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# Antibacterial Potency of Lichen *Teloschisthes flavicans* From Kepahiang District Against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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## ABSTRACT

Lichen is one of the organisms with a high level of biodiversity and rich of antibacterial secondary metabolites compounds. One species of lichenes found in Kepahyang Indonesia is *Teloschisthes flavicans*. This study aims to find out an antibacterial effectiveness of *Teloschisthes flavicans* extract against the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa. T. flavicans* samples are obtained from the barks of African trees (Maesopsis eminii Engl.), it was then extracted using 96% (1:10) ethanol for 5 days. antibacterial activity was tested using the disc diffusion method with 10 treatments; those are: negative control (DMSO 10%), positive control (chloramphenicol 0.10%), and *T. flavicans* extract with concentration of 25 ppm; 50 ppm; 75 ppm; 100 ppm; 200 ppm; 400 ppm; 800 ppm; and 1000 ppm. The result obtained the average diameter of the clear zone. Antibacterial activity of *T. flavicans* against the growth of *S. aureus* obtained an average diameter of clear zone at 35.30 ± 0.28 mm for the positive control treatment, it was also found the average diameter of the clear zone due to the aplication of *T. flavicans* extract from the lowest to the highest concentration was that of 8.37 ± 0.10 mm; 14.65 ± 0.91 mm; 11.17 ± 0.10 mm; 11.47 ± 0.$^{10}$ mm; 11.90 ± 0.84 mm; 12.50 ± 0.7 mm; 12.22 ± 0.24 mm; and 12.57 ± 0.17 mm. Meanwhile, the result of antibacterial activity of *T. flavicans* extract against the growth of *P. aeruginosa* was the average diameter of the clear zone of 37.77 ± 1.02 mm for the positive control, and the clear zone respectively at; 7.02 ± 0.03 mm; 9.00 ± 1.06 mm; 9.97 ± 1.94 mm; 10.95 ± 0.56 mm; 11.50 ±100 ppm 1.27 mm control, and 11.62 ± 1.30 mm; 11.60 ± 0.21 mm; and 12.17 ± 1.02 mm at samples treated by *T. flavicans* extract from the lowest to the highest concentration. Data was analyzed by ANOVA, followed by the Duncan test using SPSS 16.0. The most effective concentration of *T. flavicans* extract in inhibiting the growth of *S. aureus* is 50 ppm. While the most effective concentration of *T. flavicans* extract in inhibiting the growth of *P. aeruginosa* was 100 ppm.

***Keywords**: Inhibition zone, Pseudomonas aeruginosa, Staphylococcus aureu, Teloschisthes flavican*

## 1. INTRODUCTION

Most diseases in Indonesia are caused by several factors, one of which is a pathogenic bacterial infection. These bacteria attacking people through foodstuffs and the environment as a substrate of its growth process [1]. Some examples of pathogenic bacteria in humans such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* which are capable of producing exotoxins that cause skin infections, infections of the urinary tract, and respiratory tract [2]. Infection by *S. aureus* and *P. aeruginosa* is

characterized by tissue damage accompanied by an abscess. Some infectious diseases caused by *S. aureus* are ulcers, acne, impetigo, and wound infections. More severe infections include pneumonia, mastitis, phlebitis, meningitis, urinary tract infections, osteomyelitis, and endocarditis. *S. aureus* is also the leading cause of nosocomial infections, food poisoning, and toxic shock syndrome [3].

Indonesia has abundant biodiversity, the abundance of biodiversity can be utilized as raw materials of modern and traditional medicine, but still, few are utilized as raw materials of modern medicine, one of which is a lichen [4]. Lichen variety reaches 18000 species spread around the world, both in macro and microform [5], while lichen in Indonesia reaches ± 17000 [6]

Lichen is a symbiont organism between the fungi (mycobiont) of the Ascomycetes and Basidiomycetes groups, with algae (Phycobiont) from the Cyanobacteria or Chlorophyceae group. Algae is a nutrient-contributing organism for lichen, while fungi serve to provide algae with the necessary water and mineral supplies [7]. Lichen also has antibacterial activity against various species of pathogenic microbes. The large utilization of lichen in the field of health, especially medicinal materials, is related to the substance contained in it because lichen has a number of unique secondary metabolites and many of them are bioactive compounds [8].

One of the lichens that have a very large compound content is *Teloschistes flavicans*. Based on research conducted by [9], Lichen *T. flavicans* can inhibit the growth of *Bacillus subtilis* and *Staphylococcus aureus* bacteria with an average diameter of 19.25 ± 1.48 mm in B. subtilis and 17.75 ± 0.433 mm in *S. aureus*. Research conducted by [10], lichen *T. flavicans* extract is capable of inhibits the growth of *Aspergillus flavus* fungus, with the highest inhibitory zone data at a concentration of 1000 mg $mL^{-1}$ with a clear zone diameter of 11 mm. Based on this research it is known that lichen extract has the ability to inhibit and kill pathogenic bacteria and fungi.

Until now, research on lichen in Indonesia is still a few. Lichen himself has potential to be researched and utilized as an antibacterial. The potential of lichen as an antibacterial is based on the large content of secondary metabolite compounds contained in lichen. One of the types of lichen found in Indonesia is lichen *T. flavicans*. Based on this, this research was conducted aimed at testing the effectiveness of antibacterial lichen *T. flavicans* in inhibiting the growth of *S. aureus* and *P. aeruginosa*

## 2. MATERIALS AND METHODS

The Study was conducted from July to September 2020. Lichen samples were taken from Tangsi Duren Village, Kaba Wetan District of Kepahiang Regency, and samples preparation for antibacterial testing conducted in Microbiology Laboratory of Basic Science Building, Department of Biology, Faculty Mathematics and Natural Sciences, University of Bengkulu.

The tools used in this study were Erlenmeyer, beaker glass, glass funnel, petri dish, hot plate, micropipette, analytical scales, autoclave, incubator, laminar airflow, vortex mixer, shaker. Ingredients used in this study, lichen *T. flavicans*, *S. aureus*, *P. aeruginosa*, ethanol 96%, alcohol 70%, disc paper, Tryptic soy agar (TSA), Tryptic soy broth (TSB), DMSO 10%, aqua dest, chloramphenicol, filter paper, plastic, aluminum foil, cotton foil

The manufacture of lichen extract is done by cleaning 400 gr lichen *T. flavicans* then dried in the shade for 2 days, then smoothed until it becomes powder.Lichen powder as much as 200 gr is maserated using ethanol solvent 96% as much as 2 liters ( with a ratio 1:10) for 5 days and stirred daily. After the next 5 days, it is filtered and evaporated using a rotary evaporator.

Antibacterial testing uses a disc diffusion method using test bacteria rejuvenated into TSB media and in incubation for 24 hours. Bacterial cultures that have been in incubation for 24 hours are taken as much as 1 ml then put in TSA media then homogenized for 2 minutes and put in a petri dish, wait until compacted, then put the disc paper into a petri dish and drip extract using micropipettes with different treatment concentrations, chloramphenicol, DMSO 10%, and lichen extract with a concentration of 25 ppm, 50 ppm, 75 ppm, 100 ppm, 200 ppm, 400 ppm, 800 ppm, 1000 ppm. After that the media that has been treated is incubated at a temperature of 37°C for 24 hours, then a clear zone measurement is performed. The data obtained were analyzed with ANOVA, followed by the Duncan test using SPSS 16.0

## 3. RESULTS AND DISCUSSION

Based on antibacterial activity test results lichen *T. flavicans* extract shows that each concentration of lichen *T. flavicans* extract has a different clear zone based on the established clear zone. Zone the average

diameter of the clear zone obtained from the diameter of the clear zone is reduced by the diameter of the disc paper

Negative control (DMSO 10%) incapable inhibits the growth of *S. aureus* bacteria and *P. aeruginosa* bacteria. Positive control (chloramphenicol 0.10%) in lichen *T. flavicans* extract testing was shown to inhibit the growth of *S. aureus* by forming a clear zone of 35.30±0.28 mm and was able to inhibit the growth of *P. aeruginosa* by forming a clear zone of 37.77±1.02 mm. In the treatment with lichen extract *T. flavicans* the average diameter of the smallest inhibition zone and the largest inhibition zone that can inhibit the growth of bacteria *S. aureus* is at concentrations of 25 ppm and 50 ppm while in bacteria *P. aeruginosa* is at concentrations of 25 ppm and 1000 ppm. At concentrations of 25 ppm and 50 ppm can inhibit the growth of bacteria *S. aureus* with an average diameter of the clear zone of 8.37 ± 0.10 mm and 14.65 ± 0.91 mm. The average diameter of the slave zone that can inhibit the growth of bacteria *P. aeruginosa* at concentrations of 25 ppm and 1000 ppm is 7.02 ± 0.03 mm and 12.17 ± 1.02 mm.

The above buffer zone diameter data is analyzed using ANOVA one-way variant analysis with significance value ( ) 0.05 and it is known that da treatment of *S. aureus* bacteria value F calculates > F table (873.623 > 3.02), while on the treatment of bacteria *P. aeruginosa* value F calculates > F table (175.935 > 3.02) which means the data is significant or there is a difference in treatment, so Duncan tests continue to find out the most effective extract concentrations to inhibit the growth of bacteria *S. aureus* and *P. aeruginosa*.

Duncan's real difference test results (table 1.) indicates that negative control treatment in *S. aureus* bacteria and *P. aeruginosa* bacteria with (notation a) differs manifestly from all treatment, as well as a positive control both *S. aureus* and *P. aeruginosa* bacteria (notating g and e) also differs manifestly from all treatments. Treatment of *S. aureus* bacteria with concentrations of 25 ppm (b notation) and 50 ppm (notation f) differs noticeably, while the treatment in bacteria *S. aureus* concentrations of 75 ppm and 100 ppm is no different tangible to (notation c) and concentrations of 200 ppm, 400 ppm, 800 ppm, and 1000 ppm are also no different real because it has (e notation). The treatment of *P. aeruginosa* with lichen extract concentrations of 25 ppm and 50 ppm showed there are no different from (notation b) and concentrations of 75 ppm, 100 ppm, 200 ppm, 400 ppm, 800 ppm, 1000 ppm are not different from having (notation d).

Test results of antibacterial extract activity lichen *T. flavicans* with various concentrations, concentrations of 25 ppm, 50 ppm, 75 ppm, 100 pp, 200 ppm, 400 ppm, 800 ppm, and 1000 ppm show a response in inhibiting the growth of pathogenic bacteria *S. aureus* and *P. aeruginosa*. The response is in the form of a formed clear zone, wherein the clear zone is not found bacteria that grow. Treatment with a negative control in the form of DMSO does not indicate the inhibition of growth or not the establishment of a clear zone, while in the treatment with positive control formed zones inhibition that can hinder the growth of bacteria from both bacteria.

Treatment with positive control in the form of antibiotic chloramphenicol concentration, 0.10% is used to compare the ability of lichen *T. flavicans*

**Table 1.** Observation of the diameter of the slave zone of *T. flavicans* extract

| No. | Treatment | Average Clear Zone Diameter (mm)±SD | |
|---|---|---|---|
| | | ***S. aureus*** | ***P. aeruginosa*** |
| 1 | Negative control (DMSO) | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ |
| 2 | Positive control (Chloramphenicol) | $35.30 \pm 0.28^{g}$ | $37.77 \pm 1.02^{e}$ |
| 3 | Concentration 25ppm | $8.37 \pm 0.10^{b}$ | $7.02 \pm 0.03^{b}$ |
| 4 | Concentration 50 ppm | $14.65 \pm 0.91^{f}$ | $9.00 \pm 1.06^{bc}$ |
| 5 | Concentration 75 ppm | $11.17 \pm 0.10^{c}$ | $9.97 \pm 1.94^{cd}$ |
| 6 | Concentration 100 ppm | $11.47 \pm 0.10^{cd}$ | $10.95 \pm 0.56^{cd}$ |
| 7 | Concentration 200 ppm | $11.90 \pm 0.84^{cde}$ | $11.50 \pm 1.27^{d}$ |
| 8 | Concentration 400 ppm | $12.50 \pm 0.7^{e}$ | $11.62 \pm 1.30^{d}$ |
| 9 | Concentration 800 ppm | $12.22 \pm 0.24^{de}$ | $11.60 \pm 0.21^{d}$ |
| 10 | Concentration 1000 ppm | $12.57 \pm 0.17^{e}$ | $12.17 \pm 1.02^{d}$ |

**Description**: different notations (a, b, c, d, e, f) in the same column indicate that the treatment differs manifestly based on the Duncan test with the same level of trust and the same notation shows no real difference

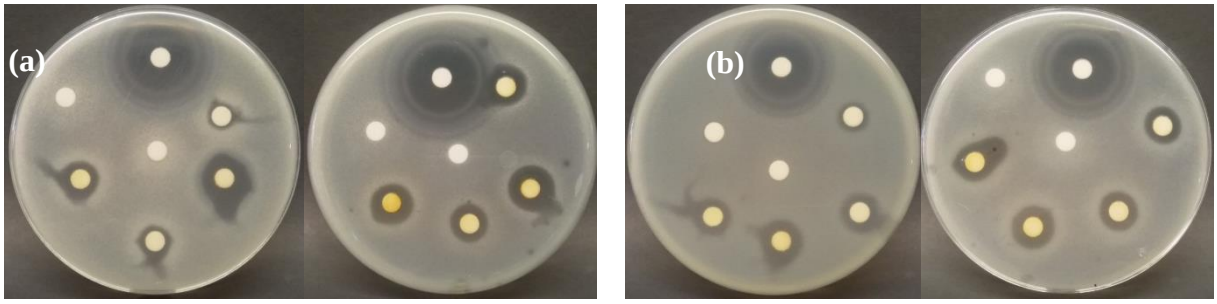


**Figure 1.** Antibacterial activity of lichen extract *T. flavicans* (a) *S. aureus* (b) *P. aeruginosa*.

extract with those antibiotics in inhibiting the growth of bacteria *S. aureus* and *P. aeruginosa*. Chloramphenicol antibiotics are broad-spectrum antibiotics (active against Gram-positive and Gram-negative bacteria) and can inhibit bacterial growth [11]. chloramphenicol inhibits growth bacteria by interfering with the binding of new amino acids to the newly emerged peptide chain, in part because chloramphenicol inhibits peptidyl transferase. If the new cluster is amino acids are not formed then will resulting in impaired protein synthesis, while bacterial cells need to synthesize proteins for its survival. Disruption of protein synthesis will result in inhibited enzyme formation as well as structural proteins in the membranes and walls of bacterial cells, resulting in the metabolism becoming disrupted and bacteria experiencing death [2].

Based on the research that has been done by [12], lichen *T. flavicans* extract has the ability to inhibit bacterial growth, *Staphylococcus aureus*, and *Bacillus subtilis*. Other research on the antibacterial capabilities of lichen *T. flavicans* has also been conducted [9], which shows that Lichen *T. flavicans* extract may inhibit bacterial growth of *Bacillus subtilis* and *S. aureus* with an average diameter of 19.25 ± 1.48 mm in *B. subtilis* and 17.75 ± 0.433 mm in *S. aureus*. The area of the inhibition zone that is formed also shows a lot of bacteria that can be inhibited by lichen extract *T. flavicans*. The larger the diameter of the inhibition zone that is formed, the more bacteria can be inhibited, and vice versa the smaller the diameter of the resulting inhibitory zone indicates that the bacteria are inhibited less.

In this study the most effective form of barrier zone by lichen *T. flavicans* extract against the growth of bacteria *S. aureus* is 50 ppm with a diameter of 14.65 mm and in bacteria, *P. aeruginosa* is 100 ppm with a diameter of *10.95 mm*. The formation of clear zone diameters according to [13] can be classified based on bacterial growth resistance response which is a very strong category if the diameter of the > zone is 20 mm, the strong category when the diameter of the clear zone ranges from 10-20, the medium category when the diameter of the clear zone ranges from 5-10 mm, and the weak category when < 5 cm. This category is based on the types of bacteria that are pathogenic bacteria and compounds that can inhibit the growth of pathogenic bacteria namely flavonoids, alkaloids, saponins, steroids, terpenoids, pens, dapsone, benzyl ether, xanthone, aliphatic esters, essential oils, and pulvinate acid derivatives. Based on that classification the lowest concentration has a strong buffer zone diameter of lichen *T. flavicans* extract, with a concentration of 50 ppm in *S. aureus* bacteria and *P. aeruginosa* bacteria with a concentration of 100 ppm have strong inhibitory power. So, it can be concluded that the ability of lichen *T. flavicans* extract in inhibiting bacterial growth has the potential to be a natural compound that allows it to be used as an antibacterial. Antibacterial capabilities are thought to come from secondary metabolites compounds contained in them. Based on phytochemical tests it has been known that lichen *T. flavicans* extract contains flavonoid compounds, saponins, and steroids.

Lichen *T. flavicans* extract is known to contain lipophilic flavonoid compounds that can damage bacterial membranes. Flavonoid compounds form hydrogen bonds that coincide with extracellular and dissolved proteins resulting in damage to bacterial cell walls and the exit of intracellular compounds [14]. Flavonoids can also inhibit energy metabolism using inhibits the use of oxygen by bacteria. Energy needed bacteria for biosynthesis macromolecules, so that if the metabolism is blocked, the bacterial molecules are not can develop into molecules that are complex [15]. Moreover, in flavonoids there are also phenol compounds that can interfere with the growth of bacteria *S. aureus* and *P. aeruginosa*. Phenols are alcohol that is acidic so that has the ability to denaturation proteins and damage bacterial cell membranes [16].

Mechanism of action of steroids as an antibacterial in inhibiting the growth of *S. aureus* and *P. aeruginosa* are related to lipid membranes and sensitivity to steroid components that cause leakage

in bacterial liposomes [13]. Steroids can interact with membranes phospholipid cells that are permeable to lipophilic compounds so that causing membrane integrity to decrease as well as cell membrane morphology changes causing fragile cells and lysis.

Mechanism of action of saponins as antibacterial i.e. by causing leakage proteins and enzymes from inside bacterial cells *S. aureus* and *P. aeruginosa* [17]. Saponins are active substances that can be membrane permeability so that hemolysis occurs in cells. When saponin interact with bacterial cells, the bacteria rupture or lysis [18].

Test results of antibacterial extract activity *T. flavicans* against the bacteria *S. aureus* and *P. aeruginosa*, indicating a noticeable difference in the test results of both bacteria. The difference lies in the average diameter of the clear zone of *S. aureus* and *P. aeruginosa*. *P. aeruginosa* bacteria have an average clear zone diameter smaller than the average clear zone diameter in *S. aureus*. The difference in results caused by a difference in type between the two bacteria that affect differences in cell wall structures, plasma membranes and, pathogenic capabilities possessed by both bacteria.

*P. aeruginosa* bacteria belonging to Gram-negative bacteria have complex cell wall structures with structures consisting of three layers namely the outer, middle, and inner layers. The outer layer consists of lipoproteins, the middle layer is thick peptidoglycan, and the inner layer is lipopolysaccharide. Meanwhile, the *S. aureus* bacterial cell wall structure included in gram-positive bacteria has only a simple cell wall structure consisting of one layer of peptidoglycan [19].

Gram-negative bacteria have double plasma membranes with a thickness > 80 nanometers protected by an outer membrane that is permeable and has a lipid content that is High. But on the contrary, Gram-positive bacteria have only a single plasma membrane, low lipid content, cell wall thickness 20-80 nanometers, and cell walls surrounded by peptidoglycans [20]. This condition causes groups of bacteria to Gram-positives are easier to enter by compounds antibacterial so that the compound is easier to find targets that will inhibit or disrupted by its metabolic processes compared to cell wall structure complex owned by a group of bacteria Gram-negative. In addition, the Gram bacterial group has the ability to pathogenic lower than the Gram-negative bacteria [21].

## 4. CONCLUSION

Based on the results of the study can be concluded that the extract lichen *T. flavicans* can inhibit the growth of *S. aureus* bacteria and *P. aeruginosa*. The most effective concentration in inhibiting the growth of bacteria *S. aureus* is 50 ppm and *P. aeruginosa* is a concentration of 100 ppm.

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