EFFECT OF GEL FORMULATION OF METHANOLIC EXTRACT OF Leucaena leucocephala LEAVES ON Propionibacterium acnes AND Staphylococcus epidermidis

**ABSTRACT/ABSTRAK**

White lead tree (*Leucaena leucocephala*) leaf extract includes antibacterial phytochemical compounds such as lupeol and flavonoids. The study aimed to determine the antibacterial activity of white lead tree methanolic extract and gel formulation against acne-causing bacteria *Propionibacterium acnes* and *Staphylococcus epidermidis*, also the physical quality of the preparation. The treatments included a methanolic extract of the White lead tree and extract gel formulations. The study used a completely randomized design (CRD) with five replications. ANOVA analyzed data at a 95% confidence level (p < 0.05) by SPSS software. Gel formulations were used in five concentrations (3.12, 6.25, 12.50, 25.00 and 50.00 %). The antibacterial activity test utilized was the agar diffusion method. The results showed the white lead tree leaf extract contains flavonoids with TFC as much as 12.33 ± 0.02 mgQE.g⁻¹. The antibacterial activity test of white lead tree leaf extract at all concentrations showed the most effective antibacterial activity against *S. epidermidis* with a zone of inhibition of 2.17 cm², with no inhibitory zone against *P. acnes*. The gel preparation extract at all concentrations had no inhibitory activity against *P. acnes*, but the gel formulations inhibit *S. epidermidis* effectively at 50.00 % concentrations with a zone of inhibitions 0.82±0.30 cm². The gel quality parameters looked homogeneity for 28 days; ph and stickiness were matched to the National Standardization Agency of Indonesia (NSI); however, the dispersibility and viscosity were not matched to NSI. In conclusion, plant leaf methanolic extract demonstrated antibacterial activity against the acne-causing bacterium *S. epidermidis*. Further study is needed on the potential of white lead tree leaves as a natural anti-acne.

Ekstrak daun petai cina (*Leucaena leucocephala*) mengandung zat fitokimia seperti lupeol dan phytol, keduanya merupakan kelompok flavonoid yang bersifat antibakteri. Tujuan penelitian ini untuk mengetahui aktivitas antibakteri ekstrak metanol dan campuran ekstrak dalam sediaan gel terhadap bakteri penyebab jerawat *Propionibacterium acnes* dan *Staphylococcus epidermidis*, serta menguji kualitas fisik sediaan. Perlu bahwa dilakukan dengan pemberian ekstrak metanol daun petai cina dan formulasi sediaan ekstrak dalam bentuk gel. Penelitian menggunakan Rancangan Acak Lengkap (RAL) dengan lima ulangan. Data dianalisis menggunakan Anova pada tingkat kepercayaan 95 % (p < 0,05) dengan SPSS. Sebanyak lima konsentrasi ekstrak sediaan gel yang digunakan yaitu 3,12; 6,25; 12,50; 25,00; dan 50,00 %. Uji aktivitas antibakteri menggunakan metode difusi agar. Hasil penelitian menunjukkan ekstrak metanol daun petai cina mengandung senyawa flavonoid dengan TFC sesuai 12.33 ± 0.02 mgQE.g⁻¹. Uji aktivitas antibakteri ekstrak daun petai cina pada semua konsentrasi menunjukkan aktivitas antibakteri paling efektif terhadap *S. epidermidis* dengan luas zona hambat sebesar 2.17 cm², namun tidak memperlihatkan zona hambat terhadap *P. acnes*. Ekstrak sediaan gel pada semua konsentrasi tidak memiliki daya hambat terhadap *P. acnes*, namun konsentrasi 50,00 % memiliki aktivitas antibakteri paling efektif terhadap *S. epidermidis* dengan luas zona hambat sebesar 0,82±0,30 cm².

* Alamat Korespondensi : boy.sidharta@uajy.ac.id

DOI : http://dx.doi.org/10.21082/bullitro.v32n2.2021.75-85

0215-0824/2527-4414 @ 2017 Buletin Penelitian Tanaman Rempah dan Obat

This is an open access article under the CC BY-NC-SA license (http://creativecommons.org/licenses/by-nc-sa/3.0/)

Accreditation Kemenristekdikti Number : 30/E/KPT/2018
INTRODUCTION

Acne vulgaris is a condition of unhealthy skin due to hyperproliferation of the follicular epidermis that causes a follicular blockage, overproduction of sebum, and inflammation (Zaenglein 2018). A bacterial infection influences acne vulgaris severity (Lynn et al., 2016; Zaenglein, 2018). Pathogenic bacteria which played important roles as causative of acne vulgaris were Propionibacterium acnes (Beylot et al., 2014) and Staphylococcus epidermidis (Claudel et al., 2019). These two bacteria were found in many acne patients. Therefore, these bacteria were commonly utilized in many types of research in anti-acne compounds tests (Beylot et al., 2014; Walsh et al., 2016; Claudel et al., 2019).

Earlier research on leaf extract of L. leucocephala showed potential antibacterial and antifungal activities. Extracts of leaves of L. leucocephala were able to inhibit the growth of both Gram-negative and positive bacteria such as Bacillus cereus, B. subtilis, Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, and Mycobacterium tuberculosis as well as inhibit fungal species namely Candida albicans, Aspergillus niger, Mucor miehei, and Rhizopus oligosporus (Sartinah et al., 2010; Mohammed et al., 2015; Ghotekar et al., 2018). However, there is a shortage of publications on the leaves extract of L. leucocephala against pathogenic bacteria as causative agents of acne, such as Propionibacterium acnes and Staphylococcus epidermidis. Therefore, this present research proposes to determine the antibacterial activity of the leaf extract of L. leucocephala against P. acnes and S. epidermidis.

Antibiotic usage to eradicate acne vulgaris is very common. However, long-term antibiotic utilization may cause bacterial resistance (Walsh et al., 2016). Tetracycline is one of the effective antibiotics against acne bacterial infections and has become commonly prescribed by dermatologists (Chopra & Roberts, 2001; Leyden & Del Rosso, 2011; Bienenfeld et al., 2017). Many studies have been done to find bioactive compounds from plants that showed antibacterial activities and may be utilized as an alternative to anti-acne therapy (Saviuc et al., 2017). For example, white lead tree (Leucaena leucocephala) leaves extracts showed potential antibacterial activities in inhibiting bacterial growth such as Escherichia coli, Staphylococcus aureus (Sartinah et al., 2010), Klebsiella pneumoniae (Umari et al., 2018), and Pseudomonas aeruginosa, and Salmonella typhimurium (Mohammed et al., 2015).

Flavonoids were presumed to be the potential antibacterial compound found in the extract of white lead tree leaves (Zayed & Samling, 2016). Previous research reported that some different types of bioactive compounds as flavonoid member, might have antibacterial activity, namely lupeol (Sartinah et al., 2010) and phytol (Umari et al., 2018). Methanol was used as the solvent due to its characteristics such as a polar substance and was reported to be the best for extracting flavonoids (Sartinah et al., 2010; Chew et al., 2011; Prakash et al., 2020).

Therefore, determining the antibacterial activities of methanolic extract of white lead tree leaves in gel formation against acne’s causative bacteria, i.e., P. acnes and S. epidermidis, further discovering the gel's physical qualities formulations were the objectives of this present study. Gel formulations of methanolic extract of white lead tree leaves have never been studied; hence the research results may give novel findings in the field of medicinal plants in Indonesia.

MATERIALS AND METHODS

The present research was done in the Teknobilio-Industry Laboratory, Faculty of Biotechnology, Atma Jaya University, Yogyakarta, from January to March, 2020. The leaves were harvested in the morning (around 09.00 am) and selected from the lower part of a five-year-old tree, considered old leaves, at Sleman District, Yogyakarta Province. The plant species was certified by a plant taxonomist from Gadjah Mada University. The bacteria were obtained from the culture collection of the Microbiology Section, Faculty of Biotechnology Atma Jaya University, Yogyakarta.
Leaf Extraction

One thousand grams of dried white lead tree leaves were ground, and 10 grams of leaf powder were macerated in 100 ml methanol at 30°C for 24 hours within a shaker incubator. Next, the extracts were filtered and put in a rotary evaporator at 60°C with 60 rpm speed to evaporate the solvent. Finally, the filtrates were dried in a water bath at 60°C to have condensed extracts (Zayed & Samling 2016).

Qualitative and Quantitative Phytochemical Analyses

Qualitative flavonoid analysis was done by adding some drops of concentrated H₂SO₄, and a positive reaction was shown by a yellow color appearance (AOAC 1990; Alfian et al., 2018). Quantitative flavonoid analysis was measured in total flavonoid concentration (TFC) utilizing standard solution quercetin in UV-Vis Spectrophotometer at 415 nm wavelength (Pranowo et al., 2016; Yulianti et al., 2016). The total flavonoid content in the sample was estimated by Chang et al. (2002). The measurements were carried out with a spectrophotometer UV-Vis Spectrophotometer (Shimadzu). The sample volume of 0.25 ml was diluted to 1.25 ml with distilled water. 75 μl of 5 % sodium nitrite was added after six minutes. 0.5 ml of 0.1M NaOH was added after 5 min and made up to 2.5 ml with distilled water. The solutions were mixed well, and the absorbance was read at 415 nm along with distilled water. 75 μl of 5 % sodium nitrite was added after 5 min and made up to 2.5 ml with distilled water. The solutions were mixed well, and the absorbance was read at 415 nm along with standard quercetin at 5 - 25 μg concentrations. The results are expressed as mg of flavonoids as quercetin equivalent per gram of dried sample. TFC was calculated using the formula:

$$\text{TFC} = \frac{(c \times n \times v)}{g \text{ dried samples}}$$

Note:
- TFC = Total Flavonoid Concentration (mg·g⁻¹ dried samples)
- c = flavonoid concentration (mg·L⁻¹)
- n = dilution factor
- v = volume of solvent for extraction (L)

Gel Preparation

As much as 50 ml aqua dest was heated to 80°C, and 2 g hydroxypropyl methylcellulose (HPMC) powder was slowly dispersed in the preheated water. The entire HPMC solution was stirred until a homogenous appearance was attained. Afterward, 2 ml glycerin, 10 ml propylene glycol, and 1 ml triethanolamine (TEA) were added and stirred until they reached a homogenous appearance. The extract concentration variations were as follows 3.12; 6.25; 12.50; 25.00 and 50.00 % (Sikawin et al., 2018).

Antibacterial Test

One loop of pure culture of each bacteria was suspended in 10 ml nutrient broth media (Asri & Fahril 2019). Each bacterial culture was inoculated into nutrient agar media perforated with seven wells. Each well was filled with treatment concentrations, i.e., 3.12; 6.25; 12.50; 25.00 and 50.00 %, positive control (tetracycline antibiotic), and negative control (distilled water). The agar plates were incubated for 48 hours at room temperature (28°C) and anaerobic conditions for *P. acnes* and 37°C and aerobic conditions for *S. epidermidis*. The inhibition diameters formed were measured utilizing calipers, and the zone of inhibitions was calculated using the formula (Bienenfeld et al., 2017):

$$\text{Inhibition Zone} = 3.14 \times \left[ \left( \frac{d_2}{2} \right)^2 - \left( \frac{d_1}{2} \right)^2 \right]$$

Note:
- d1 = diameter of wells (cm)
- d2 = average of inhibition diameter (cm)

Gel Formulation Quality

Quality of gel formulations was analysed according to National Standardization Agency (Badan Standarisasi Nasional, 1996), such as organoleptic, homogeneity, pH, viscosity, dispersibility, and stickiness. The organoleptic test was done by physically observing textures, colors, and aroma. In contrast, the homogeneity test was done by smearing the gel formulations on the object glass and following whether there was coarse grain. Furthermore, good quality gel formulations must be homogenous and show no coarse grain (Departemen Kesehatan RI 1985).

**pH test**

The pH test of the gel formulations was done by diluting the gel into distill water with a 1:5 ratio
and then stirring until homogenous. Then, the pH meter was dipped into the solution and observed until the pH value was constant (Ardana et al., 2015). The pH test was done sequentially on days 1, 3, 5, 7, 14, 21, and 28 (Badan Standarasisasi Nasional 1996).

**Viscosity**

Fifteen ml of gel formulations were taken for viscosity test and were put into a beaker glass. Viscosity was measured using a viscosimeter with spindle #6 and a velocity of 10 rpm (Badan Standarasisasi Nasional, 1996). Viscosity measurement was done sequentially on days 1, 3, 5, 7, 14, 21, and 28 (Badan Standarasisasi Nasional 1996).

**Dispersibility**

A dispersibility test was done to determine the dispersal of the gel formulations on the skin. One-half gram of gel formulations was taken for dispersibility, put into two glass plates (20 x 20 cm), given a load of 125 g, and observed for one minute. Gel dispersal diameters were measured utilizing calipers (Wijayanti et al., 2015). Dispersibility measurement was done sequentially on days 1, 3, 5, 7, 14, 21, and 28 at room temperature (28°C) (Badan Standarasisasi Nasional 1996).

Gel formulations were put on the object glass for a stickiness test, and another object glass was placed on top of it and was given a load of 85 g for five minutes. Times were noted when the two object glasses were separated (Dewantari & Sugihartini, 2015). Stickiness measurement was done sequentially on days 1, 3, 5, 7, 14, 21, and 28 (Badan Standarasisasi Nasional 1996).

The experimental design applied in this study was a complete randomized design, and all treatments were done in quintuplicates. Data were analyzed using Anova with a 95% confidence level (p < 0.05) by SPSS software. If there are significant differences between treatments, it will be continued with Duncan Multiple Range Test (Lee & Lee 2018).

**RESULTS AND DISCUSSION**

**Phytochemical Analysis**

Qualitative phytochemical analysis showed that methanolic extract of white lead tree leaves contained flavonoid compounds (Table 1). The results were similar (Dewantari & Sugihartini, 2015; Zayed & Samling, 2016). A previous study reported some bioactive compounds as flavonoids member that might have antibacterial activity, namely lupeol (Sartinah et al., 2010) and phytol (Umaru et al., 2018). However, there has been no additional study on the antibacterial activity of the two chemicals against bacteria that cause acne.

Quantitative phytochemical analysis revealed that methanolic extract of white lead tree leaves contained TFC (Total Flavonoids Content) about 12.33 ± 0.02 mgQE.g⁻¹. This result was higher compared to the previous study by Zayed & Samling (2016), as much as 0.015 mg QE.g⁻¹ (Table 1). Total flavonoid content, including phenolic compounds in some plants, was influenced by temperature, nutrition, water availability, and soil where the plants lived (Nur 2019). In addition, (Pangestuti et al., 2017) reported that extraction method, type of solvent, and extraction times affected the amount of the bioactive compounds extracted from the plants.

**Antibacterial Activity**

**Methanolic Extract**

The zone of inhibition of methanolic extract of white lead tree leaves against *S. epidermidis* showed that the higher concentration of the extract, the higher the inhibition zone (Table 2). Furthermore, the increase in extract concentrations was followed by increased bioactive concentrations in the extract (Fitri & Widiyawati 2017); hence the antibacterial activity was increased subsequently. However, there was no zone of inhibition of methanolic extract of white lead tree leaves revealed against *P. acnes* (Table 2). Therefore, it was

<table>
<thead>
<tr>
<th>Method</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qualitative</td>
<td>positive, yellow color</td>
</tr>
<tr>
<td>Quantitative</td>
<td>12.33 ± 0.02 mgQE.g⁻¹</td>
</tr>
</tbody>
</table>
Table 2. Zone of inhibition of methanolic extract of white lead tree leaves against *P. acnes* and *S. epidermidis*

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>S. epidermidis</th>
<th>P. acnes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline</td>
<td>7.25 ± 1.02a</td>
<td>5.52 ± 0.45a</td>
</tr>
<tr>
<td>50.00</td>
<td>2.17 ± 0.58c</td>
<td>0b</td>
</tr>
<tr>
<td>25.00</td>
<td>1.53 ± 0.47d</td>
<td>0b</td>
</tr>
<tr>
<td>12.50</td>
<td>1.01 ± 0.49d</td>
<td>0b</td>
</tr>
<tr>
<td>6.25</td>
<td>0.84 ± 0.63c</td>
<td>0b</td>
</tr>
<tr>
<td>3.12</td>
<td>0.26 ± 0.23bc</td>
<td>0b</td>
</tr>
<tr>
<td>Aquadestilata</td>
<td>0b</td>
<td>0b</td>
</tr>
</tbody>
</table>

Note: Numbers with the same letters in the same column are not significantly different at a 95% level of confidence (p < 0.05).

Angka yang diikuti oleh huruf yang sama pada kolom yang sama menunjukkan tidak berbeda nyata dengan tingkat kepercayaan 95%.

was presumed that *P. acnes* is resistant to the plant extract compared to *S. epidermidis* (Mulyani et al., 2017; Yusufu et al., 2019). A different *P. acnes* strain may show different responses to the leaves’ extract (Yusufu et al., 2019). It will be interesting to continue the study in this field by combining *L. leucocephala* extract with other plant species such as *Phyllanthus niruri* (Fitri & Widyawati 2017) to obtain the best result.

The bioactive compounds extracted from white lead tree leaves that showed antibacterial activity was assumed as flavonoids (Zayed & Samling, 2016; Utami et al., 2019). Complex compounds will be formed on the extracellular proteins by flavonoids. Thus, the proteins become denaturized, and bacterial cell membranes are damaged (Utami et al., 2019).

Tetracycline antibiotic was utilized as positive control and revealed inhibition zones as high as 7.25 cm² against *S. epidermidis* and 5.52 cm² against *P. acnes* (Table 2). Tetracycline was selected because it was the potential to inhibit the growth of acne’s causative bacteria such as *P. acnes* and *S. epidermidis* (Bienvenfeld et al., 2017). The active site of tetracycline will bind to aminoacyl-tRNA on the bacterial ribosome so that the protein synthesis processes will be halted (Walsh et al., 2016). No antibacterial activity showed on control negative treatment because distilled water is required by living cells. The results showed that white lead tree concentration extracts treated with acne’s causative bacteria were still low compared to the antibiotic tetracycline. This might be due to the fact that the extracts were not in pure conditions, and the concentrations utilized were considered as low, i.e., less than 50%. Therefore, further study is needed to determine the bioactive compound found in the extracts.

**Gel Formulations**

The zone of inhibitions of the gel formulations against *S. epidermidis* was lower compared to the zone of inhibitions of the methanolic extract of white lead tree leaves (Table 3). It was assumed that the effectivity of the bioactive compounds in the extract became lesser because of additional ingredients in the gel formulations (Asri & Fahril 2019). (Sekar & Jalil 2017) reported that antibacterial activity of *Muntingia calabura* leaves extract showed a zone of inhibitions higher compared to the extract in cream formulations. A similar result was also revealed by (Hendrawati et al., 2020) that the zone of inhibitions of masker gel peel-off formulations was lower compared to the antibacterial activity of *Ziziphus spina-christi* leaves extract.

Zone of inhibitions of commercial gel as positive control was higher compared to the gel formulations viz. 2.56 cm² for *S. epidermidis* and 1.30 cm² for *P. acnes* (Table 3). It was presumed that commercial gel contained the synthetic compound benzoyl peroxide which inhibits many bacterial species (Ismarani et al., 2014; Yang et al., 2020). There was no inhibition zone in both extract and gel formulations against *P. acnes* (Tables 2 and 3). This may happen because of the differences between these two bacteria responding to antibacterial compounds (Mulyani et al., 2017). (Beylot et al., 2014) studied on three Gram-positive bacteria, namely *P. acnes*, *S. epidermidis*, and *Staphylococcus aureus*, they revealed that each...
species showed different responses to antibacterial compounds treated, mostly related to physical and chemical characteristics of the compounds. Gram-positive bacteria such as *Staphylococcus aureus* and *Streptococcus pyogenes* were inhibited by the methanolic extract of white lead tree leaves with concentrations of as much as 73.39 and 80.28 %, respectively (Alfian *et al*., 2018).

*Propionibacterium acnes* did not show any zone of inhibitions in the extract and the gel formulations, which may be due to strain differences. (Yusufu *et al*., 2019) revealed in their study that the ten strains of *P. acnes* studied showed different resistance to the antibiotic. Several strains of *P. acnes* are resistant to tetracycline, trimethoprim, and rifampicin (Walsh *et al*., 2016). Genetic mutation on the resistance gene in some strains has been studied. Hence it will be harder to inhibit the growth of *P. acnes* in clinical practices.

**Gel Formulations Quality**

The homogeneity test showed no coarse grain in the gel formulations (Table 4), which means the gel formulations were homogeneously prepared (Sekar & Jalil 2017). The higher extract concentration added the gel formulations' color became darker (very dark brown). A similar result was reported by Dewantari & Sugihartini (2015) that the color of HPMC was transparent. Thus the higher concentration of extract added, the darker the color of the gel formulations (Table 4). The textures of gel formulations were classified as soft in all concentrations, meaning the gel's texture was good when applied to human skin (Dewi *et al*., 2018) and fulfilled the National Standardization Agency of Indonesia/NSI (Badan Standarisasi Nasional, 1996).

The higher extract concentrations added, the more increased dispersibility obtained. The gel formulation with 50.00 % extract fulfilled the standard criteria of NSI with an average value of 5.80 cm. Other gel formulations and control treatments were not fulfilled the usual criteria (Figure 1) (Badan Standarisisasi Nasional 1996). The shelf life of the gel formulations for 28 days showed dispersibility as relatively stable. However, there was a bit decrease on day 28 due to storage factors such as temperature, humidity, and type of container (Figure 1). A similar result was reported by Wijayanti *et al*., 2015.

### Table 3. Antibacterial activity of white lead tree gel formulae against *P. acnes* and *S. epidermidis*

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>S. epidermidis</th>
<th>P. acnes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial Gel</td>
<td>2.56 ± 1.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.30 ± 0.39&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>50.00</td>
<td>0.82 ± 0.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>25.00</td>
<td>0.52 ± 0.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>12.50</td>
<td>0.44 ± 0.20&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>6.25</td>
<td>0&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>3.12</td>
<td>0&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gel base (no extract)</td>
<td>0&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: Numbers with the same letters in the same column are not significantly different at a 95 % level of confidence (p < 0.05)

### Table 4. Organoleptic test of white lead tree gel formulae

<table>
<thead>
<tr>
<th>Organoleptic</th>
<th>Concentration (%)</th>
<th>Control</th>
<th>3.12</th>
<th>6.25</th>
<th>12.50</th>
<th>25.00</th>
<th>50.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odor</td>
<td>Odorless</td>
<td>Odorless</td>
<td>typical extract</td>
<td>typical extract</td>
<td>typical extract</td>
<td>typical extract</td>
<td>typical extract</td>
</tr>
<tr>
<td>Color</td>
<td>Transparent</td>
<td>Transparent</td>
<td>Light brown</td>
<td>Dark brown</td>
<td>Dark brown</td>
<td>Very dark brown</td>
<td>Very dark brown</td>
</tr>
<tr>
<td>Texture</td>
<td>Soft</td>
<td>Soft</td>
<td>Soft</td>
<td>Soft</td>
<td>Soft</td>
<td>Soft</td>
<td>Soft</td>
</tr>
</tbody>
</table>

<sup>a</sup>NSI: National Standardization Agency of Indonesia
A stickiness test was done to specify the gel formulations' duration on the skin, which gave enough time for the bioactive compounds to penetrate the skin (Wibowo et al., 2017). The overall value of stickiness of the gel formulations ranged from 1.34 to 7.06 seconds (Figure 2). The higher concentration of extract added in the gel formulations, the lower the stickiness value. Because when the gel viscosity is reduced, it will decrease the stickiness value (Dewantari & Sugihartini 2015). A similar result was reported by Sunnah et al. (2018) that viscosity and stickiness were directly proportional; as a consequence, the lower viscosity will decrease the stickiness value of the gel formulations. The shelf life for 28 days did not significantly affect the stickiness but showed an increase on the 28th day (Figure 2). This was presumed due to the physical characteristics of hydroxypropyl methylcellulose (HPMC) as the main ingredient in the gel formulation (Zhang et al., 2017).
A pH test was done to establish the gel formulations' acidity level to know the gel's safety when applied to the skin (Sekar & Jalil 2017). The pH value ranged from 5.44 to 6.41 (Figure 3), fulfilling the NSI standard (Badan Standarisasi Nasional 1996). The shelf life for 28 days did not affect the pH value, although there was a slight decrease on day 28 (Figure 3).

A viscosity test was done to determine the consistency of the gel formulations. Gel with an extract concentration of 50.00% gave average viscosity values of as much as 502.29 cP, indicating that it did not fulfill the S standard (Figure 4) (Badan Standarisasi Nasional 1996). Extract concentration of 50.00% contained the highest plant extract compared to other treatments; as a result, the additional extract may decrease the consistency of the gel. A similar result was reported by Alfian et al. (2018) that showed the higher extract concentration of white lead tree leaves added the lower consistency of the gel. The longer time of shelf life, the lower of viscosity of the gel (Figure 4), which was presumed affected by glycerin contained in the formulations. Glycerin has hygroscopic characteristics that may adsorb water from the environment; thus, the water content in the gel formulation became higher.
CONCLUSION

The methanolic extract of a white lead tree only inhibited the growth of *S. epidermidis* at concentrations ranging from 3.12-50%, but *P. acnes* were not inhibited. The gel formulation containing 50% extract effectively inhibited bacterial activity against *S. epidermidis*. The quality of the formulated gel, such as homogeneity, pH, and stickiness parameters, fulfilled the Indonesian Standard Nasional. However, it is not suitable for dispersibility and viscosity. The shelf-life of the gel lasted for 28 days. Further study is needed to extend the self-life of the gel. A combination of a white lead tree extract with another herbal for *P. acnes* might be interesting to study.

ACKNOWLEDGEMENT

The authors expressed gratitude to the Head of the Teknobio-Industry Laboratory and laboratory technicians for providing and facilitating this study.

CONFLICT OF INTEREST

The researchers declare that there is no conflict of interest in publishing the study's data.

CONTRIBUTORSHIP

AM did the main research, BRS did the microbiological assay, and EM did the gel formulations. All authors contributed equally to this study.

DAFTAR PUSTAKA


