Evaluation of Antimicrobial Activity and Phytochemical Screening of Chloroform Extract of Usnea sp.

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Abstract; The medicinal plants represent an enormous reservoir of potential phytochemical compounds that could be useful as an alternative to develop of drugs. In the present study, chloroform extract of usnea sp. were analyzed for phytochemical constituents and tested for antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* by agar diffusion method with MHA media (Muller Hinton Agar) using tetracycline as a positive control and DMSO as a negative control. In the chloroform extract of usnea sp. was detected the presence of saponin, polifenol, flavonoid and tannins. Based on the antibacterial activity test, chloroform extract was the greatest potential as an antibacterial, with an average of 20.600 mm diameter inhibitory effect on *Staphylococcus aureus* and 19.640 mm on *Escherichia coli* at a concentration of 5% (m/v).
Keywords: phytochemistry, antibacterial, Usnea sp.

INTRODUCTION

The chemical natural product research of Indonesian tropical plants should be encouraged because of there are potential chemical compounds. Many of new secondary metabolites chemical compounds that are bioactive useful in the fields of health, agriculture and industry has been is found. Therefore, tropical plants can be seen as a source of new molecular model diverse.[1]

The potential to the found of various types of chemical natural product useful to humans is still have a great opportunity. There are so many types of tropical plants in Indonesia which as drugs so has the ability to metabolize and produce chemical compounds have a bioactivity but have not been studied. The Exploration of secondary metabolites as bioactive compounds in plants is not limited to leaf, but overall include stems, fruits and roots including epiphytic plants attached to their host plants such as lichen species.[2] Lichens u*snea* sp. used for the treatment of asthma, bronchitis, stomach pain, allergies, diarrhea, colds, cramps, and fever before modern medicine known in America. [3]

Lichens are a composite live attached to plant stems so able to adopt metabolize metabolism of host plants. This symbiotic potentially generate sources of compounds (metabolites) for medicines derived from nature, or a metabolite provider for the pharmaceutical industry. Lichens produce metabolites proven useful in the medical community.[4]

Most metabolites produced by lichens are structurally and functionally similar to broad-spectrum antibiotics while few are associated respectively to antiseptic similarities. Usnic acid is the most commonly studied metabolite produced by lichens and has been associated with the suppression of tuberculosis. It has also proven bactericidal against *Escherichia coli* and *Staphylococcus aureus* and is considered an antimicrobial agent. It is still unclear if the antimicrobial processes derived from lichens are strictly due to their metabolites or their symbiotic relationship with the fungi.[5]

The tropical rain forest in Lompobattang mountain areas there are diversity of plant species, including species of lichen discovered utilized by local people as a source of medicines. One example is *Usnea* sp. used as a cure leprosy, cough and depilatory warts.[2] Seeing the efficacy of the lichen species are thought to contain a variety of chemical compounds that are useful for health. Therefore, in the research aims to evaluation of antimicrobial activity and phytochemical screening of lichens *usnea* sp. on Lompobattang mountains.

MATERIALS AND METHODS

Collection of plant materials

The Lichens usnea sp. were collected from Sinjai Borong district South Sulawesi Indonesia and specimens has been identified from LIPI Bogor Indonesia.

Preparation of plant extracts

Collected lichens usnea sp. were cleaned, shade dried and ground as powder form. Then the samples were extracted by using chloroform in macerator apparatus and concentrated by using rotary evaporator.

Phytochemical Test

Alkaloid test; ten milliliters of the extract was acidified by adding 1.5% v/v of HCL and a few drops of Wagner s reagent. The yellow formation or brown precipitate confirmed the presence of alkaloid. Wagner s reagent: Iodide, 1.2 g and 2.0 g of potassium iodide were dissolved in 5 mL of sulphuric acid and the solution was diluted to 100ml.

The extract (1.2 mL) was taken in a test tube to which 0.2 mL of dilute hydrochloric acid and 0.1 mL of Mayer s reagent was added. Formation of yellowish buff coloured precipitate indicate presence of alkaloids. Mayer s reagent: The mercuric chloride (1.36) was dissolved in 60 mL of distilled water and 5g of potassium iodide in 10 mL of water. The two solutions were mixed and diluted to 100 mL with distilled water.

Saponins test; the first test, extract was diluted with 20 mL of distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of 1cm layer of foam shows the presence of saponins. The second test, extract 1ml was treated with 1% lead acetate solution. Formation of white precipitate indicates the presence of saponins.

Polyphenols test; Samples extracted heat water is then cooled. After it was added 5 drops of 10% NaCl and filtered. The filtrate divided into 3 sections A, B, and C. The filtrate A was used as a blank, to the filtrate was added 3 drops of reagent B FeCl3, and into the filtrate C plus salt gelatin. The color change indicates the presence polyphenols.

Flavonoids test; in a test tube containing 0.5 mL of extract 5-10 drops of dilute HCl and small piece of zinc chloride or magnesium were added and the solution was boiled for few minutes. In the presence of flavonoids, reddish pink colour was produced.

Triterpenoids test; The extract, 10 mg was dissolved in 1 ml of chloroform; 1ml of acetic anhydride was added following the addition of 2 mL of Conc.H2SO4. Formation of reddish violet colour indicates the presence of triterpenoids.

Test for Tannins; in a test tube containing about 5 ml of the extract and a few drops of 1% lead acetate was added. A yellow precipitate was formed, indicates the presence of tannins.

Antibactrial test

Bacterial were maintained at 4°C on nutrient agar slants. Amongst two bacterial investigated Gram positive bacteria were *Staphylococcus aureus* while gram negative bacteria were *Escherichia coli.* The antimicrobial assay was performed by agar well diffusion method; Muller-Hinton agar and nutrient agar were poured on to sterile petri plates. When the media solidified, 0.1mL of inoculum with 0.5 OD was poured over feeder layer and spread evenly with a sterile spreader. Wells of 6 mm size were made into the agar set plates containing the bacterial culture and the lower portion was sealed with a little molten agar media. Concentration (5%, 3% and 2%) of the crude extract was allowed to diffuse for about 2 h. The plates were incubated at wells 37 ^{o}C for 18 – 24 h. The zone of inhibition was measured and expressed in millimeter. Inhibition zone categorized based on the strong and weak. [6]

RESULT AND DISCUSSION

Chloroform extract of lichen usnea sp. as a medicinal plant was subjected to preliminary phytochemical screening of various constituents such as flavonoids, alkaloids, tannins, saponins, triterpenoids, steroid and polyphenols. The results of screening shown in Table 1.

Table 1. The Phytochemical analysis of chloroform extract of *Usnea sp.*

Phytochemical test	Reaction identification results	
Alcaloid	no changes	(-)
Flavonoid	gren	(+)
Saponin	formed foam	(+)
Tannins	blackish green	(+)
Steroid	no changes	(-)
polyphenols	yellowish green	(+)

- = Absence, + = Presence.

Antibacterial activity extract of usnea sp. against the investigated bacterial strains (*S. Aureus* and *E. coli*) shown in Table 2 and 3.

Tabel 2. Antibacterial activity chloroform extract of *Usnea sp. Against S. aureus*

Sample	Average of inhibition zone (diameter in mm) from various of extract concentration usnea sp.			
	5%	4%	3%	2%
Chloroform extract	20.60	16.22	15.305	13.35
Tetracycline 1%			25.41	
DMSO			0.00	

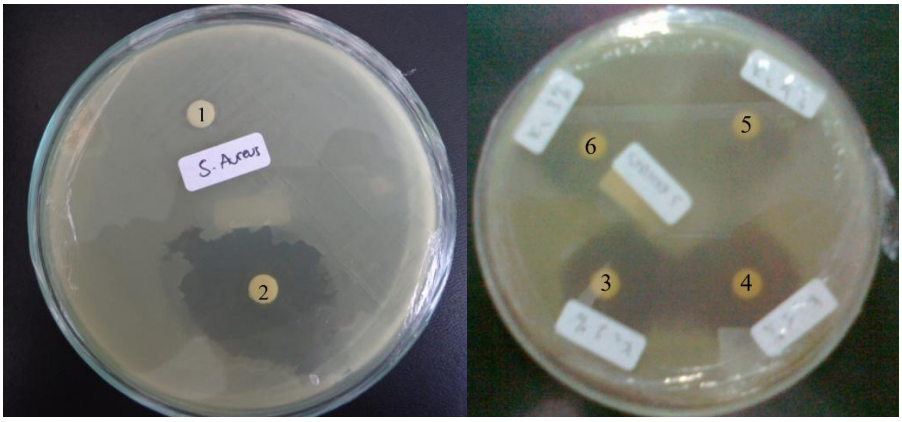
Tabel 3. Antibacterial activity chloroform extract of *Usnea sp. Against E. coli*

Sample	Average of inhibition zone (diameter in mm) from various of extract concentration usnea sp.			
	5%	4%	3%	2%
Chloroform extract	19.64	17.55	12.01	8.61
Tetracycline 1%			19.56	
DMSO			0.00	

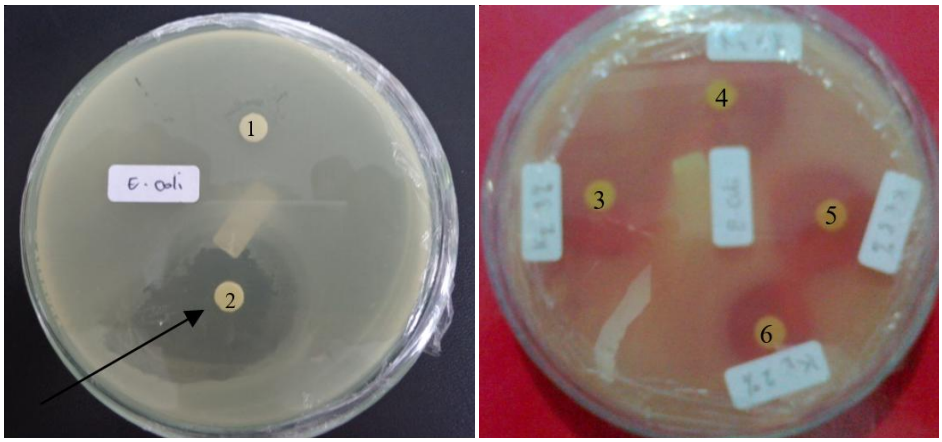
Available literature review showed that medicinal plants are the backbone of traditional medicine and antibacterial activity of plant extracts for the presence of chemicals in the extracts of which are classified as antimicrobial compounds. Compounds such as acid usnat, contortin, viresik acid, xanthones and avertrhin on lichen usnea responsible for the antibacterial activity.[7]

Chloroform used to extract samples for screening antibacterial activity usnea sp. show evidence of chloroform extracting chemical components from a sample as antibacterial. Figure 1 and Table 2 and 3, shows broad inhibition zone of chloroform extract usnea sp. containing tannins, flavonoids, saponins and polyphenols and showed inhibition zone classified into a very strong inhibition zone.[6]

Also describe the diameter of the inhibition of chloroform extract usnea sp. against the bacteria *S. aureus* and *E. coli* increased with increasing concentrations of each extract. This proves that the higher the concentration of the extract is given the greater the diameter of inhibition formed around the paper disk.



(A)



(B)

Figure 1. Antibacterial activity of chloroform extract of usnea sp. against (A) *S. aureus* and (B) *E. Coli;* 1(DMSO); 2(tetracycline); 3, 4, 5, 6(concentration of extract usnea sp respectively; 5%, 4%, 3% and2%).

The tannins compound can form irreversible complexes with proteins that so inhibited the synthesis of cell proteins. These results have been tested on the extract containing tannin. Tannins an important role in plant cells to the process stabilization and as antioxidant.[8]

Then phenolic flavonoid which is a structure containing a carbonyl group also supports the antibacterial properties of the extract. The mechanism of flavonoids against antimicrobial properties shown on the formation of complex flavonoids with extra cellular proteins that can dissolve the cell walls of bacteria that have a high as antibacterial. Another mechanism of flavonoids as antibacterial is formed complexes with extracellular proteins and soluble so can damage the cell membrane of bacteria and is followed by the release of intracellular compounds.[9,10]

The results indicate the presence of saponins from Forth test evidenced by the formation of foam. The form of foam on the Forth test showed glycosides that have the ability to form foam in water that is hydrolyzed to glucose.[11] Mechanism of saponin as antibacterial to pressing the surface with increase the permeability of cell or leakage of intracellular cell.[12]

Saponins have molecules as hydrophilic that can dissolve fat or lipophilic so as to reduce the surface tension of the cells that ultimately to the destruction of the bacteria cell.[13] Saponin compound diffuses through the outer membrane and the cell wall are vulnerable, and then bind the cytoplasmic membrane and disrupt and reduce the stability. This causes the cytoplasm to leak out of the cell resulting in cell death, so called as an antimicrobial agent which disrupt the cytoplasmic membrane is bactericidal.[14]

Activity as antimicrobial uncommon to many extract against both gram positive and gram negative bacteria by the sample plant may be indicative of the presence of broad spectrum of antibiotic compounds. But in this study can be determined exactly one class of compound as antibacterial in the usnea sp. extract. To know for certain classes of compounds are active as antibacterial there should be further examination of each class compounds. Without the classes of compounds identified in research, it is possible that the presence of the other active compounds in the usnea sp. unidentified and potentially as antibacterial.

SUMMARY

Based on result and discussion can be concluded that:

1. Chloroform Extract Usnea sp. has a strong antibacterial activity.
2. Usnea sp. Contains flavonoids, polyphenols, tannins and saponins.
3. The 2% of concentration of chloroform extract still inhibits so as to allow still able to inhibit at concentrations lower.

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