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Antimicrobic Activity of IPPU Padang (*Ammannia octandra* L.f.) Leaves Ethanol Extract against Skin Pathogenic Microbials

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Abstract

Ippu Padang plant *(Ammannia octandra L.f.)* belongs to the family of *Lythraceae*, a hardy plant that can grow to a height of 50 cm. According to previous research, Ippu Padang leaves contain glycosides, alkaloids, flavonoids and tannins. The presence of alkaloid compounds, flavonoids and tannins is predicted to have potential as an antifungal agent against fungi and antibacterial agent. This research used an experimental method. The steps included collecting plant material, plant identification, processing plants into Simplicia powder, phytochemical screening of Simplicia powder, extracting Simplicia by maceration method using ethanol solvent, antifungal and antibacterial activity test using agar diffusion method and determination of inhibition diameter of leaf ethanol extract. The results of phytochemical screening showed that ippu Padang leaves contained secondary metabolites, namely alkaloids, flavonoids, glycosides, anthraquinone glycosides, tannins, saponins and steroids. The results also revealed that the concentration with the largest inhibitory diameter was 400 mg/ml, namely 12.4 mm against the Candida albicans, 17.46 mm against the Dermacoccus nishinomiyaensis bacteria, 18.53 mm against the Micrococcus luteus bacteria, 19.38 mm against the Pseudomonas aeruginosa bacteria, and 17.71 mm against Staphylococcus epidermidis bacteria. It was concluded that the ethanol extract of ippu Padang leaves could inhibit the growth of the Candida albicans, the bacteria *Dermacoccus nishinomiyaensis*, Micrococcus luteus, Pseudomonas aeruginosa and Staphylococcus epidermidis.

Keywords: *Ammannia octandra L.f.*; Candida albicans; *Dermacoccus nishinomiyaensis*; Micrococcus luteus; Pseudomonas aeruginosa; Staphylococcus epidermidis

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INTRODUCTION

Skin is the largest organ that covers the entire surface of the body. It is often in contact with safe and unsafe objects that can cause skin diseases. Skin disease is a skin disorder caused by interactions between fungi, bacteria, viruses, and others. This disease is common in society due to several factors such as climate, environment, unclean living habits, allergies and others.[1]

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The *Candida albicans* fungus, as the cause of candidiasis, is commonly found in the oral cavity, digestive tract, reproductive tract and skin.[2] The *Dermacoccus nishinomiyaensis* bacteria, previously known as *Micrococcus nishinomiyaensis*, is a gram-positive bacterium that exhibits the implication of peritonitis associated with peritoneal dialysis, catheter-use bacteremia, polymicrobial infections of the skin and urinary tract.[3] *Micrococcus luteus* is an opportunistic pathogenic bacterium or bacteria that will not cause disease if another disease does not precede it. This bacterium is one of the gram-positive bacteria that can cause complex dermatitis.[4] The *Pseudomonas aeruginosa* bacterium will become a pathogen if it is in a place with abnormal resistance, such as damaged skin due to tissue damage. These bacteria include gram-negative bacteria that can cause secondary infections in wounds, burns and soft tissue infections.[5] *Staphylococcus epidermidis* bacteria mostly live as normal flora on human skin. These bacteria include gram-positive bacteria that can cause acne when the amount is excessive on the skin[6].

Diseases caused by fungi and bacteria are very common and can be treated with antifungals and antibacterials. However, excessive antifungal and antibacterial drugs and inappropriate antifungals and antibiotics can lead to resistance. Resistance occurs when microorganisms change to turn infection drugs to become ineffective. Resistance causes the microbe to fail to respond to the given drug, leading to the prolongation of the disease.[7]

Ippu Padang leaves have secondary metabolites of glycosides, alkaloids, flavonoids, and tannins secondary metabolites. Secondary metabolites which are estimated to inhibit microbial growth are flavonoid alkaloids and tannins,[8] but it is known that there has been no research on the activity of ethanol extract of ippu Padang leaf extract against fungi and pathogenic skin bacteria; therefore, researchers are interested in conducting this research.

METHOD

An antimicrobial activity test with the agar diffusion method was used to determine the activity of antimicrobial agents. A petri dish containing an antimicrobial agent was placed on an agar medium where microorganisms grew, which would diffuse into the agar medium. It was later incubated for 48 hours at a temperature of 25 ± 2°C for fungi and incubated for 24 hours at a temperature of 35 ± 2°C for bacteria. The clear zone indicated the growth inhibition of microorganisms on the surface of the agar medium.[9]

RESULTS AND DISCUSSION

Determining the water content in the simplicia leaves of ippu Padang showed 5.5%. Fulfilling the requirements for simplicia content was carried out as the water content was not more than 10%. Simplicia with excess water content stored for a long time will produce enzymes that can affect the content of chemical compounds and turn them into other products that may not have a pharmacological effect like the original compound.[10]

The results of phytochemical screening showed that the ethanol extract of ippu Padang leaves contained secondary metabolites, which were positive for alkaloids, flavonoids, glycosides, anthraquinone glycosides, tannins, saponins and steroids. Table 1 shows the

results of phytochemical screening on ippu Padang leaves.

Table 1. Phytochemical Screening

No	Screening	Reagent	Observation	Results
1	Alkaloids	Dragendorf	Chocolate precipitate	+
		Bouchardat	Chocolate precipitate	
		Mayer	Chocolate precipitate	
2	Flavonoids	Zn + HCl	Red	+
		Mg + HCl	Red	
3	Glycosides:			+
	Sugar	Molisch	White precipitate	
		Fehling A+ B	Brick red precipitate	
	Non-Sugar	Acetic acid anhydride + Sulfuric acid	Purple	
4	Anthraquinone Glycosides	NaOH	Red	+
5	Tannins	$FeCl_3$ 5%	Blackish green	+
6	Saponins	Hot water, shaken + HCl	1.5 cm high foam	+
7	Cyanogenic glycosides	Na. Picrate	Yellow	-
8	Steroids-Triterpenoids	Acetic acid anhydride + Sulfuric acid	Blackish green	+

Description:
(+) Contains the test compound
(-) Does not contain the test compound

An antimicrobial activity test was conducted to measure the response of microbial growth to antimicrobial materials. One of the uses of antimicrobial activity testing is to obtain an effective and efficient treatment system. The effectiveness of an antibacterial agent in inhibiting microbial growth depends on the nature of the test bacteria, concentration and length of contact time. Measurement of antimicrobial activity was carried out using the agar well diffusion method, characterized by the formation of a clear part around the well (inhibitory diameter) if the tested extract could inhibit microbial growth. The area of the clear part was then measured in diameter.[11] The

diameter of the inhibition was divided into several categories based on the diameter of the clear part around the well, including weak (<5 mm), medium (5-10 mm), strong (10-20 mm) and very strong (>20 mm).[12]

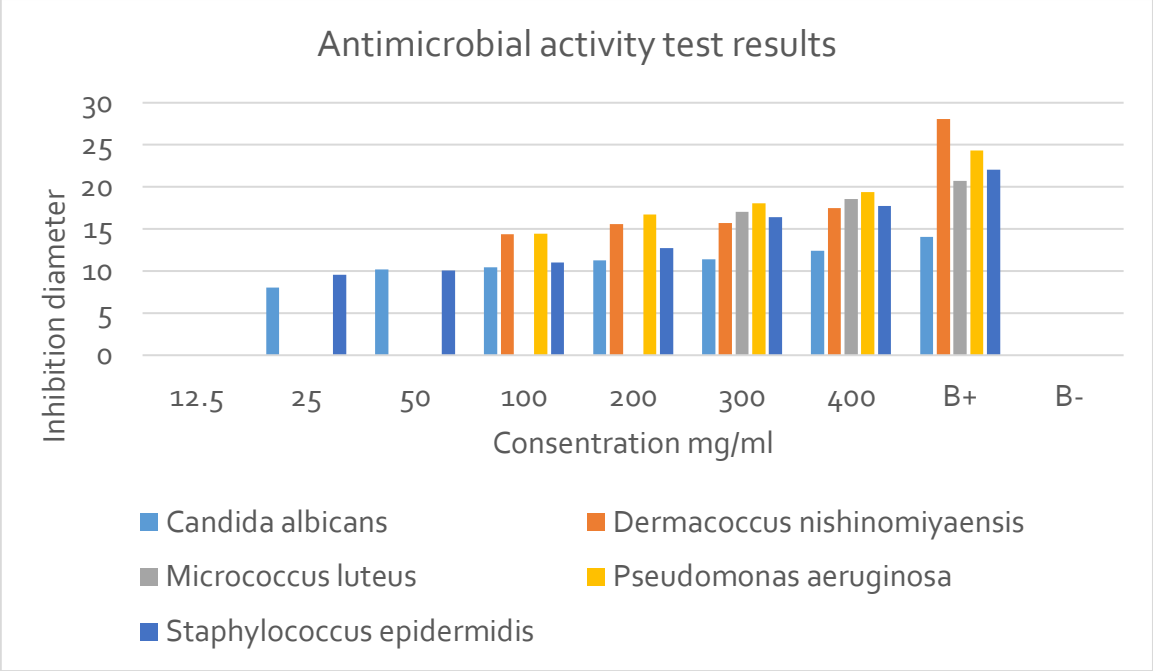

Figure 1. Antimicrobial activity test results

Based on Figure 1, B- (Negative blank) does not have a diameter of inhibition for testing all microbes. The ethanol extract of ippu Padang leaves tended to have an inhibitory diameter at a certain concentration even though it had no inhibitory diameter of 12.5 mg/ml. It indicated that the B- used DMSO: ethanol (3:4) could not inhibit microbial growth. The concentration of 400 mg/ml had the largest inhibitory diameter among all the tests carried out where the diameter of inhibition was almost the same as the diameter of the inhibitory B+ (positive blank) used, namely nystatin for fungi and chloramphenicol for bacteria. Based on Figure 1, it can be observed that the higher the concentration is, the larger the diameter of the formed inhibition will be. In line with it, according to the statement of Surjowardojo *et al.* (2015), the greater the concentration is, the greater the interaction of the extract with the tested microbes will be. It causes a larger diameter of the inhibition as the extract with a large concentration contains a large number of chemical compounds that affect microbial growth.[13]

Furthermore, alkaloids in ippu Padang leaves can damage proteins that can harm enzyme activity and cause death in microbial cells. Alkaloids can also arrange microbial cell walls so that they cannot be formed completely and cause death in these microbial cells, leading to the formation of an inhibitory diameter.[14] Flavonoids, as antifungals, work by damaging proteins, inhibiting the enzyme system, interfering with the formation of the end of hyphae, and constricting the cell wall so that the fungal cell wall died.[15] Flavonoids as antibacterial work by inhibiting the synthesis of nucleic acids, inhibiting cell membranes' function and inhibiting the metabolic energy of the bacteria.[14]

The mechanism of action of tannins as an antimicrobial is to inhibit the activation of the enzyme and disrupt transport protein in the cell's lining so that the antimicrobial cell cannot be formed. In addition, the antimicrobial effect of tannins also can be

through reaction with cell membranes and inactivation of the function of the genetic material of.[14]

CONCLUSION

Based on the results of this research, it can be concluded that the results of phytochemical screening of ippu Padang leaf Simplicia powder showed the presence of alkaloids, flavonoids, glycosides, anthraquinone glycosides, tannins, saponins and steroids. Ippu Padang leaf ethanol extract had antimicrobial activity against the *Candida albicans*, the *Dermacoccus nishinomiyaensis, Micrococcus luteus, Pseudomonas aeruginosa* and *Staphylococcus epidermidis bacteria*

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