**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 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 antifungal against fungi and antibacterial. The method used in this research is experimental. The steps involved in this research include the collection of plant material, plant identification, processing of plants into simplicia powder, phytochemical screening of simplicia powder, extraction of 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. ippu field. The results of phytochemical screening showed that ippu padang leaves contain secondary metabolites, namely alkaloids, flavonoids, glycosides, anthraquinone glycosides, tannins, saponins and steroids. The results showed that the concentration with the largest inhibitory diameter was 400 mg/ml, namely 12.4 mm against the fungus Candida albicans, 17.46 mm against the bacterium Dermacoccus nishinomiyaensis, 18.53 mm against the bacterium Micrococcus luteus, 19.38 mm against the bacterium Pseudomonas aeruginosa, 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 fungus 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.*

**Introduction**

The skin is the largest organ that covers the entire surface of the body so that the skin is often in contact with various objects, including objects that are not harmful or objects that can cause skin diseases. Skin disease is a disorder of the skin that can be caused due to 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 others1.

The fungus *Candida albicans*as the cause of candidiasis is commonly found in the oral cavity, digestive tract, reproductive tract and skin2. The bacteria *Dermacoccus nishinomiyaensis*or previously known as *Micrococcus nishinomiyaensis is*a gram-positive bacterium that exhibits implication of peritonitis associated with peritoneal dialysis, catheter-use bacteremia, polymicrobial infections of the skin and urinary tract3. The bacteria *Micrococcus luteus*is an opportunistic pathogenic bacterium or bacteria that do not cause disease if it is not preceded by another disease before. This bacterium is one of the gram-positive bacteria that can cause complex dermatitis4. The bacterium *Pseudomonas aeruginosa*becomes 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 infections5. *Staphylococcus epidermidis*bacteria mostly live as normal flora on human skin. These bacteria include gram-positive bacteria which if the amount is excessive on the skin can cause acne6.

Diseases caused by fungi and bacteria are very common, can be treated with antifungals and antibacterials. However, excessive use of antifungal and anti-bacterial drugs and inappropriate use of antifungals and antibiotics can lead to resistance. Resistance is what occurs when microorganisms change so that they have the ability to make drugs to treat infections ineffective. Resistance causes the microbe to fail to respond to the given drug, which can lead to prolongation of the disease7.

Ippu padang leaves have secondary metabolites of glycosides, alkaloids, flavonoids and tannins. Secondary metabolites which are estimated to inhibit microbial growth are flavonoid alkaloids and tannins8, but it is known that there has been no research on the activity of ethanol extract of ippu padang leaf extract against fungi and skin pathogenic bacteria, therefore researchers are interested in conducting this research.

**Methods**

**Materials and tools**

**1. Materials**

Ippu padang leaves (*Ammannia octandra* L.f.). aquadest, reagent for phytochemical screening,

Mueller Hinton Broth (MHB) (Himedia®), Nutrient Agar (NA) (Merck®), Sabouraud Dextrose Agar (SDA) (Himedia®), Sabouraud Dextrose Broth (SDB) (Himedia®), fungus culture *Candida albicans* ATCC 10231, bacterial culture *Dermacoccus nishinomiyaensis* clinical isolate, *Micrococcus luteus* clinical isolate, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus epidermidis* clinical isolate.

**2. Tools**

Glass blender (Phillips), Bunsen, incubator (Memmert), caliper, gas stove (Rinnai), refrigerator (Panasonic), drying cabinet, oven (Memmert), water bath, metal spare 6mm diameter, digital scale (Vibra AJ) and vortex (Wiggens).

**Preparation of Plant Materials**

**1. Making Ippu Padang Leaf Simplicia Powder**

The collection of plant material was carried out purposively or without comparing with the same plant from different areas. Plants were collecter from rice fields in Sitampa Village, Batang Angkola District, South Tapanuli Regency, North Sumatra. The part of the plant used is the leaf. Making simplicia begins with wet sorting, where the leaves are separated from the stems. then washed with running water. A total of 2,500 grams of fresh leaves were dried in a drying cabinet at a temperature of 40-50oC until brittle (dry), then the simplicia leaves were sorted dry. The dried leaf simplicia was mashed using a blender and weighed, then stored in a tightly closed container to avoid sunlight and humidity9.

**2. Determination of Water Content**

The assay is done gravimetric water is by heating the porcelain dish in the oven at 105oC for 30-60 minutes and cooled in a desiccator for 30-60 minutes and then the cup is weighed. Put 2 g of the sample into a porcelain dish, then put it in the oven at a temperature of 105oC for 5 hours. After 5 hours, transfer it to a desiccator and cool it for 1 hour, weigh it, then calculate the moisture content of10, calculated by the formula:

Water content = $\frac{\left(a+b\right)-c}{b}$ x 100%

Description: a = weight of porcelain cup after drying (g)

 b = sample weight (g)

 c = weight of cup + contents (g)

**3. Phytochemical Screening of Ippu Padang Leaf Simplicia Powder**

Phytochemical screening was carried out to qualitatively identify the chemical compounds contained in the studied plants. Phytochemical screening carried out in this study included chemical compounds of alkaloids, flavonoids, glycosides, anthraquinone glycosides, tannins, saponins, cyanogenic glycosides and steroid-triterpenoids11.

**4. Preparation of Extracts**

The extraction method used is maceration and the solvent used is ethanol pro analysis. Simplicia powder as much as 200 g was put into a maceration container, then ethanol was added until the simplicial powder was completely submerged and left for 3 days while shaking occasionally. After 3 days, filtered using whatman filter paper, the filtrate is taken and then evaporated on a water bath until thick12. After that, it was weighed and placed in a closed glass container.

**5. Preparation of Ippu Padang Leaf Ethanol Extract Concentration Solution**

The ethanol extract of the leaves of ippu padang was weighed as much as 4 g, dissolved with 3 ml of dimethylsulfoxyde (DMSO) and made up to 10 ml of ethanol to obtain an extract concentration of 400 mg/ml. Extract concentration of 400 mg/ml was diluted to obtain extract concentration of 300 mg/ml, 200 mg/ml, 50 mg/ml, 2.5 mg/ml and 6.25 mg/ml.

**Preparation of Antimicrobial Activity Test**

**1. Sterilization of Tools and Materials**

Sterilization is carried out to avoid contamination of microorganisms originating from the tools and materials used during the test. The whole appliance and heating resistant material that are not quantitative sterilized in the oven at 170oC for 1 hour, while the tools and materials that can not withstand high heat and quantitative nature sterilized in an autoclave at 121oC for 15 minutes. The ose tool is sterilized over a bunsen fire before and after use13.

**2. Making SDA and NA**

The SDA and NA media used were made according to the procedures and sterilization listed on each media label.

SDA media was used as a medium for rejuvenation and antifungal activity test. A total of 65 g of SDA powder was dissolved in 1 L of sterile distilled water, then heated until clear. Sterilize in an autoclave at 121oC for 15 minutes. SDA which will be used for rejuvenation is transferred into a test tube and then left in an inclined position (30-45o) (the media is tilted). Media that has not been used can be stored in the refrigerator.

NA media was used as a medium for rejuvenation and antibacterial activity test. A total of 20 g of NA powder was dissolved in 1 L of sterile distilled water, then heated until clear. Sterilize in an autoclave at 121oC for 15 minutes. SDA which will be used for rejuvenation is transferred into a test tube and then left in an inclined position (30-45o) (the media is tilted). Media that has not been used can be stored in the refrigerator.

**3. Making SDB and MHB Media**

The SDB and MHB media used were made based on the procedures and sterilization listed on each media label.

SDB media was used as a fungal inoculum medium. A total of 30 g of SDB powder was dissolved in 1 L of sterile distilled water, then heated until clear. Move into a 10 ml test tube to each test tube, and then sterilized in an autoclave at 121oC for 15 minutes. Media that has not been used can be stored in the refrigerator.

MHB media was used as a bacterial inoculum medium. A total of 21 g of MHB powder was dissolved in 1 L of sterile distilled water, then heated until clear. Move into a 10 ml test tube to each test tube, and then sterilized in an autoclave at 121oC for 15 minutes. Media that has not been used can be stored in the refrigerator.

**4. Microbial Rejuvenation**

The microbial cultured used were the fungus *Candida albicans,* the bacteria *Dermacoccus nishinomiyaensis, Micrococcus luteus, Pseudomonas aeruginosa* and *Staphylococcus epidermidis.* The microbial culture used for the antimicrobial activity test was rejuvenated every 2 days at a temperature of 25±2oC for 48 hours for fungi and a temperature of 35±2oC for 24 hours for bacteria.

One ose cultured microbes were taken using ose sterile, then scratched on the surface of an agar medium slant SDA to culture fungi and NA for bacteria13, then incubated at 25±2oC for 48 hours for fungi and a temperature of 35±2oC for 24 hours for bacteria.

**5. Making Microbial Inoculum**

Microbial inoculum was made by taking microbial colonies from the last rejuvenation using an ose needle and then put into a test tube containing 10 ml of SDB for fungi and 10 ml of MHB for bacteria and then homogenized using a vortex. Microbial inoculum was incubated at 25±2oC for 48 hours for fungi and 35±2oC for 24 hours for bacteria. After the incubation period was completed, the inoculum was homogenized again and then compared its turbidity with a standard solution of Mc. Farland No. 0.5 or equivalent to 1.5 x 108 CFU/ml14.

**6. Antimicrobial Activity Test**

The antimicrobial activity test included determining the inhibitory diameter for the ethanol extract of ippu padang leaves. The inhibition diameter was determined by the agar well diffusion method using a sterile metal backing with a diameter of 6 mm. The base layer was made by pouring 10 ml of SDA medium for fungi and 10 ml of NA for bacteria into sterile petri dishes, allowed to solidify. After solidification, 0.1 ml of microbial inoculum suspension was poured and added 25 ml of SDA medium for fungi and NA for bacteria, then homogenized. Metal backers are immediately placed and arranged on the surface of the media and spaced. After the agar medium was solid, the metal backing was removed so that wells were formed, 0.1 ml of the concentration of the ethanol extract of the leaves of ippu padang were added into the wells, a negative blank (DMSO:ethanol) and a positive blank of nystatin for fungi and chloramphenicol for bacteria13. Petri dishes were closed and allowed to stand for 30 minutes, then incubated at a temperature of 25±2oC for 48 hours for fungi and a temperature of 35±2oC for 24 hours for bacteria. Observations were made by measuring the clear area formed around the well using a caliper, so that the diameter of the inhibition of microbial growth was known in millimeters (mm), the data was taken from three treatments.

**Results and Discussion**

The result of determining the water content in the simplicia leaves of ippu padang was 5.5%, fulfilling the requirements for simplicia content because it was not more than 10%. Simplicia with excess water content and then 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 compound15.

Phytochemical screening was carried out qualitatively to determine the content of chemical compounds contained in the simplicia powder of Ippu Padang leaves.

**Table 1.** Phytochemical Screening

|  |  |  |
| --- | --- | --- |
| **No.** | **Skrining** | **Hasil Skrining** |
| 1 | Alkaloid | (+) Alkaloid |
| 2 | Flavonoid | (+) Flavonoid |
| 3 | Glikosida | (+) Glikosida |
| 4 | Glikosida Antrakuinon | (+) Glikosida Antrakuinon |
| 5 | Tanin | (+) Tanin |
| 6 | Saponin | (+) Saponin |
| 7 | Sianogenik Glikosida | (-) Sianogenik Glikosida |
| 8 | Steroid-Triterpenoid | (+) Steroid |

Description:

(+) Contains the test compound

(-) Does not contains the test compound

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 is then measured in diameter16. The diameter of the inhibition is 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)17.

**Figure 1.**Antimicrobial activity test results

Based on Figure 1, B- (Negative blank) does not have a diameter of inhibition for testing all microbes that have been tested. The ethanol extract of ippu padang leaves tends to have an inhibitory diameter at a certain concentration even though at a concentration of 12.5 mg/ml it has no inhibitory diameter. This indicates that the B- used DMSO:ethanol (3:4) is not able to inhibit microbial growth. Concentration of 400 mg/ml had the largest inhibitory diameter among all the tests that had been 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. Viewed from Figure 1, it can be observed that the higher the concentration, the larger the diameter of the inhibition formed, according to the statement of Surjowardojo *et al* (2015), that the greater the concentration, the greater the interaction of the extract with the tested microbes, causing the larger the diameter of the inhibition formed because the extract with a large concentration contains a large amount of chemical compounds that affect microbial growth18.

Alkaloids contained in ippu padang leaves are able to damage proteins so that they damage 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, this is what causes the formation of an inhibitory diameter19.

Flavonoids as antifungals work by damaging proteins, inhibits the enzyme system which can interfere with the formation of the end of hyphae and constrict the cell wall so that the fungal cell wall die20. Flavonoids as antibacterial works by inhibiting the synthesis of nucleic acids, inhibits the function of cell membranes and inhibit the metabolic energy of the bacteria19.

Mechanism of action of tannins as antimicrobial is to inhibit the activation of the enzyme and disrupt transport protein in the lining of the cell so that the cell antimicrobial can not be formed, in addition to the antimicrobial effect of tannins also can through reaction with cell membranes and inactivation of the function of the genetic material of19.

**Conclusion**

Based on the results of the research that has been carried out, 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 has antimicrobial activity against the fungus *Candida albicans*and the bacteria *Dermacoccus nishinomiyaensis, Micrococcus luteus, Pseudomonas aeruginosa*and *Staphylococcus epidermidis.*

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