Antibacterial evaluation of Spray Gel Formula of Angsana Leaf Extract (Pterocarpus indicus Willd.) Using Combination Base of Carbopol 940 and HPMC

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Abstract. The 70% ethanol extract of 70% angsana leaves known have antibacterial activity. The aim of this study was to determine the inhibitory activity produced by a spray gel using a combination base of carbopol 940 and HPMC against Staphylococcus aureus as one of bacteria causes of skin infections. In this study, 3 formulas were prepared with various base concentrations then tested for its antibacterial activity by disc diffusion method. On initial study showed that concentration of 30% angsana leaves extract gave best inhibition so it was used in formula. The base concentration at F1 was 0.4% carbopol 940 and 0.4% HPMC, F2, 0.2% carbopol 940 and 0.4% HPMC and F3, 0.4% carbopol at 10.5, 11mm, and 11mm. Combination gelling agent used in formula didn’t showed significance difference in antibacterial activities, but the formulas need further study to evaluated the performance.

Keywords: Angsana leaves; spray gel; Staphylococcus aureus, Pterocarpus indicus Willd.

1 Introduction

The angsana plant (Pterocarpus indicus Willd.) is one of the plants that grow in Indonesia. The angsana plant is widely cultivated in the tropics. In some countries, this plant is known as a protective plant along the road and can also be used as decoration. Angsana plants are also commonly used as medicine. Traditionally, the bark of this plant can treat thrush, kidney stones, and can lower blood pressure. Then, the leaves of this plant can be used to treat diabetes and ulcers[1]. In a study conducted by Fatimah (2004), angsana leaf extract can inhibit the growth of gram-positive bacteria Staphylococcus aureus where these bacteria can cause skin infections such as furuncle, carbuncle, acne, and folliculitis[2]. Spray gel is a form of gel development. The advantages of this form include that it allows the preparation to be delivered to a specific target or destination without contact with the cotton swab, thereby minimizing waste, reducing the possibility of contamination or infection and patient trauma. One of the polymers that can be used as a base for spray gel is carbopol which is also widely used as a gelling agent. The results of spray gel preparations based on carbopol is not optimal because carbopol has a high viscosity[3]. Therefore, it is combined with HPMC which can be used as a stabilizing agent in topical gels and ointments [4]. The advantage of carbopol and HPMC is that they form a clear, water-soluble gel. This combination is expected to be applied as a gelling agent in spray gel formulations by forming a diffuse spray pattern. Present study, the inhibitory activity produced by a spray gel using a combination base of
carbopol 940 and HPMC against *Staphylococcus aureus* as one of bacteria causes of skin infections were evaluated.

2 Method

2.1 Materials

Angsana leaves (*Pterocarpus indicus* Willd.) collected from Rajeg sub-district, Tangerang regency, Banten. Harvested in December 2018.

2.2 Chemicals

Aquadest, H\textsubscript{2}SO\textsubscript{4}, BaCl\textsubscript{2}, Karbopol 940 (CV. Total Equipment Pharmacy), Hydroxy Propyl Methyl Cellulose (Total Equipment Pharmacy), Propylenglycol (Total Equipment Pharmacy), Triethanolamine (Total Equipment Pharmacy), Methyl Parabens (Total Equipment Pharmacy), Propyl Parabens (Total Equipment Pharmacy), NaCl (CV. Total Equipment Pharmacy), *Staphylococcus aureus* bacteria isolate (Clinical Microbiology Laboratory, Faculty of Medicine, University of Indonesia, Jakarta), nutrient agar (Angkasa Abadi) media, Mueller hinton agar (Angkasa Abadi).

2.3 Plant Extraction

Angsana leaf powder (*Pterocarpus indicus* Willd.) was macerated with 70\% ethanol, left for 48 hours, then filtered and concentrated using a rotary vacuum evaporator at a temperature of not more than 40\(^\circ\)C until a viscous extract is obtained.

2.4 Preparation of Bacterial Suspensions

Culture of the test bacteria was suspended with a 0.9\% sodium chloride solution to obtain the same turbidity as the standard Mc. Farland (1 × 10\(^8\) CFU / ml). 0.1ml was taken, the bacterial suspension was added with 9.9ml of 0.9\% sodium chloride solution, shaken until homogeneous in order to obtain a bacterial suspension of 10 CFU / ml [2].

2.5.1 Preparation of Nutrient Agar

The nutrient media for 20 grams is weighed then dissolved in 1 L of aquadest, heated until dissolved. Then, sterilized using autoclave. Thus, the media is placed into a test tube to make it tilted [5].

2.5.1 Rejuvenation of *Staphylococcus aureus*

The rejuvenation of bacteria was carried out by planting the *Staphylococcus aureus* bacteria onto the agar medium for nutrient agar that had been prepared by scraping a loop of bacterial isolate, then incubating it at 37\(^\circ\)C-38\(^\circ\)C for 24-48 hours [6].

2.6 Preparation of Mueller Hinton Agar

A total of 38 grams of MHA were dissolved in 1 L of aquadest then heated and stirred using a magnetic stirrer until homogeneous. The media was sterilized by using autoclave at 121\(^\circ\)C for 15 minutes. After being sterilized, it is put into a petri dish as much as ± 15 ml which will be used as a medium in the antibacterial test [7].
2.7 Antibacterial activity assays
Various extract concentrations were made, each of 5%, 10%, 20%, 30%, 40%. Each of these extracts was dissolved using DMSO solvent. After that, 30 µl of the angsana leaf extract which has been dissolved with these various concentrations, is dropped into an empty sterile dish disc, then 2 controls are made, namely solvent and clindamycin with a concentration of 10 μg / ml, then left for 15 minutes, and left to stand in an incubator at 35 oC for 24 hours. The incubation results were then viewed and clear areas were measured [7].

2.8 Spray Gel Formulation
Angsana extract is formulated following the method presented by Kamishita (1992) with a view of modifications.

| Table 1. Composition of Angsana Leaf Extract Spray Gel Formula |
|---------------------------------|----------------|----------------|
| **Material** | **Formulas (%)** | **Function** |
| Angsana leaf extract | 30 | 30 | 30 | Test samples |
| Carbopol | 0.4 | 0.4 | 0.4 | Gelling agent |
| HPMC | 0.4 | 0.4 | 0.2 | Gelling agent |
| TEA | 8 drops | 8 drops | 8 drops | Base |
| Propilenglicol | 15 | 15 | 15 | Humectan |
| Methyl p-hydroxy benzoate | 0.05 | 0.05 | 0.05 | Preservative Basics |
| Propyl p-hydroxy benzoate | 0.05 | 0.05 | 0.05 | Preservative |
| Ethanol | 20 | 20 | 20 | Extract solvent |
| NaCl 3.5% | 5ml | 5ml | 5ml | Viscosity regulator |
| Aquadest | Ad 100 | Ad 100 | Ad 100 | Solvent |

2.9 Preparation of The Angsana Leaf Extract Formula
The extract was dissolved with ethanol, carbopol 940 was dispersed in hot water until carbopol 940 was completely dispersed. After that, TEA is added to form a transparent gel mass, the weighed HPMC is dispersed in cold water and warm water is added until the HPMC is completely dispersed and becomes a clear liquid with a thick enough consistency, propyl paraben and methyl paraben are homogenized with the propylenglycol provided, HPMC and carbopol 940 which have been dispersed are mixed together, then stirred until homogeneous, after homogeneity is added with homogeneous methylparaben, propylparaben and propylenglycol, then stirred until homogeneous, the homogeneous preparation is sprinkled with NaCl solution until the viscosity drops to slightly liquid, then the process is stopped, after that, the diluted extract is added, then stirred until it is homogeneous, after that, the preparation is added with aquadest until the weight reaches 100, then stirred until it is homogeneous.

2.10 Antibacterial test of formula against Staphylococcus aureus
This test was performed by using the swab method. Culture of bacteria in a swab above ± 15 ml of MHA that has been solid. The gel solution was prepared by dissolving 100 mg of spray gel in 10 ml of DMSO. 0.03 ml of ready-to-take gel spray solution using a micropipette is dropped on sterile disc paper then planted on solid MHA medium in a petri dish. After that it was incubated for 24 hours at 37°C. Next, measure the formed inhibition zone [8].
3 Result and Discussion

3.1 Antibacterial test of extract against Staphylococcus aureus

In this study, the inhibition test of the ethanol extract of 70% angsana leaves against *S. aureus* bacteria with a concentration of 5%, 10%, 20%, 30% and 40%. The average inhibition generated at each concentration, namely, 8.5 mm, 8.5 mm, 14 mm, 16 mm and 17 mm. Where the inhibition power at a concentration of 5% and 10% is included in the moderate inhibition zone category. Whereas at concentrations of 20%, 30% and 40% are included in the strong inhibition zone category [9]. The positive control used in this study was the clindamycin disk which produced an average inhibitory power of 40 mm. As well as the negative control used, namely, the solvent to dissolve the extract that did not produce inhibition in this study. The extract concentration selected for inclusion in the preparation was 30% extract concentration.

<table>
<thead>
<tr>
<th>Concentrations (b/v)</th>
<th>Inhibition Diameter ± SD (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>8.5 ± 0.547</td>
</tr>
<tr>
<td>10</td>
<td>8.5 ± 0.547</td>
</tr>
<tr>
<td>20</td>
<td>14 ± 0.632</td>
</tr>
<tr>
<td>30</td>
<td>16 ± 0.632</td>
</tr>
<tr>
<td>40</td>
<td>17 ± 0.632</td>
</tr>
<tr>
<td>Positive control</td>
<td>40 ± 0.632</td>
</tr>
<tr>
<td>Negative control</td>
<td>0</td>
</tr>
</tbody>
</table>

3.2 Angsana Leaf 70% Ethanol Extract Spray Gel Formulation

In the formulation the ingredients used included 70% ethanol extract of angsana leaves as the active substance, carboxol 940 and HPMC were used as a gelling agent, TEA was used as a base, propylene glycol as a humectant, methyl paraben and propyl paraben were used as ingredients, ethanol is used as a solvent for extracts and solvent for preservatives, NaCl is used as a viscosity regulator and aquadest is used as a solvent.

In this formulation, three formulas were made with various concentrations between carboxol 940 and HPMC as a gelling agent. Where at F1 the concentration of carboxol was 0.4% and HPMC was 0.4%, F2 was 0.2% and HPMC was 0.4% and F3 was 0.4% and HPMC was 0.2%. In each formula there is an active substance, namely, 30% of angsana leaves ethanol extract. Where the concentration is taken from the initial experiment in the extract inhibition test.

3.3 Antibacterial test of formula against Staphylococcus aureus

The test for antibacterial activity on the ethanol extract spray gel preparation of 70% angsana leaves aims to determine the ability of the preparation to inhibit bacterial growth. In this study, *S. aureus* bacteria were used, which are gram-positive bacteria. This antibacterial activity test was carried out by using the disc diffusion method. The activity test of the spray gel preparation against *S. aureus* bacteria was carried out in three formulas. The activity test was carried out in duplicate. The inhibition test was carried out using the disc diffusion method in LAF aseptically. The results of the bacterial inhibition test on the three formulas for the spray gel preparation were as follows.
Table 3. Diameter of inhibition and standard deviation of preparation

<table>
<thead>
<tr>
<th>Inhibition diameter ± SD (mm)</th>
<th>Spray gel base</th>
<th>Spray gel formula</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F1</td>
<td>F2</td>
</tr>
<tr>
<td>6.5 ±</td>
<td>0.577</td>
<td>0.577</td>
</tr>
<tr>
<td>11 ±</td>
<td>0.577</td>
<td>0.577</td>
</tr>
</tbody>
</table>

The table 3 shows the presence of antibacterial activity on the base of the preparation. This may be due to the presence of preservatives and alcohol in the formula. Whereas propyl p-hydroxy benzoic and methyl p-hydroxy benzoic have antimicrobial activity on a broad spectrum [4]. In addition, alcohol also has activity as a bactericidal, which can kill bacteria in its vegetative form. The average diameter of the inhibition for the three basic formulas is 6.5 mm. The table shows that the F1, F2 and F3 spray gel preparations provide inhibition of *S. aureus* bacteria growth in F1 with 10.5 mm of inhibition, F2 with 11 mm of inhibition, and F3 with 11 mm of inhibition, respectively. Each formula has the same inhibition zone strength category. Where the activity test results of spray gel preparations on the three formulas are included in the strong inhibition zone category [9].

Carbomer is a non-particular name for a gathering of polymers known as Carbopol. They thicken at higher pH (around 5 or 6) and will similarly swell in liquid game plan of that pH to as much as 1000 times their extraordinary volume [10]. HPMC produces an elastic and tough coating which is highly consistent, economical, printable, non-allergenic, non-calorigenic, micro structural and are more resistant to microbial attack [11]. Due to solubility problems, most of the lipophilic drugs cannot be formulated directly as a hydrogel. Otherwise, emulgel provides better stability and release of the lipophilic drug in comparison with simple hydrogel base [12]. Various novel polymers are being building up nowadays which are demonstrating multi works basically as thickeners and emulsifiers [10]. Noor *et al.* (2019) found that metronidazole benzoate emulgel formulations prepared with Carbopol 940 (F4) showed acceptable physical properties, drug content, and drug release which deliver about 9% of drug within 5 h compared to formulation prepare with HPMC [13]. This study showed that difference in the concentration of the gelling agent in each formula did not give a significant difference to the inhibition of *S. aureus* bacteria. Formula needs further characterization to determine the effect of the emulgel on the performance of the preparation.

4 Conclusion

The ethanol extract of 70% angsana leaves can be formulated as a spray gel using a variety of gelling agent concentrations. The difference in the concentration of the gelling agent did not result in a significant difference in antibacterial activities but the formula requires further study to determine the performance of the formulas.
5 References


