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Antibacterials Activity of *Escherichia coli* and *Salmonella typhi* by Acetone Extract of the Lichen *Usnea* sp.

Andi Nafisah Tendri Adjeng[1], Akhmad Darmawan[2], Prima Endang Susilowati[1], Besse Vika[3], Andi Musdalifah[4], Usman Usman[5], Nurlansi Nurlansi[6], Muhammad Nurdin[4] and Maulidiyah Maulidiyah[4,a)]

[1]*Department of Pharmacy, Faculty of Medicine, Universitas Lampung, Lampung, Indonesia*
[2]*Research Center for Chemistry, Indonesian Institute of Sciences, Jakarta, Indonesia*
[3]*Department of Pharmacy, Faculty of Sciences and Technology, Institut Teknologi dan Kesehatan Avicenna, Kendari, Indonesia*
[4]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Halu Oleo, Kendari, Indonesia*
[5]*Department of Chemistry Education, Faculty of Teacher Training and Education, Universitas Mulawarman, Samarinda, Indonesia*
[6]*Department of Chemistry Education, Faculty of Teacher Training and Education, Universitas Halu Oleo, Kendari, Indonesia*

[a)] *Corresponding author: maulid06@yahoo.com*

Abstract. Lichen organisms have been used by the community as traditional herbal ingredients, especially lichens of the genus *Usnea* sp.. In this study, we focused on examining the effect of the acetone extract lichen *Usnea* sp. in inhibiting the growth of *Escherichia coli* and *Salmonella typhi* bacteria. The sample of *Usnea* sp. lichen was extracted using the maceration method with acetone solvent for 3×24 hours. An antibacterial test was carried out by measuring the diameter of the inhibition zone using a caliper. The results of the antibacterial test on inhibition zones at concentrations of 1.25%, 2.5%, and 5% were 21.25 mm, 26.33 mm, and 30.5 mm (*E. Coli*) and 23.08 mm, 26.41 mm, and 33.0 mm (*S. typhi*), respectively. The lichen organism *Usnea* sp. has very potential for treating diarrhea and fever.

INTRODUCTION

Indonesia has abundant biodiversity and can be used as raw materials for medicines or traditional medicines, such as lichen. The existence of lichen in Indonesia is very much, but the people have not explored and know much about its benefits. The diversity of lichen species reaches 18,000 species, while lichen in Indonesia reaches ± 17,000 species [1, 2]. Lichen has unique secondary metabolites and many of them are bioactive compounds [3, 4].

Based on empirical experience, lichen genus *Usnea* sp. has properties that can treat several diseases such as diarrhea, bloody stools, canker sores, colds, abdominal pain, difficulty urinating, menstrual disorders, dizziness, and fever [5]. In recent years, the bioactivity of the *Usnea* sp. have been reported, such as antimicrobial [6], antioxidant [7,8], antibacterial [9, 10], antifungal [11], antiviral [12], antimalarial [13], cytotoxic activity [14], and antidiabetic [15].

Most diseases in the world are caused by several factors, one of which is infection with pathogenic bacteria. *E. coli* and *S. typhi* are pathogenic bacteria that cause diarrhea and pyoderma with a high prevalence rate [16]. *E. coli* bacteria are bacteria from the Gram-negative group that have a capsule-lined cell wall and normally live in the digestive tract of humans and animals [17]. The impact of these bacteria often causes diarrhea. *S. typhi* is a gram-negative rod bacterium that causes typhoid fever [18, 19].

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Until now, research on the *Usnea* sp. as an antibacterial of *E. Coli* and *S. typhi* has not been widely reported. We have previously reported the antibacterial activity of lichen in *Klebsiella pneumoniae* [20], and *Staphylococcus aureus* [21,22]. The potential of lichen as an antibacterial is based on the amount of secondary metabolite compounds present. In this focus study, we reported of test the antibacterial effectiveness of the acetone extract of lichen *Usnea* sp. in inhibiting the growth of *E. Coli* and *S. typhi* bacteria. The sample in this study was the lichen organism *Usnea* sp. obtained from the Enrekang Regency, South Sulawesi Province, Indonesia. The process of bacterial inhibition will be described in detail in this article.

MATERIAL AND METHODS

Materials

The materials used were acetone (70%), distilled water, hydrochloric acid, dragendorff's reagent, iron (III) chloride ($FeCl_3$), barium chloride ($BaCl_2$), sulfuric acid (H_2SO_4), chloramphenicol, nutrient agar, pure culture of *E. coli* and *S. typhi* obtained from Sigma-Aldrich.

Extraction of Usnea sp.

The *Usnea* sp. sample was obtained from the mountainous area of Latimojong Village, Pasui District, Enrekang Regency, South Sulawesi Province, Indonesia. *Usnea* sp. samples were extracted using the maceration method with acetone for 3×24 hours, where after 1×24 hours, the solvent was changed. Then the macerate was filtered, the liquid macerate was evaporated using a vacuum evaporator at a temperature of 50°C at a speed of 65 rpm to obtain a thick extract. The crude extract was placed on a water bath until a dry, viscous extract was obtained. The extract obtained was then weighed, and the percentage yield was calculated against the weight of the initial simplicia.

Antibacterial Test

The antibacterial testing method refers to research [21]. Determination of the ability of antibacterial activity is carried out aseptically using the paper disc diffusion method. 10 mL of nutrient agar was taken into a test tube, then 1 mL of bacterial suspension (density $1.5x10^8$/mL) was added and then homogenized. Media containing bacteria was poured into sterile Petri dishes. Disc paper containing lichen extract with concentrations of 1.25%, 2.5%, and 5% was placed on the surface of the solidified media, followed by control (+) and control (-), incubated for 1×24 hours at 37°C in an incubator. Furthermore, it was removed from the incubator and observed the area of the inhibition zone for bacterial growth in the form of a clear area and the inhibition zone formed was measured using a caliper.

RESULTS AND DISCUSSION

Extraction

The maceration technique has several advantages is not need heating. Extraction of lichen sample powder as much as 500 grams using acetone resulted in a thick extract of 18.23 grams, so that the percent yield obtained was 3.64%. The yield of this extract can be used as a reference for the extract to be obtained with the required amount of simplicia. This shows that the use of acetone as a solvent is quite good in attracting the compounds contained in the lichen *Usnea* sp.

Antibacterial Activity

Determination of the ability of antimicrobial activity was carried out aseptically using the paper disc diffusion method. Antibacterial testing used a positive control as a comparison antibiotic, namely chloramphenicol. Tests were also carried out on acetone solvent as a negative control aimed at comparing the results of the inhibition zone formed, not from the solvent used. Tests are carried out in triples to obtain data with a high level of precision [23].

The results of the antibacterial activity test are shown in Fig. 1**.** The antibacterial activity showed that there was a clear zone formed in the test of *E. coli* and *S. typhi* bacteria. The antibacterial test against *E.coli* showed the effectiveness of lichen *Usnea* sp. of each concentration as a very strong antibacterial (Table 1) based on the category of inhibition [24]. The diameter of the inhibition zone formed against *E.coli* bacteria was at a concentration of 1.25% (21.25 mm), a concentration of 2.5% (26.33), and at a concentration of 5% (30.5 mm). Antibacterial activity of *Usnea* sp. against *S. typhi* showed better activity than *E. coli*. The diameter of the inhibition zone formed from each concentration was greater, namely the concentration of 1.25% (23.08 mm), 2.5% (26.41 mm), and 5% (31.25 mm) (Table 2). The comparative activity of chloramphenicol showed better effectiveness than the lichen extract of *Usnea* sp. The acetone solvent activity did not show any inhibition against the two test bacteria.

Based on the data obtained, the higher the concentration used, the more potential the lichen extract of *Usnea* sp. in inhibiting bacterial growth. The active compounds contained in the extract work synergistically in inhibiting bacterial growth by forming complex bonds with the bacterial cell wall and damaging the cell membrane without being repaired. The active compound in the extract which is still complex can penetrate to the bacterial cell wall compared to a single pure compound.

TABLE 1. *E. Coli* inhibition zone

Sample test	Concentration (%)	Inhibition zone diameter (mm)	Inhibition category
Usnea sp. extract	1.25	21.25	Very strong
	2.5	26.33	Very strong
	5	30.5	Very strong
Positive control (Chloramphenicol)	5	32.5	Very strong
Negative control (aseton)	10	0	Not active

TABLE 2. *S. typhi* inhibition zone

Sample test	Concentration (%)	Inhibition zone diameter (mm)	Inhibition category
Usnea sp. extract	1.25	23.08	Very strong
	2.5	26.41	Very strong
	5	31.25	Very strong
Positive control (Chloramphenicol)	5	32.33	Very strong
Negative control (aseton)	10	0	Not active

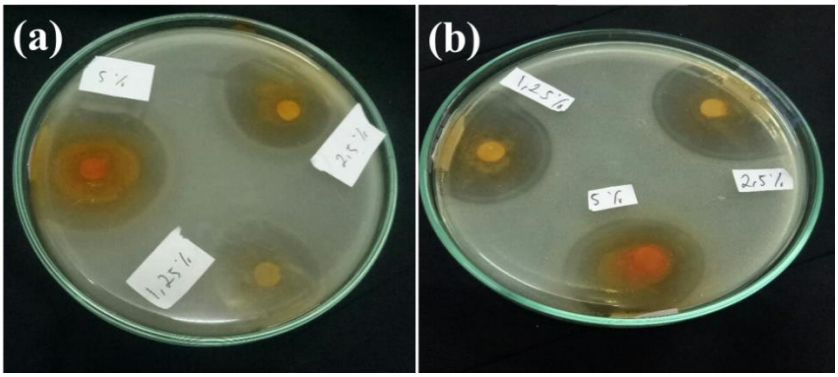

FIGURE 1. Inhibition zone of bacterial (a) *E. Coli*, and (b) *S. typhi*

CONCLUSION

The sample of *Usnea* sp. has been successfully extracted using acetone solvent. An antibacterial test was carried out by measuring the diameter of the inhibition zone using a caliper. The results of the antibacterial test on inhibition zones at concentrations of 1.25%, 2.5%, and 5% were 21.25 mm, 26.33 mm, and 30.5 mm (*E. Coli*) and 23.08 mm, 26.41 mm, and 33.0 mm (*S. typhi*), respectively.

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