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Antibacterial Activity of Ethanol Extract Gel of Nangka Leaves (*Artocarpus heterophyllus* LMK.) ON *Propionibacterium acnes*

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ABSTRACT

Introduction: Artocarpus heterophyllus LMK. leaves contain flavonoids, tannins, and saponins, which have the potential to be antibacterial. **Aims**: This study aims to test the antibacterial activity of A. heterophyllus leaves ethanol extract, which is formulated in a gel preparation. **Methods**: Antibacterial testing was done on the acne-causing bacteria Propionibacterium acnes. The gel formula is designed with an extract concentration of 5, 10, or 15% with Carbopol 940 and HPMC as the bases. The antibacterial activity test was carried out using the well diffusion method. **Result**: The results of the study showed that the concentration of the extract affected pH, adhesive power, removability, and viscosity. Antibacterial activity tests showed that the gel of A. heterophyllus extract could inhibit Propionibacterium acnes bacteria. The higher the concentration of the extract, the greater the antibacterial activity. **Conclusion**: It can be concluded that the gel with an extract concentration of 15% gave the strongest antibacterial activity with an inhibition zone of 10.9 ± 1.18 mm.

KEYWORDS: *Artocarpus heterophyllus*, antibacterial, gel, *Propionibacterium acnes*, flavonoids.

INTRODUCTION

Acne vulgaris is a skin disorder characterized by comedones, papules, abscesses, protrusions, or fading caused by sebum hyperproduction or the propagation of Propiobacterium acnes and Staphylococcus aureus. Acne can occur at any age, but the majority (80%) occur in adolescents during skin (Nawarathne, Wijesekera, Wijayaratne, & Napagoda, 2019).

Acne vulgaris is a skin disorder characterized by comedones, papules, puberty (Qureshi et al., 2021). *P. acnes* is an anaerobic microorganism responsible for the development of inflammatory acne because of its work in activating complement and metabolizing sebaceous triglycerides to fatty acids, which then chemotactically attract neutrophils. While Staphylococcus species usually cause superficial infections in the abscesses, protrusions, or fading caused by sebum hyperproduction or the propagation of *Propiobacterium acnes* and *Staphylococcus aureus*. Acne can occur at any age, but the

majority (80%) occur in adolescents during puberty (Qureshi et al., 2021). *P. acnes* is an anaerobic microorganism responsible for the development of inflammatory acne because of its work in activating complement and metabolizing sebaceous triglycerides to fatty acids, which then chemotactically attract neutrophils. While Staphylococcus species usually cause superficial infections in the skin (Nawarathne, Wijesekera, Wijayaratne, & Napagoda, 2019).

Artocarpus heterophylus, the local name, is known as jackfruit. This plant is rich in phenolic compounds and flavonoids. The pharmacological activity of *A. heterophyllus* leaves has been widely reported, including as an antioxidant, anti-bacterial (Khan et al., 2003), and anti-inflammatory (Wei et al., 2005). These activities are needed to treat acne. The use of extracts as medicine is more applicable if they are made in pharmaceutical dosage forms. There has been no report on the antibacterial activity of the gel formula with *A. heterophyllus* extract, especially as an anti-acne agent. This seafltudy aims to design a gel formulation of *A. heterophyllus* leaves ethanol extract and evaluate its effectiveness against *P. acnes*, an acne-causing bacteria.

MATERIAL AND METHODS

Herbal and chemical Materials

A. heterophyllus leaves were collected from Indralaya, South Sumatra. *P. acnes* bacteria (Microbiology Laboratory, ITB), ethanol (Brataco), n-hexane (Brataco), Carbopol 940, triethanolamine, propylene glycol, methyl paraben, propyl paraben, distilled water, aqua rose, ethyl acetate, zinc, nutrient agar (NA), and Nutrien Broth (NB). The chemicals are purchased from Merck (Germany).

Extract Preparation

A. heterophyllus leaves ethanol extract was prepared by the maceration method. As much as 600 g of jackfruit leaves powder was soaked in 96% ethanol (3 L) for 48 hours. Then re-maceration was carried out twice (2 L x 24 hours). The ethanol filtrate obtained was collected and evaporated using a rotary evaporator at 60 oC to obtain a thick extract. The concentrated ethanol extract (50 g) was fractionated using a separating funnel using 96% ethanol:n-hexane (1:1), then shaken and allowed to stand until the two solutions were completely separated. Once separated, pour the ethanol extract into a glass beaker. The fractionation process was continued until the n-hexane solution became clear. Furthermore, the ethanol extract obtained was concentrated using a rotary evaporator (Yamato®) (Fitrya et al., 2022a).

Gel Formulation of *A. heterophyllus* leaves extract

The gel formula was designed with three extract concentrations (Table 1) (Fitrya et al., 2022b; Tambunan & Sulaiman, 2018). Gel preparation was prepared by dispersing carbopol 940 in distilled water for 24 hours and was homogenized with a magnetic stirrer at 150–250 rpm until a clear solution was

Tabel 1**.** Formula of *Artocarpus heterophyllus* ethanol extract gel

Component	F1 (g)	F2 (g)	F3 (g)
Ethanol extract	5	10	15
Carbopol 940	0.5	0.5	0.5
HPMC	2	2	2
Triethanolamin	0.25	0.25	0.25
Methyl paraben	0.09	0.09	0.09
Propyl paraben	0.01	0.01	0.01
Propilene glycol	10	10	10
Aqua rose	q.s	q.s	q.s
Distilled water up to	50	50	50

obtained. Then add tri ethanolamine (TEA) (Phase 1). The HPMC was dispersed in hot water until it swelled, then stirred until it was homogeneous and transparent (Phase 2). Then, mix the two phases, stirred until homogeneous and form a gel base. The extract of *A. heterophyllus* was added to the base until homogeneous gel base was formed. Methylparaben and propylparaben which were dissolved in propylene glycol and then mixed with the gelling agent. Add aqua rose and then stirred at 150- 250 rpm until a homogeneous and clear (Febriyenti et al., 2020).

Evaluation of the physical and chemical properties of the gel

Evaluation of the the prepared gel included organoleptic, pH, homogeneity, spreadability, adhesive power, washability, viscosity and stability tests. Organoleptic examination, pH, homogeneity refers to (Shahtalebi et al., 2018). Spread ability tests (Gupta & Gupta, 2017), adhesive power, wash ability and viscosity refer to (Febriyenti et al., 2020) and stability tests according to (Bolla et al., 2020).

Rejuvenation of *Propionibacterium acnes* Bacteria

Propionibacterium acnes bacteria were grown on NA media by streaking a pure culture of bacteria onto the medium obliquely using an ose needle. Then incubate the media that has been streaked with bacteria at 37ºC for 48 hours.

Preparation of Bacterial Suspension

The culture of *P. acnes* in NA medium was taken aseptically in one loop, put in 12 mL of NB medium and shaker until homogeneous. The number of *P. acnes* cells in the suspension was measured until it reached 105-108 cells/mL using a hemocytometer (Muharni 2017).

Antibacterial Activity Test

Antibacterial activity testing was carried out using the well diffusion method. The positive control used Nourish Skin Acne Gel® and the gel base as negative control. A total of 0.2 mL of the test bacterial suspension was put into a test tube containing 20 mL of NA medium, then shaken until homogeneous. The

agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 5 mm was punched aseptically with a sterile Durham tube, and 0.1 g of the gel preparation was introduced into the well. Then, agar plates are incubated for 24 hours at 37ºC After incubation period, the inhibition zone was measured (Shahtalebi et al., 2018).

Data analysis

Statistical analysis of physical characteristics and in-vitro diffusion data was performed with SPSS 24 software. The normality of data was checked with Shapiro-Wilk and followed by one-way ANOVA. Then proceed with the Tukey and LSD post-hoc tests to find out the differences between groups. Differences were considered significant at $p < 0.05$ and results are reported as the mean ± standard deviation (SD) of the three measurements.

RESULTS AND DISCUSSION

A. heterophyllus leaves ethanol extract is known to contain flavonoids and phenolic compounds with various pharmacological activities. Fractionation using n-hexane was carried out to remove chlorophyll, fat and wax. The dosage form chosen in this study was hydrogel because it is easy in utilization, has a high water content and spreads easily on the skin and has a longer contact time. The high water content in the gel can suppress inflammation due to accumulation of lipids in the pores of the skin with acne (Shahtalebi et al., 2018). The gel base used was a hydrophilic base, namely carbopol 940 and HPMC. Carbopol can affect the increase in pH and viscosity, has better properties in releasing active substances compared to other gel bases. In addition, in relatively small concentrations ranging from 0.02 - 2%, Carbopol was easily dispersed in water because it is a hydrophilic carbomer group. HPMC could affect the increase in spreadability and adhesion of preparations (Tambunan & Sulaiman, 2018). According to (Patel & Patel, 2009) this base has a cooling effect, good spread on the skin and does not clog the pores on the skin and good drug release. The use of triethanolamine (TEA) can increase the dispersion and the viscosity and stability of carbopol (Gupta & Gupta, 2017). Additionally, TEA also functions as a wetting agent so that the gel can reach a ideal pH for topical preparation, namely 5 - 6.5 (Saryanti & Zulfa, 2017).

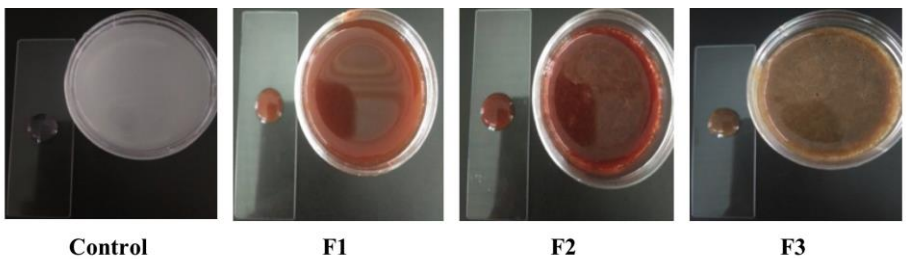

Figure 1. Organoleptic Gel of *A. heterophyllus* Ethanol Extract

Table 2. The physical characteristics of gel of *A. heterophyllus* extract

Characteristic	Control	F1(5%)	F2 (10%)	F3 (15%)
Organoleptic	Clear, colorless	Clear, reddish	Reddish brown	Brownish green
pH	5.82 ± 0.07	5.98 ± 0.03	6.13 ± 0.02	6.23 ± 0.02
Homogeneity	Homogeneous	Homogeneous	Inhomogeneous	Inhomogeneous
Spreadability (cm)	3.70 ± 0.10	3.83 ± 0,47	4.00 ± 0.20	4.37 ± 0.12
Adhesive power (s)	80.67 ± 9,07	67.33 ± 4,93	32.67 ± 2,08	20.33 ± 4,16
Washability (mL)	9.87 ± 0.70	8.87 ± 0.25	6.20 ± 0.56	4.27 ± 0.55
Viscosity (cPs)	3930 ± 226.49	3410 ± 96.44	2930 ± 105.36	770 ± 256.32
Stability	Stable	Stable	Stable	Stable

Values expressed are mean ± SD of three measurements.

The organoleptic the prepared gel showed that each formula had a distinctive aroma of rose. The results of the evaluation of the physical and chemical characteristic of the gel are shown in Figure 1 and Table 2.

Based on the normality test for the parameters pH, adhesive power, wash ability and viscosity of the preparation, it yields a significant value > 0.05. It mean that the data is normally distributed. The results of analysis of data pH, adhesive power, washability and viscosity with ANOVA resulted a significant value <0.05. Post-hoc follow-up test showed a significant value <0.05. This indicated that the addition of extract concentration has an effect on the pH value, adhesive power, washability and viscosity. There was no significant effect of the extract concentration on the spread ability ($p>0.05$).

The resulting gel preparation looks homogeneous, but has different colors as shown in Figure 1. This color and homogeneity difference swas influenced by the difference in the concentration of the extract. (Tambunan & Sulaiman, 2018). Inhomogeneity of F2 and F3 caused by the high concentration of the extract not to be dispersed in the gel base. The pH value of formulas F1 - F3 are in the skin pH criteria, namely in the interval 4.5 – 6.5 (Saryanti & Zulfa, 2017). The pH of the gel must be close to the skin pH to avoid irritation (Gupta & Gupta, 2017). Topical preparations with a pH value > 7 will irritate the skin because alkaline ingredients cause the skin to dry out (Sayuti, 2015).

The spreadability of the gel is out of the range in good spreadability range, namely 5-7 cm (Garg et al., 2002). The spreadability that does not meet these specifications is due to the viscosity of the gel being too thick or too dilute, but F3 is close to the expected viscosity. All gels prepared showed good adhesion, which was not less than 4 seconds (Putranti et al., 2019). The study showed that there was an increase in the spreading power with each addition of extract concentration, on the other hand the adhesive power was decreased. This is due to the increase in the extract concentration causes the viscosity of the preparation to decrease, furthermore the low viscosity will increase the spreadability of the gel preparation. Based on the results of the stability test, the gel of A. heterohyllus ethanol

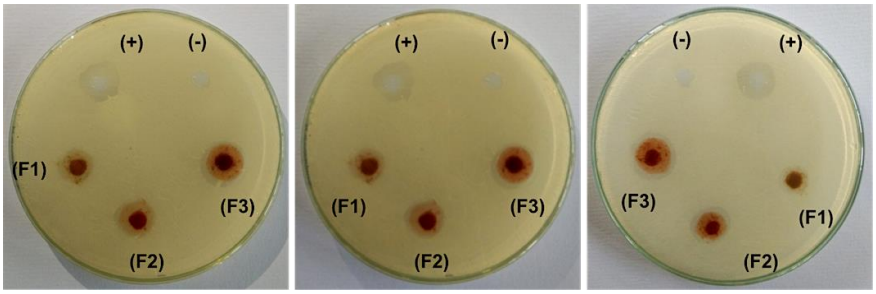

Figure 2. Zone of inhibition of gel against *Propiobacterium acnes.*

extract have good stability to changes in temperature and mechanical stress.

Antibacterial Activity of Gel

The results of measuring the inhibition zone of gel preparations are shown in Table 3 and Figure 2. The normality test of antibacterial activity showed that the data were normally distributed (> 0.05). Furthermore, the one-way ANOVA test and post-hoc follow-up test showed that there was an effect of the addition of concentration of extract on the inhibition zone diameter (<0.05).

The antibacterial activity testing of gel *A. heterophyllus* ethanol extract was carried out using the well diffusion method. The Nourish Skin Acne Gel® was used as a positive control to compare the activity of the ethanol extract of *A. heterophyllus* with the marketed formula. The gel base was used as a negative control to ensure that the antibacterial activity came from the extract, not of the gel base.

Table 3. Antibacterial activity of *A. heterophyllus* gel against *P. acnes*

Formula	Zone of inhibition (mm) ± SD
Negative control	--
Positive control	16.23 ± 0.59
F 1 (5%)	4.16 ± 2.30*
F 2 (10%)	7.37 ± 0.68*
F 3 (15%)	10.9 ± 1.18*

*p<0.05 compare to positive control (*Nourish Skin* Acne Gel®). Values expressed are mean ± SD of three measurements.

Based on Table 3 it can be seen that the gel of *A. heterophyllus* extract has antibacterial activity. The positive control produces a strong inhibition diameter zone because Nourish Skin Acne Gel® contains Aloe barbadensis extract with saponin and acemanan compounds which are antibacterial. The prepared gel F1, F2 and F3 showed an increase in inhibition diameter that was consistent with an increase in extract concentration. It is indicated that the inhibition zone depends on the concentration of the extract (Dange et al., 2020). The gel with 15% of extract concentration produces strong antibacterial activity. According to (Khan et al., 2003), the methanolic extract of the Artocarpus heterophyllus leaves exhibited a broad spectrum of antibacterial activity.

The antibacterial activity shown in this study is related to the antioxidant and anti-inflammatory activity of the extract. The antioxidant activity works to prevent damage due to lipid peroxidation (Ajiboye et al., 2020; Omar et al., 2011) and the anti-inflammatory activity works to inhibit inflammatory mediators such as COX-2 (Wei et al., 2005). Thus, the using of the extract as a herbal

medicine for acne is scientifically acceptable. The *A. heterophyllus* leaves extract was reported to contain flavonoid compounds. The flavonoids work as antibacterial by forming complex with extracellular proteins that damage bacterial cell membranes (Shahtalebi et al., 2018). In addition, there are also saponin that work by lowering surface tension so that cell permeability increases and causes intracellular fluid to diffuse out through the vulnerable outer membrane, then binds to the cytoplasmic membrane. This causes cytoplasmic leakage and results in cell death (Shahtalebi et al., 2018).

The antibacterial mechanism of the extract is related to the structure of the cell wall of the *P. acnes* bacteria which contains polysaccharides and teichoic acid. Teichoic acid is a water-soluble phosphate polymer. Because this water-soluble nature indicates that the bacterial cell wall is more polar. Flavonoids, tannins and saponins are polar compounds that make it easier to penetrate the cell wall layer of the *P. acnes* bacteria by forming complexes with teichoic acid.

CONCLUSION

Based on the analysis of the physical characteristic and stability of the gel of *A. heterophyllus* ethanol extract, the formula produced a stable preparation. The gel of *A. heterophyllus* extract with a concentration of 15% produced strong antibacterial activity but not as good as the positive control. For further development, optimization of gel base

Antibacterial activity of nangka leaves concentration will be carried out to produce a stable formula with the best physico-chemical characteristics.

CONFLICT OF INTEREST

There is no conflict of interest associated with this study.

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