perceptible, 2 = clear raised edge, 3 = 1 mm, 4 = >1 mm.

Stability Test

The results of the stability test, as presented in Table 6, show that formulas F0, F1, and F2 remained stable. However, formula F3 showed phase separation from day 21 to day 28, indicating that the stability of the formulation was compromised at the higher concentration of lime peel extract.

Table 6. Stability Test Results

Formula	Color	Scent	Texture	Homogeneity	Notes
F0	White	Odorless	Soft semi-solid cream	No separation	Very stable
F1	Light yellow	Characteristic lime extract smell	Soft semi-solid cream	Homogeneous, stable	Stable
F2	Brown	Characteristic lime extract smell	Soft semi-solid cream	No separation	Stable
F3	Dark brown	Characteristic lime extract smell	Soft semi-solid cream	Separation from day 21 to 28	Less stable

Notes:

All formulas showed good stability up to day 28, except for F3, which experienced phase separation from day 21 to 28.

Antibacterial Test

The results of the antibacterial test, as shown in Figure 8, indicate that all formulas (F1, F2, and F3) exhibited the ability to inhibit bacterial growth, with formula F3 (15% extract) showing the largest inhibition zone. This result suggests that as the concentration of lime peel extract increases, the antibacterial efficacy improves.

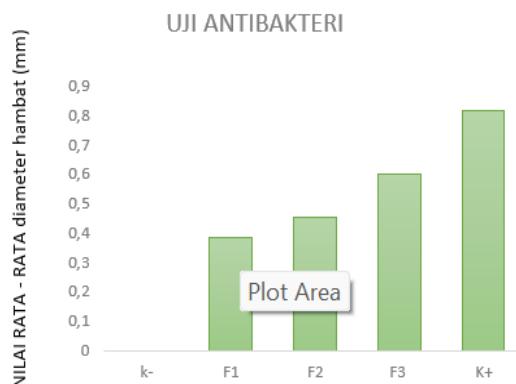


Figure 8. Antibacterial Test Results

Evaluation of Hair Mask Formulation with Lime Peel Extract

The evaluation results indicate that lime peel extract has strong potential as an active ingredient in anti-dandruff hair mask formulations. Increasing the concentration of lime

lime peel extract caused changes in the color and scent of the formulation, but all formulas retained a good cream texture. Formulas F0 to F2 remained stable, while F3, with the highest concentration of lime peel extract, experienced phase separation, making it less stable. The antibacterial test results showed that the effectiveness of the formulations improved with higher concentrations of lime peel extract, with the inhibition zone against *Staphylococcus aureus*, the bacteria responsible for dandruff, increasing. Formula F3, containing the highest concentration of extract, exhibited the largest inhibition zone, indicating higher antibacterial efficacy. However, the irritation test on rabbit skin showed that formula F3 could cause severe irritation, with an increase in erythema scores. This suggests that while lime peel extract demonstrates strong antibacterial activity, higher concentrations may cause skin irritation, highlighting the need for careful balance in formulation concentration.

DISCUSSION

This study aimed to evaluate the effectiveness of a hair mask formulated with lime peel extract (*Citrus aurantifolia*) for dandruff treatment. The results showed that the hair mask formulations, particularly those with lime peel extract concentrations of 5%, 10%, and 15%, demonstrated a consistent cream texture, with slight color changes depending on the extract concentration. The antibacterial activity of the hair mask formulations was significant, especially for formula F3 (15% lime peel extract), which exhibited the largest inhibition zone against *Staphylococcus aureus*, the bacteria responsible for dandruff. Stability testing revealed that formulations F0, F1, and F2 remained stable for 28 days, while F3, the formulation with the highest concentration of lime peel extract, experienced phase separation after 21 days. Additionally, the irritation test indicated that the higher concentration of lime peel extract in F3 caused severe skin irritation in rabbits.

The results of this study suggest that the effectiveness of lime peel extract in the hair mask formulation increases with the concentration of the extract, as observed in the antibacterial test. The higher concentration of lime peel extract likely leads to a more potent antibacterial effect, which aligns with the increase in the inhibition zone observed in formula F3. However, the phase separation and the skin irritation observed in the higher concentration formula (F3) indicate that while lime peel extract is effective, it also presents challenges when used in high concentrations. The occurrence of irritation could be due to

the chemical composition of lime peel extract, such as its citric acid content, which may irritate sensitive skin at higher concentrations.

The findings of this research are consistent with previous studies that explored the antibacterial properties of lime peel extract. Hidayah ([Hidayah, 2010](#)) and Sreekanth and Kaithavalappil ([Sreekanth & Kaithavalappil, 2016](#)) demonstrated the antifungal potential of lime peel extract, though they also noted that it was less effective compared to synthetic agents like ketoconazole. In this study, while lime peel extract demonstrated antibacterial activity, it was also found to cause skin irritation at higher concentrations, a limitation not fully explored in previous studies. This study adds to the literature by exploring the stability and irritation potential of hair mask formulations containing lime peel extract, showing that higher concentrations of the extract lead to greater antibacterial efficacy but also compromise the formulation's stability and cause skin irritation.

The results of this study provide valuable insights into the use of lime peel extract in hair care products, particularly as a natural alternative to synthetic anti-dandruff agents. The significant antibacterial properties of lime peel extract suggest its potential as a safe and effective ingredient for dandruff treatments, particularly for individuals seeking natural and eco-friendly products. However, the study also highlights the need to balance efficacy with stability and skin safety, as higher concentrations of lime peel extract may cause undesirable side effects like irritation.

The positive implications of this research are evident in its contribution to the development of natural, safe, and effective anti-dandruff formulations. Lime peel extract has shown promise in addressing dandruff, and with further refinement of the formulation, it could become a viable alternative to synthetic products. However, the negative implications, such as the risk of irritation with higher concentrations, suggest that careful formulation adjustments and concentration limits are necessary. This study also underscores the importance of considering not only the active ingredient's effectiveness but also its impact on product stability and user safety.

Based on the findings, it is recommended that future research focus on optimizing the concentration of lime peel extract in hair care products to maximize its antibacterial effects while minimizing the risk of skin irritation. Additionally, further studies should explore the combination of lime peel extract with other natural ingredients to enhance its stability and efficacy. Regulatory agencies and cosmetic formulators should consider establishing guidelines for the safe use of natural extracts like lime peel in cosmetic formulations to

ensure both effectiveness and safety for consumers. Furthermore, manufacturers should prioritize the development of more stable formulations to ensure longer shelf lives and consistent performance of natural hair care products.

CONCLUSION

The results of this study demonstrate that lime peel extract (*Citrus aurantifolia*) has significant potential as an active ingredient in anti-dandruff hair mask formulations. The hair mask formulations with lime peel extract showed good antibacterial efficacy, particularly with the 15% concentration (Formula F3), which exhibited the largest inhibition zone against *Staphylococcus aureus*. However, the study also revealed that higher concentrations of lime peel extract (F3) may cause phase separation and skin irritation, highlighting the need to balance efficacy with safety in formulation development.

This study contributes to the growing body of knowledge on the use of natural ingredients, specifically lime peel extract, in cosmetic formulations. It provides valuable data on the physicochemical properties, stability, and antibacterial effects of lime peel extract when used in hair care products. The research highlights the potential of lime peel extract as a natural alternative to synthetic anti-dandruff agents, offering a more eco-friendly and safe option for consumers. Furthermore, this study adds to the understanding of the limitations and challenges of using natural extracts, particularly in terms of product stability and skin irritation, which is crucial for future research and product development.

While the study successfully demonstrated the potential of lime peel extract in anti-dandruff formulations, it also faced several limitations. One limitation was the focus on only one bacterium, *Staphylococcus aureus*, and additional studies exploring a broader range of dandruff-causing microorganisms are necessary. Another limitation was the challenge of balancing the extract concentration for maximum efficacy without compromising the stability and safety of the product. Future research should further investigate the optimal concentration of lime peel extract, explore the synergistic effects of combining lime peel extract with other natural ingredients, and examine the long-term effects of the hair mask formulations on scalp health.

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