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Research Article

ANTIMICROBIAL ACTIVITY OF ACETONE EXTRACT AND USNIC ACID CONSTITUENT OF LICHEN *USNEA LONGISSIMA* (ACH.)

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ABSTRACT

Acetone extract of lichen *Usnea longissima* (Ach.) and its usnic acid constituent (concentrations of 0.1, 0.5; 1.0 and 10.0 mg/mL) was investigated in order to examine the activity of antimicrobial test i.e. *Escherichia coli* ATCC 35218, *Salmonella typhi* ATCC 13311, *Klebsiella pneumoniae* ATCC 70603, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 14579, *Candida albicans*, and *Aspergillus flavus* as pathogenic fungus. The usnic acid constituent was tested by means of extensive Retention factor (Rf) compared with data reported in the literatures. High-inhibitory activity test using acetone extract showed that it can inhibit activity of *E. coli*, *S. typhi*, *K. pneumoniae*, *S. aureus*, *B. cereus*, and *C. albicans* with averages diameter zone of 9.22; 6.32; 15.32; 10.65; 4.92; and 6.82 mm, while the usnic acid as antimicrobial material were found with diameter zone of 18.57; 18.12; 11.27; 8.65; 23.65; and 6.5 mm, respectively. The low inhibitory activity was occured to the *A. flavus* that was exhibited by using acetone extract and usnic acid constituent from lichen *U. longissima*.

Keywords: *Usnea longissima*, Lichen, antimicrobial, usnic acid

INTRODUCTION

Indonesia is one of richest biodiversity and megadiversity countries[1]. Approximately of 30,000 plants species were very helpful and had been used by the peoples as medicine ingredients, consumed sustainably, and hereditary for a long time based on the traditional experiences[2]. The potential for biological diversity in Indonesia had been explored maximally as a natural medicinal compound[3,4].

Lichen is a medicinal plant which categorized unique and low organism. Lichen is classified into two organism's symbiosis mutualisms between fungus and algae[5,6]. Lichen produces very useful secondary metabolite substances and have bioactivity including antibiotic or antibacterial agents[7-9]. The increased prevalence of antibiotic-resistant bacteria due to the extensive use of antibiotics may render the current antimicrobial agents insufficient to control bacterial diseases. The investigation of new bioactive compounds is an importance to antibiotic-resistant microorganism control[10]. Lichen was proven to contain secondary metabolite compounds can prevent infection and cure illness. This infection due to the bacterium infected in the human body such as *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumonia*, *Staphylococcus aureus*, and *Bacillus cereus*[11-14]. Several of researchers have reported of lichen organism that widely used as traditional medicines in several countries i.e. Lichen *Cetraria islandica* (L.) Ach as a cough medicine and *Lobaria pulmonaria* (L.) Hoffm. was cultivated as medicine for lung disease[15]. In the last decade, Americans native was used lichen for natural drugs examples *U. longissima* as the smooth skin drug and *Letharia vulpina* as a yellow dye[16,17]. In India, the lichen was used to cure some diseases, i.e. asthma, bronchitis, stomach pain, allergy, the cracking of bones, liver, and blood disorders[18,19]. In Indonesia countries, Lichen from *Usnea* genus was sold in dry formed used as herbal medicine to treat diarrhea, dysentery, and muscle cramp. It is also used to treat wounds and swelling, as well as to overcome lung and tuberculosis infections.

Based on the literature the advantages of lichen for antimicrobial activity which usually infected in the human body (*E. coli, S. typhi, K. pneumoniae, S. aureus, B. cereus, and albicans*, and *A. Flavus*)[20]. The acetone extract was extracted and purified by thin layer chromatography (TLC) and gravity column chromatography (GCC) to obtain the usnic acid constituent. The antimicrobial test was conducted by comparing the acetone extract and usnic acid constituent from *U. longissima.* Subsequently, this study aims to identify the secondary metabolites from lichen *U. longissima* originating from Sulawesi-Indonesia and its bioactivity testing as antimicrobial agent such as *Escherichia coli* ATCC 35218, *Salmonella typhi* ATCC 13311, *Klebsiella pneumoniae* ATCC 70603, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 14579, *Candida albicans*, and *Aspergillus flavus* as pathogenic fungus.

MATERIAL AND METHODS

The thallus of lichen *U. longissima* was collected from Enrekang region of South Sulawesi Province, Indonesia, acetone (Merck, Germany), ethyl acetate (Merck, Germany), n-hexane (Merck, Germany), chloroform (Merck, Germany), ethanol 95% (Merck, Germany), Whatman 42 filter paper (Merck, Germany), GF254 silica gel (Merck, Germany), G.60 silica gel (Merck, Germany), sea sand, $CeSO_4$ (Sigma-Aldrich), disk paper, media of Nutrient Agar (NA) (Sigma-Aldrich), media of Sabaroud Dextrose Agar (SDA) (Sigma-Aldrich), antibiotics (chloramphenicol) (Sigma-Aldrich), antifungal fluconazole (Sigma-Aldrich), microbial culture of *E. coli* ATCC 35218, *S. aureus* ATCC 25923, *S. typhi* ATCC 13311, *K. pneumoniae* ATCC 70603, *B. cereus* ATCC 14579, and *C. albicans*.

Extraction and purification

The thallus of *U. longissima* were collected, washed, and dried at ambient temperature. Then, the sample was pulverized to obtain the sample powder. 700 grams *U. longissima* macerated with 5.5 L acetone solvent for 3 × 48 hours. Subsequently, the acetone extract was evaporated by using rotary evaporator vacuum to obtain crude extract. The crude extract was eluted by using TLC to determine the solvent composition to get the best separation. The crude extract was separated by GCC in stationary phase of silica gel 60.G that eluted by gradiently with *n*-hexane and ethyl acetate solvents. The pure compound was carried out using TLC with three solvents. Samples were taken slightly and dissolved in n-hexane and TLC tested with various eluent comparisons. TLC results were observed under UV light and sprayed with $CeSO_4$ stain removal agents. If the stain on the TLC plate remains single, then the compound can be said to be pure and is called an isolate. The purity test was repeated by bottling the isolate and acid compounds which had previously been isolated (Maulidiyah et al., 2016; 2019) as companion side by side on the TLC plate[21,22].

Test of antimicrobial activities test

The antimicrobial test was used agar diffusion with disc paper, which inoculated 1.0 mL of each bacterial or fungal suspension into 15.0 mL Nutrient Agar (NA) media (for bacteria) and Sabaroud Dexrose Agar (SDA) media (for fungus) have been melted in a sterile petri dish let to be solid. The disc paper that was dropped by each of the test material was placed on the media surface and left to stand for 30 minutes at the ambient temperature before being put into an incubator at the temperature of 37°C. The acetone extract and isolate compound (usnic acid constituent) were prepared in several concentrations: 0.1; 0.5; 1.0; and 10.0 mg/mL. The negative control of chloroform and positive control 1.0 mg/mL chloramphenicol antibiotic for bacteria and 1.0 mg/mL fluconazole for fungus. The result of antimicrobial test based on the measurement of diameter inhibitory area growth around the disc paper.

RESULTS AND DISCUSSION

Isolation of Secondary Metabolite Compounds

Lichen *U. longissima* was cleaned and dried at ambient temperature, then crushed using a blender to obtain the lichen powder. The extraction was done using maceration technique by acetone solvent. The 700 g lichen powder was soaked in 5.5 L acetone solvent for 3 × 48 hours so that yielding of 13.5 g acetone extract. Subsequently, the acetone extract was partitioned by n-hexane solvent in the separating funnel three times and concentrated using a rotary evaporator.

(a) (b) (c)

Figure 1: Sample preparation, (a) Tallus *U.longissima*, (b) Gravity Column Chromatography, and (c) maceration technique

The separation of isolate compound (usnic acid constituent) was contained in lichen extract by using GCC and TLC. TLC test was performed to determine eluent which can be used as reference on GCC. Establishment of the eluent system using n-hexane and ethyl acetate solvent in various ratios to increase the polarity as well as compound will be separated according to polarity. The chromatogram results show different spots for each eluent ratios. The eluent system produced good separation with comparison of n-hexane: ethyl acetate (8:2) eluents. It is becoming a reference to the next fractionation stage using GCC[23]. The GCC was performed to obtain the yielded 58 fractions, and each fraction tested by TLC using n-hexane and ethyl acetate in the ratio of 8:2. Based on the stain pattern formed on the chromatogram, fractions have a stain pattern and the same Rf value are combined, assuming the fractions contain the same compound. The combined fractions that form the yellow crystals are further purified by recrystallization. Recrystallization is carried out by adding n-hexane solvent which can dissolve impurities. Furthermore, TLC test with some eluent system to purity test.

From the TLC test results with some eluent systems, the resulting chromatogram showed a single stain, so it can be said that the compound isolate has been pure. To confirm the type of compound isolate estimated by usnic acid as it exhibits the same characteristics as usnic acid, another TLC test was performed using an usnic acid compound previously isolated by Maulidyah

et al. (2019) as a compound[22]. The TLC results show that the isolate compound has a single spot and the Rf value is the same as the isolate compound so it can be ascertained that the compound successfully isolated is usnic acid[24].

Antimicrobial Activity Test of Acetone Extract and Isolate Compound

Antimicrobial activity test was conducted from acetone extract and usnic acid as isolate compound by using disc diffusion method. The inhibition zone results have obtained by measuring the clear zone formed around the disc paper. The results of the inhibitory test of acetone extract are presented in Table 1.

Table 1: Inhibition zone of acetone extract and usnic acid against bacteria and fungus

Bacteria/Fungus		Concentration of Extract/Isolate (mg/mL)	Inhibition Zone of Acetone Extract (mm) (Mean±SD)	Inhibition Zone of Isolate (mm) (Mean±SD)
Gram-negative	*E. coli*	10.0	11 ± 2.6 (a)	24.5 ± 5.8 (a)
		1.0	9.7 ± 2.2 (a)	18.4 ± 0.8 (b)
		0.5	8.2 ± 1.0 (a)	17.6 ± 1.2 (b)
		0.1	8.0 ± 1.0 (a)	13.8 ± 2.4 (c)
		Average	9.22	18.57
		Control (+)	23.2 ± 1.1 (b)	23.2 ± 1.1(a)
		Control (-)	0.0	0.0
	S. typhi	10.0	9.0 ± 1.0 (a)	28.5 ± 1.3 (a)
		1.0	6.7 ± 1.1 (a)	20.6 ± 3.1 (b)
		0.5	5.3 ± 2.3 (a)	13.8 ± 4.4 (b)
		0.1	4.3 ± 1.1 (b)	9.6 ± 6.3 (b)
		Average	6.32	18.12
		Control (+)	23.8 ± 0.3 (c)	23.8 ± 0.3 (b)
		Control (-)	0.0	0.0
	K. pneumoniae	10.0	18.3 ± 1.5 (a)	15.8 ± 0.7 (a)
		1.0	16.4 ± 0.3 (a)	12.9 ± 3.8 (a)
		0.5	14.0 ± 1.8 (b)	10.9 ± 7.1 (a)
		0.1	12.6 ± 2.0 (b)	5.5 ± 1.8 (b)
			15.32	11.27
		Control (+)	17.5 ± 0.8 (a)	19.8 ± 3.4 (c)
		Control (-)	0.0	0.0
Gram-positive	*S. aureus*	10.0	16.4 ± 0.6 (a)	12.3 ± 0.5 (a)
		1.0	13.8 ± 0.2 (b)	10.8 ± 0.7 (b)
		0.5	10.3 ± 2.0 (c)	9.0 ± 0.5 (c)
		0.1	8.5 ± 1.4 (c)	4.8 ± 0.2 (d)
		Average	10.65	8.65
		Control (+)	10.0 ± 0.4 (c)	10.0 ± 0.4 (b)
		Control (-)	0.0	0.0
	B. cereus	10.0	5.8 ± 0.7 (a)	35.3 ± 7.6 (a)
		1.0	6.0 ± 0.5 (a)	24.6 ± 1.6 (b)
		0.5	4.9 ± 0.1 (b)	20.6 ± 3.2 (b)
		0.1	3.0 ± 0.1 (c)	14.1 ± 5.7 (b)
		Average	4.92	23.65
		Control (+)	22.3 ± 1.0 (d)	22.3 ± 1.0 (b)
		Control (-)	0.0	0.0
Fungus	*C. albicans*	10.0	12.4 ± 3.3 (a)	9.9 ± 0.6 (a)
		1.0	6.8 ± 0.5 (b)	7.7 ± 1.0 (b)
		0.5	5.7 ± 0.8 (b)	6.9 ± 0.8 (b)
		0.1	2.4 ± 0.5 (c)	1.5 ± 0.4 (c)
		Average	6.82	6.50
		Control (+)	3.0 ± 0.8 (c)	3.0 ± 0.8 (d)
		Control (-)	0.0	0.0
	A. flavus	All of concentration	0.0	0.0
		Average	0.0	0.0
		Control (+)	22.7 ± 1	22.7 ± 1
		Control (-)	0	0

Control (+) bacteria: chloramphenicol 1 mg/mL, fungi: fluconazole 1 mg/mL, *A. flavus*: policresulen 26%. Control (-): chloroform
The numbers followed by the same letter, did not differ significantly with the Mann-Whitney U test at $p < 0.05$

The inhibitory test results that acetone extract in 10.0 mg/mL growth of five bacteria types. It is showed the inhibit zone was highest to *S. typhi* growth of 18.3 mm, *K. pneumoniae* (16.4 mm), *E. coli* (11 mm), *S. aureus* (9 mm), and *B. cereus* (5.8 mm) while the inhibitory test against *C. albicans* fungus has produced inhibition zone of 12.4 mm. The inhibitory zone produced from usnic acid compound against various bacterial and fungal species, was the largest inhibition zone to *B. cereus* (35.3 mm), *S. aureus* (28.5 mm), *E. coli* (24.5 mm), *S. typhi* (15.8 mm), *K. pneumoniae* (12.3 mm), and the smallest of *C. albicans* (9.9 mm).

Based on the antibacterial activity test was conducted on the five types of bacteria, both acetone extract and usnic acid have potential to inhibit bacteria in the broad spectrum of both Gram-

positive and Gram-negative bacteria. In this study, acetone extract and usnic acid compound did not inhibition of *A. flavus* growth. Several types of pathogenic bacteria tested in this study, were classified into 2 groups of bacteria related to nature of the pathogenicity Gram-positive and Gram-negative bacteria. The Gram-positive bacteria tested were *S. aureus* and *B. cereus*, whereas Gram-negative bacteria were *S. typhi, K. pneumoniae,* and *E. coli*.

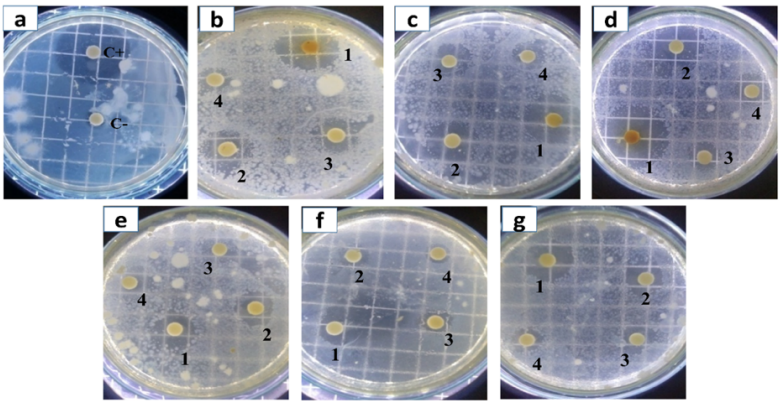

Figure 2: (a) Inhibition zone of positive and negative controls against *S. typhi,* (b)(c)(d) Inhibition zone of acetone extract against *S. typhi* (triplo) ; (e)(f)(g) Inhibition zone of usnic acid against *S. typhi* (triplo)
The concentration of extract/isolate: 1 = 10 mg/mL = 1 mg/mL; 3 = 0.5 mg/mL; 4 = 0.1 mg/mL

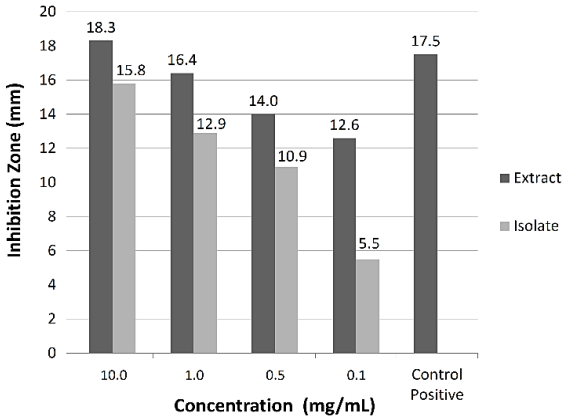

Figure 3. Inhibition zone compared of *U. logissima* extract and usnic acid against *S. typhi*

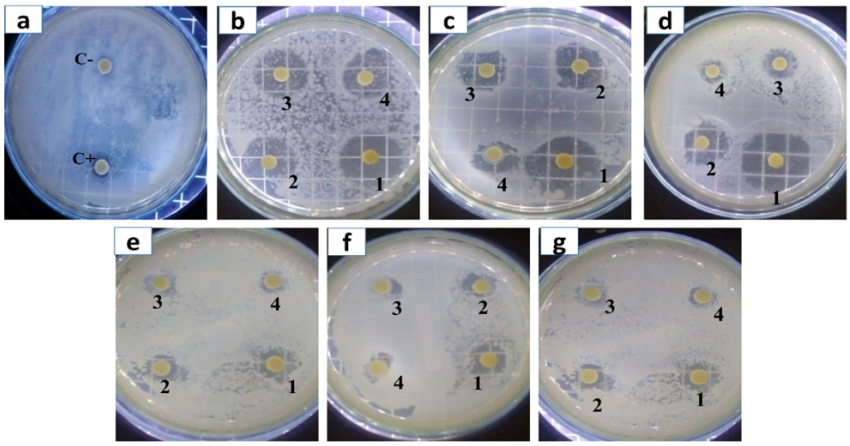

Figure 4: (a) Inhibition zone of positive and negative controls against *K. pneumoniae,* (b)(c)(d) Inhibition zone of acetone extract against *K. pneumoniae* (triplo) ; (e)(f)(g) Inhibition zone of usnic acid against *K. pneumoniae* (triplo)
The concentration of extract/isolate: 1 = 10 mg/mL = 1 mg/mL; 3 = 0.5 mg/mL; 4 = 0.1 mg/mL

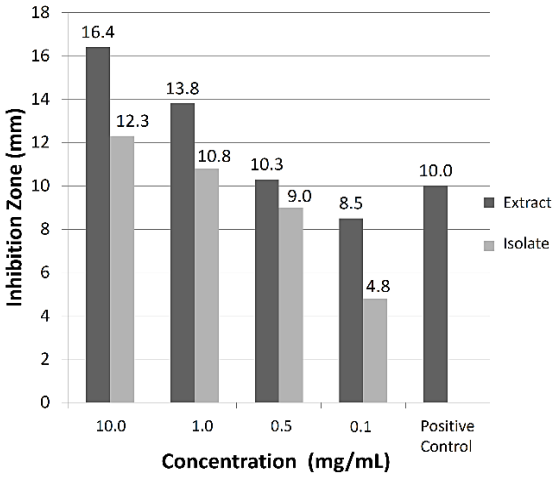

Figure 5: Inhibition zone compared of acetone extract and usnic acid against *K. pneumoniae*

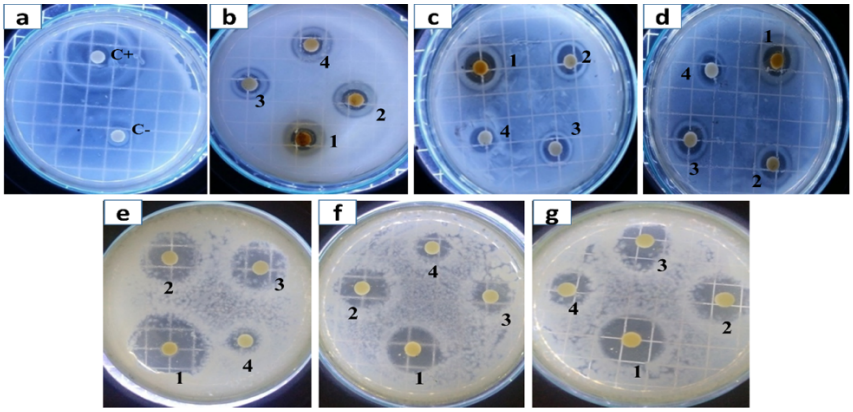

Figure 6: (a) Inhibition zone of positive and negative controls against *E. coli,* (b)(c)(d) Inhibition zone of acetone extract against *E. coli* (triplo) ; (e)(f)(g) Inhibition zone of usnic acid against *E. coli* (triplo)
The concentration of extract/isolate: 1 = 10 mg/mL; 2 = 1 mg/mL; 3 = 0.5 mg/mL; 4 = 0.1 mg/mL

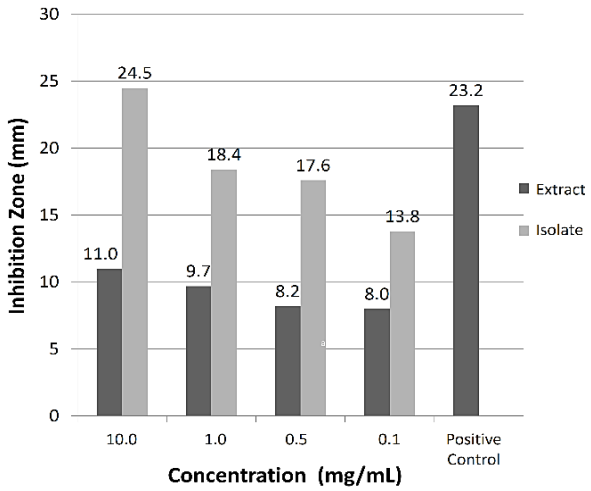

Figure 7: Inhibition zone compared of acetone extract and usnic acid against *E.coli*

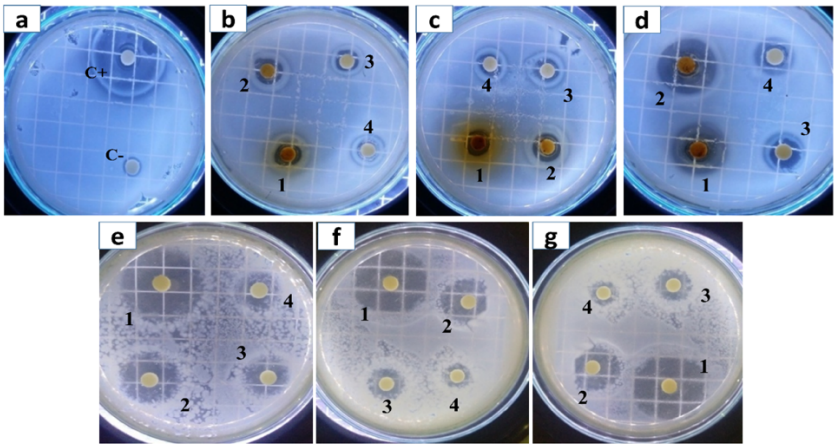

Figure 8: (a) Inhibition zone of positive and negative controls against *S. aureus*, (b)(c)(d) Inhibition zone of acetone extract against *S. aureus* (triplo) ; (e)(f)(g) Inhibition zone of usnic acid against *S. aureus* (triplo)
The concentration of extract/isolate: 1 = 10 mg/mL = 1 mg/mL; 3 = 0.5 mg/mL; 4 = 0.1 mg/mL

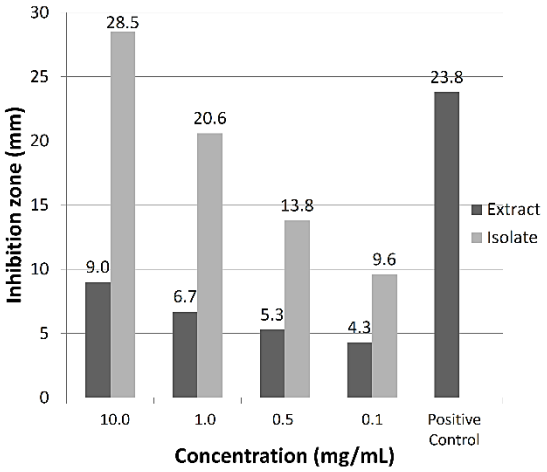

Figure 9: Inhibition zone compared of *U. logissima* extract and usnic acid against *S. aureus*

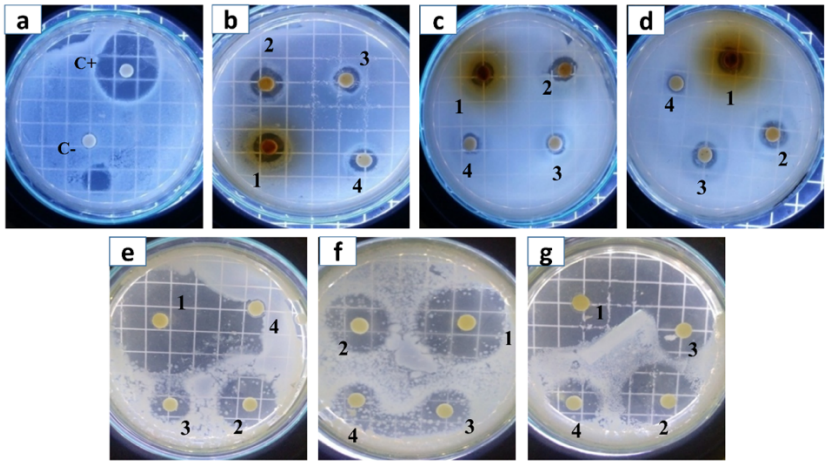

Figure 10: (a) Inhibition zone of positive and negative controls against *B. cereus*, (b)(c)(d) Inhibition zone of acetone extract against *B. cereus* (triplo) ; (e)(f)(g) Inhibition zone of usnic acid against *B. cereus* (triplo)
The concentration of extract/isolate: 1 = 10 mg/mL = 1 mg/mL; 3 = 0.5 mg/mL; 4 = 0.1 mg/mL

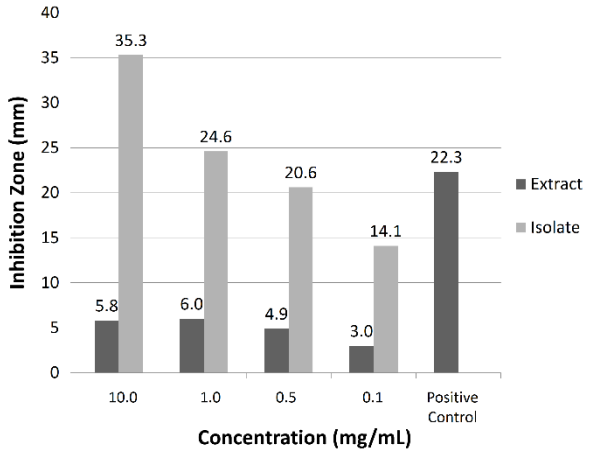

Figure 11: Inhibition zone compared of *U. logissima* extract and usnic acid against *B. cereus*

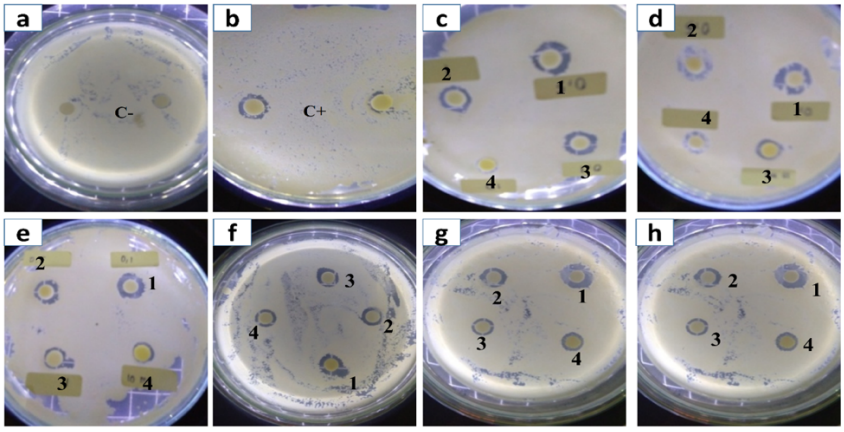

Figure 12: (a) Inhibition zone of negative control, (b) Inhibition zone of positive control (c)(d)(e) Inhibition zone of acetone extract against *C. albicans* (triplo) ; (f)(g)(h) Inhibition zone of usnic acid against *C. albicans* (triplo)
The concentration of extract/isolate: 1 = 10 mg/mL = 1 mg/mL; 3 = 0.5 mg/mL; 4 = 0.1 mg/mL

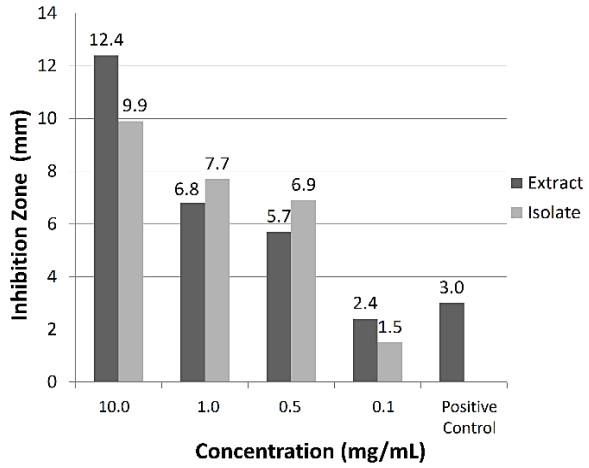

Figure 13: Inhibition zone compared of *U. logissima* extract and usnic acid against *C. albicans*

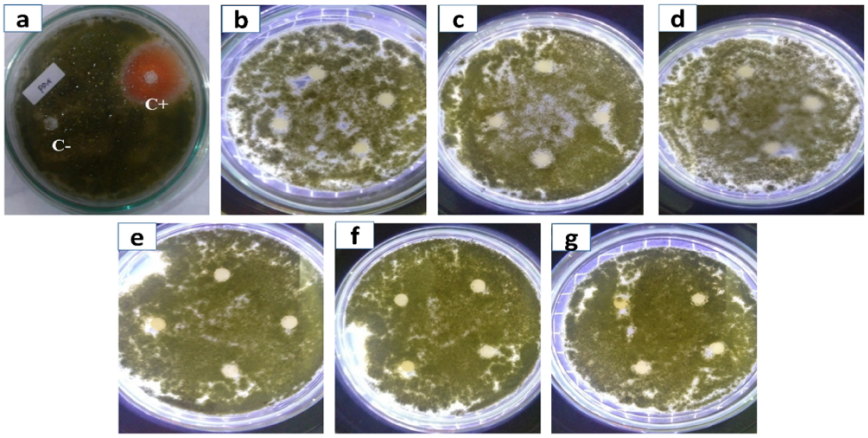

Figure 14: (a) Inhibition zone of positive and negative controls against *A. flavus*, (b)(c)(d) Inhibition zone of acetone extract against *A. flavus* (triplo) ; (e)(f)(g) Inhibition zone of usnic acid against *A. flavus* (triplo)
The concentration of extract/isolate: 1 = 10 mg/mL = 1 mg/mL; 3 = 0.5 mg/mL; 4 = 0.1 mg/mL

Inhibition of gram-negative bacteria

Inhibition of *S. typhi* bacteria (Figure 2) was performed by triplo technique to obtain the average inhibition zone of bacterial activity. Fig. 2b,c,d shows the inhibition zone produced from acetone extract in concentrations of 10.0 ; 1.0 ; 0.5 ; and 0.1 mg/mL. The same result was produced by the usnic acid that the inhibitory zone forms in each concentration. However, the inhibitory zone comparison indicates that acetone extract has good activity compared to usnic acid. When compared with positive control, they were significantly smaller as lower antibiotic potency than chloramphenicol. The difference of the inhibitory zone produced by acetone extract and usnic acid can be seen in Figure 2.

Figure 3 shows that the acetone extract was produced a relatively larger zone compared to the inhibition zone produced by the usnic acid. The data exhibited show significant differences with Mann Whitney-U Test at $p < 0.05$.

In inhibition of *K. pneumonia* (Figure 4), the inhibitory zone of extract was highest at concentrations of 10.0 mg/mL showed that significant differences in concentrations of 1.0 ; 0.5 ; and 0.1 mg/mL, respectively. At concentrations of 0.5 and 0.1 mg/mL, the resulting inhibit zone did not differ significantly, and both did not differ significantly compared with positive control, suggesting the same antibiotic potential as chloramphenicol. However, the inhibitory zone of the extract of 10.0 and 1 mg/mL were significantly greater than the positive control. While the inhibitory zone of the usnic acid to *K. pneumonae* from each of the concentrations showed significant differences from the 10.0 mg/mL concentration resulting the greatest inhibit zone and subsequent lower concentrations. The 1.0 mg/mL usnic acid gave an inhibitory zone did not differ significantly with positive control against *K. pneumoniae*. Comparison of inhibit zones of acetone extract and usnic acid to *K. pneumoniae* can be seen in Figure 5.

Figure 5 exhibits the same phenomenon as *S. typhi*. The acetone extract provide a larger inhibitory zone than the isolate. At the same concentration as chloramphenicol i.e 1.0 mg/mL, extract gives a larger inhibitory zone. Gram-negative bacteria have strong pathogenic capabilities due to double walls in cell membranes, endotoxins and drug resistance mechanisms. Gram-negative bacteria are the causative agent for various respiratory infections, generative infectious diseases, gastrointestinal diseases, and also the main cause of nosocomial (health-related) infections. In addition, these bacteria develop and get multidrug resistance through various gene transfer mechanisms. Unique external membranes that provide antibiotic resistance as well as drug resistance cause difficulties in treating Gram-negative bacterial infections. However, certain antibiotics including streptomycin, chloramphenicol, and certain cephalosporins offer effective treatment options. In many cases, the combination of antibiotics used to treat the infection.

Related with the results of this study, acetone extract is more effective in inhibiting Gram-negative bacteria (*S. typhi* and *K. pneumoniae*) because in the extract contains various types of secondary metabolite compounds that may have a combination of antibiotics that are more effective in inhibiting the synthesis of microbial cell walls, damage the integrity (permeability) of microbial cell walls, inhibit the synthesis of microbial cell proteins, inhibit enzyme activity (enzyme inactivation), inhibit nucleic acid synthesis, and damage nucleic acid cell microbes. Maulidiyah et al. (2016) reported to have isolated other than the usherate acid from lichen *U. longissima*, ie compound (5E, 6E) 5-ethylidene-7-formyl-6,7-dihydroxy methyl hept-6-enoate; which can inhibit *E. coli* and *S. typhi* but could not inhibit *S. aureus*; while chloroform extract of *U. longissima* can inhibit *E. coli, S. typhi,* and *S. aureus*[4].

Exceptions to *E. coli* (Figure 6) which is Gram-negative bacteria, in this study usnic acid compound provide zones inhibition is greater than the extract, can be seen in Figure 7. The inhibitory zone of acetone extract to *E. coli*, at various concentrations (10.0 mg/mL; 1.0 mg/mL, 0.5 mg/mL, 0.1 mg/mL) showed no significant difference, and when compared with positive control (chloramphenicol 1.0 mg/mL) the value is lower. Whereas from usnic acid at concentration 10 mg/mL showed significant difference of drag zone with concentration of 1.0 mg/mL, but at concentration 1.0 and 0.5 mg/mL no significant difference, and zones tend to be smaller significantly at 0.1 mg/mL concentration. Compared with chloramphenicol 1 mg/mL as positive control, 10.0 mg/mL usnic acid inhibition zone was not significantly different; shows the same potential of antibiotics at greater concentrations.

Figure 7 shows significant differences between inhibit zones of acetone extract and usnic acid in all variations of concentration against *E. coli*, in which usnic acid provides a relatively larger inhibit zone. But to be able to provide a zone of inhibition that is

not significantly different from chloramphenicol 1.0 mg/mL, a greater usnic acid concentration is required 10.0 mg/mL.

Inhibition of gram-positive bacteria

The inhibitory zone formed against *S. aureus* of 10.0 mg/mL usnic acid was significantly greater than the concentration of 1.0 mg/mL. At the lower concentration variations were 1.0 mg/mL; 0.5 mg/mL; and 0.1 mg/mL inhibit zone were not significantly different and each of these concentrations did not differ significantly when compared with positive control. The difference of inhibit zone produced by extract and isolate of *U. longissima* to *S. aureus* can be seen in Figure 8 and 9.

Figure 9 depicts that against *S. aureus*, the usnic acid provides a larger inhibitory zone than the acetone extract at all concentrations. At a concentration of 1.0 mg/mL, the usnic acid provides no significant inhibitory zone; showed antibiotic potency equal to chloramphenicol 1.0 mg/mL, whereas at 10.0 mg/mL concentration the inhibition zone was relatively larger.

The inhibitory zone of acetone extract to *B. cereus* (Figure 10) at various concentrations shows significantly smaller differences than positive control. At 10.0 and 1.0 mg/mL concentrations the resulting inhibit zone did not differ significantly and began to differ at 0.5 mg/mL concentrations. At 10 mg/mL usnic acid giving the largest inhibit zone, significantly different with the lower concentration of 1 mg/mL; 0.5 mg/mL, and 0.1 mg/mL. Where in the above three concentration variations, the resulting inhibit zone did not differ significantly and also did not differ significantly with the positive control zone inhibition; shows the potential of antibiotics equal to chloramphenicol at concentration 1.0 mg/mL; 0.5 mg/mL, and 1.0 mg/mL. The difference of inhibit zone produced by extract and isolate to *B. cereus* can be seen in Figure 11.

Based on Figure 11 shows that the usnic acid provides a relatively large extent of *B. cereus* inhibition zone compared to acetone extract. At 1.0 and 0.5 mg/mL concentrations the usnic acid gives no inhibitory zone significantly of 1.0 mg/L chloramphenicol. Jawetz et al. mentioned differences in sensitivity of Gram-positive and Gram-negative bacteria to antibacterial agents possibly due to differences in cell wall structures, such as number of peptidoglycans, lipids, crosslinking and enzyme activity, which determines penetration, binding and antimicrobial activity[25]. *S. aureus* has cell wall structures containing polysaccharides and antigenic proteins and has a low lipid content (1-4%), whereas *E. coli*, including Gram-negative bacteria, has cell walls with lipid content high (11-22%) and multilayer cell wall structures i.e. lipoproteins, outer membranes of phospholipid and lipopolysaccharides. The outer membranes of the phospolipid can reduce the entry of antibacterial substances into cells.

It was reported that usnic acid inhibits the growth of a number of Gram-positive bacteria, while it has no effect on Gram-negative species[24]. Research Dorszyńska et al. reported that usnic acid can inhibit the synthesis of RNA and DNA rapidly and strongly in *S. aureus* and *Bacillus subtilis* representing Gram-positive bacteria, while not in *E. coli* which is a Gram-negative bacteria[26]. Whereas in other Gram-negative bacteria *Vibrio harveyi* happened a little inhibition of RNA synthesis. Indirect inhibition of *S. aureus* and *B. subtilis* is done by delaying protein synthesis (possibly through transcriptional disorders) in the process of translation by usnic acid. Interestingly, in *S. aureus* and *B. subtilis* the synthesis of DNA stopped rapidly, suggesting a disturbance in the process of elongation of DNA replication by usnic acid, a mechanism proposed by Dorszyńska et al. as the mechanism of inhibition of usnic acid directly against *S. aureus* and *B. subtilis*[26].

In this study, inhibitory zone exhibited by usnic acid against *B. cereus* and *S. aureus* were Gram-positive bacteria. The usnic acid compounds which are single active compounds more effectively inhibit the growth of Gram-positive bacterial cells than acetone extract containing more than one active compound.

Inhibition of acetone extract and isolate compound against fungus

Inhibition of *C. albicans*, inhibitory zone of 10.0 mg/mL extract showed significantly greater difference than positive control (fluconazole 1.0 mg/mL). At 10.0 and 1.0 mg/mL concentrations showed significant differences, while at both lower concentrations (1.0 and 0.5 mg/mL) no significant difference was observed. At the lowest concentration of 0.1 mg/mL, the inhibit zone differed significantly lower with the above concentrations, but when compared with the positive control did not differ significantly.

Figure 12 illustrates that the usnic acid of 10.0; 1.0, and 0.5 mg/mL indicates a significantly higher inhibition zone than the 1.0 mg/mL fluconazole inhibition zone as a positive control. At 10.0 and 1.0 mg/mL concentrations showed significant differences, while at both lower concentrations (1.0 and 0.5 mg/mL) no significant difference, but significantly different with 0.1 mg/mL concentration. Comparison of inhibition zone of extract and isolates *U. longissima* against *C. albicans* can be seen in Figure 13.

Figure 13 shows that the usnic acid produced inhibitory zones did not differ significantly from extract on all concentration variations and when compared with the positive control (fluconazole 1.0 mg/mL), the usnic acid at the lowest concentration of 0.1 mg/mL the inhibitor did not differ significantly and at concentration of 0.5; 1.0, and 10 mg/mL resulted in a relatively larger inhibition zone.

Other types of fungi tested in this study were *A. flavus* (Figure 14), a kind of mold that is multicellular fungi group structured like filaments or threads. Both the usnic acid compound and the acetone extract at all concentrations, did not show any inhibition of the *A. flavus.*

CONCLUSION

Antimicrobial bioactivity test using disc diffusion method indicates that both acetone extract and usnic acid have potential to inhibit Gram-positive bacteria (*B. cereus* and *S. aureus*) and Gram-negative bacteria (E. *coli, S. typhi,* and *K pneumoniae*) and *C. albicans* but cannot inhibit the growth of *A. flavus*. The usnic acid produced a larger inhibitory zone than the extract against the Gram-positive bacteria (*S. aureus* and *B. cereus*), while the acetone extract produced a larger inhibitory zone against Gram-negative bacteria (*S. typhi* and *K. pneumoniae*). It is therefore advisable to examine more in depth about the antibiotic potential of extract as well as usnic acid compounds as medicines for various infectious diseases, more specifically the potential study of antibiotic combinations of lichen extracts to fight Gram-negative pathogenic bacteria and potential antibiotic of usnic acid against Gram-positive bacteria.

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