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PHYTOCHEMICAL SCREENING OF LICHENS EXTRACT Usnea sp. ON PINES IN THE BARRIAN HILL FOREST, NORTH SUMATRA

Ashar Hasairin[1], Ahmad Shafwan S Pulungan[2], Adi Hartono[1]
Program Studi Magister Pendidikan Biologi, Universitas Negeri Medan
[2]Program Studi Biologi, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Negeri Medan
* Corresponding author : asharhasairin@unimed.ac.id

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ABSTRACT

Lichen is an organism that has a high level of biodiversity and contains secondary metabolites. This study aims to determine the secondary metabolites of lichen extract Usnea sp. on Pine. Qualitative descriptive research method. Preparation of Usnea sp. extract. carried out by maceration method using 96% ethanol (1:10) for 5 days. The results of the study based on the phytochemical screening of the ethanol extract of lichen Usnea sp. contains alkaloids, flavonoids, saponins and tannins and triterpenoids. The results of the phytochemical screening showed a positive (+) result indicated by a change in color or the formation of a precipitate or the formation of foam after adding reagents to the test extract. Meanwhile, a negative result (-) is indicated by the absence of a change in color or the formation of precipitate or the formation of foam after adding reagents to the test extract.

Introduction

Indonesia has a fairly high biodiversity. The high level of biodiversity makes Indonesia a place of exploration to utilize natural materials that are still pure and not widely known by the public. One of the organisms that has a high level of biodiversity in Indonesia is lichen, reaching ± 17,000 of the total number of lichen species in the world which reaches ± 100,000 (Negi, 2003).

Lichen is a symbiotic mutualism between fungi and algae or cyanobacteria. Fungi provide habitat protection or ecological niches to algae, while algae provide organic compounds or photosynthetic products, and vitamins for fungi. These symbiosis have the ability to combine to form a thallus so that morphologically and physiologically it is a new individual unit (Nash, 2008). Lichens live as epiphytes on trees, on rocks or other substrates and do not require complicated living conditions and are resistant to lack of water. Lichens can be found from the coast to the mountains (Galloway, 1991). Lichens can grow well in very extreme environmental conditions. According to Vashishta (2007) lichens are sensitive to air pollution and are able to absorb toxic materials in the air by

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showing characteristic symptoms for these toxic materials, which is why lichens can be used as bioindicators of air pollution in an environment. In more humid environmental conditions, lichens can live better and thrive, so that the absorption of water, minerals and accumulation of pollutant materials becomes more effective and more abundant.

The Forest Area is a very potential area for the growth habitat of lichens. One type of forest found in North Sumatra is Tahura (Great Forest Park) Tongkoh, Karo District. In these forests, not much research has been conducted on lichens secondary metabolites found in tree stands, especially pine or tusam trees (Pinus merkusii). The lichen's ability to survive in hot, cold, or dry conditions is of course inseparable from the secondary metabolites it produces, so that lichens can protect themselves from the environment, microbes, predators, and competitors (Shrestha and St. Clair, 2013). Lichen is a natural source for discovering new active compounds, both from direct secondary metabolite compounds and chemical modifications of compounds isolated from natural materials (Balaji et al., 2007). Lichen produces secondary metabolites consisting of many classes including amino acid derivatives, pulvinic acid, peptides, sugar alcohols, terpenoids, steroids, carotenoids, aliphatic acids, monocyclic phenols, depsides, dibenzofurans, anthraquinones, xanthones, usnic acids and other compounds. others (Huneck, 1999).

Therefore, it is necessary to conduct research on phytochemical screening of lichen extract Usnea sp. found in pine trees in TAHURA Bukit Barisan, North Sumatra. Determination of phytochemical screening was carried out to determine the class of metabolites in the ethanol extract of lichens in the form of alkaloids, flavonoids, saponins, triterpenoids, and tannins.

Materials and Methods

This research was conducted at the Biology Laboratory of the Faculty of Mathematics and Natural Sciences, Medan State University. Lichnes sampling was carried out at TAHURA Bukit Barisan, North Sumatra. Lichens Usnea sp. which grows on the bark of trees (corticolous) on Pinus merkusii. Descriptive research method. Types of stands of Pinus merkusii by purposive sampling. Determination of species was carried out by identifying the characteristics and morphology of lichens Usnea sp. The tools used in this study were analytical balances, blenders, sieves, a set of glassware, maceration bottles, stir bars, rotary vacuum evaporators, test tubes, dropper pipettes. Phytochemical screening was carried out to determine secondary metabolites from lichen extract Usnea sp. The secondary metabolites that were tested qualitatively included: alkaloids, flavonoids, saponins, triterpenoids, and tannins. The results of the phytochemical screening showed a positive (+) result indicated by a change in color or the formation of a precipitate or the formation of foam after adding reagents to the test extract. Meanwhile, a negative result (-) is indicated by the absence of a change in color or the formation of precipitate or the formation of foam after adding reagents to the test extract.

Results and Discussion

Usnea sp. The family Usneaceae belongs to the fructicose species which grows epiphytically on tree trunks and branches, is shaped like the roots of a plant, is green in color, and has a holdfast which is used to attach it to the substrate. Based on Hasnunidah (2009), the species Usnea sp. also known as wind wood (resi beard). This lichen species is beard-like, grey-green in color, has a thallus and rhizoids, and has dichotomous branches.

Usnea sp. flat cylinders or ribbons that hang from the surface of the substrate. The size of the talus varies long or short, cylindrical or beard-like "Moss". The usnea filipendula has a pendent growth type, namely the main talus to the fibrils hanging down. The distance between the thallus is tight so that it also looks like a bush attached to the bark of a

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tree trunk. Soredia and tubercles radiate over the surface of the talus.

Usnea sp. also useful because it produces an usnin acid antibiotic which is useful for fighting tuberculosis. Lichens belonging to the same family tend to share the compounds they contain or generally contain other characteristic constituents that are structurally related. Lichens that are taxonomically related have a tendency to contain compounds that are related to one another (Markham, 1988). Morphology of lichen Usnea sp. can be seen in Figure 1 below.

Figure 1. Results of Usnea sp lichen sample collection. Sticking To Pines

Usnea sp. has a light green thallus color with an irregular thallus shape, uneven thallus surface, size 4 cm and how it grows the thallus is attached and dangling on the substrate. The size of the thallus varies long or short, cylindrical or like a "Beard Moss" beard that hangs or stands upright with short branches. Usnea cornuta has a talus erect (upright) growth type, which is an upright growth form, the direction of growth is to the side not tufting downwards. The distance between the talus is slit, tenuous and not too tight. The soredia are on the granular margins of the thallus. There are papillae that grow on the surface of the main talus.

Phytochemical Screening of Lichen Usnea sp.

The results of the manufacture of lichen ethanol extract Usnea sp. 45.88 g of viscous extract was obtained with a yield value of 11.47%. The results of lichen ethanol extract Usnea sp. brown in color, thick consistency and has a characteristic odor. Based on the research results, it was found that the ethanol extract of lichen Usnea sp. contains a class of secondary metabolites, namely alkaloids, flavonoids, saponins, tannins and triterpenoids (Table 1).

Table 1. Results of the Phytochemical Screening of the Ethanol Extract of Lichen Usnea sp.

No	Secondary Metabolites	Reactor	Test results	Information
1	Alkaloid	Dragendroff	(-)	No precipitate formed
		Bouchardat	(+)	red/orange
				A brown precipitate formed
		Mayer	(+)	A white precipitate formed

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2	Flavonoid	Sserbuk Mg + Amil Alkohol + HClp	(+)	A deep orange color is formed
3	Saponin	Air panas/dikocok	(+)	Form 1 cm high foam stable
4	Tanin	FeCl3	(+)	Formed in blue Black
5	Triterpenoid/ Steroid	Lieberman-Bourchat	(+)	Formed purple red color

Note: (+) positive for compounds, (-) does not contain compounds

Testing for alkaloid compounds uses three reagents, namely Dragendroff reagent, Bouchardat reagent and Mayer reagent. Testing for alkaloid compounds showed positive results which were indicated by the formation of precipitate or turbidity in at least two of the three experiments, namely: the formation of a brown precipitate when reacted with Bouchardat reagent, a white precipitate when reacted with Mayer reagent and no red or orange precipitate when the extract was reacted with Dragendorf reagent which showed negative results, this test only produced a clear brown solution when added Dragendorff reagent.

Figure 2. (a) Concentration of the extract using a rotary evaporator (b) Phytochemical screening of lichen extract Usnea barbata

Alkaloids are compounds that contain nitrogen atoms and are basic in nature so that to extract them requires the addition of hydrochloric acid. The addition of hydrochloric acid aims to extract alkaline alkaloids using an acid solution (Jones and Kinghorn, 2006). In this study, alkaloid testing was carried out using 3 reagents, namely Dragendorff, Bouchardat, and Mayer. According to Sangi et al. (2008), the principle of this analytical method is the precipitation reaction that occurs due to the replacement of ligands. Nitrogen atoms that have lone pairs of electrons in alkaloids can replace iodo ions in the reagents. If a compound contains alkaloids, then on testing with Dragendorff's reagent it will form a red or orange precipitate, because the alkaloids will interact with tetraiodobismutat (III) ions.

In the Bouchardat test, a positive result is indicated by the formation of a brown to black precipitate. The precipitate that is formed occurs due to the coordination covalent bond between K+ metal ions and alkaloids so that a precipitated potassium-alkaloid complex is formed. Bouchardat's reagent contains iodine and potassium iodide. In testing with Mayer's reagent, a positive result will form a white to yellowish precipitate. Alkaloid compounds will interact with tetraiodomercurate (II) ions to form complex compounds and precipitate. This is

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because mercury ions are heavy metal ions which are capable of precipitating alkaline alkaloid compounds (Svehla, 1990).

The results showed positive for containing alkaloid compounds with the formation of precipitate or turbidity in at least two of the three experiments, namely the formation of brown precipitate when reacted with Bouchardat reagent and white precipitate when reacted with Mayer reagent, while the extract reacted with Dragendorf reagent showed negative results. this test only produces a clear brown solution on the addition of Dragendorff reagent.

Testing for flavonoid compounds showed positive results which were indicated by the formation of two layers in the solution, where the top layer, namely amyl alcohol, changed color to dark orange after adding Mg powder and concentrated HCl. In the saponin test, positive results were obtained with the formation of 1 cm high foam which lasted for ± 10 minutes and after adding 1 drop of HCl 2 N the foam that formed did not disappear.

Flavonoids were tested for the presence of Mg and concentrated HCl. The addition of Mg and HCl was carried out on lichen extract Usnea sp. and formed a deep orange color. This indicates that the sample contains flavonoids. Concentrated HCl is used to hydrolyze the flavonoids into their aglycones. According to Harborne (1987), flavonoid compounds will be reduced by concentrated Mg and HCl so that they will produce red, yellow or orange colors.

The results of the examination carried out on the samples showed the presence of tannin compounds in the Usnea sp. extract, indicated by the addition of 1% FeCl3 to the diluted filtrate resulting in a change in the color of the solution to blackish blue indicating the presence of tannin compounds. The triterpenoid test also showed a positive result which was indicated by a change in the color of the evaporated filtrate when it was dropped by LB reagent (Lieberman-Buchard) to a purplish-red color.

Tannins are polar compounds due to the presence of OH groups. Tannins are divided into two groups and each group gives a different color reaction to 1% FeCl3. Hydrolyzed tannins will produce a black-blue color and condensation tannins will produce a black-green color. At the time of its addition, it was estimated that FeC13 would react with one of the hydroxyl groups present in the tannin compound. The result of the reaction is what eventually gives rise to a color. FeCI3 reagent is widely used to identify phenolic compounds including tannins, therefore there is a possibility that positive results can also be given by other phenolic compounds in the sample (Sangi et al., 2008). The results of the research resulted in a change in the color of the solution to blackish blue which indicated that it contained tannin compounds positively.

The results of the phytochemical screening test of the lichen ethanol extract of Usnea barbata are in accordance with the research of Hazimi et al. (2018), regarding the phytochemical screening of several medicinal plants, which stated that angina wood positively contains secondary metabolites in the form of flavonoids, terpenoids, saponins, and tannins. Early Research et al. (2017), stated that phytochemical screening on lichen lichen extract Usnea sp showed that the extract contained flavonoids, alkaloids, saponins and tannins. The group of active compounds such as flavonoids, alkaloids, saponins and tannins contained in the lichen extract is a group of compounds that have antibacterial properties.

The results of this study were also supported by research (Amalia and Trimulyona, 2018) stating that the lichen extract Usnea subfloridana contains secondary metabolites in the form of usnic acid, alkaloids, phenolics, steroids, triterpenoids, saponins, and tannins which act as antimicrobial substances, lichen with a family of are likely to have similarities in the compounds they contain or generally contain other characteristic constituents that are structurally related.

Saponin is a glycoside form of

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sapogenin so it is polar. Saponins have glycosyl which functions as a polar group and steroid and triterpenoid groups as nonpolar groups. Compounds that have polar and nonpalar groups are surface active so that when shaken with water saponins can form micelles. In the micelle structure, the polar group faces outward while the nonpolar group faces inward. This situation looks like foam, because in this analysis the ability of the sample to form foam is seen (Sangi et al., 2008), so that the results of the study obtained positive results for saponins.

Phytochemical screening is a qualitative method used to determine the presence of secondary metabolites in the ethanol extract of lichen Usnea sp. The process of extracting chemical compounds contained in lichen Usnea barbata was carried out by maceration method using 96% ethanol solvent. The solvent used in the extraction process is selected based on its polarity. The compounds to be extracted in this study are phenol derivatives which are polar in nature so that a polar solvent is needed as well. One good type of polar solvent is ethanol, ethanol is widely used to extract the polar components of a natural substance known as a universal solvent. In this study, 96% ethanol was used which had a dielectric constant of 24.30° and a boiling point of 78.4°. The higher the dielectric constant of a solvent, the better its ability to extract active compounds from the sample.

In the preparation of the extract, 45.88 g of thick extract was obtained with a yield value of 11.47%. These results meet the requirements of the Indonesian Herbal Pharmacopoeia, namely the yield of not less than 7.2% (Ministry of Health RI, 2000). The yield value is related to the amount of bioactive content contained, the higher the yield the higher the content of substances attracted to a raw material. The results of the phytochemical screening of lichen ethanol extract Usnea sp. obtained in this study indicate that lichen Usnea sp. Has a class of secondary metabolites, namely alkaloids, flavonoids, saponins, tannins and triterpenoids.

Triterpenoid testing was carried out by the Liebermann-Burchard test. In the Liebermann-Burchard test, if a red or purple color is formed it indicates the presence of triterpenoids, whereas if a green color is formed it indicates the presence of steroids (Depkes RI, 1995). The principle of the reaction in this test mechanism is the condensation or release of H2O and combining with carbocations. The test results showed the formation of a purplish red color, the color change formed due to the oxidation of triterpenoid compounds through the formation of conjugated double bonds.

Conclusions

Lichen extract Usnea sp. Pinus contains a class of secondary metabolite compounds consisting of alkaloids, flavonoids, saponins, tannins and triterpenoids. The results of the phytochemical screening showed a positive (+) result indicated by a change in color or the formation of a precipitate or the formation of foam after adding reagents to the test extract. Meanwhile, a negative result (-) is indicated by the absence of a change in color or the formation of precipitate or the formation of foam after adding reagents to the test extract.

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