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Formulation of a sheet mask essence incorporating Betel leaf (*Piper betle* L.) ethanol extract and its antibacterial efficacy against *Propionibacterium acnes*

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ABSTRACT

Betel leaf (*Piper betle* L.) encompasses a multitude of bioactive constituents, including tannins, saponins, flavonoids, alkaloids, and terpenoids, which possess significant antibacterial properties. An ethanol extract derived from betel leaf was meticulously formulated and assessed for its potential as an antibacterial agent specifically targeting *Propionibacterium acnes*, a bacterium associated with the etiology of acne. This investigation was undertaken with the aim of mitigating the antibiotic resistance commonly observed with traditional acne therapies. The ethanol extract of betel leaf was incorporated into a sheet mask essence, resulting in the development of four distinct formulations (F0, F1, F2, and F3), which were subsequently subjected to comprehensive evaluations of their physical quality and stability. The formulation exhibiting optimal physical characteristics and stability was further scrutinized for its antibacterial efficacy against *Propionibacterium acnes*. The antibacterial performance of the selected formulation was benchmarked against a positive control comprising a sheet mask infused with clindamycin gel, as well as another containing tea tree oil. Empirical results identified Formula 2, containing 1.5% ethanol extract of betel leaf, as the most efficacious formulation. Formula 2 demonstrated pronounced antibacterial activity against *Propionibacterium acnes*, with an inhibitory zone measuring 24.67 mm. Although this inhibitory zone was slightly less extensive than that produced by the clindamycin gel sheet mask (32.00 mm), it was comparable to the tea tree oil sheet mask, which exhibited an inhibitory zone of 23.00 mm. In conclusion, the selected sheet mask formulation (Formula 2) exhibits significant antibacterial activity against *Propionibacterium acnes*, surpassing the efficacy of commercially available tea tree oil sheet masks, thereby presenting a promising alternative for acne management with a diminished risk of promoting antibiotic resistance.

Keywords: sheet mask essence, betle leaf (*Piper betle* L.), antibacterial, *Propionibacterium acnes*

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INTRODUCTION

Acne vulgaris, colloquially known as acne, represents a chronic dermatological disorder that originates within the pilosebaceous units and is predominantly marked by inflammatory reactions. It is identified as the second most common dermatologic condition, with only dermatitis occurring more frequently. Remarkably, this condition affects over 85% of adolescents worldwide, and its manifestations can persist into adulthood (Aslan Kayiran et al., 2020; Cong et al., 2019). In Indonesia, the prevalence of acne is observed to range from 80% to 85% among adolescents, with the highest incidence occurring between the ages of 15 and 18 years. Additionally, the prevalence is reported to be 12% among women over the age of 25 years, and 3% in individuals aged 35 to 44 years (Xu & Li, 2019). The severity and persistence of acne are profoundly influenced by a multitude of factors, encompassing environmental determinants (such as ultraviolet radiation), hormonal variations, genetic susceptibility, psychological stressors, lifestyle behaviors, and bacterial colonization (Dreno et al., 2018; Moradi Tuchayi et al., 2015). Among the numerous contributing factors, the colonization by the bacterium *Propionibacterium acnes* is hypothesized to play a crucial role in the pathogenesis of acne, primarily by eliciting an inflammatory response and subsequent cutaneous inflammation.

Acne management can be effectively achieved through the reduction of bacterial populations using both topical and systemic antibiotics. Topical antibiotics, such as erythromycin and clindamycin, are utilized for their potent bactericidal activity against *Propionibacterium acnes*. Simultaneously, oral antibiotics, including tetracycline, azithromycin, and trimethoprim/sulfamethoxazole, demonstrate dual functionalities, encompassing both anti-inflammatory properties and antimicrobial efficacy (Luk et al., 2013; Xu & Li, 2019). The administration of antibiotics has been concomitant with the emergence of resistance. In 1979, antibiotic resistance in *Propionibacterium acnes* was first documented in the United States. Subsequently, a multitude of other countries have reported an escalating trend in antibiotic resistance. Among the studies documented, resistance to erythromycin and clindamycin was most prevalent, followed by resistance to tetracycline (Luk et al., 2013). In Indonesia, the prevalence of antibiotic resistance in *Propionibacterium acnes* is reported to be 45.2% for erythromycin, 61.3% for clindamycin, and 12.9% for tetracycline (Luk et al., 2013).

In light of the burgeoning issue of antibiotic resistance, there is an imperative need to reassess the reliance on antibiotics as the principal therapeutic strategy for acne treatment, in order to curtail the proliferation of resistant bacterial strains. Alternative therapeutic approaches, particularly those derived from natural sources such as betel leaf (*Piper betle* L.), are gaining attention as viable substitutes. Betel leaf, a historical staple in traditional medicinal practices, has been well-documented for its potent antibacterial properties. Notably, a study entitled "Antibacterial Activity Test of Purified Extract of Green Betel Leaf (*Piper betle* L.) against *Propionibacterium acnes*" demonstrated that a purified extract at a concentration of 20 mg/mL exhibited significant antibacterial efficacy against *Propionibacterium acnes* (Dahal et al., 2017). The antimicrobial efficacy of betel leaf can be ascribed to the presence of a diverse array of bioactive constituents, such as tannins, flavonoids, alkaloids, and terpenoids (Nayaka et al., 2021). However, the direct topical application of betel leaf extract encounters practical impediments. Consequently, it is essential to develop a formulation that augments its usability.

Sheet masks are contoured facial sheets saturated with a nutrient-dense solution referred to as essence. Their application necessitates only 15-20 minutes. Sheet masks have emerged as one of the most recent trends, garnering widespread popularity across Asia. When compared to other mask formulations, sheet masks demonstrate enhanced absorption capabilities, provide efficient and sanitary single-use packaging, and obviate the necessity for cleansing following application (Perugini et al., 2018). The development and enhancement of mask products incorporating a thin, dry layer, often termed as sheet masks, present substantial advantages owing to their customizable cut and shape. This study aimed to develop a sheet mask essence utilizing the ethanol extract of betel leaf and evaluate its antibacterial efficacy against *Propionibacterium acnes*.

MATERIALS AND METHOD

Materials

In this study, a fine powder of betel leaves, characterized by its yellowish-brown color and distinctive betel leaf aroma, was procured from CV. Lansida Group Yogyakarta, Indonesia, was utilized. The reagent employed to prepare the betel leaf ethanol extract was 96% ethanol p.a., purchased from PT. Brataco Purwokerto, Indonesia. Additional substances included polyethylene glycol (PEG)-40 hydrogenated castor oil (CV. Sentana Sempurna Makassar, industrial grade), butylene glycol (CV. Sentana Sempurna Makassar, industrial grade), glycerin (PT. Brataco Purwokerto), xanthan gum (CV. Sentana Sempurna Makassar, industrial grade), methyl paraben (PT. Brataco Purwokerto), triethanolamine, rose oil (CV. Sentana Sempurna Makassar), distilled water (PT. Brataco Purwokerto), and dimethyl sulfoxide (DMSO, PT. Brataco Purwokerto). The compressed sheet masks were sourced from Miniso®, and the Mueller Hinton Agar (MHA) from Oxoid®. The positive controls comprised clindamycin gel and tea tree oil sheet masks. All materials required for the antibacterial activity assays were supplied by the Microbiology Laboratory, Faculty of Medicine, and the Faculty of Biology at Universitas Jenderal Soedirman, Purwokerto, Indonesia. The materials also included McFarland solution 0.5, 0.9% NaCl solution, and *Propionibacterium acnes* suspension.

Methods

Extraction of betel leaf

Betel powder was subjected to maceration utilizing 96% ethanol p.a. at a ratio of 1:7. The maceration process was executed over a duration of 48 hours, followed by at least two additional remaceration steps. The resultant macerate was subsequently filtered and evaporated using a water bath until a concentrated betel leaf extract was achieved.

Formulation of sheet mask essence

The extract was solubilized in ethanol (referred to as mass I). Methylparaben was dissolved in water at 70°C (designated as mass II). Butylene glycol, glycerin, and PEG-40 hydrogenated castor oil were thoroughly homogenized (termed as mass III). Masses I, II, and III, along with triethanolamine, were combined and mixed until a homogeneous solution was achieved. Subsequently, distilled water was incorporated, and the mixture was stirred using a magnetic stirrer at 60°C and 800 rpm for 10 minutes. Following this, xanthan gum was dispersed in distilled water and stirred with a magnetic stirrer at 60°C and 1000 rpm for 20 minutes.

Table 1. The formulation of the material for the sheet mask essence

Material	Function	Concentration (%)			
		F0	F1	F2	F3
Betel leaf ethanol extract	Active substance	0	1	1.5	2
PEG-40 Hydrogenated Castor Oil	Surfactant	0.2	0.2	0.2	0.2
Butylene glycol	Humectant	5	5	5	5
Glycerin	Humectant	5	5	5	5
Xanthan gum	Viscosity-increasing agent	0.2	0.2	0.2	0.2
Triethanolamine	Alkalizing agent	4	4	4	4
Methylparaben	Preservative	0.3	0.3	0.3	0.3
Ethanol	Cosolvent	3	3	3	3
Rose oil	Perfume	1 drop	1 drop	1 drop	1 drop
Distilled water (up to)	Solvent	100	100	100	100

The xanthan gum mixture was then integrated, and one drop of rose oil was added. The volume was adjusted to 100 mL with distilled water. This solution was further stirred with a magnetic stirrer for 20 minutes until a homogeneous mixture was achieved. Subsequently, 15 mL of each formulation was dispensed into separate containers. The compressed sheet masks were immersed in the respective essence formulations and allowed to absorb the solution. The compositions of the sheet mask essences are detailed in Table 1.

Physical quality testing

All formulations were meticulously stored and systematically subjected to a battery of tests on days 0, 7, 14, 21, and 28. The parameters evaluated in this comprehensive study encompassed organoleptic properties, homogeneity, pH levels, and viscosity.

Organoleptic tests

This study endeavors to meticulously assess the physicochemical properties of the formulation, encompassing its morphological features, chromatic characteristics, and olfactory attributes. An optimal formulation is defined as one that maintains its stability and exhibits no discernible alterations throughout the storage period (Perugini et al., 2020).

Homogeneity tests

This investigation aims to assess the uniformity of the preparation. An aliquot of the preparation, when applied to a glass slide or an equivalent transparent substrate, should demonstrate a homogeneous and consistent composition, free from any discernible granularity. The evaluation is performed by dispensing 1 mL of the sheet mask essence onto two transparent glass slides, followed by a meticulous examination of its uniformity (Perugini et al., 2020).

pH tests

This investigation endeavors to determine the safety profile of the formulation, specifically assessing its potential to induce dermal irritation upon application. The evaluation is meticulously conducted utilizing a calibrated pH meter. Accurately measure 2 mL of the sheet mask essence, dissolve it in 20 mL of distilled water, and permit the pH meter to stabilize until a constant reading is obtained (Perugini et al., 2020).

Viscosity tests

The primary aim of this experiment is to determine the viscosity of the formulation. The measurement was performed employing a Brookfield viscometer. The essence of the sheet mask was transferred into a beaker and subsequently assessed using spindle number 63 at a rotational speed of 12 rpm until a stable viscosity reading was obtained (Leblanc et al., 1999).

Physical stability testing

The primary aim of the stability assessment is to determine the resilience and robustness of the formulation. The formulations were subjected to a stability evaluation utilizing the freeze-thaw methodology, conducted across six cycles (Chen et al., 2021). Each formulation was stored at 4°C for a period of 48 hours, followed by 40°C for another 48 hours, comprising one complete cycle. Upon the conclusion of each cycle, the preparation was meticulously scrutinized for any changes in morphology, color, and odor.

Antibacterial activity testing

Twenty milliliters of Mueller-Hinton Agar (MHA) medium were aseptically dispensed into a sterile Petri dish. The bacterial suspension was uniformly inoculated onto the surface of the MHA medium using a sterile cotton swab. Subsequently, the sheet mask was immersed in the specified formulations,

which included the positive control, negative control, and extract solution in 10% dimethyl sulfoxide (DMSO). The assay involved placing the saturated sheet mask onto the MHA medium for a period of three minutes. The inoculated culture media were subsequently incubated at 37°C for a duration of 24 hours. The antibacterial efficacy was assessed by observing the presence of an inhibition zone encircling the sheet mask specimens. The experiment was conducted in triplicate (Balouiri et al., 2016).

Data Analysis

All graphical representations and statistical analyses were conducted utilizing GraphPad Prism version 8.0.1 for Windows. The results are expressed as means ± standard deviations, calculated from three independent experimental replicates. The assessment of data normality was conducted using the Kolmogorov-Smirnov test, and intergroup variations were examined through one-way analysis of variance (ANOVA).

RESULT AND DISCUSSION

Organoleptic tests

The results demonstrated that Formula 0 exhibited a high degree of fluidity, while Formulas 1, 2, and 3 maintained a consistent liquid state. The chromatic characteristics of Formulas 1, 2, and 3 varied from brown to dark brown, a result of the intrinsic blackish-brown coloration of the extract utilized. Moreover, Formulas 1, 2, and 3 emitted a distinctive betel leaf aroma, with each formulation presenting a unique olfactory profile. The observed variation in scent intensity exhibits a direct correlation with the concentration of betel leaf extract utilized, whereby elevated concentrations result in a markedly enhanced fragrance. Over the 28-day storage period, all organoleptic formulations exhibited stability in terms of shape, color, and odor, thereby affirming their adherence to the established organoleptic standards. Comprehensive results of the organoleptic evaluations are provided in Table 2, with observational data illustrated in Figure 1.

Table 2. Results of the organoleptic observation of the essence sheet mask containing ethanol extract of betel leaves

Formula	Organoleptic data		
	Shape	Color	Odor
0	Very liquid	Colorless	Odorless
1	Liquid	Brown	Typical
2	Liquid	Brown	Typical
3	Liquid	Dark brown	Typical

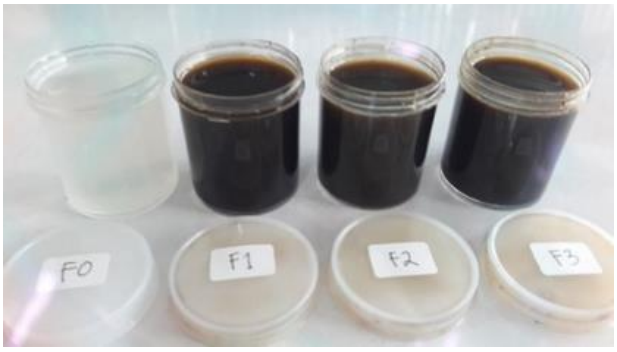

Figure 1. Results of the organoleptic observation of essence sheet masks with ethanol extract of betel leaf

Homogeneity tests

Homogeneity evaluations were performed to determine the accuracy of blending each formulation during the preparatory phase. This verification is essential to ensure the uniform dispersion of active ingredients. The results demonstrated that formulations 0, 1, 2, and 3 exhibited homogeneity with no observable granules. Therefore, it can be concluded that the sheet mask essence incorporating betel leaf ethanol extract met the necessary homogeneity standards (Perugini et al., 2018). The outcomes of the homogeneity analyses are illustrated in Figure 2.

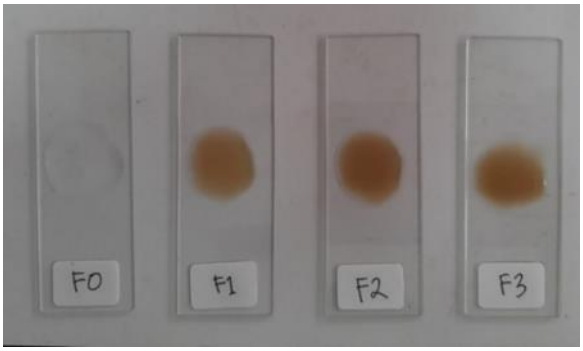

Figure 2. The results of the homogeneity observations of the essence sheet mask with ethanol extract of betel leaves

pH tests

The results demonstrated that the pH of the sheet mask essence incorporating ethanol extract of betel leaf exhibited a decrement over the 28-day storage interval. Nonetheless, it persisted within an acceptable pH spectrum, taking into account the physiological pH of the skin, which ranges from 4.5 to 6.5, as well as the pH stipulations for cosmetic formulations, which span from 5 to 8 (Secretariat, 2014). Consequently, it was deemed safe for topical application. The findings indicated a variation in pH levels among different formulations of the sheet mask essence. This variation can be ascribed to the differing concentrations of betel leaf extract, which possesses intrinsic acidity. As a result, higher concentrations of the extract corresponded with lower pH values. Statistical analysis revealed that the data for all formulations adhered to a normal distribution, with a p-value of 0.111 (>0.05), signifying homogeneity of variances. Subsequently, a one-way ANOVA test was performed, yielding a p-value of 0.483 (>0.05), suggesting that there was no significant difference in pH values across the various formulations. The pH test results are delineated in Table 3.

Table 3. The results of pH observations on the essence sheet mask containing ethanol extract of betel leaves

Days	Average value of pH			
	F0	F1	F2	F3
0	5.57 ± 0.06	5.50 ± 0.10	5.33 ± 0.06	5.40 ± 0.10
7	5.47 ± 0.06	5.30 ± 0.10	5.33 ± 0.06	5.27 ± 0.06
14	5.43 ± 0.06	5.23 ± 0.06	5.37 ± 0.12	5.37 ± 0.12
21	5.33 ± 0.06	5.40 ± 0.10	5.17 ± 0.12	5.33 ± 0.15
28	5.17 ± 0.12	5.13 ± 0.15	5.10 ± 0.17	4.97 ± 0.06

Viscosity tests

The results revealed that the viscosity of the sheet mask essence, which included ethanol extract of betel leaf, exhibited a decline over a 28-day storage period. Nonetheless, it remained within the optimal viscosity range for formulation, specifically between 200 and 400 cP (Gaspar et al., 2022). The statistical

analysis demonstrated that the data for all formulations conformed to a normal distribution, evidenced by a p-value of 0.080, and exhibited homogeneity of variance, as indicated by a p-value of 0.080 (>0.05). Consequently, one-way ANOVA tests were conducted. The results yielded a p-value of 0.082 (>0.05), indicating that there was no statistically significant difference in the viscosity among the various formulations. The viscosity assessment results are illustrated in Figure 3.

Physical stability evaluation

The outcomes of the freeze-thaw test, conducted over six cycles, revealed no changes in the formulation's morphology, color, or odor, thereby affirming the stability of all formulations throughout the storage period. Figure 3 illustrates a graph showing the viscosity, which demonstrated a reduction over the 28-day storage duration. Linear regression analysis performed using MS Excel yielded the slope viscosity values as follows: F0 = -11.33, F1 = -20.33, F2 = -14.33, and F3 = -27.67. The detailed results of the physical stability assessments are presented in Table 4.

Table 4. The results of the physical stability observation of the essence sheet mask containing ethanol extract of betel leaves after undergoing freeze-thaw testing for six cycles

Evaluation	Stability			
	F0	F1	F2	F3
Color	Colorless	Brown	Brown	Dark brown
Odor	Odorless	Typical	Typical	Typical
Shape	Very liquid	Liquid	Liquid	Liquid
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous
pH	5.17 ± 0.21	5.00 ± 0.10	5.10 ± 0.20	5.00 ± 0.00
Viscosity	206.67 ± 5.77	193.33 ± 11.55	196.67 ± 5.77	230.00 ± 0.00

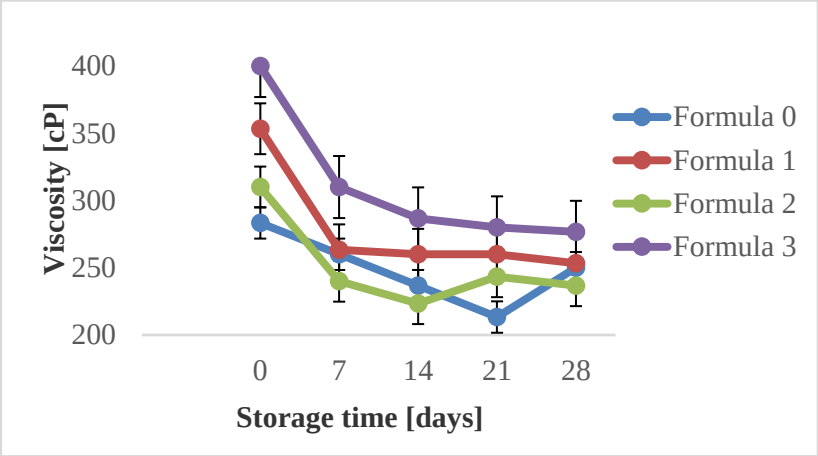

Figure 3. The relationship between the duration of storage and the viscosity characteristics of sheet mask essence infused with ethanol extract of betel leaf

The gradient value functions as a pivotal parameter in evaluating the stability of the formulation. A gradient value nearing zero denotes minimal variations, thus indicating a heightened degree of stability (Dwivedi, 2022). The research findings indicate that Formula 2 exhibits a linear slope in comparison to the other formulas; thus, Formula 2 is deemed the most optimal formulation. Therefore, Formula 2 was selected for subsequent assessment of its antibacterial effectiveness against *Propionibacterium acnes*.

Antibacterial activity evaluation

Based on the results of the physical quality and stability assessments, Formula 2 emerged as the optimal formulation. Subsequently, Formula 2 was subjected to evaluation for its antibacterial efficacy against *Propionibacterium acnes*. The testing protocol encompassed sheet masks incorporating the selected formulation (Formula 2), a positive control consisting of a sheet mask with clindamycin gel and a tea tree oil sheet mask, a negative control (Formula 0), and a positive control of extract solution in 10% DMSO. Antibacterial inhibition was categorized into four criteria: very strong (clear zone >20 mm), strong (clear zone 10-20 mm), moderate (clear zone 5-10 mm), and weak (clear zone <5 mm). The antibacterial activity results are depicted in Figure 4.

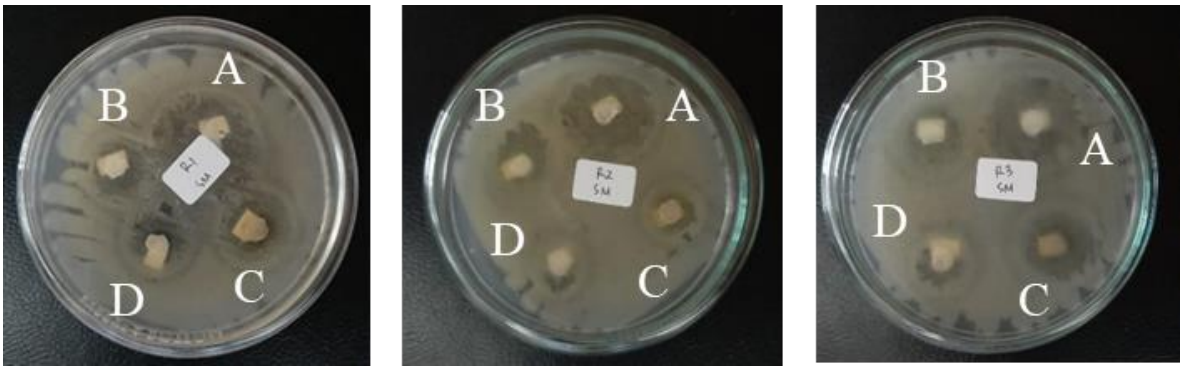

Figure 4. Results of the antibacterial cctivity assay of the essence sheet mask containing ethanol extract of betel leaves. (A) positive control group (clindamycin gel), (B) negative control group (Formula 0), (C) experimental group with the selected formulation (Formula 2), and (D) 1.5% extract solution in 10% DMSO

Upon a detailed examination of the antibacterial activity assays (Figure 4), it was evident that the sheet masks embedded with the selected formulation, positive control, negative control, and extract solution exhibited inhibitory effects against *Propionibacterium acnes*. The data revealed that the sheet mask containing the positive control, clindamycin gel, demonstrated substantial antibacterial efficacy against *Propionibacterium acnes*, as indicated by an inhibition zone measuring 32.00 mm, as elucidated in Table 5.

Table 5. The results of antibacterial activity testing of the essence sheet mask

Sheet mask	Inhibition zone diameter (mm)			Average (mm)
	R1	R2	R3	
C	34	32	30	32.00 ± 2.00
TTO	22	23	24	23.00 ± 1.00
F0	21	19	22	20.67 ± 1.53
F2	24	25	25	24.67 ± 0.58
E	20	22	25	22.33 ± 2.52

Note: C: clindamycin gel, TTO: tea tree oil, F0: formula 0, F2: formula 2, E: extract

The positive control of clindamycin gel demonstrates bacteriostatic effects by facilitating the dissociation of peptidyl-tRNA through its interaction with the 50S ribosomal subunit during the translocation phase, thereby impeding the synthesis of bacterial proteins (Leccia et al., 2015). The results obtained from the examination of sheet masks containing the negative control (Formula 0) revealed an antibacterial effect against *Propionibacterium acnes*, as evidenced by an inhibition zone of 20.67 mm. The detected antibacterial activity in the negative control can be ascribed to the inclusion of methylparaben and ethanol, both well-established antimicrobial preservatives (Sheskey et al., 2017). The observational results for sheet masks infused with a 1.5% extract solution in 10% DMSO exhibited

notable antibacterial activity, as evidenced by the formation of a clear inhibition zone with an average diameter of 22.33 mm. These findings are in stark contrast to those from a prior study, which documented that the antibacterial activity assay of a 1.5% concentration of purified ethanol extract of betel leaf (*Piper betle* L.) against *Propionibacterium acnes* produced an inhibition zone measuring 13.25 mm (Omar et al., 2021). The evaluation of the antibacterial efficacy of the selected formulation (F2) sheet mask, incorporating betel leaf ethanol extract, against *Propionibacterium acnes* revealed an inhibition zone with a diameter of 24.67 mm. In comparison, the positive control sheet mask, containing tea tree oil, demonstrated an average inhibition zone diameter of 23.00 mm. Table 6 provides a detailed comparison of the distinguishing characteristics between the sheet masks formulated with betel leaf (*Piper betle* L.) and tea tree oil (*Melaleuca alternifolia* L.).

Table 6. Properties of sheet masks enriched with Betel Leaf (*Piper betle* L.) and tea tree oil (*Melaleuca alternifolia* L.)

Characteristics	Betel leaf (*Piper betle* L.)	Tea tree oil (*Melaleuca alternifolia* L.)
Average diameter of inhibition zone against *P. acnes*	24.67 mm	23.00 mm
Main compounds	Phytol, chavibetol, 4-chromanol, eugenol, β-caryophyllene, methyl eugenol, isoeugenol, piperol A and B, hydroxychavicol, allylpyrocatechols (Carson et al., 2006; Nayaka et al., 2021).	Terpinen-4-ol, α-terpinene, 1,8-cineole, ρ-cymene, α-pinene, α-terpineol, limonene (Carson et al., 2006).
Minimum Inhibitory Concentration (MIC)	Allylpyrocatechols provided the strongest activity against *P. acnes* with an MIC of 4.0% (Lubis et al., 2020).	Terpinen-4-ol and α-terpineol provided the strongest antimicrobial activity against *P. acnes* with an MIC of 2.5% (Carson et al., 2006).

The principal components of betel leaf extract include phytol, chavibetol, 4-chromanol, eugenol, β-caryophyllene, methyl eugenol, isoeugenol, piperol A and B, hydroxychavicol, and allylpyrocatechols (Nayaka et al., 2021; Syahidah et al., 2017). The mechanism underlying the action of betel leaf can be ascribed to its abundant composition of phenolic compounds, flavonoids, saponins, and tannins, all of which demonstrate significant antibacterial properties (Meinisasti et al., 2020; Nayaka et al., 2021). Phenolic compounds are omnipresent metabolites that can inhibit microbial proliferation due to the functional properties of the carboxyl group within aromatic hydrocarbons. These groups participate in the formation of complexes with bacterial extracellular proteins, ultimately resulting in a reduction of the bacteria's pathogenic potential (Lubis et al., 2020). Tannins and flavonoids are also recognized for their contribution to the antibacterial efficacy of betel leaf extract. The underlying mechanism of this antibacterial activity entails the perturbation of intracellular potassium ion homeostasis in Gram-positive bacteria, culminating in membrane dysfunction (Farhadi et al., 2019).

Flavonoids demonstrate a diverse and complex inhibitory potential, characterized by their ability to impede nucleic acid synthesis, disrupt energy metabolism, inhibit microbial adhesion and biofilm formation, block porin channels within cellular membranes, modify membrane permeability, and diminish pathogenic virulence. In contrast, the antimicrobial activity of tannins is primarily attributed to tannic acid, which exerts its inhibitory effects through the suppression of enzyme production and the inhibition of enzymatic reactions (Lubis et al., 2020; Xie et al., 2015). Tea tree oil is predominantly composed of terpinen-4-ol, α-terpinene, 1,8-cineole, ρ-cymene, α-pinene, α-terpineol, and limonene as its chief constituents. The antimicrobial mechanism of tea tree oil is characterized by the disruption of

bacterial cell walls and membranes, resulting in cytoplasmic leakage and thereby inhibiting bacterial proliferation (Xie et al., 2015). Furthermore, tea tree oil has garnered recognition for its potent anti-inflammatory properties. Inflammation is pivotal in the pathogenesis of acne, with evidence demonstrating that inflammatory alterations occur both antecedent to and subsequent to the colonization of pilosebaceous follicles by *Propionibacterium acnes*. Following follicular colonization, *Propionibacterium acnes* instigates a cascade of inflammatory responses, including the secretion of cytokines by host tissues and the activation of the innate immune system via Toll-like receptor 2 (TLR2) (Lee et al., 2019). A multitude of studies have elucidated that terpinen-4-ol, the principal component of tea tree oil, possesses substantial anti-inflammatory attributes. This efficacy is ascribed to its capacity to suppress cytokine synthesis by monocytes and macrophages. Furthermore, terpinen-4-ol has been evidenced to attenuate interleukin-8 production by keratinocyte cells. Therefore, tea tree oil emerges as a potent anti-inflammatory agent, particularly in the pathogenesis of inflammation associated with acne (Hammer, 2015).

The results of this study revealed that the selected formulation, specifically the sheet mask essence containing 1.5% ethanol extract of betel leaf, demonstrated notable efficacy, categorized within the strong criteria (24.67 mm). Comparatively, the positive control, which utilized a sheet mask with clindamycin gel, exhibited superior efficacy within the very strong criteria (32.00 mm). The tea tree oil sheet mask, serving as another positive control, similarly achieved efficacy within the strong criteria (23.00 mm). The negative control formulation, which lacked the betel leaf ethanol extract, was also classified within the strong criteria (20.67 mm), as was the extract solution itself (22.33 mm). These findings suggest that the sheet mask essence incorporating betel leaf ethanol extract holds promise as a potential anti-acne treatment, offering a viable alternative in the dermatological field.

CONCLUSION

The selected sheet mask essence formulation, referred to as Formula 2, demonstrated the most linear viscosity slope, indicative of its exceptional stability. Formula 2 adhered to the necessary physical quality and stability standards. Over a 28-day storage period, no perceptible alterations in shape, color, or odor properties were observed; the formulation remained homogeneous, maintained a pH range of 5.10 to 5.33, exhibited a viscosity between 223.33 and 310.00 cP, and demonstrated resilience under freeze-thaw testing conditions. The formulation designated as Formula 2 comprises a 1.5% ethanol extract derived from betel leaf. Significantly, Formula 2 demonstrated substantial antibacterial efficacy against *Propionibacterium acnes*, evidenced by an inhibition zone measuring 24.67 mm. Although this result differed from the positive control sheet mask containing clindamycin gel, which exhibited an inhibition zone of 32.00 mm, it was not significantly different from the positive control sheet mask containing tea tree oil, which exhibited an inhibition zone of 23.00 mm.

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