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

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Abstract. Angsana leaves ethanol extracts known to provide antibacterial activity. The aim of this study was to determine the inhibitory activity produced by spray gel using a combination base of carbopol 940 and HPMC against *Staphylococcus aureus* as one of the bacteria causes of skin infections. Three 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 was 0.2% carbopol 940 and 0.4% HPMC and F3 was 0.4% carbopol at 10.5, 11mm, and 11mm. The combination of gelling agent used in the formula did not show a significant difference in antibacterial activity, but the formula needs to be studied further to evaluate its performance.

Keywords: Angsana leaves; spray gel; *Staphylococcus aureu*s, *Pterocarpus indicus* Willd.

1 Introduction

The angsana plant (*Pterocarpus indicus* Willd.) 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, its bark use to treat thrush, kidney stones, and lower blood pressure. Hence, the leaves was used to treat diabetes and ulcers[1]. Previous study conducted by Fatimah (2004), angsana leaf extract can inhibit the growth of gram-positive bacterium *Staphylococcus aureus* which can cause skin infections such as furuncles, boils, acne, folliculitis [2].

Spray gel is a form of gel development with the advantage of allowing preparations to be delivered to specific targets or destinations without contact with cotton, thereby minimizing waste, reducing the possibility of contamination or infection and trauma to the patient. 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 its 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

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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, Banten in December 2018.

2.2 Chemicals

Aquadest, H_2SO_4, $BaCl_2$, carbopol 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), isolate of *Staphylococcus aureus* (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 leaves 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 40ºC until a thick 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 9.9 ml 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

20 grams nutrient agar were dissolved in 1 L of aquadest, heated until the solution was clear. 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 transfering the bacteria onto the medium that had been prepared by scratching a loop of bacterial isolate, then incubating it at 37º C-38ºC for 24-48 hours [6].

2.6 Preparation of Mueller Hinton Agar

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 121ºC 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 disc, with a clindamycin as a positive control with a concentration of 10 µg/ ml, then left for 15 minutes, and left to stand in an incubator at 35ºC for 24 hours. Antibacterial activity was determined by measuring the diameter of the clear area formed [7].

2.8 Spray Gel Formulation

Ansana 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

Materials	Formulas (%)			Function
	A	B	C	
Angsana leaf extract	30	30	30	Test samples
Carbopol	0.4	0.2	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, 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. HPMC and carbopol 940 which have been dispersed are mixed together, then stirred until homogeneous, after homogeneous added with methylparaben, propylparaben and propylenglycol, then stirred until homogeneous, the homogeneous preparation is mixed 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 homogeneous.

2.10 Antibacterial test of formula against *Staphylococcus aureus*

This test is carried out using the swab method. Bacterial cultures were swabbed in ± 15 ml of solidified MHA. The gel solution was prepared by dissolving 100 mg of spray gel in 10 ml of DMSO. 0.03 ml of ready-to-use gel spray solution using a micropipette was dripped onto sterile disc paper and then placed on solid MHA media in a petri dish. After that, it was incubated for 24 hours at 37ºC and the inhibition zone formed was measured [8].

3 Result and Discussion

3.1 Antibacterial test of extract against *Staphylococcus aureus*

Present study, the inhibition test of the ethanol extract of angsana leaves against *S. aureus* with a concentration of 5%, 10%, 20%, 30% and 40%. The average inhibition at each concentration, respectively was 8.5 mm, 8.5 mm, 14 mm, 16 mm and 17 mm. Where the inhibition 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 of 40 mm. As well as the negative control used, did not produce inhibition in this study. The concentration of the extract chosen to be included in the formula is the extract concentration of 30%.

Table 2. Inhibition diameter of extract

Concentrations (b/v)	Inhibition Diameter ± SD (mm)
5	8.5 ± 0.547
10	8.5 ± 0.547
20	14 ± 0.632
30	16 ± 0.632
40	17 ± 0.632
Positive control	40 ± 0.632
Negative control	0

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, carbopol 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 carbopol 940 and HPMC as a gelling agent. Where at F1 the concentration of carbopol 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

Inhibition diameter ± SD (mm)					
Spray gel base			Spray gel formula		
F1	F2	F3	F1	F2	F3
6.5 ± 0.577	6.5 ± 0.577	6.5 ± 0.577	10.5± 0.577	11 ± 0.577	11 ± 0.577

Table 3 shows the presence of antibacterial activity on the base of the formulas. 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 studied need characterization further to determine the effect of the emulgel on the performance of the preparation.

4 Conclusion

The ethanol extract of 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.

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