Advances in Healthcare Research
Volume 3, Issue 2 (2025)
Website: https://advancesinresearch.id/index.php/AHR
OPEN ACCESS
e-ISSN: 2985-9808

Formulation Research and Antibacterial Activity Test of Face Mist Preparation from Ethanol Extract of Papaya Leaves (Carica Papaya L.) Against Propionibacterium Acnes

Aulia Nur Mustaanah [1] Muhammad Nurul Fadel [2*] Muhamad Khudzaifi [3]

[1] Universitas Muhammadiyah Kudus, Kudus, Indonesia. Email: aulianur301002@gmail.com
[2] Universitas Muhammadiyah Kudus, Kudus, Indonesia. Email: nurulfadel@umkudus.ac.id
[3] Universitas Muhammadiyah Kudus, Kudus, Indonesia. Email: khudzaifi@umkudus.ac.id

ARTICLE HISTORY

Received: July 15, 2025
Revised: July 27, 2025
Accepted: August 12, 2025

DOI :
https://doi.org/10.60079/ahr.v3i2.559

ABSTRACT

Purpose: The purpose of this study was to formulate a face mist preparation containing ethanol extract of papaya leaves (Carica papaya L.) at concentrations of 10%, 15%, and 20%, and to evaluate its antibacterial activity against Propionibacterium acnes, a primary acne-causing bacterium.
Research Method: This study employed a laboratory-based experimental design with a post-test-only control group. Data were collected through experimental observations. The face mist was formulated with three extract concentrations (F1: 10%, F2: 15%, and F3: 20%), with a face mist base as the negative control and clindamycin as the positive control. The preparations underwent organoleptic, pH, homogeneity, and spreadability tests. Antibacterial activity was evaluated using the disc diffusion method, and the data were analyzed using One-Way ANOVA with a significance level of $p < 0.005$.
Results and Discussion: The formulations met the physical property standards, showing a brownish-green color, liquid consistency, and a characteristic papaya leaf aroma. All samples were homogeneous, with pH values ranging from 5.08 to 5.16, and spreadability between 5.33 and 5.75 cm. The highest antibacterial activity was observed in F3 (20%) with an average inhibition zone of 16.3 mm.
Implications: The findings suggest that papaya leaf ethanol extract is a promising natural ingredient for face mist formulations with antibacterial properties, offering potential as an alternative acne treatment product.

Keywords: extract; face mist; propionibacterium acnes; antibacterial activity; herbal skincare.

Introduction

Human skin is constantly exposed to various environmental stressors that can compromise its integrity and trigger numerous dermatological conditions. Among these, free radicals, airborne dust, vehicle emissions, and prolonged exposure to ultraviolet (UV) radiation from sunlight are recognized as major contributors to skin damage (Larasati & Setyowati, 2022). These elements accelerate oxidative stress, leading to inflammation, premature aging, and the development of more persistent conditions such as acne. Acne vulgaris, a prevalent skin disorder, typically emerges during puberty and can persist

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into adulthood. The appearance of comedones, papules, pustules, and, in severe cases, nodules and cysts characterizes it. The root cause of acne is multifactorial, but one of the primary mechanisms is the colonization and proliferation of Propionibacterium acnes within the hair follicles. This bacterium initiates the formation of microcomedones, clinically invisible precursor lesions that evolve into both non-inflammatory (blackheads and whiteheads) and inflammatory lesions (papules, pustules, nodules) (McLaughlin *et al.,* 2019). The persistence of acne and its impact on skin health and self-esteem have driven interest in preventive and therapeutic skincare strategies. One widely adopted measure is maintaining facial hygiene through regular cleansing and the use of topical skincare formulations tailored to control bacterial activity and sebum production. In this context, face mists have gained popularity, especially among adolescents and young adults, due to their convenience, ease of application, and ability to refresh, hydrate, and soothe the skin. Compared to other skincare products, face mists offer portability and are quickly absorbed, making them ideal for daily use (Herliningsih & Anggraini, 2021). However, despite their growing appeal, many commercial face mists are synthetic-based and may contain irritants.

Research into skincare innovations increasingly emphasizes the use of natural ingredients with multifunctional properties, including antibacterial, anti-inflammatory, and antioxidant activities. One promising solution for acne management is the formulation of a face mist. This topical preparation not only hydrates and refreshes the skin but also provides ease of application without direct hand contact (Herliningsih & Anggraini, 2021). The antibacterial mechanism in acne prevention is fundamental, considering that Propionibacterium acnes plays a central role in the formation of acne lesions. The presence of P. acnes triggers inflammation through the activation of the immune response in pilosebaceous units, leading to the appearance of inflammatory and non-inflammatory acne types (McLaughlin *et al.,* 2019). Among natural antibacterial agents, papaya leaves (Carica papaya L.) have attracted significant scientific attention. These leaves are rich in phytochemicals, including flavonoids, terpenoids, alkaloids, saponins, and tannins, all of which contribute to their antibacterial efficacy (Singh *et al.,* 2020). Beyond their antibacterial role, papaya leaf extracts also exhibit antimalarial, antihelmintic, and anti-inflammatory properties, as well as the ability to enhance systemic antioxidant capacity and reduce lipid peroxidation (Jati *et al.,* 2019). Experimental studies have proven that papaya leaf extracts can inhibit various pathogenic bacteria: for instance, Staphylococcus aureus with an inhibition zone of up to 21.5 mm (Maharani *et al.,* 2022), Vibrio cholerae and Salmonella typhi with inhibition zones of 10.3 mm and 10.06 mm, respectively (Utami *et al.,* 2023), and Escherichia coli with an inhibition zone of 22.3 mm at 100% extract concentration (Hasriyani *et al.,* 2021). Veronica, (2022) found that a 50% ethanol extract of papaya leaves inhibited P. acnes with an impressive 25 mm inhibition zone. These findings validate papaya leaf extract's potential as a bioactive ingredient in acne-targeted formulations. To test antibacterial efficacy, the disc diffusion method remains widely used due to its simplicity and accuracy. This method involves infusing antimicrobial substances into sterile disc paper placed on bacterial-inoculated agar, which is then incubated to observe the formation of an inhibition zone. Studies by (Nafi *et al.,* 2023; Mayefis *et al.,* 2020; Hasanah & Novian, 2020) applied this method using Nutrient Agar (NA) media to assess the inhibition of P. acnes. Kabakoran *et al.,* (2022) highlighted that NA supports bacterial viability and diffusion activity, making it a preferred medium for this purpose. Rinihapsari *et al.,* (2023) similarly confirmed the suitability of NA in cultivating P. acnes for antibacterial testing.

Despite the growing body of evidence supporting the antibacterial properties of papaya leaf (Carica papaya L.) extracts, most existing studies have focused primarily on laboratory-scale testing of

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the extract against various bacterial strains, including Propionibacterium acnes, in isolation rather than in formulated cosmetic products. While studies by (Veronica *et al.,* 2023; Hasriyani *et al.,* 2021; Utami *et al.,* 2023) confirm the significant inhibitory zones of papaya leaf extract against P. acnes and other pathogens, these findings are often limited to crude extract applications in controlled agar environments. The formulation aspect, particularly integration into face mist products, and its performance in practical, consumer-oriented applications remain underexplored. There is a lack of empirical data assessing how the antibacterial efficacy of papaya leaf extract translates into topical formulations such as sprays or mists, mainly when used in varying concentrations under common usage conditions. From a theoretical perspective, previous studies tend to emphasize the phytochemical composition and in vitro effectiveness without adequately addressing the formulation science involved in creating stable, functional skincare products. This limits the generalizability and practical application of their findings. There is also minimal discussion on the specific benefits of face mist as a delivery system for natural antibacterials, despite its advantages in hygiene, usability, and absorption (Herliningsih & Anggraini, 2021). This gap calls for an integrated study that not only confirms antibacterial activity through standard microbiological methods, such as disc diffusion, but also formulates and tests papaya leaf extract in a practical, consumer-ready format.

The novelty of this study lies in the development and evaluation of a face mist formulation containing ethanol extract of papaya leaves (Carica papaya L.) in varying concentrations (10%, 15%, and 20%) specifically aimed at inhibiting the growth of Propionibacterium acnes. Unlike previous research that focused solely on the antibacterial activity of the extract in isolation, this study integrates the extract into a topical cosmetic preparation that aligns with current consumer preferences for natural, easy-to-use skincare products. By utilizing the disc diffusion method on Nutrient Agar (NA) media, the study not only assesses antibacterial efficacy but also bridges the gap between in vitro testing and practical application. The purpose of this research is to formulate a natural-based face mist and empirically evaluate its antibacterial performance against P. acnes, offering a scientifically validated, hygienic, and user-friendly skincare alternative that addresses both aesthetic and dermatological concerns in the context of rising demand for herbal cosmetics.

Literature Review and Hypothesis Development

Papaya Leaf Extract

Papaya leaf extract (Carica papaya L.) is derived from the extraction of bioactive compounds present in papaya leaves. It is widely recognized for its diverse pharmacological potentials, including antibacterial, antioxidant, anti-inflammatory, antifungal, and anticancer activities. The primary secondary metabolites contained in papaya leaf extract include flavonoids, alkaloids, tannins, saponins, and terpenoids. These compounds function through various mechanisms to inhibit the growth of pathogenic microorganisms. For instance, flavonoids are known to disrupt the integrity of bacterial cell membranes, interfere with DNA synthesis, and inhibit vital enzymatic activity within bacterial metabolism. Alkaloids act by hindering bacterial protein synthesis, whereas tannins damage the cell wall and precipitate bacterial proteins. Sharma *et al.,* (2020) reported that papaya leaf extract exhibits strong antibacterial efficacy against Gram-positive bacteria, including Propionibacterium acnes, the primary causative agent of acne. This efficacy is attributed to the ability of phenolic compounds in papaya leaves to interact directly with the bacterial cell membrane, leading to cell lysis and death. Ethanol extract from papaya leaves also exhibits antioxidant activity capable of reducing free radical levels and lipid

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peroxidation in the body, which ultimately accelerates wound healing and reduces skin inflammation (Yuslianti, 2018). Furthermore, a study by Chaijan *et al.,* (2025) demonstrated that the maturity level of the leaves influences the concentration of active compounds, with older leaves containing higher levels of antibacterial metabolites. This factor is crucial in selecting raw materials to ensure the optimal efficacy of the final formulation.

The use of papaya leaf extract in topical formulations is gaining increased attention in the pharmaceutical and cosmetic industries, particularly in the development of natural skincare products. Its application as an antimicrobial agent in topical preparations is supported not only by the strength of its bioactive compounds but also by its relatively safe profile, with minimal risk of skin irritation. Nascimento *et al.,* (2024) demonstrated in their study that an ointment containing 1% papaya leaf extract had comparable wound-healing efficacy to a 2% mupirocin topical antibiotic, without causing irritation or allergic reactions. These findings reinforce the potential of papaya leaf extract as a key component in antibacterial cosmetic formulations, such as face mist. Natural extract-based face mists offer numerous advantages, including ease of application, broad spreading capability, and reduced risk of contamination due to non-contact usage. Agarwal *et al.,* (2021) further highlighted that formulations containing papaya leaf extract can create herbal matrices that not only support tissue regeneration but also exhibit antibacterial activity against various strains of skin-related bacteria. This indicates that the potential of papaya leaf extract extends beyond acne prevention. Siddiqui *et al.,* (2025) also emphasized that the papaya leaf, in addition to its seeds and fruit, contains significant bioactive compounds with strong potential in antimicrobial therapy, both topically and systemically. Therefore, the integration of papaya leaf extract into face mist formulations offers not only a natural solution for acne treatment but also opens new opportunities for the development of eco-friendly, safe, and effective skincare products.

Propionibacterium Acnes

Propionibacterium acnes (currently reclassified as Cutibacterium acnes) is a Gram-positive, anaerobic bacterium that resides naturally in the human skin microbiome, particularly within the pilosebaceous units where hair follicles and sebaceous glands converge. While generally considered a commensal organism under normal physiological conditions, P. acnes can transition into an opportunistic pathogen under specific environmental or physiological imbalances. Its pathogenic potential lies in its ability to trigger localized inflammation, adhere to follicular walls, and produce pro-inflammatory mediators such as lipases, proteases, and porphyrins, which contribute to the disruption of the follicular epithelium. These activities can lead to hyperkeratinization and excessive sebum production, two key features in the pathogenesis of acne. Süer & Güvenir, (2019) emphasized that P. acnes is not a homogenous entity; rather, it comprises multiple phylotypes with differing genetic compositions and virulence profiles. Some of these strains exhibit higher levels of inflammatory potential and are strongly linked to the development of pustular and nodular acne. In addition to producing inflammatory mediators, P. acnes forms robust biofilms that enable it to persist within blocked follicles and on the skin surface, making it more resistant to topical treatments and immune clearance. Fournière *et al.,* (2020) demonstrated that when P. acnes dominates under dysbiosis—a disrupted state of the skin microbiota—it can activate both innate immune pathways, such as Toll-like receptor signaling, and adaptive responses, resulting in elevated cytokine production, including IL-1β and TNF-α. This mechanistic understanding shifts modern acne treatment paradigms from simply eradicating bacteria to targeting inflammation and restoring microbial balance.

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In recent years, increasing attention has been given to the issue of antibiotic resistance in Propionibacterium acnes, particularly in response to the widespread and often indiscriminate use of topical antibiotics such as clindamycin and erythromycin. This trend is especially concerning because these antibiotics have been a mainstay of acne treatment for decades. Beig *et al.,* (2024) conducted a comprehensive global review. They found a notable rise in antibiotic-resistant strains of P. acnes, indicating that the effectiveness of conventional treatment regimens is being undermined by microbial adaptation. This growing resistance crisis has led to a surge of interest in developing alternative therapeutic strategies that are both effective and sustainable in the long term. One promising avenue is the use of topical probiotics, which can suppress P. acnes through competitive inhibition and the production of antimicrobial metabolites, while also contributing to the restoration of a healthy skin barrier. Lebeer *et al.,* (2022) reported that specific Lactobacillus strains have demonstrated dual functionality—directly inhibiting P. acnes growth and enhancing epithelial barrier integrity, making them highly suitable for incorporation into cosmetic and dermatological formulations. Furthermore, plant-based compounds have shown significant potential as antibacterial agents. In an in vitro study, Blaskovich *et al.,* (2019) demonstrated that phytochemicals and novel non-antibiotic formulations were effective against antibiotic-resistant P. acnes strains, supporting the rationale for using herbal-derived ingredients in modern skincare.

Face Mist

Face mist is a liquid cosmetic formulation designed to be sprayed directly onto the face, providing hydration, refreshing the skin, and helping to balance its condition. Typically, face mists contain active ingredients such as humectants, antioxidants, botanical extracts, and thermal water, which help reinforce the skin barrier, reduce inflammation, and maintain surface hydration. The formulation works by forming a thin, even layer of moisture on the epidermis, which helps retain water while simultaneously balancing the skin's pH level after cleansing. According to Ganceviciene *et al.,* (2012), incorporating moisturizing agents such as glycerin and hyaluronic acid into face mist formulations plays a crucial role in anti-aging skincare strategies, as they help preserve skin hydration and elasticity. Additionally, research by Cacciapuoti *et al.,* (2020) demonstrated that face mists containing thermal spring water significantly improved skin barrier recovery after exposure to hard water, indicating that these products can also offer dermatological benefits beyond cosmetic refreshment. The fine misting spray mechanism enhances even distribution across the face and facilitates better absorption of active compounds. This delivery method is particularly beneficial for sensitive skin, as it reduces the need for manual application and potential irritation. The growing interest in face mists among consumers is not only a result of their sensory appeal but also of their functional efficacy in protecting and revitalizing the skin, especially in environments with high levels of air conditioning, pollution, or sun exposure, which can rapidly dehydrate the skin and disrupt its natural defense mechanisms.

Support for the benefits of face mist has also been reinforced by industry experts and cosmetic formulators who emphasize its increasing relevance in daily skincare routines. The use of face mist has evolved from being a simple refreshing spray to a multifunctional product used before, during, and after makeup application to maintain moisture and skin vitality throughout the day. Commercial innovations such as The Revitalizing Mist by La Mer illustrate how face mists can be enhanced with marine nutrients and microemulsion technologies, offering instant soothing and revitalizing effects on irritated skin. Consistent use of face mist can brighten the complexion, improve hydration levels, and reduce dullness,

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particularly when used multiple times daily in urban or dry environments. Beyond hydration, recent advances have revealed the potential of botanical-based face mists as natural alternatives in skin treatment. For instance, natural ingredients such as aloe vera, rose water, and papaya leaf extract have demonstrated anti-inflammatory and antibacterial properties, making them ideal for inclusion in formulations targeting acne-prone or sensitive skin types. These findings underscore the utility of face mist not only as a hydrating agent but also as a safe and effective delivery system for active compounds, eliminating the need for physical contact and thereby minimizing contamination risk. Therefore, the integration of papaya leaf extract into face mist formulations addresses the growing demand for natural, multifunctional, and convenient skincare solutions suitable for modern lifestyles and sensitive skin care.

Research Method

This study employed a laboratory-based experimental design, where the research subjects were exposed to controlled artificial conditions specifically tailored to meet the research objectives. This design enabled researchers to control all variables within the experiment, ensuring the accuracy and reliability of the results (Rosyad *et al.,* 2024). A post-test-only control group design was utilized, which involves conducting observations exclusively at the end of the intervention or treatment (Setyowati *et al.,* 2024). The primary objective of this study was to formulate a face mist preparation containing an ethanol extract of papaya leaves (Carica papaya L.) and to evaluate its antibacterial activity against Propionibacterium acnes. The research was categorized as laboratory experimental research, focusing on the formulation process and antibacterial testing in a controlled environment. The population in this study consisted of 10 kilograms of fresh papaya leaves (Carica papaya L.) collected from the Margoyoso District, Pati Regency. The selected leaves exhibited consistent characteristics, including a single leaf shape, smooth and hollow petioles, a large size, dark green coloration on the upper surface, and light green on the underside. From this population, a 1-kilogram sample of papaya leaves was processed into simplicia and used to prepare extracts. The extract was produced using a 1:10 ratio of maceration with 70% ethanol solvent. This extract was later incorporated into three face mist formulations at varying concentrations: F1 (10%), F2 (15%), and F3 (20%).

Data collection was carried out through experimental observation, specifically to evaluate the physical properties and antibacterial performance of the face mist formulations. The extract preparation involved the maceration method using ethanol 70%, after which the concentrated extract was formulated into face mist preparations. These were tested through several physical quality assessments, including organoleptic evaluation, pH measurement, homogeneity observation, and spreadability tests. For antibacterial activity, the disc diffusion method was applied. This involved impregnating sterile paper discs with the face mist samples and placing them on Nutrient Agar (NA) plates inoculated with Propionibacterium acnes. The plates were then incubated to allow the diffusion of the active substances and the formation of inhibition zones, which were measured to assess antibacterial effectiveness. The data obtained from antibacterial activity tests were recorded in tabular form and analyzed using One-Way Analysis of Variance (ANOVA) with a significance threshold set at $p < 0.005$. This statistical technique was used to determine whether there were significant differences in antibacterial activity among the three face mist formulations with extract concentrations of 10%, 15%, and 20%. The analysis enabled a comparative evaluation of the formulations' effectiveness in inhibiting the growth of Propionibacterium acne, thereby supporting conclusions about the optimal concentration for antibacterial use.

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Results and Discussion

Analysis Result

Plant Determination

The papaya plants obtained were then identified to ensure that they were indeed papaya plants (Carica papaya L.). The purpose of this plant determination is to provide certainty and accuracy that the plants being studied are papaya plants (Carica papaya L.) to prevent errors when collecting research materials (Fadel *et al.,* 2024). Determination was carried out by comparing the morphological characteristics of the plant with those in the authorized literature at the Biology Laboratory at Ahmad Dahlan University (UAD). According to the determination results, the plant used was the papaya plant (Carica papaya L.). The results obtained were valid, with evidence of a determination letter numbered 063/Lab.Bio/B/I/2025 issued by the Biology Laboratory at Ahmad Dahlan University (UAD).

Making Simple Papaya Leaf Drugs (Carica papaya L.)

The processing of papaya leaf samples began with the collection of 5 kg of fresh papaya leaves. The papaya leaves selected were old, intact leaves from the Carica papaya L. type, without holes. After that, the leaves were separated from the other parts of the plant, as only the leaves were used as research samples. After separation, the papaya leaves were wet-sorted to separate the simplicia from other impurities, such as soil. After that, the papaya leaves were cleaned with running water to remove any existing impurities. The papaya leaves were then shredded to increase their surface area, as this increases the rate of the drying process. (Maslahah, 2024). Papaya leaves are then dried in the sun using a woven bamboo container with the top covered in black cloth. Drying is performed to reduce the water content in herbal medicine, preventing it from being easily damaged when stored for an extended period. The use of a woven bamboo container aims to ensure that air flows smoothly from top to bottom (Handoyo & Pranoto, 2020). Additionally, covering the herbal medicine with a black cloth is also intended to prevent the active substances from being damaged by sunlight (Fadhilah *et al.,* 2022). To remove the silica that is too damaged during drying, it is necessary to do dry sorting and then smooth it with a blender. After being smoothed, use sieve no. 40 or 80 to make the powder fine and homogeneous. The purpose of the sieving process is to increase the surface area of the simplicia to facilitate the process of osmosis and diffusion of active compounds in the simplicia, because if the surface area is larger, it will be easier to experience contact with the solvent (Widwiastuti *et al.,* 2022).

Water Content Test

Measuring the water content of the simple drug is necessary because high water content causes the active substances in the extract to break down through enzymatic reactions. Therefore, measuring the water content is crucial for the quality and stability of the extract (Sambode *et al.,* 2022). The water content in the simplicia was tested using a moisture balance by placing 2 grams of simplicia evenly throughout the aluminum so that the needle points to the neutral position. The light was turned on and the temperature was set to 100°C. After that, it was left for 15 minutes, and then the light was turned off. The results of the water content test were then recorded. rded (Dhina *et al.,* 2019). To maintain the quality of the herbal medicine, the standard water content used is no more than 10% (Wandira *et al.,* 2023).

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Table 1. Water Content Test Results

Identification	Replication	Water Content Test Results	Average	Information
Water content test	1	9.11%	8.58%	Qualify
	2	7.67%		
	3	8.98%		

Papaya Leaf Extract (Carica papaya L.)

Papaya leaf simplicia that has been tested for water content is then extracted to extract compounds in the simplicia. Papaya leaf extraction utilizes the maceration method because it is a simple process that does not require heating, thereby minimizing damage to existing compounds. ents (Hasanah & Novian, 2020). The solvent used in maceration is 70% ethanol with a ratio of 1:10. The selection of 70% ethanol solvent is based on the fact that it is more selective towards the compound to be extracted, bacteria and molds are also difficult to grow, in addition, the presence of 30% water content in 70% ethanol is considered to affect the wetting of the simplicia because the solvent can easily penetrate the cell walls of the simplicia (Maharadingga et al., 2021). A ratio of 1:10 during the maceration process can produce more extract because the greater the amount of solvent, the greater the pressure applied, which can increase the maceration process. cess (Asworo & Widwiastuti, 2023). One thousand grams of papaya leaf simplicia powder is soaked in 10 liters of 70% ethanol and left for 3 days, with stirring every 6 hours. The macerate is then concentrated with a rotary evaporator to separate the solvent and the resulting extract. After that, the extract is evaporated using a water bath to remove the remaining solvent, resulting in a thick papaya leaf extract (Kurnia *et al.,* 2023). The results of the extract yield are presented in Table 2.

Table 2. Extract Yield Results

Fresh Plant Weight	Sample Weight	Extract Weight	Yield	Information
10 kg	1 kg	162.7075 g	16,270%	Qualify

Based on Table 2, the results of the thick extract obtained were 162.7075 grams with a yield value of 16.270%. The yield value is according to the literature, where the yield of the extract is known as kentall, yesling, Balbig, iclrnyalHalrus >10% (Ministry of Health of the Republic of Indonesia, 2017).

Ethanol Free Test

The ethanol-free test is conducted to ensure that the extract produced is a pure extract with no ethanol content (Khudzaifi *et al.,* 2022). The absence of ethanol content in the sample can prevent positive results in subsequent treatments due to its antibacterial properties. ties (Sukadiasa *et al.,* 2023). To conduct an ethanol-free test, start by adding 2 mL of acetic acid and 2 mL of H2SO4 to 0.5 g of extract, then heating it. If the ester aroma is no longer detected, it indicates a positive reaction; however, if the ester aroma persists, it means that the esterified ethanol remains (Priamsari & Rokhana, 2020).

Based on Table 3, the test results show that the smell of esterified ethanol is no longer detected. This indicates that the papaya leaf extract (Carica papaya L.) no longer contains 70% solvent.

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Table 3. Ethanol Free Test Results

Identification	Reagent	Extract Name	Results	Information
Ethanol-free test	2 ml CH3COOH + 2 ml H2SO4	Papaya leaf extract (Carica papaya L.)	No ester odor	Qualify

Phytochemical Screening

The extract obtained is then subjected to phytochemical screening, including the flavonoid test, saponin test, alkaloid test, and tannin test. The flavonoid test begins by adding papaya leaf extract, three drops of concentrated hydrochloric acid (HCl), and 2 mg of magnesium powder, then shaking. After shaking, the reaction that occurs is observed. If yellow, orange, and red colors are formed in the solution, it means the sample is positive for flavonoids (Tutik *et al.,* 2021). The saponin test begins by adding 1 mL of papaya leaf extract to 1 mL of warm distilled water, then shaking vigorously for approximately 1 minute. The formation of a stable foam indicates that the extract is beneficial for sap. onin (Adjeng et *al.,* 2019). To perform the alkaloid test, papaya leaf extract is added with two drops of Dreagendroff reagent, and the color change is observed after 30 minutes. The test result is considered positive if it produces an orange precipitate with the Drechsel reagent (Tutik *et al.,* 2021). To test tannins, 0.5 mg of papaya leaf extract was added to a test tube with a few drops of a 1% FeCl3 solution. The reaction that was formed was then observed. If it produces a greenish-black or dark blue color, then the sample is detected to contain tannin compounds (Tutik *et al.,* 2021).

Based on the test results in Table 4, it is evident that the papaya leaf extract is positive for flavonoids, as it produces an orange color. If the flavonoid compound is reduced in the extract with Mg2+ and concentrated HCl, a complex [Mg(OAr)6]4- will form, causing an orange color to appear in the solution (Oktavia & Sutoyo, 2021). The reaction equation is shown in Figure 1.

Table 4. Phytochemical Screening Results

Compound Groups	Reagent	Initial Color	Results	Information
Flavonoid	3 drops of concentrated HCl + 2 mg of Mg powder	Brownish Green	Brownish orange	Positive (+)
Saponins	Warm distilled water + shake vigorously	Brownish Green	Stable Foam	Positive (+)
Alkaloid	Dragendorff	Brownish Green	Orange sediment	Positive (+)
Tannin	FeCl3 1%	Brownish Green	Greenish black	Positive (+)

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Advances in Healthcare Research
Volume 3, Issue 2 (2025)
Website: https://advancesinresearch.id/index.php/AHR
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e-ISSN: 2985-9808

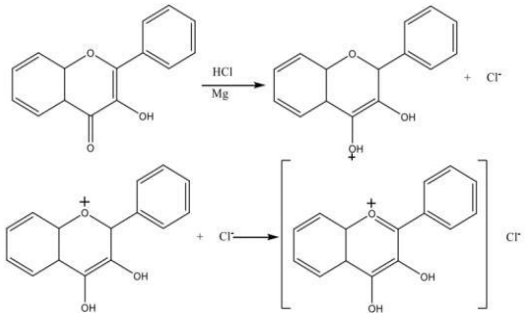

Figure 1. Flavonoid Test Reaction (Oktavia & Sutoyo, 2021)

Based on the test results in Table 4. It is known that the papaya leaf extract is positive for saponin because it forms stable foam with an average height of 0.8 cm. The formation of stable foam is attributed to the ability of glycosides to create a foam in water and subsequently hydrolyze it into glucose and other compounds (Oktavia & Sutoyo, 2021). The reaction equation is shown in Figure 2.

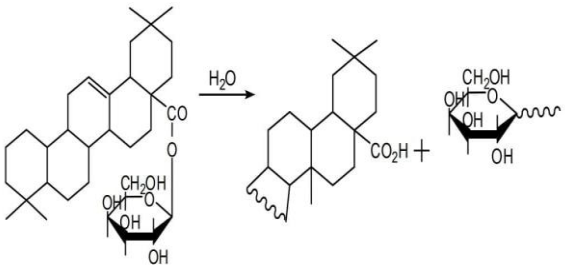

Figure 2. Saponin Test Reaction (Oktavia & Sutoyo, 2021)

Based on the test results in Table 4. It is known that the papaya leaf extract is positive for alkaloids because it produces orange deposits. The formation of potassium-alkaloid complexes causes the deposits. In alkaloid reagents, the free electron pairs on the alkaloid nitrogen atoms will form bonds with K+ ions (Oktavia & Sutoyo, 2021). The reaction equation is shown in Figure 3.

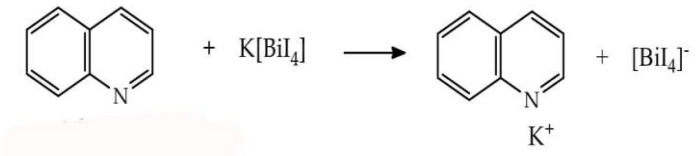

Figure 3. Alkaloid Test Reaction(Oktavia & Sutoyo, 2021)

Based on the test results in Table 4. It is known that papaya leaf extract contains tannin, as it produces a greenish-black color. This greenish-black color is due to the complex compound formed by the reaction of tannin with Fe3+ ions, resulting from the reaction of tannin with polyphenols and ferric chloride (Sulasmi *et al.,* 2019). The reaction equation is in Figure 4.

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Volume 3, Issue 2 (2025)
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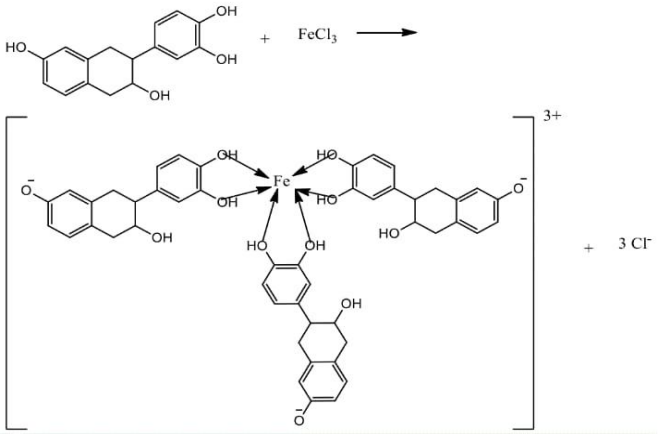

Figure 4. Tannin Test Reaction(Oktavia & Sutoyo, 2021)

Making Face Mist Preparations

The preparation of face mist preparations begins by weighing each ingredient for each formula. For F1 (10%) using 10 grams of extract, F2 (15%) using 15 grams of extract, F3 (20%) using 20 grams of extract, and K- (face mist base) without using extract. For the additional ingredients in F1 (10%), F2 (15%), F3 (20%), and K (face mist base), the following quantities are used: 20 mL of glycerin, 4 grams of PVP, 0.02 grams of methyl paraben, and 100 mL of distilled water. After that, each extract concentration is dissolved in a small amount of distilled water, making it easy to homogenize when added later. A hotplate is prepared to heat the glycerin, because PVP and methyl paraben are easily dissolved in hot glycerin. In the face mist preparation, glycerin functions as a moisturizer (Rowe *et al.,* 2023). After the glycerin has cooled, PVP and methyl paraben are added gradually while stirring with a magnetic stirrer. PVP itself in the preparation functions as an adhesive, while methyl paraben functions as a preservative (Rowe *et al.,* 2023). After being homogeneous, the extract is then added little by little to each formula, and the hotplate temperature is lowered to prevent damage to the chemical compounds in the extract. After everything is homogeneous, 100 mL of distilled water is added while stirring until the mixture is homogeneous. Each formula is then put into a spray bottle, and the physical properties of the preparation are tested.

Figure 5. Face Mist Preparation

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Physical Properties Test of Face Mist Preparations

Organoleptic Test

Organoleptic testing utilizes the human senses to assess the color, shape, and aroma of the preparation (Cendana *et al.,* 2021). The organoleptic test results are presented in Table 5.

Table 5. Organoleptic Test Results

Formulation	Observation result		
	Color	Form	Aroma
F1 (10%)	Brownish green	Liquid	Papaya leaf specialties
F2 (15%)	Brownish green	Liquid	Papaya leaf specialties
F3 (20%)	Brownish green	Liquid	Papaya leaf specialties
K- (face mist base)	Clear	Liquid	No scent

Based on the results obtained (Table 5), F1 (10%), F2 (15%), and F3 (20%) have the same color, shape, and aroma, namely brownish green according to the color of papaya leaf extract, liquid form, and the distinctive smell of papaya leaves. While K- (face mist base) has a transparent color because it contains no additional extracts, it is in liquid form and has no aroma. Face mist preparations are typically made in liquid form, allowing for easy application to the face by spraying. If the form is thick, the face mist may feel sticky, making it uncomfortable to use (Aspia *et al.,* 2024).

pH Test

To measure the pH of the face mist, use a pH meter that has been calibrated using standard buffer solutions of pH four and pH 7 (Muzayanah *et al.,* 2024). The results of the pH test on preparation are presented in Table 6.

Table 6. pH Test Results

Formulation	Results			Average	Condition	Information
	Replication 1	Replication 2	Replication 3			
F1 (10%)	5.09	5.05	5.10	5.08	4.5-6.5	Qualify
F2 (15%)	5.19	5.17	5.12	5.16	4.5-6.5	Qualify
F3 (20%)	5.09	5.07	5.09	5.08	4.5-6.5	Qualify
K- (face mist base)	3.68	3.47	3.42	3.52	4.5-6.5	Not eligible

Based on the results obtained (Table 6), the pH levels in F1 (10%), F2 (15%), and F3 (20%) have met the skin pH requirements, which range between 4.5 and 6.5. Meanwhile, K- (face mist base) does not meet the requirements because it lacks additional extracts and serves only as a negative control in the test. Face mist must meet the skin pH standards, which are between 4.5 and 6.5 (Herliningsih & Anggraini, 2021). A pH value that is too low can irritate the skin, while a pH that is too high can cause dry and itchy skin (Aspia *et al.,* 2024).

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Homogeneity Test

The homogeneity of the preparation is tested by applying it to a glass object and observing whether any remaining substances are homogeneous (Arnandea & Murrukmihadii, 2020). The results of the homogeneity test are presented in Table 7.

Table 7. Homogeneity Test Results

Formula	Results	Condition	Information
F1 (10%)	Homogeneous	Homogeneous	Qualify
F2 (15%)	Homogeneous	Homogeneous	Qualify
F3 (20%)	Homogeneous	Homogeneous	Qualify
K- (face mist base)	Homogeneous	Homogeneous	Qualify

Based on the results obtained (Table 7), F1 (10%), F2 (15%), F3 (20%), and K- (face mist base) did not contain coarse grains; therefore, it can be said that the four preparations have met the homogeneity requirements. This demonstrates that the four formulas are thoroughly distributed, with no components that clump or remain stable during preparation (Aspia *et al.,* 2024).

Spread Power Test

To test the spreadability, the preparation is sprayed at a distance of 5-7 cm on mica plastic, then the spreadability area is measured with a ruler. The spreadability for a good face mist preparation is 5-7 cm (Hayati *et al.,* 2019). The results of the spreadability test are presented in Table 8.

Table 8. Spreadability Test Results

Formula	Replication	Result (cm)	Average (cm)	Terms (cm)	Information
F1 (10%)	1	5.90	5.75	5-7	Qualify
	2	5.25			
	3	6.10			
F2 (15%)	1	5.35	5.33	5-7	Qualify
	2	5.15			
	3	5.50			
F3 (20%)	1	5.45	5.50	5-7	Qualify
	2	5.15			
	3	5.90			
K- (face mist base)	1	5.40	5.56	5-7	Qualify
	2	5.60			
	3	5.70			

Based on the results obtained (Table 8), the four formulas have met the requirements for spreading power in face mist preparations, producing a circular shape and spreading evenly. This demonstrates that the applicator effectively delivers the desired amount of face mist preparation formula, which can be adjusted for each spray (Aspia *et al.,* 2024).

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Identification of Bacteria

Identification of bacteria aims to facilitate their observation microscopically, as well as to clarify their shape (Wardani *et al.,* 2023). Propionibacterium acnes staining begins with one drop of distilled water being dripped onto a glass slide. Then, the bacterial colony is placed on the glass slide and homogenized with distilled water. After that, fixation is carried out. Then, the bacterial colony is stained with crystal violet and left for 3-5 minutes. It is then washed with distilled water and stained with iodine for 1 minute. The resulting color is rinsed with alcohol and then flooded with distilled water. After that, re-staining is carried out with fuchsin (safranin) for 1 minute and rinsed with distilled water. After being rinsed with distilled water, it is then dried and observed under a microscope through the objective lens. (Ritonga et al., 2025). Based on the results of observations using a microscope with 100x magnification, the bacteria are identified as gram-positive due to their purple color. In addition, isolates in the form of bacilli also appeared, meaning that the bacteria have been identified as Propionibacterium acnes. Propionibacterium acnes is a gram-positive bacterium that has a thick cell wall because it consists of layers of peptidoglycan polymers. The thick cell wall prevents crystal violet and iodine from escaping the cell when washed with alcohol, resulting in a purple color because it retains its initial dye (Nurhaini et al., 2024).

Antibacterial Activity Test

The antibacterial activity test begins by first sterilizing all the equipment. The aim is to ensure that the equipment to be used is clean from harmful microorganisms and to prevent contamination (Rizki *et al.,* 2021). After that, NA media was made for bacterial growth media. The selection of NA media itself is because NA media consists of peptone, yeast extract, agar, and water, which will later support the growth of various types of bacteria, including Propionibacterium acnes (Agustina *et al.,* 2025). Additionally, NA media is one of the most frequently used growth media for testing antibacterial activity. (Chezar *et al.,* 2025). After the NA media is prepared, bacterial rejuvenation is carried out on the slant agar, which aims to renew bacterial cells, maintain nutrient availability, and prevent changes in the bacterial culture's characteristics to maximize bacterial growth. (Kantari & Ariyanti, 2024). After that, Mc. The Farland solution was prepared by dissolving 9.95 mL of 1% H2SO4 with 0.05 mL of 1% BaCl2 solution—the function of Mc. The Farland solution is a standard for adjusting the turbidity of bacterial suspensions, ensuring they fall within the specified standard range. nge (Sangkoy et al.,, 2023). A bacterial suspension was then prepared by transferring bacterial colonies cultured from slant agar into 0.9% Sodium Chloride Solution. The suspension was then equalized with McFarland solution until the turbidity was the same. After that, an antibacterial activity test was conducted using the disc diffusion method, in which 20 mL of Nutrient Agar (NA) was poured into a petri dish and allowed to solidify. The choice of the disc diffusion method was based on its simple working method, and it is widely used to test antibiotic sensitivity in quality control programs (Kabakoran *et al.,* 2022). After the media solidifies, the bacterial inoculum is then evenly applied to the media using a sterile cotton swab. After that, the disc paper (6 mm) is placed in a petri dish, each containing a face mist formulation of papaya leaf ethanol extract at concentrations of 10%, 15%, and 20%. For the positive control, a disc of paper containing the antibiotic clindamycin was inserted into each petri dish. For the negative control, a disc of paper containing a face mist base was inserted into each petri dish. The selection of clindamycin as a positive control is due to its frequent use as a topical antibiotic for treating acne vulgaris (Rizki *et al.,* 2021).

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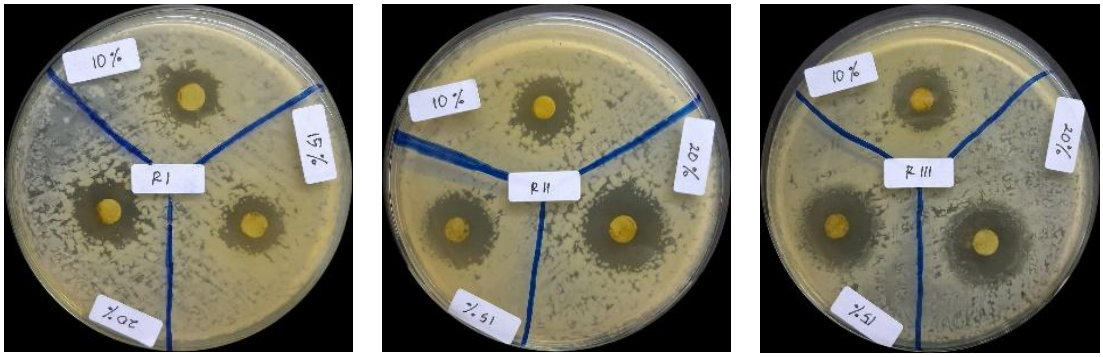
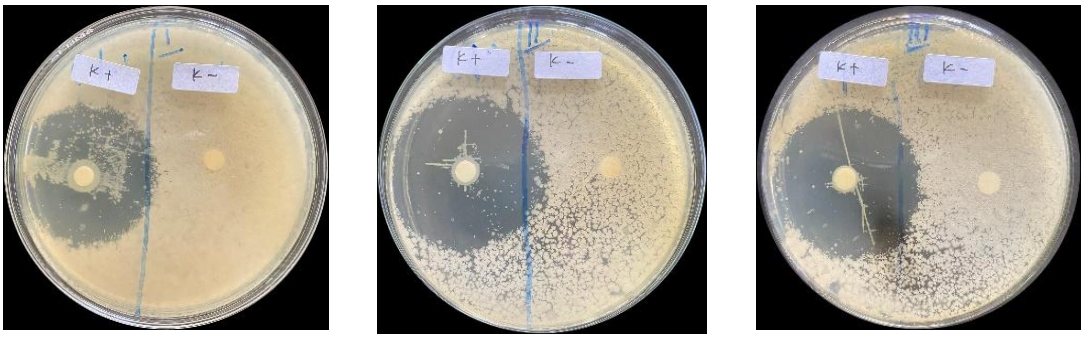
Incubation in this test was carried out for 24 hours at a temperature of 37°C. The incubation temperature of 37°C produced the highest growth compared to temperatures of 30°C and 35°C, which correspond to the optimal growth temperature for Propionibacterium acnes bacteria, similar to the human body temperature (Agustina *et al.,* 2025). After incubation is complete, the inhibition zone is observed and measured to determine its diameter. The presence of a clear area around the disc paper indicates antibacterial activity. The classification of bacterial growth inhibition response is as follows: a diameter of <5 mm indicates weak inhibition, a diameter of 5-10 mm indicates moderate inhibition, a diameter of 10-20 mm indicates potent inhibition, and a diameter of ≥20 mm indicates potent inhibition (Rahmah *et al.,* 2020).



(a) (b) (c)

Figure 6. Results of Antibacterial Test of Face Mist Preparation (a) replication 1 (b) replication 2 (c) replication 3



(d) (e) (f)

Figure 7. Antibacterial Test Results K+ and K- (a) replication 1, (b) replication 2, (c) replication 3

Based on the results obtained (Table 9.) shows that F1 (10%) has moderate inhibition with an average value of the inhibition zone diameter of 9.33 mm, F2 (15%) has potent inhibition with an average value of the inhibition zone diameter of 12.67 mm, F3 (20%) has potent inhibition with an average value of the inhibition zone diameter of 16.33 mm. While the positive control exhibits potent inhibition, with an average inhibition zone diameter of 37.6 mm, the negative control shows no inhibition. These results indicate that papaya leaves have been empirically proven to possess antibacterial properties, as evidenced by their ability to inhibit Propionibacterium acnes bacteria. Although the inhibition is not greater than that of the positive control (Clindamycin), the inhibition value is still considered strong, indicating potential for development as a face mist product with natural ingredients. The antibacterial

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activity in papaya leaves is attributed to the presence of secondary metabolite compounds, including alkaloids, flavonoids, tannins, and saponins, which can inhibit the growth of bacterial cells.

Table 9. Results of Calculation of Antibacterial Test Inhibition Zone

Formula	Observation of Inhibition Zone (mm)			Average	Information
	Replication 1	Replication 2	Replication 3		
F1 (10%)	8.5mm	9.0mm	9.5mm	9mm	Medium inhibition
F2 (15%)	11.5mm	13.0 mm	14.5mm	13mm	Strong inhibition power
F3 (20%)	15.0 mm	16.5mm	17.5mm	16.3 mm	Strong inhibition power
K+ (clindamycin)	37.0 mm	39.5 mm	36.5 mm	37.6 mm	Powerful inhibition force
K- (face mist base)	0 mm	0 mm	0 mm	0 mm	Does not produce inhibition zones

Alkaloids have a substantial ability to inhibit the formation of proteins and nucleic acids in bacterial cells. This interaction causes DNA and RNA to undergo structural changes, preventing them from functioning as standard templates for the biological functions of bacteria. Additionally, alkaloids can alter the permeability of bacterial cell membranes. Because alkaloids such as 8-hydroxyquinoline are highly lipophilic, they can cross bacterial cell membranes to reach their target sites and exert antibacterial effects. Disruption of bacterial metabolic pathways is also an essential aspect of alkaloid antibacterial activity (Thawabteh *et al.,* 2024). Flavonoids are important secondary metabolites that are widely distributed in various plants, and many of them have varying degrees of inhibitory activity against many pathogenic bacteria. Some of them can enhance the antibacterial activity of antibacterial agents. Various antimicrobial mechanisms of flavonoids include inhibition of DNA, protein, and cell envelope biosynthesis, as well as damage to cell membranes. Flavonoids are the primary site of action against Gram-positive bacteria, targeting the cell membrane and causing damage to the phospholipid bilayer, as well as inhibiting the respiratory chain (Yan *et al.,* 2024). Saponins are compounds produced from secondary plant metabolism and act as a chemical barrier between plants and pathogens. According to many researchers, saponins are similar to detergents, possessing not only antibacterial, antiprotozoal, and insecticidal properties, but also anticancer properties. Saponins can increase the hydrophobicity of cell surfaces and reduce the fluidity of cell membranes by changing the fatty acid composition of cell membranes. In addition, saponins also bind to lipids, increasing the permeability of the outer membrane of bacterial cell walls and facilitating the penetration of antibiotics into bacterial cells. (Alina et al.,, 2023). Tannins are antibacterial agents that disrupt cell metabolism and penetrate the bacterial cell wall to reach the internal membrane, thereby destroying the bacteria. Tannin activity increases rapidly in Gram-positive bacteria, whereas in Gram-negative bacteria, tannin activity is slowed down due to the presence of a double-layered membrane. Tannins also inhibit bacterial attachment to the surface. Lack of bacterial attachment to the surface causes bacteria to become lysed. Additionally, tannins also inhibit the absorption of sugars and amino acids, thereby inhibiting bacterial growth (Kaczmarek, 2020).

The data obtained were then analyzed, including a normality test, a homogeneity test, an ANOVA test, and a Post Hoc test. A normality test was conducted to determine whether the data had a normal distribution (Monoarfa *et al.,* 2022). The normality test using Shapiro-Wilk is a test used because

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the sample in this study was <50. A significance figure greater than 0.05 indicates that the data is usually distributed, while a significance figure smaller than 0.05 indicates that the data is not normally distributed (Khudzaifi *et al.,* 2024). Analysis of antibacterial activity test data using a normality test reveals that the three formulas, positive control, and negative control have a significance value of more than 0.05, indicating that the data have a normal distribution. After determining that the data is normally distributed, a homogeneity test is then conducted to verify that the sample group originates from a population with the same variation (Monoarfa *et al.,* 2022). This study employs the Levene statistical test, where a significance level of>0.05 indicates homogeneous data variation, while a significance level of <0.05 indicates non-homogeneous data variation (Khudzaifi *et al.,* 2024). The results of the antibacterial activity test data analysis, using a homogeneity test, showed a significance value of greater than 0.05, indicating that the data variation is considered homogeneous. After the data is typically distributed and homogeneous, an ANOVA test is then conducted to determine whether two or more groups exhibit significant differences (Monoarfa *et al.,* 2022). The results are considered statistically significant if the p-value is less than 0.05 (Fadel *et al.,* 2024). The results of data analysis with the ANOVA test showed a significance value of <0.05, so it can be said that the average antibacterial activity in each formulation has a significant difference. After conducting the ANOVA test, a Post Hoc test was then performed to determine whether there were significant differences among three or more groups. This Post Hoc test uses the Tukey test, where the data group is considered to have a substantial difference if the significance value is less than 0.05 (Nurmasliah *et al.,* 2022). The results of the analysis with the Post Hoc Test showed that each data point had a significance value of <0.05, so it can be said that the average antibacterial activity in each formulation has a significant difference.

Conclusion

This study was designed to formulate a face mist preparation using ethanol extract of papaya leaves (Carica papaya L.) at varying concentrations (10%, 15%, and 20%) and to evaluate its potential antibacterial activity against Propionibacterium acnes. Through a structured experimental design and a series of laboratory tests, the research successfully answered its core question—whether papaya leaf extract can be effectively incorporated into a topical spray formulation while maintaining favorable physical properties and demonstrating antibacterial potential. The study followed a systematic approach, including formulation development, organoleptic testing, pH and spreadability evaluation, and antibacterial activity analysis, thereby fulfilling its objective of producing a natural-based skincare solution that targets acne-causing bacteria.

This research makes both theoretical and practical contributions to the growing field of herbal cosmetic innovation. Scientifically, it confirms the feasibility of transforming raw plant-based antibacterial compounds into a functional and consumer-friendly skincare product. Practically, it offers a novel solution for individuals seeking natural alternatives to synthetic acne treatments, with the added benefits of user convenience and hygienic application. From a managerial perspective, the findings open opportunities for cosmetic producers and formulators to develop environmentally friendly and market-relevant skincare products, particularly face mists that meet consumer demands for efficacy, safety, and ease of use. The originality of this study lies in its integration of papaya leaf extract into a formulated spray rather than evaluating the extract in isolation.

This study had several limitations. It was conducted entirely under laboratory conditions, without in vivo or consumer-based testing, which may limit the generalizability of its findings to real-

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world applications. Additionally, the study did not explore the long-term stability or potential side effects of the formulation. Future research is encouraged to expand on this study by including broader microbiological evaluations, conducting dermatological testing in clinical settings, and exploring additional concentrations or formulation enhancements. It is also recommended to examine the product's shelf life, sensory acceptability, and effectiveness over time, to ensure it can be safely and effectively commercialized as a natural anti-acne solution.

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Corresponding author

Muhammad Nurul Fadel can be contacted at: nurulfadel@umkudus.ac.id

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