Antimicrobial activity of secondary metabolite compounds from lichen Teloschistes flavicans

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Abstract. Exploration of lichen natural compounds has attracted a lot of attention from researchers as potential medicinal raw materials because chemical compounds contained in lichen have many properties. The potential for developing secondary metabolite compounds of lichen has become a trend for various benefits, for example, in cosmetics, agriculture, health, and food. Lichen Teloschistes flavicans was a concern to study because there were not many reports of secondary metabolites that have antimicrobial activity. The research objective was to examine antimicrobial activity using 3-[1′-(2″,3″-dihydroxy-phenyl)-propyl]-7hydroxy-chroman-4-one compound from lichen Teloschistes flavicans. The antimicrobial activity test was a well diffusion method. Bioactivity was determined by calculating the clear zone formed around the well. Antimicrobial activity testing indicated the inhibition of bacterial growth at a concentration of 500 ppm and 1000 ppm with the respective inhibition zones namely E. coli (10-11 mm), S. Typhi (2-5 mm), K. pneumonias (11-13 mm), and S. aureus (10-13 mm). Also, B. cereus bacteria and C. albicans fungi were only inhibited at a concentration of 1000 ppm with an inhibition zone of 6 mm each. This research provides scientific knowledge about the potential development of lichen T. Flavicans as an antimicrobial.

1. Introduction
The exploration of chemical compounds from natural materials continues today as technology develops. The objectives are very diverse, namely the development of science, economic benefits, the discovery of new drugs, and the development of traditional medicine. Chemical compounds contained in natural materials, namely primary metabolites, secondary metabolites, macromolecules, and genetic compounds [1–3]. Thus far, the potential for developing secondary metabolite compounds has become a trend for researchers for various benefits, such as for cosmetics, agriculture, health, and food [4].

The lichen organism is interesting research to be studied at this time. Researchers have reported that lichen chemical compounds have a lot of potentials to be developed into raw materials in the health sector, namely single chemical compounds or mixtures. The benefits of lichen are shown from the widely reported bioactivity results, namely anti-inflammatory [5], antimicrobial [6], antioxidants [7], antidiabetic [8], and anticancer [9]. The reported variety of lichen species is that around 18,500 have been identified that have various shapes, colors, and habitats [3]. Lichen has four forms of the thallus, namely crustose, foliose, fruticose, and squamulose. The difference in lichen morphology makes the constituent chemical compounds different for each type [10–13].
Lichen is known as a unique organism because the unique components of its body are formed by algae and fungi, as well as chemical compounds that are rarely found in other natural sources [14,15]. Generally, the secondary metabolite compounds produced by lichen are the depside, depsidone, dibenzofurans group [16,17]. About 1050 chemical structures of lichen have been reported [18]. Several secondary metabolite compounds that have been reported from lichen Usnea are usnic acid [6] and atranorin [19]. Secondary metabolites of lichen from the Teloschistes type, namely viciancin [20], caloplocin [21], teloschistin [22], and emedin [21].

Lichen Teloschistes was first described by Norman and the species was classified in the 18th century. Several species of the Teloschistes have bright colors, such as yellow and orange [23]. Species of Teloschistes that have been identified are T. flavicans, T. exilis, T. chrysophthalmus, T. lacunosus, and T. villosus [24]. Teloschistes is a concern to study because there are not many reports of secondary metabolites that have biological activity. This study is a continuation of the exploration of secondary metabolites from mosses of the Teloschistes flavicans type. Previous studies have reported the results of isolation from T. flavicans moss, namely compounds 3-[1'-(2''3''-dihydroxy-phenyl)-propyl]-7hydroxy-chroman-4-one have antifungal activity [25].

Based on the literature, another bioactivity of the lichen T. flavicans mixture, namely methanol extract can inhibit the growth of A. aegypti larvae [26], chloroform extract has a growth-inhibiting activity of Fusarium solanum [27], and acetone extract has growth-inhibiting activity against Aspergillus flavus [25]. Based on the literature, the compound activity of lichen is very reactive to microbial growth, this is supported by empirical results that lichen is used as a traditional medicine to treat diseases caused by various microbes, such as water flea disease, dysentery, thrush, and warts [28]. Therefore, in this study, we report secondary metabolites of T. flavicans to determine the biological activity against gram-positive, gram-negative bacteria, and C. albicans fungi.

2. Materials and methods

2.1 Materials

The materials used were lichen T. flavicans collected from South Sulawesi Indonesia, acetone (Merck, Germany), ethyl acetate (Merck, Germany), methanol pa (Merck, Germany), n-hexane (Merck, Germany), silica gel G.60 (0.063-0.200 mm), sulfuric acid (Sigma-Aldrich), cerium sulfate (Sigma-Aldrich), BaCl₂ (Sigma-Aldrich), NaCl (Sigma-Aldrich), antiseptic alcohol (Sigma-Aldrich), NA medium (Sigma-Aldrich), PDA medium (Sigma-Aldrich), tetracycline (Merck), nystatin (Sigma-Aldrich), C. albicans fungi, E. coli, S. Typhi, S. aureus, K. pneumoniae and B. cereus.

2.2 Isolation of lichen T. flavicans

The T. flavicans samples were cleaned and mashed first. The extraction used is maceration. Maceration was carried out for 5x24 hours with 1 L acetone in succession, every 1 x 24 hours the extract was filtered and then macerated. The filtrate obtained is combined and evaporated to obtain concentrated acetone extract.

The impregnated extract was slowly fed into the column then flattened. The sample was eluted in the chromatography column with various eluent mixtures in comparison. The elution process begins with a 100% n-hexane eluent and then a gradient mixture of n-hexane: ethyl acetate and ethyl acetate: methanol. Eluate that comes out of the column is collected every 250 mL in a container then evaporated. The eluate was then recrystallized using methanol and tested its purity by TLC.

2.3 Antimicrobial activity test

Firstly, the tools used for testing and the media are sterilized. The media was prepared by following the instructions of the Sigma-Aldrich product. The antimicrobial test used a well diffusion method with a well-made diameter of 6 mm. The microbial density used in the test was 1.5 x 10⁷/mL bacterial suspension and 1.4 x 10⁶/mL fungal suspension, which is equivalent to the standard Mc. Farland 0.5 [23]. The test solution was made into 4 levels of concentration, each well filled with 15 μL of the test sample solution. The incubation process was carried out for 24 hours for bacteria and 48 hours for C.
*albicans* with a temperature of 37 °C. Determination of the activity of the test sample is done by calculating the diameter of the inhibition area that is formed around the well.

### 3. Results and discussion

#### 3.1 Isolation of lichen *T. flavicans*

The results of lichen *T. flavicans* extraction obtained concentrated acetone extract with a mass of 57.27 grams. The results of the separation of concentrated extract using CCG obtained pure eluate as shown in Figure 1. Separation of chemical compounds occurs during the elution process; chemical compounds experience the interaction between the stationary phase and the mobile phase. Compounds that have the same polarity as the eluent are attracted and follow the eluent out of the gravity column. A chemical compound that has the same polarity as the stationary phase will be stuck in the column [25,29,30]. The TLC purity test using three different eluent systems shows that the spots appear to remain single, this indicates that the eluate contains only one chemical compound. Purification of eluate by recrystallization obtained orange needle-shaped crystal eluate shown in Figure 2.

![Figure 1. Chromatogram test for eluate purity under 254 nm UV lamp (a) n-hexane eluent: ethyl acetate (8: 2) v / v; (b) Eluent n-hexane: CHCl₃: MeOH (8: 1: 1) v / v; (c) n-hexane eluent: acetone (1: 1) v/v](image1)

![Figure 2. Crystal from eluate lichen *T. flavicans* acetone extract](image2)

In previous studies, we have reported that compound 3-[1’-(2”,3”-dihydroxy-phenyl)-propyl]-7hydroxy-chroman-4-one has been isolated from lichen *T. flavicans*. The structural formula can be seen in Figure 3.

![Figure 3. Structure of the compound 3-[1’-(2”,3”-dihydroxy-phenyl)-propyl]-7hydroxy-chroman-4-one [25]](image3)
Table 1. Elucidation data of isolate compounds [25]

| Spectra Data                        | Molecular weight ([M+]+ (m/z)) | UV (λmax, (nm)) | IR (μm, (cm^-1); functional groups) | 1H-NMR (δ_H (ppm) (ΣH, mult, J (Hz)) |
|-------------------------------------|-------------------------------|-----------------|-------------------------------------|--------------------------------------|
| -                                  | 315                           | 270             | 3619, OH; 3559, OH; 2932, Csp^3-H; 1740, C=O; 1625, C=C; 1037, C-O dan 978, C-O | 7.612 (1H, d, 1.7); 7.296 (1H, d 1.6); 7.196 (1H, d, 1.69); 6.892 (1H, d, 1.6); 4.353 (2H, s); 3.784 (3H, s); 2.966 (2H, t, 7.18); 1.59 (1H, q); 1.337 (2H, m); 0.876 (3H, t, 7.12) |

3.2 Antimicrobial activity test

Determination of antimicrobial activity was carried out by observing the activity of the test sample on microbial growth in the petri dish by observing and measuring the diameter of the inhibition area around the well after incubation which is shown in Table 2.

Table 2. Table of antimicrobial activity test

| Bacteria/Fungus | Concentration of Acetone Isolat (ppm) | Inhibition Zone of Isolat (mm) |
|-----------------|---------------------------------------|--------------------------------|
| E. coli         |                                       |                                |
| Control (+)     | 1000                                  | 11                             |
| Control (-)     | 500                                   | 10                             |
|                 | 100                                   | 4                              |
|                 | 10                                     | 0                              |
| Control (+)     | 500                                   | 25                             |
| Control (-)     | 0                                     | 0                              |
| S. typhi        |                                       |                                |
| Control (+)     | 1000                                  | 5                              |
| Control (-)     | 500                                   | 2                              |
|                 | 100                                   | 0                              |
|                 | 10                                     | 0                              |
| Control (+)     | 500                                   | 5                              |
| Control (-)     | 0                                     | 0                              |
| K. pneumoniae   |                                       |                                |
| Control (+)     | 1000                                  | 13                             |
| Control (-)     | 750                                   | 12                             |
|                 | 500                                   | 11                             |
|                 | 100                                   | 10                             |
| Control (+)     | 500                                   | 24                             |
| Control (-)     | 0                                     | 0                              |
| S. aureus       |                                       |                                |
| Control (+)     | 1000                                  | 13                             |
| Control (-)     | 500                                   | 10                             |
|                 | 100                                   | 0                              |
|                 | 10                                     | 0                              |
| Control (+)     | 500                                   | 22                             |
| Control (-)     | 0                                     | 0                              |
| B. cereus       |                                       |                                |
| Control (+)     | 1000                                  | 6                              |
| Control (-)     | 500                                   | 0                              |
|                 | 100                                   | 0                              |
|                 | 10                                     | 0                              |
| Control (+)     | 500                                   | 24                             |
| Control (-)     | 0                                     | 0                              |
| C. albicans     |                                       |                                |
| Control (+)     | 1000                                  | 0                              |
| Control (-)     | 500                                   | 0                              |
|                 | 100                                   | 0                              |
|                 | 10                                     | 0                              |
| Control (+)     | 500                                   | 17                             |
| Control (-)     | 0                                     | 0                              |

Table 2 shows that T. flavicans isolates had a response to bacterial growth inhibition and increased with increasing concentration. Antimicrobial activity testing indicated the inhibition of bacterial growth
at a concentration of 500 ppm and 1000 ppm with the respective inhibition zones namely *E. coli* (10-11 mm), *S. typhi* (2-5 mm), *K. pneumoniae* (11-13 mm), and *S. aureus* (10-13 mm). In addition, *B. cereus* bacteria and *C. albicans* fungi were only inhibited at a concentration of 1000 ppm with an inhibition zone of 6 mm each. The resistance response of the isolates to the growth of *E. coli*, *K. pneumonia* and *S. aureus* bacteria was better than other microbes. The hydroxyl group in the isolate is a reactive functional group that binds to form a hydrogen bond chain. Proteins are very sensitive to physical and chemical influences, so they are prone to deformation or denaturation [28]. The formation of protein hydrogen chain bonds on the lowering of the microbial cell wall, thereby decreasing the biological activity of protein and protein solubility.

4. Conclusion
Isolation of lichen *T. flavicans* using CCG and TLC obtained pure compounds 3-[1'-(2".3"-dihydroxy-phenyl)-propyl]-7-hydroxy-chroman-4-one. Antimicrobial activity testing indicated the inhibition of bacterial growth at a concentration of 500 ppm and 1000 ppm with the respective inhibition zones namely *E. coli* (10-11 mm), *S. typhi* (2-5 mm), *K. pneumoniae* (11-13 mm), and *S. aureus* (10-13 mm). In addition, *B. cereus* bacteria and *C. albicans* fungi were only inhibited at a concentration of 1000 ppm with an inhibition zone of 6 mm each. This research provides scientific knowledge about the potential development of lichen *T. Flavicans* as an antimicrobial.

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