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Original Research Article

Differential Testing of the Effectiveness of Anti-Acne Agents Niacinamide and Melaleuca Alternifolia on the Growth of Propionibacterium Acnes

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Abstract: ***Introduction*:** Acne Vulgaris is a multifactorial disorder affecting various parts of the pilosebaceous unit and ranks second in the occurrence of skin diseases. The primary line of therapy for acne vulgaris involves antibiotics. However, prolonged and monotherapy use of antibiotics may lead to resistance. Therefore, an alternative approach is necessary for managing acne vulgaris, such as utilizing anti-acne compounds. ***Aims*:** The aim of this study is to evaluate the differences in the effectiveness of the anti-acne compounds niacinamide and melaleuca alternifolia in inhibiting the growth of Propionibacterium acnes, both as individual agents and when combined with other anti-acne compounds. ***Method*:** This research employs a true experimental design with a posttest-only control group design. The statistical analysis utilized is the One Way ANOVA test, with the diffusion disc method employed for testing. ***Results*:** The results of antibacterial activity testing indicate that both melaleuca alternifolia and niacinamide are effective in inhibiting the growth of Propionibacterium acnes. The One Way ANOVA analysis yields a p-value of 0.000, smaller than $\alpha = 0.005$, signifying a significant difference in mean values among the sample groups. ***Conclusion*:** The anti-acne compounds niacinamide and melaleuca alternifolia exhibit antibacterial activity in inhibiting the growth of Propionibacterium acnes. Nevertheless, melaleuca alternifolia demonstrates higher effectiveness compared to niacinamide in inhibiting the growth of Propionibacterium acnes.

Keywords: Acne Vulgaris, Anti Acne, Bacterial Skin Infection, Niacinamide, Melaleuca Alternifolia, Propionibacterium Acnes.

INTRODUCTION

Acne vulgaris, commonly known as acne, is the most frequently encountered skin disorder and may present with both inflammatory and non-inflammatory lesions. Acne vulgaris is a multifactorial disorder involving various components of the pilosebaceous unit. (Shalita *et al*., 2011) According to data from the Global Burden of Disease (GBD) 2013, acne vulgaris ranks as the second most prevalent skin disease worldwide and occupies a dominant position among dermatological conditions. (Chen *et al*., 2022)

The management of acne vulgaris consists of topical and systemic therapies. Topical antibiotic therapy is considered a first-line treatment for mild to moderate acne due to its activity against *Propionibacterium acnes* and its anti-inflammatory effects. However, both topical and oral antibiotic therapies are not recommended as monotherapy, as they may lead to antimicrobial resistance and are not advised for long-term use. Acne treatment commonly begins with facial cleansing using anti-acne cleansers containing various active ingredients such as salicylic acid, benzoyl peroxide, and triclosan. (Sibero *et al*., 2019) In addition to these agents, other anti-acne ingredients with comparable efficacy in the management of acne vulgaris include niacinamide and Melaleuca alternifolia (tea tree).

Compared to *Melaleuca alternifolia*, which has long been recognized for its extensive medical applications, niacinamide (vitamin B3) plays a central

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role in numerous biochemical reactions, including cellular energy production, glucose metabolism, and lipid synthesis. Niacinamide is known to enhance skin barrier function by increasing ceramide and free fatty acid levels and possesses broad anti-inflammatory activity. (Boo, 2021; Farris & Lain, 2022) A study conducted by Walocko *et al.*, (2017) in Michigan investigating the role of niacinamide in acne management demonstrated that both topical and oral niacinamide significantly reduced acne severity compared with other topical or oral therapies. (Walocko *et al*., 2017)

In a study conducted by Sartini and Karim (2018) in Medan, the effectiveness of six anti-acne facial cleansers on the growth of *Propionibacterium acnes* was evaluated. The study found that facial cleansers containing salicylic acid and Melaleuca alternifolia exhibited the greatest inhibitory effect on the growth of *Propionibacterium acnes*. (Sartini & Karim, 2018)

These findings motivated the authors to investigate the comparative effects of *Melaleuca alternifolia* (tea tree) and niacinamide as anti-acne agents, as well as the effectiveness of both ingredients when combined with other anti-acne components in various anti-acne facial cleansing products. Therefore, the scope of this study is to experimentally evaluate and compare the antibacterial activity of niacinamide and *Melaleuca alternifolia* against *Propionibacterium acnes* under controlled in vitro conditions, using an agar diffusion method and a posttest-only control group design.

This research encompasses a comparison between pure active compounds and commercially formulated anti-acne facial cleansers, with antibacterial effects assessed across multiple experimental and control groups. The novelty of this study lies in its direct comparative analysis between formulated products and their corresponding pure compounds, providing insight into how formulation with additional anti-acne ingredients influences antibacterial effectiveness and offering a bridge between laboratory-based compound evaluation and real-world anti-acne product formulations.

METHODS

This study was conducted at the Laboratory of the Medical Education Study Program, Universitas Nusa Cendana, from August to October 2023. The study employed a true experimental design using a posttest-only control group design. The antibacterial activity was evaluated using the agar diffusion method. This study did not involve human participants or experimental animals; therefore, ethical committee approval was not required.

Bacterial Strain and Test Materials

The bacterial strain used in this study was *Propionibacterium acnes*, which was obtained from the Center for Health Laboratory (Balai Besar Laboratorium Kesehatan), Surabaya, Indonesia. The strain was used as the test organism to evaluate antibacterial activity. Two commercially available anti-acne facial cleansers and two pure anti-acne compounds were used in this study. The tested materials consisted of anti-acne facial cleansers containing *Melaleuca alternifolia* (tea tree) and niacinamide, as well as pure powdered *Melaleuca alternifolia* and niacinamide. The facial cleansers were purchased from several supermarkets in Kupang City, East Nusa Tenggara, Indonesia. The pure anti-acne compounds were obtained from CV Biotech Chemical and were diluted using sterile distilled water prior to testing.

The positive control used in this study was clindamycin antibiotic. Clindamycin was finely ground and diluted in sterile distilled water before application. Sterile distilled water was used as the negative control. Sterile distilled water is purified water obtained through a distillation process and does not possess antibacterial activity.

Table 1 : Anti-acne Active Ingredients Used

Product Name	Type of Product	Anti-acne Active Ingridients
TT	Facial cleanser	*Melaleuca alternifolia*
Melaleuca alternifolia	Pure powder	*Melaleuca alternifolia*
CC	Facial cleanser	*Niacinamide*
Niacinamide	Pure powder	*Niacinamide*

Sample Size

The sample size in this study was divided into six separate groups, consisting of two control groups and four experimental test groups. The determination of the number of repetitions for each group was calculated using Federer's formula, which indicated that four repetitions were required for each group, with a total of six treatment groups. Therefore, the total number of samples required was 24 samples.

Experimental Procedure

The experimental procedure began with obtaining official permission for laboratory use. This was followed by the preparation of all instruments and materials, including sterilization procedures to ensure aseptic conditions throughout the study.

Subsequently, bacterial confirmation testing was performed using Gram staining to verify the identity and purity of the *Propionibacterium acnes* strain. Culture

media were then prepared, and bacterial rejuvenation was conducted to obtain actively growing bacterial cultures.

Preparation of the positive and negative control solutions was carried out, followed by the preparation of the experimental samples, which consisted of four test groups. A bacterial suspension was prepared and standardized using McFarland densitometry to ensure uniform bacterial concentration.

The antibacterial activity test was conducted using the disc diffusion method. After incubation, the results were observed and the inhibition zones were measured. Finally, the data obtained were analyzed statistically, and the research findings were compiled and documented.

RESULTS

In the initial phase of this study, a bacterial confirmation test was performed to ensure the purity of the bacterial strain used in the experiment. The Gram staining results showed that the test bacteria appeared purple with a rod-shaped (bacillary) morphology, which is consistent with the characteristics of *Propionibacterium acnes*, namely Gram-positive bacteria with a pleomorphic rod-shaped morphology (16).

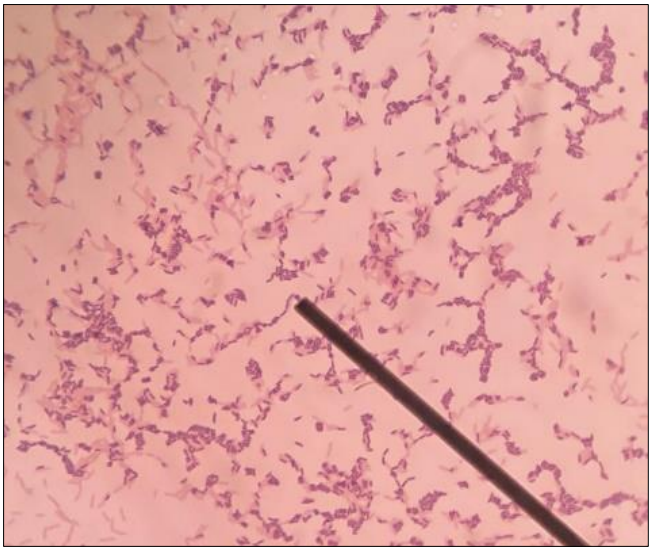

Figure 1 : Gram Staining Test of Propionibacterium acnes

In the subsequent stage of this study, the antibacterial activity of anti-acne facial cleansers containing *Melaleuca alternifolia* (TT) and pure *Melaleuca alternifolia*, as well as anti-acne facial cleansers containing niacinamide (CC) and pure niacinamide, was evaluated. The positive control used in this study was topical clindamycin, while the negative control was sterile distilled water. Antibacterial activity was indicated by the diameter of the inhibition zone formed or by the absence of bacterial growth surrounding the paper discs (formation of a clear zone). The results of the antibacterial activity testing of the anti-acne ingredients niacinamide and *Melaleuca alternifolia* are presented in the figure below.

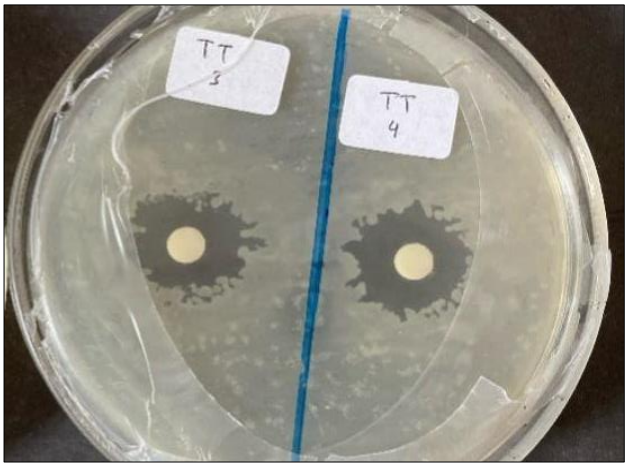

Figure 2 : Results of Antibacterial Activity Test Anti-acne Facial Cleanser Containing Melaleuca alternifolia (TT)

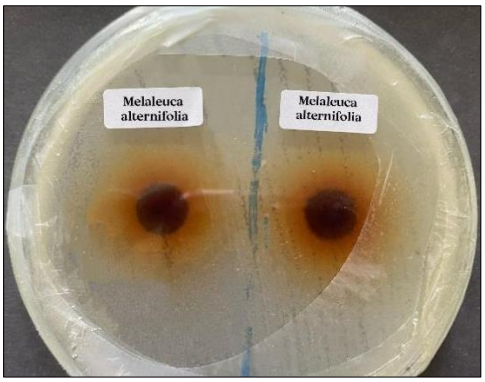

Figure 3 : Results of Antibacterial Activity Test Pure Melaleuca alternifolia

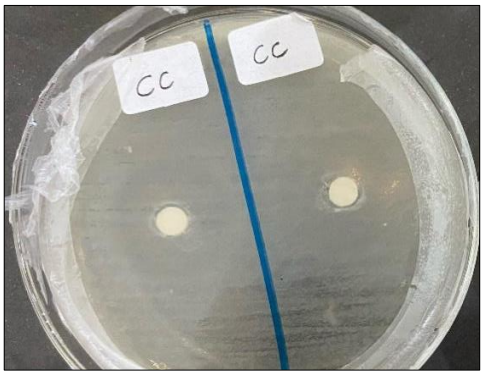

Figure 4 : Results of Antibacterial Activity Test Anti-acne Facial Cleanser Containing Niacinamide (CC)

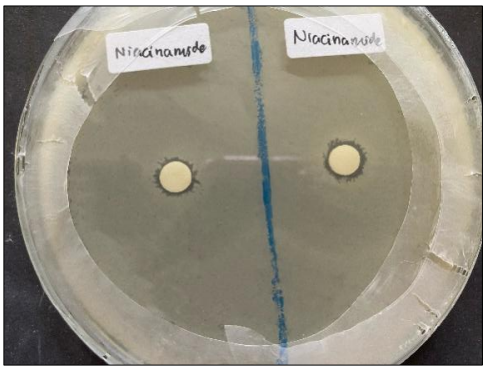

Figure 5 : Results of Antibacterial Activity Test Pure Niacinamide

After measuring the diameter of the inhibition zones against the growth of *Propionibacterium acnes* for each test group, the results of the antibacterial activity assessment of niacinamide and *Melaleuca alternifolia* are presented in the following table.

Table 2 : Results of Inhibition Zone Diameter Measurements of Anti-acne Ingredients Niacinamide and Melaleuca alternifolia Against the Growth of Propionibacterium acnes

Type of Product	Inhibition Zone Diameter (mm)				
	1	2	3	4	Mean
Control (+)	28,85 mm	27 mm	30 mm	28,2 mm	28,5 mm
Sample 1	9,6 mm	10,06 mm	14,3 mm	14,4 mm	12,09 mm
Sample 2	7,5 mm	7,5 mm	7,2 mm	6,3 mm	7,12 mm
Sample 3	7,2 mm	5,18 mm	4 mm	5,2 mm	5,4 mm
Sample 4	4,3 mm	0	5,2 mm	4,8 mm	3,6 mm
Control (-)	0	0	0	0	0

Notes:
Sample 1: Anti-acne Facial Cleanser Containing Melaleuca alternifolia (TT)
Sample 2: Pure Melaleuca alternifolia
Sample 3: Anti-acne Facial Cleanser Containing Niacinamide (CC)
Sample 4: Pure Niacinamide

Data Analysis

The data analysis phase of this study began with univariate analysis, specifically the Shapiro–Wilk normality test, due to the sample size being fewer than 50. (Quraisy, 2020) The results of the normality test indicated that all data were normally distributed, with p-values greater than 0.05. Based on the normal distribution of the data, statistical analysis was continued using the One-Way Analysis of Variance (One-Way ANOVA). (Muhson, 2016)

The results of the One-Way ANOVA showed a p-value of 0.000, which was lower than the significance level of $\alpha = 0.05$. This finding indicates that there were statistically significant differences in mean inhibition zone diameters of the anti-acne ingredients niacinamide and *Melaleuca alternifolia* against the growth of *Propionibacterium acnes*.

Subsequently, a homogeneity of variance test was performed using Levene's test, which yielded a p-value < 0.05, indicating that the data were not homogeneous. Therefore, a post hoc analysis was conducted using the Dunnett T3 test to identify groups with statistically significant differences in mean values.

The results of the Dunnett T3 test demonstrated significant differences ($p < 0.05$) in several group comparisons, namely between sample 1 and sample 4, positive control, and negative control; between sample 2 and positive and negative controls; between sample 3 and positive and negative controls; between sample 4 and sample 1 and negative control; between the negative control and samples 1, 2, 3, and the positive control; as well as between the positive control and all sample groups and the negative control.

DISCUSSION

Based on a study conducted by Walocko *et al*., (2017) in Michigan regarding the role of niacinamide in acne management, positive outcomes were observed in both topical and oral formulations. Niacinamide was shown to significantly reduce acne severity when compared with other topical or oral therapies. (Walocko *et al*., 2017) These findings were further supported by a study by Shih *et al*., (2021), which investigated the activation of Deoxyribonuclease I by niacinamide as a single agent and demonstrated its ability to reduce the thickness of tetracycline-resistant *Propionibacterium acnes* biofilms to a certain extent. (Shih *et al*., 2021)

Niacinamide is the amide form of vitamin B3 and is water-soluble. It functions as a precursor of the cofactors NAD and NADP, which play crucial roles in biochemical reactions, including cellular energy production, glucose metabolism, and lipid synthesis. Unlike retinoids and hydroxy acids, niacinamide is non-irritating. It is also known to enhance skin barrier function by increasing ceramide and free fatty acid levels. (Boo, 2021; Farris & Lain, 2022) In vitro studies have shown that niacinamide can reduce cytokine secretion, including interleukin-8, produced by keratinocytes in response to pathogens such as *Propionibacterium acnes*. (Farris & Lain, 2022)

In a study conducted by Sartini and Karim (2018) in Medan, the effectiveness of six anti-acne facial cleansers against the growth of *Propionibacterium acnes* was evaluated. The results demonstrated that facial cleansers containing salicylic acid and *Melaleuca alternifolia* exhibited the most significant inhibitory effects on the growth of *Propionibacterium acnes*. (Sartini & Karim, 2018) These findings were further supported by a literature review conducted by Shamshad Ahmad *et al*., (2019), which reported that the active component of *Melaleuca alternifolia*, particularly terpinen-4-ol, possesses broad-spectrum antimicrobial activity. In addition, *Melaleuca alternifolia* has various pharmacological properties, including antibacterial, antifungal, anti-inflammatory, antioxidant, antitumor, and immunomodulatory effects. (Ahmad *et al*., 2019)

Compounds derived from this plant, namely terpinen-4-ol and α-terpineol, exhibit high solubility and demonstrate significant antibacterial activity. (Carson *et al.,* 2006) Terpinen-4-ol has been shown to reduce the production of interleukin-1 (IL-1), IL-8, IL-10, prostaglandin E2, and tumor necrosis factor (TNF). These water-soluble compounds are also capable of suppressing superoxide production by monocytes. Furthermore, terpinen-4-ol has the ability to modulate vasodilation and plasma extravasation. (Ahmad *et al*., 2019; Boo, 2021) These mechanisms explain why both niacinamide and *Melaleuca alternifolia* are able to inhibit the growth of *Propionibacterium acnes*.

CONCLUSION

Based on the findings of this study, it can be concluded that Melaleuca alternifolia and niacinamide exhibit greater antibacterial effectiveness when combined with other anti-acne ingredients in anti-acne facial cleansers compared to their performance as single agents. In this context, Melaleuca alternifolia demonstrated superior antibacterial activity, both in its pure form and when formulated in facial cleansing products, compared with niacinamide.

Furthermore, there were significant differences in the mean diameters of inhibition zones between the pure forms of Melaleuca alternifolia (tea tree) and niacinamide and their respective combinations with other anti-acne ingredients in various anti-acne facial cleansers. The measured inhibition zone diameters indicated that anti-acne facial cleansers containing Melaleuca alternifolia exhibited strong antibacterial activity (12.09 mm), while pure Melaleuca alternifolia showed moderate activity (7.12 mm). In comparison, anti-acne facial cleansers containing niacinamide demonstrated moderate antibacterial activity (5.4 mm), whereas pure niacinamide exhibited weak antibacterial

activity (3.6 mm). These findings highlight the importance of formulation strategies in enhancing the antibacterial efficacy of anti-acne products.

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