Leaf extract of *Strychnos ligustrina* Blume inhibited *Propionibacterium acnes* growth in vitro

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**Abstract.** *Propionibacterium acnes* plays an important role in inducing skin inflammatory mediators and comedogenic acne. This study aimed to evaluate the antimicrobial effect of *Strychnos ligustrina* Blume leaf extract against *P. acnes*. This study was an in-vitro experiment with a completely randomized design (CRD) testing five concentrations of ethanolic extract of *S. ligustrina* leaves. The control treatment was the antibiotic Kindamicin. The anti-bacterial evaluation of the extract was carried out on Mueller-Hinton agar media by using the diffusion method. The inhibition zone was assessed according to NCCLS (National Committee for Clinical Laboratory Standards). Analysis and interpretation of the results used 95% ANOVA (α: 0.05). The results of the study showed that *S. ligustrina* leaf extract inhibited the bacterial growth of *P. acnes*. The minimum concentration of the extract that inhibited the growth of *P. acnes* was 25% with a diameter inhibition zone of 4.13 ± 0.13 mm, while the highest concentration of the undiluted extract was 7.88 ± 0.67 mm. Compared to the standard antibiotic Kindamycin (positive control), the ethanolic leaf extract of *S. ligustrina* was less effective having an inhibition zone of 24.81 ± 0.54 mm. The study suggests that *S. ligustrina* leaf extract has antimicrobial effects on *P. acnes* and may be further studied as an herbal remedy for acne.

1 Introduction

Acne (*Acne vulgaris*) is one of the skin diseases that occurs due to blockage of follicles, sebum, and inflammation caused by acne-causing bacteria such as *S. aureus*, *S. epidermis*, and *P. acnes* [1]. *P. acnes* is referred to as a Gram-positive bacterium that will result in tissue inflammation so that the skin feels itchy and painful. This bacterium will increase sebum production because fat is the main food for *P. acnes* [2]. The use of chemicals can stimulate the occurrence of bacterial resistance, therefore an alternative to natural materials is needed.

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Strychnos ligustrina leaves can be used as a natural antibiotic that is proven to treat acne-prone skin. Traditionally, S. ligustrina leaves are commonly used as a skin moisturizer, addressing beauty issues, antimicrobial, anti-inflammatory, antifungal, and antioxidant [3]. Suriaman and Khasanah [4] reported that it can inhibit the bacteria S. aureus and has an antimicrobial effect on E. coli and C. albicans [5]. Various studies have shown that S. ligustrina extract is beneficial because it has active compounds that can anchor the growth of acne-causing bacteria. In addition, there are still few who explore the antibacterial effectiveness of S. ligustrina leaves, especially in P. acnes bacteria. Thus, research was carried out on antibacterial activity regarding S. ligustrina leaf extract against acne-causing bacteria Propionibacterium acnes. This study aims to determine the phytochemical profile of S. ligustrina leaf extract in a quality and quantitative manner, look at antibacterial activity, and determine the smallest concentration in inhibiting the growth of P. acnes. The results of this study can provide natural antibacterial alternatives sourced from S. ligustrina leaf extract which can be developed into health and beauty products and can be a consideration for the use of these two plants in treating acne based on their antibacterial effectiveness.

2 Materials and methods

Researchers at the Indonesian Spice and Medicinal Plants Research Institute's disease laboratory in March – June 2022. This study used a laboratory experimental method using a Complete Randomized Design (CRD) with 4 repeats. Data analysis was carried out using One Way ANOVA method and Duncan's follow-up test at a 95% confidence level.

2.1 Strychnos ligustrina extract

S. ligustrina leaves are obtained from the Grobogan Regency of Central Java. The leaves are dried for 7 days at room temperature, then a size reduction was carried out using a blender. The powder is then sifted using a sieve with a size of 40 mesh. The extraction of S. ligustrina leaves was carried out using ethanol solvent 750 × 5 mL by the maceration method. Maceration is carried out for 2 × 24 hours with occasional stirring. Then the extract is filtered with a vacuum filter and concentrated using a vacuum rotary evaporator at a temperature of 50°C, speed of 50 rpm, for ± 2 hours until no solvent drips.

2.2 Water content analysis

Water content measurement of the powder preparation was carried out using the gravimetric method, three repetitions. The powder was heated at a temperature of 105°C for 30 min and then cooling it in a desiccator for 15 min (W₀). A sample of 1 gram was put into a saucer and re-weighed (W₁). It was then oven to a temperature of 105°C for 1 hour, cooled again in a desiccator, and then weighed (W₂) [6]. The moisture content is calculated using the following equation:

\[
\text{Water content (\%)} = \frac{W₁ - W₂}{W₁ - W₀} \times 100\% \quad (1)
\]

2.3 Extract yield

The extraction results of S. ligustrina leaves are calculated as the yield value to see the amount of extract obtained [7]. The calculation of the extract amendment obtained is as follows:
Yield (%) = \( \frac{\text{extract weight (g)}}{\text{sample weight (g)}} \times 100\% \) \hspace{1cm} (2)

2.4 Active compound testing

Testing of active compounds was carried out qualitatively and quantitatively. In qualitative analysis, an extract has added a reagent, if there is a change in color, the formation of a precipitate, or the formation of foam, it shows results (+) meaning that plants contain a class of active compounds. The results that showed positive were followed by quantitative testing to determine the levels of active compounds [8]. Here is the quantification equation of the total content of phenolic compounds (mg GAE/g), flavonoids (mg QE/g), tannins (mg TAE/g), and saponins (mg DE/g).

\[ \text{Total active compounds (mg/g)} = \frac{c \times V \times df}{m} \] \hspace{1cm} (3)

Notes:
c = concentration of active compounds (ppm or mg/L), V = volume of solvent used for extraction (L), df = dilution factor in quantification. m = weight of extracted dry matter (g)

2.5 Antibacterial activity

The antibacterial activity test of S. ligustrina extract was carried out using pathogenic bacteria, namely Propionibacterium acnes by disc diffusion method on MHA (Mueller Hinton Agar) media. The concentration of extracts used was 5%, 10%, 25%, 50%, and 100%, as well as aqueous as a negative control and clindamycin at 1% as a positive control. In this test, the value of the minimum inhibitory concentration or the lowest concentration of the extract was sought in inhibiting the growth of P. acnes. According to Toy et al., [9] the formula for calculating the inhibition zone is as follows:

\[ \text{inhibition zone (mm)} = \frac{(D_v-D_c)+(D_H-D_c)}{2} \] \hspace{1cm} (4)

Notes:
\(D_v\) = vertical diameter of the clear zone on media (mm), \(D_H\) = the horizontal diameter of the clear zone on the media (mm), \(D_c\) = disc paper diameter (mm).

3 Results and discussion

3.1 Water content

The moisture content of the S. ligustrina leaf powder ranged from 8.87-9.13% with an average of 9.02 ± 0.12% was 9.02% (Table 1). Therefore, it agreed with the Indonesian National Standard (SNI) of less than 10% [10]. Having a moisture content of less than 10% will extend the quality and shelf life of the powder. Dried leaves with higher moisture content are vulnerable to the growth of microbes, such as fungi and mold, that cause enzymatic processes that will change the active compounds of the herbs and affect pharmacological activity [11].
Table 1. The results of testing the water content of the S. ligustrina leaf powder.

<table>
<thead>
<tr>
<th>Replication</th>
<th>Water content</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.87%</td>
</tr>
<tr>
<td>2</td>
<td>9.13%</td>
</tr>
<tr>
<td>3</td>
<td>9.07%</td>
</tr>
<tr>
<td>Average</td>
<td>9.02% ± 0.12</td>
</tr>
</tbody>
</table>

3.2 Extract yield

The extract of S. ligustrina leaves has a blackish-green color, a characteristic odor, and a viscous concentration (Table 2). The concentrated extract yield was 15.41%. This extract yield was higher than that obtained in a previous study by Jannah of 10.6% using an 80% ethanol solvent [12].

Table 2. The yield of S. ligustrina concentrated leaf extract.

<table>
<thead>
<tr>
<th>Dry leaf weight (g)</th>
<th>Total simplicity (g)</th>
<th>Concentrated Extract Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>620</td>
<td>500</td>
<td>15.41</td>
</tr>
</tbody>
</table>

3.3 Active compound testing

The concentrated extract of S. ligustrina leaves extract taken from Grobogan Regency, Central Java contained flavonoids, tannin, saponin, and phenolics in high concentrations ranging from large (+++) to moderate (+) (Table 3). Therefore, the extract is suitable to be used for further study. The absence of alkaloid and triterpenoid groups in the extract might be affected by physical molecular tend to be semipolar for alkaloid (strychnine, novacine, brucine and its other strychnine derivatives) [13].

Table 3. Phytochemical characteristics of S. ligustrina leaf extract.

<table>
<thead>
<tr>
<th>Phytochemical Test</th>
<th>References</th>
<th>Leaf Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>Red-orange precipitate</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>Black, reddish, yellow, orange</td>
<td>++</td>
</tr>
<tr>
<td>Tanin</td>
<td>Green-blue (catechol tannins), blue-black (pyrogallol tannins)</td>
<td>+++</td>
</tr>
<tr>
<td>Saponin</td>
<td>Foam forms on the surface</td>
<td>++</td>
</tr>
<tr>
<td>Phenolic</td>
<td>Bluish-green or blackish blue</td>
<td>++</td>
</tr>
<tr>
<td>Triterpenoid</td>
<td>Brown/violet ring (terpenoids), turquoise ring (steroids)</td>
<td>-</td>
</tr>
</tbody>
</table>

Notes: the sign (+) indicates the level of color intensity and the sign (-) indicates the compound does not contain

Table 4 shows the results of the test of the levels of active compounds of the phenolic group with a standard curve of gallic acid obtained a regression equation with a coefficient of determination proportional to 99.16%. Phenolic levels \( y = 0.0081x + 0.004 \) of \( R^2 = 0.9916 \). S. ligustrina leaf extract was 102.82 ± 0.42 mg GAE/g. Phenolics can enter the cell wall and damage hydrophobic bonds (phospholipids and proteins) which can cause cell nutrients to leak so that enzyme biosynthesis and bacterial metabolic activity are disturbed [14].
Table 4. The determination of the active compound content of *S. ligustrina* leaf extract.

<table>
<thead>
<tr>
<th>No</th>
<th>Test Parameters</th>
<th>mg/gram</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Total phenolic gallic acid equivalent</td>
<td>102.82±0.42</td>
</tr>
<tr>
<td>2.</td>
<td>Total flavonoids equivalent to quercetin</td>
<td>148.59±0.09</td>
</tr>
<tr>
<td>3.</td>
<td>Total Tannins equivalent to tannic acid</td>
<td>36.87±0.05</td>
</tr>
<tr>
<td>4.</td>
<td>Total Saponins equivalent to diosgenin</td>
<td>103.58±0.03</td>
</tr>
</tbody>
</table>

The results of the quantitative test of total flavonoid levels with a quercetin standard curve obtained a linear regression equation with a coefficient of determination proportional to 99.35%. Based on the regression equation, flavonoid levels $y = 0.0043 + 0.0174x$ of $R^2 = 0.9935$. *S. ligustrina* was obtained by $148.59 ± 0.09$ mg QE / g. The flavonoid compounds can inhibit bacterial growth by forming complex bonds with proteins, inhibiting the synthesis of DNA and RNA, and inhibiting the process of respiration and energy metabolism in bacteria [15].

Quantitative testing of tannins using the tannic acid standard curve obtained a regression equation with a coefficient of determination proportional to 96.94%. The total tannin content of $y = 0.0211x + 0.0429R^2 = 0.9694$. *S. ligustrina* leaves are $36.87 ± 0.05$ mg TAE/g. Tannin group compounds can also help inhibit the growth of bacteria. Tannins will enter the cell to disintegrate the genetic material, inhibit DNA topoisomerase, and disrupt protein metabolism in the cell so that the bacteria will not live [16]. The standard diosgenin curve in saponin testing has a linear regression equation $y = 0.0009x - 0.0119$ with a coefficient of determination proportional to 99.62%. Based on the results of this study, it can be concluded that the total saponin compounds of $R^2 = 0.9962$. *S. ligustrina* leaves are $103.58 ± 0.03$ mg DE / g. Saponins have a role to lower surface tension and increase cell wall permeability [17].

3.4 Antibacterial activity

Measurement of antibacterial activity of *S. ligustrina* leaf extract with concentrations of 5%, 10%, 25%, 50%, and 100% has different inhibitory values. Table 5. shows that of the five concentrations of *S. ligustrina* leaf extract, only three concentrations of extracts can inhibit the growth of *P. acnes* bacteria. Akuades as a negative control used as a solution for dilution of the extract does not affect inhibiting the growth of *P. acnes*, it can be seen from the non-formation of clear zones or inhibitory zones. This means that the death of *P. acnes* bacteria comes from the extract, there is no influence from other ingredients.

Table 5. Antibacterial activity of *S. ligustrina* leaf extract.

<table>
<thead>
<tr>
<th>Extract Concentration (%)</th>
<th>Inhibition Zone Diameter (mm) Average+SD</th>
<th>Inhibition Zone Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.000&lt;sub&gt;a&lt;/sub&gt;</td>
<td>None</td>
</tr>
<tr>
<td>10</td>
<td>0.000&lt;sub&gt;a&lt;/sub&gt;</td>
<td>None</td>
</tr>
<tr>
<td>25</td>
<td>4.13±0.13&lt;sub&gt;b&lt;/sub&gt;</td>
<td>Weak</td>
</tr>
<tr>
<td>50</td>
<td>4.75±0.31&lt;sub&gt;c&lt;/sub&gt;</td>
<td>Weak</td>
</tr>
<tr>
<td>100</td>
<td>7.88±0.67&lt;sub&gt;d&lt;/sub&gt;</td>
<td>Moderate</td>
</tr>
<tr>
<td>Clindamycin 1% (+)</td>
<td>24.81±0.54&lt;sub&gt;e&lt;/sub&gt;</td>
<td>Very strong</td>
</tr>
<tr>
<td>Sterile water(-)</td>
<td>0.000&lt;sub&gt;a&lt;/sub&gt;</td>
<td>None</td>
</tr>
</tbody>
</table>

Notes: data has been reduced disc diameter of 6.5 mm; Different notation letters show significant differences according to Duncan's test results with a 95% confidence level.
The results of antibacterial testing of *S. ligustrina* leaves show that not all concentrations can inhibit the contamination of *P. acnes* bacteria. *S. ligustrina* leaf extracts with concentrations of 5%, 10%, 25%, 50%, and 100% have an average inhibition zone diameter of 0 mm, 0 mm, 4.13 mm, 4.75 mm, and 7.88 mm, respectively. Concentrations of 5% and 10% of *S. ligustrina* leaf extract did not show bacterial inhibitory power, concentrations of 25% and 50% were included in the weak category, and 100% concentrations belonged to the moderate category. The minimum inhibitory concentration value formed from testing *S. ligustrina* leaf extract is at a concentration of 25% with an average inhibitory zone diameter of 4.13 mm ± 0.13 which is included in the weak category.

Differences in the type of material or simplisia will affect the inhibitory power formed because the levels of active ingredients contained in a simplisia vary. Brock et al. stated that the higher the concentration of the extract, the more active ingredient content in the sample serves as antibacterial [18]. A high concentration of the extract will accelerate diffusion so that the diameter of the antibacterial inhibition zone formed will be even greater. This is following the results of the study that *S. ligustrina* extract with an extract concentration of 100% has the greatest inhibitory power. Duncan's follow-up test results on *S. ligustrina* leaf extract, negative control group, concentrations of 5%, and 10% did not differ significantly because no inhibitory zone was formed. Whereas concentrations of 25%, 50%, 100%, and positive controls differed significantly. Positive control differs very markedly from other concentrations because positive control is an antibiotic to treat acne bacteria. As per the statement, subsequent studies can select extract concentrations that differ significantly against the average resulting inhibitory zone yield.

**4 Conclusion**

*S. ligustrina* leaf extract contains active compounds of flavonoids, tannins, saponins, and phenolics. These compounds help inhibit the growth of *P. acnes* bacteria. *S. ligustrina* leaf extract has antibacterial activity against *P. acnes* with a minimum inhibitory concentration value of 25%. Further study is required to develop a herbal cosmetic based on the *S. ligustrina* leaf extract for treating *P. acnes*.

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