ANTIMICROBIAL ACTIVITY TEST OF 96% ETHANOL EXTRACT OF FLOWERS, LEAVES, AND STEM BARK OF TIGARUN (*Crateva magna* DC.) AGAINST *Staphylococcus aureus* and *Malassezia furfur*

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ABSTRACT

Staphylococcus aureus and *Malassezia furfur* One of the microbes that can cause folliculitis is the inflammation of the skin in the hair follicles. The use of natural ingredients as antimicrobials must be developed as an alternative to overcome the resistance and side effects of chemical drugs. Tigarun (*Crataeva magna* DC.) is a typical plant in South Kalimantan that can be developed as a natural antimicrobial agent for the treatment of skin infections. Previous studies have reported that tigarun has antimicrobial activity, but no studies have reported its potential antimicrobial activity against folliculitis-causing microbes. Therefore, this study aimed to determine the antimicrobial activity of 96% ethanol extracts of the flowers, leaves, and stem bark of tigarun (*Crataeva magna* DC.) against microbes that cause folliculitis, including *Staphylococcus aureus* and *Malassezia furfur*. Extraction was performed by the maceration method using 96% ethanol. The antimicrobial activities of the extracts were tested using the agar diffusion method. Based on the results of the antimicrobial activity test using the agar diffusion method, the tigarun flower extract showed better antimicrobial activity than the leaf and tigarun bark extracts. Flower extract activity at 200 mg/mL concentrations against *Staphylococcus aureus* (10.65 ± 0.25) and the fungus *Malassezia furfur* (7.40 ± 0.40) with moderate inhibition zone ketogenic

Keywords: *Folliculitis, tigarun, antimicrobial, agar diffusion*

INTRODUCTION

The skin is a vital organ that functions in protection, absorption, excretion, perception, regulation of body temperature (thermoregulation), pigment formation, vitamin D, and keratinization. The skin is sometimes infected by pathogenic bacteria during protection of the body. Infection is a disease caused by the entry of pathogenic microorganisms into the body, causing certain symptoms. Skin disease is one of Indonesia's most common health problems and ranks fourth out of all diseases affecting almost a third of the world's population (Campo *et al*., 2021). Various pathogens, including bacteria, fungi, viruses, and parasites can cause skin infections. Personal hygiene, environmental hygiene, immunity, and environmental exposure can influence skin infection likelihood (Tumwine *et al*., 2022). According to the 2020 Indonesia Health Profile, Indonesia has a prevalence rate of 0.49 (49%) cases per 10,000 population and new skin disease cases of 4.2 per 100,000 population. This figure is included in the high prevalence group. Therefore, it is important and needs to be a concern, and prevention is carried out early and special treatment for those infected (Indonesia Health Profile, 2021). A type of skin disease that bacteria or fungi can cause is folliculitis

Folliculitis is skin inflammation in hair follicles that are infected by bacteria or fungi. *Staphylococcus aureus* is a gram-positive bacterium that can be found on the human skin and mucous membranes. These bacteria cause various types of skin infections ranging from mild to serious. *Staphylococcus aureus* most commonly infects the skin, causing superficial folliculitis, which results in the formation of pustules or erythematous papules on the hair-covered parts of the scalp (Griffiths *et al*., 2016; S Jappa and R Kutre, 2020). Folliculitis can also be caused by the fungus *Malassezia furfur*, which causes increased sebaceous gland activity or pityrosporum folliculitis. *Malassezia furfur* is a lipophilic fungus found on the human skin. However, under certain conditions, overgrowth of this fungus can lead to certain skin infections. This fungal infection is characterized by white, reddish, or brown patches on the skin (Suzuki *et al*., 2016).

Treatment of folliculitis generally uses a synthetic chemical drug, such as an antibiotic or antifungal agent, to inhibit inflammation and kill the microbes that trigger it. However, the use of antibiotics and antifungals on the skin has side effects, such as skin irritation, and long-term use can lead to resistance. This encourages the development of research on natural materials in Indonesia (back to nature) as alternatives, one of which is tigarun (Crateva Magna DC.).

Tigarun (Crateva magna DC.) is a plant that is widespread in India, Sri Lanka, Myanmar, Malaysia, Indonesia, and China. In Indonesia, especially in South Kalimantan, Tigarun, also known as Jaruk Tigarun, is a fermented vegetable made from its flower parts. Apart from being used as a vegetable, other parts of the tigarun plant are believed to have medicinal properties. Tigarun root decoction is believed to be efficacious as a medicine for kalalah, a disease in women after giving birth, characterized by a weak body and chills. Tigarun stem bark that is boiled are believed to be efficacious in treating ambient conditions (Nazarni et al., 2016). Another study reported the use of stem bark extract to treat skin irritations, such as abscesses and other skin disorders (Bhattacharjee, Shashidhara, and Aswathanarayana, 2012).

Tigarun has been reported to have various pharmacological activities, one of which is antimicrobial activity, which was thought to be due to the presence of the compounds batulinic acid, catechins, kaempferol, and lupeol, which affect its antimicrobial activity (Kumar, Sharma and Kumar, 2020) thus, tigarun can be developed as a natural antimicrobial to treat skin infections such as folliculitis. Research on the potential of tigarun as an antibacterial agent reported that the fermented methanol extract of tigarun flowers was active against *Escherichia coli*, *Salmonella sp*., *Staphylthat reportedus*, *Pseudomonas flourescens*, and *Basillus subtilis* (Nazarni *et al*., 2016). Another study reported that the acetone extract from the bark of tigarun showed MI,C agaBacillus*herichia coli* (0.31 mg/mL) and *Klebsiella pneumoniae* (2.50 mg/mL) (Dholaria and Desai, 2018).

Plant antimicrobials provide opportunities to obtain new antibacterial alternatives and minimize the possibility of resistance to pathogenic microorganisms. Therefore, research on discovering a new generation of drugs from natural products against infection is highly desirable for developing effective, affordable, and safe treatments (Utami and Damayanti, 2022). Thus, this study aimed to determine the antimicrobial activity of flower, leaf, and stem bark extracts against *Staphylococcus aureus* and *Malassezia furfuf.*

RESEARCH METHODS

Equipment and Materials

The tools used in this study were: simplisia grinding machine, simplisia drying cupboard, soxhlet device, rotavapor (Buchi®), balance (Mettler Toledo®), petri dish (Pyrex®) caliper (Osaka®), test tube (Pyrex®), beaker glass (Pyrex®), measuring cup (Pyrex®), funnel and erlenmeyer (Pyrex®), loop needle, Bunsen lamp, incubator (WieseCube®), autoclave (PBI®), Laminar Air Flow (DottBonapace®), micropipette (Eppendorf®), UV lamp (Camag®), water bath (Memmert®), standard glassware.

The material used in this study was tigarun (*Crateva magna* DC.) which was studied in section flowers, leaves and stem bark of tigarun, ethanol, methanol, ethyl acetate, n-hexane,

reagents for phytochemical screening (amyl alcohol, 0.7% acetic acid, 2 N hydrochloric acid, sulfuric acid, distilled water, ethanol, ethyl acetate, chloroform, n-hexane, Mayer's reagent, Dagendorff reagent, Lieberman-Burchard reagent, magnesium powder), *Nutrient Agar* (NA), *Muller Hinton Agar* (MHA), *Sabouraud Dextrose Agar* (SDA), *Sabouraud Dextrose Broth* (SDB).

Research Procedure

1. Sample Collection

Tigarun (*Crateva magna* DC.) was obtained from the Tandipah River area, Sungai Tabuk District, Banjar Regency, South Kalimantan, along the river, and rice fields. The parts used in this study were the flowers, leaves, and stem bark, which were subjected to simplisia. Flowers, leaves, and stem bark of fresh tigarun were sorted wet to separate dirt or other foreign materials in the plant, then cleaned of impurities, and washed. Next, chopping reduces leaf size to accelerate drying. The material was dried using a drying cabinet at 40 °C, dried, and ground to a powder using a grinder. The obtained powder was stored in a clean, tightly closed container. Tigarun was determined at the Biology Laboratory of the Faculty of Mathematics and Natural Sciences, University of Lambung Mangkurat, Banjarbaru (South Kalimantan).

2. Extraction

Maceration was used as an extraction method. The plant parts of tigarun (*Crateva magna* DC.), including flowers, leaves, and bark (as much as 300 g), were extracted using 96% ethanol. The extract was concentrated using a rotary evaporator and tested for antimicrobial activity against *Staphylococcus aureus* and *Malassezia furfur.*

***3.* Phytochemical Screening**

Phytochemical screening aims to determine the content of secondary metabolites found in the flowers, leaves, and bark of tigarun. Phytochemical screening includes examination of alkaloids, saponins, flavonoids, tannins, quinones, steroids, and triterpenoids (Farnsworth, 1966).

4. **Specific weight**

Characterization of the extract was performed by determining the specific gravity. Weight assignment: The type of extract was determined using a 5 mL pycnometer. Previously, The pycnometer was weighed empty as Wo and then weighed when it contained the extract as W1. The extract used was 1%, and the specific gravity of the water was assumed to be equivalent to 1 g/mL (Depkes RI, 2000).

5. Antimicrobial Activity Test

a. Sterilization of Test Equipment and Media

The tools used in the experiment were sterilized first. The tool was washed, dried, and wrapped in an aluminum foil. All glassware was sterilized using an autoclave at 121°C with a pressure of 2 atm for 15 minutes. The test medium for Nutrient Agar (NA) (28 g/1 L), Muller Hinton Agar (MHA) (38 g/1 L), Sabouraud Dextrose Agar (SDA) (65 g/1 L), and Sabouraud, which was weighed, was put into the Erlenmeyer flask, and distilled water was added according to the amount of volume you wanted to make, covering the mouth of the Erlenmeyer flask with cotton. Sterilize by autoclaving at 121 °C for 15 minutes (Zahro and Agustini 2013).

b. Making Stock Culture

Colonies of bacteria and fungi were taken using an ose needle that was sterilized with a Bunsen lamp, implanted in a slanted nutrient medium by scraping, and then incubated in an incubator at ± 37°C for 18-24 hours for bacteria and at ± 35 °C for 36-48 hours for fungus. Especially for *the Malassezia furfur* fungus, 1% VCO fatty oil is added to the medium (Zahro and Agustini, 2013; Suzuki *et al.*, 2016).

c. Preparation of Inocolum

Bacterial and fungal suspensions were prepared by taking each culture stock using a sterilized loop needle and then suspending it in a test tube containing 0.9% NaCl solution until turbidity equal to 0.5. A McFarland standard was obtained using

a UV-Vis spectrophotometer at λ=625 nm with 0.9% NaCl as a blank until an absorbance of 0.08 – 0.13 (1×10^8 CFU/mL) was obtained (CLSI, 2012).

6. Agar Diffusion Method

Antimicrobial activity test using the disc diffusion method. *Staphylococcus aureus* and *Malassezia furfur* were rejuvenated first, and then a microbial suspension was prepared. mL to 0.5 McFarland added as much as 0.1 - 0.3 mL of MHA and SDA media 15 – 20 mL while homogenizing. The medium was then left at room temperature for 15-30 minutes, and a 6 mm blank disc was placed aseptically. For test concentrations of 200, 150, 100, 50, and 10 mg/mL diluted with DMSO 5%, each concentration was dripped with 10 µL of the test sample was added dropwise to each concentration. In the control plate, 30 µg/mL clindamycin disc was added as a positive control and DMSO was added as a negative control (solvent) at ± 37°C for 18-24 hours for bacteria and 35°C for 36-48 hours for fungi (CLSI, 2012).

Data Analysis

Inhibition zone diameter data from the extracts are shown as mean ± standard deviation (SD) with n = 3. The research data are in the form of qualitative data presented in tables and percentages. The inhibition zone categories were classified as follows.

Table I. Inhibition Zone Categories
(Rahmawati *et al.*, 2022)

Inhibition Zone Diameter	Growth Restraint Response
≤ 21mm	Very strong
11-20mm	Strong
6 – 10 mm	Moderate
≤ 5mm	Weak

RESULTS AND DISCUSSION

In this study, the tigarun simplisia was extracted using the maceration method for each part of the plant, including flowers, leaves, and stem bark. Maceration is a simple method and tool. It aims to avoid damage to the active ingredients of simplisia, which are not resistant to heating or evaporation. Maceration uses 96% ethanol because it is universal, so that it can attract almost all components in the simplisia: polar, semi-polar, and slightly non-polar (Nor, Rahmita, and Nashihah, 2022). Each extract yielded different results, indicating that the chemical compounds in simplisia had different abilities to be extracted in certain solvents. The results showed that the highest extract yield was 28.59% for the tigarun flower simplisia, which indicated that the chemical compounds contained in it were more concentrated in the solvent used. At the same time, the samples had the lowest extract yield value of 4.01%, which indicated that the chemical compounds were less concentrated.

Table II. Extract Yield Results

No	used part	Extract yield (%w/w)
1	Flower	28.59
2	Leaf	14.78
3	Stem Bark	4.01

The concentrated extracts obtained were tested to determine the specific gravity of the extract (1%), aiming to provide an overview of the chemical content dissolved in an extract as well as to provide a limit on the amount of mass per unit volume, which is a special parameter for liquid extracts to become a thick extract that can still be poured. Specific

gravity is also related to the purity of the extract from contamination (Depkes RI, 2000). The results for determining the specific gravity of each are listed in **Table III**.

Table III. Results of Determination of the Specific Weight of 1% Extract

No	Sample	Specific gravity (g/mL)
1	Flower	0.8928 ± 0.0028
2	Leaf	0.9026 ± 0.0012
3	Stem Bark	0.9001 ± 0.0019

Note: expressed as mean ± SD (n=3)

Phytochemical screening was conducted to determine the secondary metabolite content of the test samples, both simplisia and the extracts. Phytochemical screening of both samples revealed the presence of alkaloids, flavonoids, saponins, tannins, phenols, steroids/triterpenoids, and coumarins. Quinones were not detected in either Simplisia or extracts. Examination of quinones, flavonoids, and tannins allows for false-positive results for quinones because flavonoids have a 4'-OH group, and tannins with a phenol group can also give positive results when examining NaOH. Therefore, a re-examination was carried out using the results of the flavonoid analysis, taking the water layer to be tested again with NaOH. The results showed a red color in the tested solution. In the examination of tannins using gelatin and filtration, the filtrate was tested again with NaOH, and the results showed a red color in the solution being tested. Thus, simplisia and its extracts contain quinone (Farnsworth, 1966; Shaikh and Patil, 2020). The results of the phytochemical screening are shown in **Table IV**.

Table IV. Screening Results for Phytochemical Simplisia and Extracts

Compound	Simplisia			Extract		
	Flower	Leaf	Stem bark	Flower	Leaf	Stem bark
Alkaloids	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
tannins	+	+	+	+	+	+
Phenol	+	+	+	+	+	+
Steroids/Triterpenoids	+	+	+	+	+	+
Quinone	-	-	-	-	-	-
Coumarins	+	+	+	+	+	+

Note: (+) detected
(-) not detected

The antimicrobial activity test was performed by agar diffusion using a paper disc to identify potential barriers to the extract to be tested. The results of the diffusion antimicrobial activity tests on agar are shown in **Table V**.

Table V. Antimicrobial Activity Test by Agar Diffusion Method

Sample and concentration (mg/mL)	Inhibition zone diameter(mm)			
	Staphylococcus aureus	Inhibitory strength category	*Malassezia furfur*	Inhibitory strength category
(1) EEBT 200	10.65±0.25	moderate	7.40±0.40	moderate
(2) EEBT 150	8.38±0.19	moderate	-	-
(3) EEBT 100	6.28±0.08	moderate	-	-
(4) EEBT 50	-	-	-	-
(5) EEBT 10	-	-	-	-
(1) EEDT 200	9.53±0.15	moderate	-	-
(2) EEDT 150	7.62±0.28	moderate	-	-
(3) EEDT 100	6.35±0.05	moderate	-	-
(4) EEDT 50	-	-	-	-
(5) EEDT 10	-	-	-	-
(1) EEKBT 200	9.35±0.18	moderate	-	-
(2) EEKBT 150	7.52±0.08	moderate	-	-
(3) EEKBT 100	6.17±0.08	moderate	-	-
(4) EEKBT 50	-	-	-	-
(5) EKEBT 10	-	-	-	-
Clindamycin*	21.55±0.28	very strong	-	-
Ketoconazole*	-	-	8.60±0.20	moderate
DMSO*	-	-	-	-

Note: (-) not detected
*Clindamycin 30 µg/disc, Ketoconazole 30 µg/disc, DMSO 5%
stated as mean ± SD (n=3)
EEBT (96% ethanol extract of tigarun flower)
EEDT (96% ethanol extract of tigarun leaves
EEKBT (96% ethanol extract of tigarun stem bark)

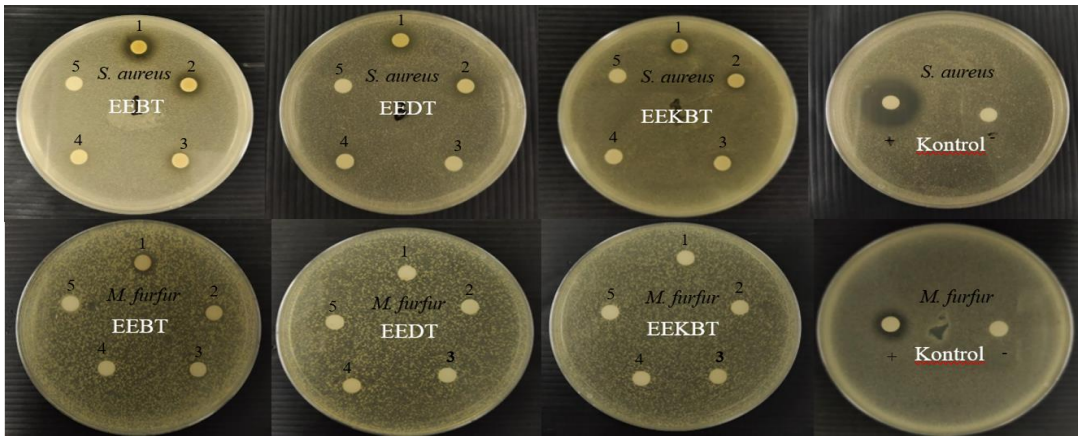

Figure 1. The results of the antimicrobial activity test with the agar diffusion method

In the antimicrobial activity test of the 96% ethanol extract of the flowers, leaves, and bark of tigarun, five concentrations (200, 150, 100, 50, and 10 mg/mL) were tested. Clindamycin (30 µg/disc) and ketoconazole (30 µg/disc) were used as positive controls and 5% DMSO was used as a negative control. A positive control was used to determine whether the tested extract had an antimicrobial effect marked by an inhibition zone around the disc

paper. The solvent used as the diluent for the extract was used as the negative control. The aim was to determine whether the solvent used did not affect the antibacterial activity, as indicated by the absence of an inhibition zone in the negative control treatment.

The results showed that the greater the concentration of food, the greater the inhibition. The size of the inhibition zone was caused by variations in the extract used for the test. According to Pelczar and Chain (1988), the higher the concentration of an antimicrobial substance, the stronger its activity. The Tigarun flower extract provided better antimicrobial activity than the leaf extract and stem bark extract, where a concentration of 200 mg/mL for each test extract had the highest average. The test results show that all test extracts are more sensitive to Staphylococcus aureus than the fungus Malassezia furfur, which only provides a sensitive response to flower extracts.

Ethanol extract (96 %) of tigarun flowers against Staphylococcus aureus at concentrations of 200 mg/mL (10.65±0.25), 150 mg/mL (8.38±0.19), and 100 mg/mL (6.28±0.08) produced moderate average inhibition zones. In comparison, the 50 and 10 mg/mL concentrations did not produce an inhibition zone. This study is in line with previous studies which stated that the fermented methanol extract of Tigarun flowers had activity against Escherichia coli, Salmonella sp., Staphylococcus aureus, Pseudomonas flourescens and Basillus subtilis (Nazarni et al., 2016). The difference from previous studies is the solvent used for extraction and the treatment for simplisia production, where previous research was carried out to manufacture simplisia by fermentation and drying in a freeze dryer. Tests on Malassezia furfur with the same concentration also produced an average inhibition zone (7.40 ± 0.40) in the moderate category. The results for the two test microbes produced the inhibition strength category. However, when viewed according to the size of the inhibition zone, the resulting inhibition was more significant for Staphylococcus aureus than for Malassezia furfur. Test results on 96% ethanol extract of tigarun leaves and stem bark also showed activity against Staphylococcus aureus, but not against Malassezia furfur.

The difference in antimicrobial activity seen from the diameter of the inhibition zone in the test results above can be influenced by the type of test microbe used, where the test microbes, both bacteria and fungi, have different sensitivities to antimicrobial compounds where a bacterium or fungus forms resistance within itself, which is a natural mechanism for survival (Ernst, 1986). The bioactive content of the test extract also influences the response to different activities, where each part of the plant contains different bioactive compounds that provide different antimicrobial activities (Rahmawati et al., 2022).

The results of the antimicrobial activity test can be attributed to the components of the compounds contained therein; tigarun, based on the positive phytochemical screening test results, contained alkaloids, flavonoids, saponins, tannins, phenols, steroids/triterpenoids, and coumarins. Alkaloids are associated with the inhibition of bacterial growth by interfering with the constituent components of peptidoglycan in bacterial cells; therefore, the cell wall layer is not completely formed and causes cell death. Flavonoids inhibit cell membrane function by forming complex compounds with extracellular proteins, thereby damaging the bacterial cell membrane (Jacky, Putri, and Azizah, 2019). Saponins play a role by influence the permeability of the cytoplasmic membrane so that microbial cells become lysed, and phenols have a protein denaturation mechanism that causes the protein structure to be damaged and disrupts the physiological functions of bacteria, resulting in bacterial cell death. Tannins can shrink cell walls or membranes, thereby interfering with bacterial cell permeability (Liu *et al.*, 2020). Steroids/terpenoids kill bacteria by damaging their cell membranes (Madduluri et al., 2013). Coumarins can interfere with the synthesis of DNA, RNA, and proteins in microorganisms need to reproduce (He *et al.*, 2022).

The results of this study are an initial exploratory stage to determine the activity of tigarun plant extracts against microbes that cause folliculitis. Further research is also carried out to explore secondary metabolites that specifically act as antimicrobials to treat skin infections such as folliculitis and possibly develop into pharmaceutical preparations for the treatment of skin infections.

CONCLUSION

The results showed that the 96% ethanol extract of tigarun flower had antimicrobial activity against *Staphylococcus aureus* and *Malassezia furfur* at an extract concentration of 200 mg/mL. The 96% ethanol extract of the leaves and stem bark of tigarun only showed activity against *Staphylococcus aureus*. The research results can be continued to the isolation stage to identify compounds that contribute to antimicrobial activity, and this research can be developed into pharmaceutical preparations intended for the treatment of skin infections.

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