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Research Article

Sungkai Leaf Extract (Peronema Canescens Jack) As Antibacterial Agent In Wound Dressing

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Abstract

The leafes of the Sungkai plant (Peronema canescens Jack) is an herbal plant that contains secondary metabolites such as alkaloids, flavonoids, and tannins. The tannins that found in Sungkai leafes act as an inhibitor in bacteria. This study aims to analyze tannin content that is found in young sungkai leafes and old sungkai leafes. And then to analyze the performance of sungkai leaf extract in the making of wound dressing and measuring the distance of Staphylococcus Aureus bacteria after the addition of sungkai leaf extract and wound dressing making materials. Using UV-VIS spectrophomete, for the highest tannin yield there is a variable mass sample of young sungkai leafes 20 grams with an extraction time of 180 minutes which is 293.03ppm in young sungkai leafes and old sungkai. For samples of old sungkai leafes with a mass variable of 20 grams and extraction time of 180 minutes, there is a tannin content of 291.65 ppm.And for the performance of sungkai leaf extraction as a modern wound dreassing against staphylococcus aureus bacteria, the best combination of liquid Sodium Alginate + Glycerol + Sungkai Extract 100 % with a greater bacterial barrier distance of 13 mm compared to the combination of liquid Sodium Alginate + Glycerol + Sungkai Extract 50 %. 0 mm. Then the extraction performance on the film layer without wound dressing combination is better with 100 % Sungkai Extract variable that is 22.5 mm with positive control comparison using Amoxicillin of 33 mm.

Keywords: Sungkai Leafes, Tannins, Extraction, UV-VIS Spectrophotometer

1. Introduction

There are many plants that can be used for health in Indonesia. One of them is Sungkai plant. Sungkai plant or teak sebrang has a scientific name, namely *Peronema canescens Jack*. This species is a native Indonesian plant that found in West Sumatra, Jambi, Bengkulu, South Sumatra, Lampung, West Java and all of Kalimantan [1]. In South Sumatra,it found in the Rambutan area, Banyuasin Regency.

Sungkai plant *(Peronema canescens Jack)* is one of the herbal medicines that found in Indonesia. Sungkai leafes contain secondary metabolites such as alkaloids, flavonoids, and tannins [2]. Sungkai leafes *(Peronema canescens Jack)* is rarely used by humans. Some people in South Sumatra and

Lampung use sungkai leafes (P. canescens. Jack) as malaria medicine and fever medicine [3]. Based on previous research, Sungkai leafes contain secondary metabolites [2] including alkaloids, terpenoids – steroids, flavonoids, and tannins.

The content of metabolites is an important factor for inhibiting the growth of bacteria. This study aims to analyze the extraction of young sungkai leafes and old sungkai leafes with the highest tannin content that produced using a UV-VIS Spectrophotometer and then to analyze the performance of sungkai leaf extraction in the making of wound dressings against Gram-positive bacteria (Staphylococcus Aureus) , to measure the distance of bacteria after the addition of extraction of sungkai leafes on the film layer and as a comparison with the film layer without the addition of sungkai leaf extraction.[4]. From previous studies, sungkai leafes extract not only could increase immunity due to the protein content but also could be used for inhibiting bacterial growth caused by the presence of secondary metabolite compounds (tannins) which are one of the compounds that used for inhibiting bacterial growth. [5].

The increased content of tannins will increase the activity of metabolites that function as antibacterial. It is based on the research that was conducted by Arsyik Ibrahim and Hadi Kuncoro in 2011 namely *Identification of Secondary Metabolites and Antibacterial Activity of Sungkai Leaf Extract (Peronema canescens Jack)*. The conclusion of their research is on the identification of secondary metabolites of extracts obtained from the group of alkaloids, terpenoids - steroids, flavanoids, and tannins.

The data obtained from previous studies of sungkai leafes extract has great potential as a support for making modern wound dressings as antibacterial [6]. The making of wound dressings from previous studies often uses essential oils as antibacterial. Because in there are many Sungkai plants Indonesia, it would be more useful if the extracts of Old and Young Sungkai leafes were analyzed with a Spectrophotometer and tested by the well method against Staphylococcus Aureus bacteria in a mixture of wound dressing materials and the sungkai extract itself. [7].

This study aims to analyze the amount of tannins found in young sungkai leafes and old sungkai leafes, to analyze the performance of sungkai leafes extract in wound dressing and to measure the distance of Staphylococcus Aureus bacteria after the addition of sungkai leafes extract and wound dressing.

2. Research Methods

The research was conducted using old sungkai leafes and young sungkai leafes *(Peronema canescens Jack)*. This research also use equipment such as beakers, erlenmeyer, measuring pipette, analytical balance, extraction tool, spatula, funnel, blender, hotplate, petri dish, filter paper, UV-VIS spectrophotometer and analytical balance.

This study used old sungkai leafes and young sungkai leafes that has weight of 10 grams and 20 grams with extract time variables of 60 minutes, 120 minutes and 180 minutes each. Sungkai leafes are sorted by the types of old sungkai leafes and young sungkai leafes. Then it cleaned with running water and dried in an oven at a temperature of ± 1000C for 15 minutes. After the sungkai leafes has been dry (young sungkai leafes and old sungkai leafes), the sungkai leafes are cut into small pieces with dimensions of ± 2 millimeters. Then it measure into 10 grams and 20 grams for variables. After that put the sample into filter paper or a sleeve that is shaped like a cylinder where the size is according to the socket used.

Installing and assembling the extraction tool and then the sample is inserted into the socket which has been assembled with a condenser and a boiling flask, then the solvent which is ethanol 200 ml is inserted into the boiling flask. Then the socket series was placed on a heater and then heated at a temperature of 80°C for 60 minutes, 120 minutes, and 180 minutes so that the extracts of young sungkai leafes and old sungkai leafes were obtained. After that, remove the filter paper containing the chopped sungkai leafes from the used sleeve.

After the extraction results with predetermined variables are obtained. Then, the tannin content was analyzed using a UV-VIS Spectrophotometer. After the analysis, the tannin content in each variable was found for old sungkai leafes and young sungkai leafes. The results of the best data will be used for the making of wound dressings.

The material used in the making of wound dressings is sodium alginate. Sodium alginate with a mass of 1 gram was first dissolved with aquadest, then 20 ml was stirred until thickening occurred. And then 4 ml of NaAlg solution was poured into 2 petri dishes each. Then it heated to a temperature of 80-100°C for 10 minutes after the heating process and then it cooled to a room temperature of 25-30°C.

After the cooling process of the sodium alginate solution, 1 ml of glycerol was added, and then it was dripped little by little so that all surfaces would be covered with glycerol and allowed to stand at room temperature. After the mixture of sodium alginate and glycerol solution has dried, then drop the sungkai leaf extract into the solution with the variable concentration of 100 % and 50 % sungkai leaf extract. [8].

The method used to test the bacterial inhibition of the combination of sodium alginate, glycerol and the concentration of sungkai leaf extract (100% and 50%) on the growth of Staphylococcus bacteria. aureus is a well diffusion method. Staphylococcus bacteria suspension. aureus was made according to Mc. Farland no.0.5, which is 108 bacterial cells/ml, and then diluted with sterile 0.9% NaCl to obtain a concentration of 1.5 x 106 bacterial cells/ml. The next stage is the suspension which has been prepared by following the 0.5 McFarland standard is put in a sterile pentri dish.

A sterile pasteur pipette which has been modified with a diameter of 5 mm, is used to make wells in the prepared media.This well will be filled with a combination infusion of each concentration to be tested, such as amoxilin positive control; ethanol negative control ; combination of sodium alginate + glycerol + sungkai leaf extract (100%); combination of sodium alginate + glycerol + sungkai leaf extract (50%); 100% sungkai leaf extract; sungkai leaf extract 50%; liquid sodium alginate + glycerol. Using a micropipette the placement of wells on the agar media has its own requirements, such as, each well must have the same distance, which is 2 cm from the edge of the cup and the distance between wells is 3 cm with the depth adjusted to the agar media made. After the whole process is complete, all the petri dishes are put into the Biological safety Cabinet at 25oC for 24 hours. Each parameter is repeated 2 times. The zone of inhibition that appeared on each agar was then measured using a caliper. Well layout sketch on MHA media. The larger the bacterial inhibition zone, the better it is as a wound dressing material.

3. Result And Discussion

Based on the research that has been done by using the extraction process with variable mass variation and the type of sungkai leafes used, it can be seen in Table 1.

Table 1. The Result of Sungkai Leafes Extract

Type of leaf	Sungkai Levaes Mass	Extraction times (minutes)	Extract Product Vol. (mL)
Muda	10	60	100
		120	80
		180	65
	20	60	95
		120	80
		180	50
Tua	10	60	100
		120	85
		180	65
	20	60	98
		120	80
		180	55

The extract obtained was then tested for tannin content using a UV-VIS spectrophotometer, and the results of the analysis of the tannin content of the sungkai leafes extract can be seen in Figure 1.

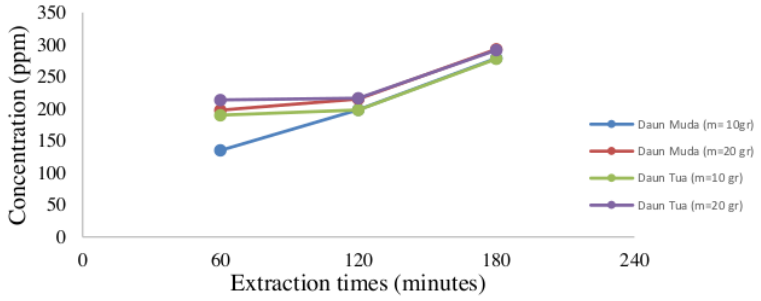

Figure 1. Tannin Content In Extracts Sungkai leafes

Based on the data from the analysis, the longer the extraction process, the greater the concentration of tannins obtained. Based on Figure 1, for a sample of young sungkai leafes at an extraction time of 180 minutes will produce tannins 278.95 ppm in 10gr mass of sungkai leafes extracted and for 20 grams of mass of sungkai leafes extracted with extraction time will contain 293.03ppm tannins. The tannin concentration obtained for the 10 gram sample was 278.01 ppm and 291.65 ppm for the tannin concentration of the 20 gram sample for the old sungkai leaf sample. The extraction process was also carried out for 180 minutes by analyzing using a UV-VIS spectrophotometer.

The results of the highest tannin content were used for the next research, namely the calculation of the inhibition zone distance of Staphylococcus aureus bacteria to sungkai leaf extract as an antibacterial in wound dressing base ingredients. In this case, the method used to obtain the results of the distance

of the bacterial inhibition zone to the film is to use the Mueller Hinton Agar (MHA) method of well media as shown in Table 2, Figures 2 and Figures 3.

Table 2. The Result of Inhibition Zone Analysis

Concentration	Inhibition Zone (mm)		
	1	2	Mean
Natrium Alginat + Gliserol	0	0	0
Natrium Alginat cair + Gliserol + Ekstrak Sungkai 50 %	0	0	0
Natrium Alginat cair + Gliserol + Ekstrak Sungkai 100 %	14.0	12.0	13.0
Ekstrak Sungkai 50 %	20.0	21.0	20.5
Ekstrak Sungkai 100 %	22.0	23.0	22.5
Amoxicillin (Control positif)	33.0	33.0	33.0
Ethanol (Control Negatif)	0	0	0

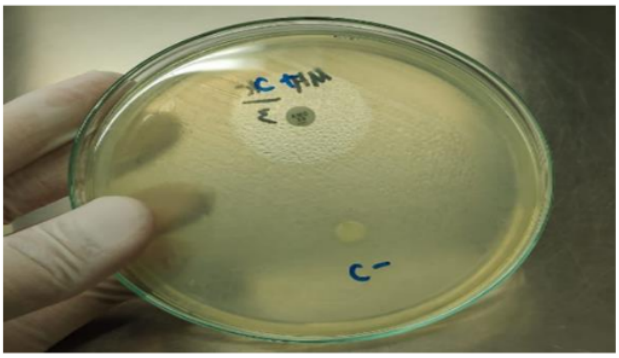

Figure 2. MHA Media on Control Positive (Amoxicillin) And Control Negative (Ethanol)

Figure 3. MHA Media on Sungkai Extract Combination.

Based on the results of the study, the negative control using ethanol did not show an inhibition zone. Ethanol can be used as a diluent solution and has no effect on antibacterial activity. Thus, the inhibition activity is ensured to come from the plant extract used, not from the dilution used or the wound dressing material used. The results showed that the concentration of sungkai leaf extract 50% had an average inhibition zone of 20.5 mm, the concentration of sungkai leaf extract 100% had the largest average inhibition zone of 22.5 mm and the combination of wound dressing materials with a concentration of 100% sungkai leaf extract had an average resistance zone 13 mm.

The inhibition of bacteria will increase as the concentration of an extract increases, because the higher the concentration of the extract will cause the antibacterial active ingredients contained to increase. The addition of the concentration of antibacterial compounds is also assumed to strengthen the penetration of antibacterial compounds that have potential to damage the cell metabolism system and caused death to the cell to the inside of microbial cells [5].

The incubation process that took long time will cause the diameter of the inhibition zone to be wider.This is because the active compounds in the extract will increase and provide a wider inhibition zone as the incubation period increases [9]. However, the speed of formation of the inhibition zone will be slow because the amount of active compound content in the extract will decrease. The thing that will be slow only occurs at the speed of formation of the inhibition zone [10].

The formation of inhibition zones at each concentration is the result of active compounds possessed by sungkai leafes such as flavonoids, saponins and tannins. These ingredients have activity that can be used as antibacterial [11, 12, 5]. Bacterial cell membranes are damaged and destroyed by flavonoids with the formation of new complex compounds against extracellular proteins, so that the damaged cell membrane cannot be repaired [11].

The function of saponins as antibacterial is to work by damaging the porin in the outer membrane of the bacterial cell wall, by forming a strong polymer bond. Bacterial growth is inhibited or dies due to damage to the porin as a bridge for the entry and exit of compounds, causing a nutritional crisis in bacterial cells [11]. Tannins as antibacterial can block the formation of bacterial cells by blocking the work of DNA topoisomerase and reverse transcriptase enzyme. Microbial cell adhesion is inhibited, enzymes are deactivated and in the inner layers of the cell protein transport is disrupted. The cell wall is formed imperfectly, because the polypeptides owned by the cell wall are damaged, so that the bacterial cell becomes lysed and dies.

Thus, sungkai leafes extract can be combined with wound dressing because it is quite effective as an inhibition of Staphylococcus aureus bacteria. This indicates that the tannin that contained in sungkai leaf extract is very good at inhibiting bacteria from living and spreading when a wound occurs on the skin and can use wound dressing with a mixture of sungkai extract.

4. Conclusion

Based on the research that has been done, it is concluded that the tannin content measured based on UV-VIS spectrophometer on young sungkai leafes and old sungkai, the highest tannin content is found in the variable mass of young sungkai leafes 20 grams with an extraction time of 180 minutes is

293.03ppm. In a sample of old sungkai leafes with a mass of 20 grams and an extraction time of 180 minutes, the tannin content was obtained 291.65 ppm.

The best performance of Sungkai leaf extraction as a modern wound dressing on Staphylococcus aureus bacteria is in the combination of liquid Sodium Alginate + Glycerol + Sungkai Extract 100% which is seen at a greater bacterial inhibition distance of 13 mm compared to the combination of liquid Sodium Alginate + Glycerol + Sungkai Extract 50% is 0 mm. While the extraction performance on the film layer without a combination of wound dressings was better with the Sungkai extract variable 100% which was 22.5 mm with a positive control comparison using Amoxicillin of 33 mm.

5. Suggestions

This research is expected to be a contribution to scientific advice and then further research can be carried out using different raw materials and variables.

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