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Secondary Metabolites and Antioxidant Properties of Lichens from Sicike-Cike Nature Park, North Sumatra

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Abstract: This study aimed to evaluate the phytochemical composition and antioxidant activity of five lichen species *Cladonia portentosa*, *Cladonia rapii*, *Letharia vulpina*, *Parmotrema hypotropum*, and *Usnea trichodea* collected from Sicike-Cike Nature Park, North Sumatra, Indonesia. Methanol extracts were prepared from dried and powdered lichen samples. Qualitative phytochemical screening using specific reagents identified the presence of alkaloids, flavonoids, tannins, saponins, terpenoids/steroids, and glycosides in varying levels among the species. Antioxidant activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, with IC50 values ranging from 42.3244 µg/mL to 86.7479 µg/mL. *Parmotrema hypotropum* demonstrated the strongest antioxidant activity (IC50 = 42.3244 µg/mL), categorized as moderate based on literature benchmarks. The strong activity is likely due to the presence of alkaloids, flavonoids, and saponins. In contrast, *Usnea trichodea* showed the weakest activity. These results suggest that certain lichens, especially *Parmotrema hypotropum*, may serve as promising sources of natural antioxidants. The findings support potential applications in pharmaceutical formulations and natural cosmetic products, particularly those targeting oxidative stress. Future studies should focus on isolating dominant compounds such as usnic acid or flavonoid derivatives and further exploring their bioactivity profiles.

Keywords: Antioxidant; Lichen; Phytochemical; Sicike-Cike Nature Park.

Introduction

Lichens are symbiotic associations of two or more different types of organisms, namely algae, fungi, and cyanobacteria (Elkhateeb et al., 2021). This association enhances the survival ability of fungi or algae in non-ideal environments due to the unique structure of the thallus, physiology, and synthesis of chemical compounds that differ from those produced by fungi or algae individually (Spribille et al., 2022). Lichens have great potential as natural sources for discovering new bioactive compounds, either through the extraction of natural substances or the direct production of secondary metabolites (Ren et al., 2023). The secondary metabolites of lichens exhibit high diversity, with more than 1,000 different compounds identified. Based on their chemical structures, these compounds can be categorized as phenolics, dibenzofurans (such as usnic acid), depsides (such as atranorin, barbatic acid), depsidones (such as salazinic acid, lobaric acid), aliphatic acids (such as protolichesterinic acid), quinones (such as parietin), pulvinic acid derivatives (such as vulpinic acid), and anthraquinone-related compounds (Silva et al., 2023).

Lichens contain various significant antioxidant compounds. These compounds can neutralize toxic free radicals due to the presence of phenolic groups. Several studies have reported that certain compounds, such as atranorin and divaricatic acid, as well as depsidones like pannarin and 1'-chloropannarin found in lichens, exhibit significant antioxidant activity (Elečko et al., 2022).

Many lichen extracts possess antioxidant properties due to their phenolic compounds. Phenolic compounds

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such as orsellinic acid, methyl orsellinate, lecanoric acid, and atranorin have shown moderate antioxidant activity. Additionally, stictic acid derivatives derived from the lichen *Usnea articulata* have also been reported to exhibit significant antioxidant activity (Khalid et al., 2024).

Recent studies have further confirmed this potential, including the identification of antioxidant activity in *Parmotrema perlatum* (Haikal, 2024) and several other lichen species (Atni et al., 2025). However, despite the growing interest, research on the antioxidant potential of lichens in tropical ecosystems—particularly those in underexplored habitats like the Sicike-Cike Nature Park in North Sumatra—remains limited.

This study focuses on five lichen species: *Cladonia portentosa*, *Cladonia rapii*, *Letharia vulpina*, *Parmotrema hypotropum*, and *Usnea trichodea*. These species were selected not only due to their accessibility and taxonomic representation but also based on preliminary reports indicating the presence of diverse and potentially bioactive secondary metabolites within these genera. By exploring their antioxidant properties, this research aims to contribute novel insights into the phytochemistry of tropical lichens and emphasize their potential application in natural antioxidant development.

Method

Sampling

The sampling method involved collecting the most abundant lichen species at the research site, ensuring enough for analysis. Sample selection was based on species abundance and accessibility in their natural habitat to ensure optimal representation in the study.

Screening of Secondary Metabolite Compounds Lichen Sample Preparation

Lichen Cladonia portentosa, Cladonia rapii, Letharia vulpina Parmotrema hypotropum, Usnea trichodea collected from the field were cleaned and air-dried at room temperature for 7 to 10 days. The dried samples were finely ground until a dry powder of 100 g was obtained.

Screening of Secondary Metabolite Compounds LichenExtraction

The collected lichens were cleaned and dried. The lichen was then ground using a blender. The lichen powder was then extracted using the maceration method in 70% methanol solvent for 7 days at room temperature with shaking at 150 rpm. The filtrate was separated from the residue using filter paper, and then concentrated using a rotary evaporator until a concentrated lichen extract was obtained. The lichen extract was then diluted to a 50% concentration using Dimethyl Sulfoxide (DMSO) solvent.

Phytochemical Component Determination

Phytochemical screening was conducted to determine the secondary metabolites of the lichen extract. The secondary metabolites tested qualitatively include alkaloids, flavonoids, saponins, triterpenoids, and tannins.

Alkaloid Test

Total 0.5 grams of the extract were dissolved in 5 mL of distilled water, then 5 drops of 2 N HCl were added and heated for 2 minutes. The solution was divided into 3 parts, and each part was tested with 2 drops of Dragendorf reagent (a positive result produces a red or orange precipitate), Bouchardat Reagent (a positive result produces a brown to black precipitate), and Mayer reagent (a positive result produces a white or yellow precipitate). Alkaloids are considered positive if at least two of the three tests produce a precipitate or turbidity.

Flavonoid Test

Total 0.5 grams of the extract were added to 20 mL of hot water, boiled for 10 minutes, and filtered while hot. To the 5 mL filtrate, 0.1 g of magnesium powder and 1 mL of concentrated hydrochloric acid were added, followed by 2 mL of amyl alcohol. The mixture was shaken and left to separate. Flavonoids are considered positive if a red, yellow, or orange color appears in the amyl alcohol layer (Farnsworth, 1966).

Saponin Test

Total 0.5 grams of the extract were dissolved in 10 mL of warm water, then shaken vigorously for about 60 seconds. The presence of saponins is considered positive if a foam layer of about 1 cm height forms and persists for approximately 10 minutes. After adding 1 drop of 2 N HCl, the foam should not disappear.

Triterpenoid Test

Total 1 gram of the extract was macerated with 20 mL of n-hexane for two hours. The resulting macerate was filtered, and the filtrate was evaporated in an evaporating dish. The remaining residue was treated with Liebermann-Burchard reagent. If a blue-green or purple-red color appears, it indicates the presence of triterpenoids/steroids.

Tannin Test

The extract was dissolved in 5 mL of distilled water, then 3 drops of FeCl3 were added. Tannins are considered present if the solution changes to a greenish-black or bluish-black color.

Antioxidant Activity Testing on Lichen

Preparation of Sample Solution and Measurement of Maximum Absorption Wavelength

Twenty-five mg of the lichen methanol extract was weighed and placed into a 25 mL volumetric flask, then dissolved in methanol and diluted to a volume that produced a concentration of 1000 ppm. A dilution was then made by adding ethanol to achieve sample concentrations of 10, 20, 40, 80, and 160 ppm. For the determination of antioxidant activity, 0.2 mL of each sample solution was pipetted into a test tube, followed by the addition of 3.8 mL of 50 µM DPPH solution. The mixture was homogenized and left for 30 minutes in the dark. The absorption was measured using a UV-Vis spectrophotometer at a wavelength of 516 nm.

Determination of DPPH Free Radical Scavenging Activity (a, a-diphenyl-β-picrylhydrazyl)

This process was determined using the DPPH free radical scavenging method, calculated using the Formula 1.

$$\% \text{ Inhibition} = \frac{(\text{Abs.ctrl} - \text{Abs.sample})}{\text{Abs.ctrl}} \text{ x } 100\% \quad (1)$$

Where:
Abs. control = Absorbance of the DPPH solution without the sample (control)
Abs. sample = Absorbance of the DPPH solution treated with the sample.

Calculation of IC50 Value (Inhibition Concentration 50%)

The IC50 value was calculated from the regression equation of the linear curve plot of concentration (ppm) on the X-axis versus % inhibition on the Y-axis. The IC50 value was obtained by calculating the value when Y = 50 using the following linear regression Formula 2.

$$Y = aX + b, \text{ at } Y = 50 \quad (2)$$

Result and Discussion

Screening of Secondary Metabolite Compounds

Table 1 presents the results of secondary metabolite screening of five lichen species, revealing variations in their bioactive compound content. Cladonia portentosa contained alkaloids terpenoids/steroids (Salkowski & Liebermann- Burchard), flavonoids ($FeCl_3$ 5% and Mg + HCl), tannins, and glycosides. Cladonia rapii exhibited alkaloids (Bouchardat Reagent) flavonoids ($FeCl_3$ 5% and Mg + HCl), saponins, and tannins. Letharia vulpina contained alkaloids (Mayer and Wagner) and flavonoids ($FeCl_3$ 5% and Mg + HCl). Parmotrema hypotropum was found to contain alkaloids (Wagner, Dragendorff, and Bouchardat Reagent), flavonoids (Mg + HCl), saponins, and glycosides but lacked tannins. Usnea trichodea showed the presence of alkaloids (Mayer, Wagner, Dragendorff, and Bouchardat Reagent), flavonoids ($FeCl_3$ 5% and Mg + HCl), tannins, and glycosides.

These findings indicate that each lichen species has a distinct composition of secondary metabolites. Among them, Cladonia portentosa exhibited the highest diversity of bioactive compounds, including alkaloids, terpenoids/steroids, flavonoids, tannins, and glycosides. In contrast, Parmotrema hypotropum contained alkaloids, flavonoids, and saponins but lackedtannin and glycosides. According to a study by (Nagar et al., 2023), Cladonia portentosa contains secondary metabolites such as perlatolic acid and usnic acid, which have been reported to possess various biological activities.

Several secondary metabolites exhibit pharmacological activities. Flavonoids, a subgroup of polyphenols, are well known for their ability to scavenge free radicals and act as potent antioxidants. They possess anti-inflammatory properties, protect cells from oxidative damage, and demonstrate strong anticancer activity. Due to these benefits, flavonoids are considered one of the most important bioactive compound groups for maintaining human health (Valsan & Raphael, 2016). Lichens produce a wide range of secondary metabolites, including flavonoid derivatives, alkaloids, terpenoids, saponins, tannins, and glycosides (Huneck, 1999). They also hold significant potential for development into modern medicines, especially with the support of biotechnological advancements to ensure sustainable biomass production (Fatima et al., 2024).

Lichens Antioxidant Activity

Antioxidant testing on lichens was conducted on the species *Cladonia portentosa, Cladonia rapii, Letharia vulpina, Parmotrema hypotropum*, and *Usnea trichodea* due to their widespread distribution and abundance in the study area. The results showed variations in antioxidant activity among the species.

Based on Table 2, the IC_{50} values of the five lichen species indicate variations in antioxidant activity. IC_{50} is a parameter that represents the concentration of a sample required to inhibit 50% of free radical activity, where a lower IC_{50} value signifies stronger antioxidant activity. Among the tested species, *Parmotrema hypotropum* exhibited the highest antioxidant activity, with an IC_{50} value of 42.3244 µg/mL, indicating that its bioactive compounds are more effective in scavenging free radicals compared to other species. *Letharia vulpina* and *Cladonia rapii* also demonstrated relatively strong antioxidant activity, with IC_{50} values of 59.9691 µg/mL and 63.5240 µg/mL, respectively.

Meanwhile, *Cladonia portentosa* had an IC_{50} of 82.2710 µg/mL, and *Usnea trichodea* showed the weakest antioxidant activity among the five species, with the highest IC_{50} value of 86.7479 µg/mL. The differences in IC_{50} values can be attributed to variations in the composition of bioactive compounds in each lichen, such as usnic acid, depsidones, or other phenolic compounds, which contribute to antioxidant capacity.

Tabel 1. Secondary Metabolite Results of Methanol Extract of Lichen in Sicike-cike Nature Park

Secondary Metabolite	*Cladonia portentosa* (Dufour) Coem.	*Cladonia rapii* A Evans.	*Letharia vulpina (L.)*	*Parmotrema hypotropum* (Nyl.) Hale	*Usnea trichodea* Ach.
Alkaloid	+	+	++	+++	++++
Terpenoid/Steroid	+	-	-	-	-
Flavonoid	++	++	++	+	++
Saponin	-	+	-	+	-
Tanin	+	+	+	+	+
Glikosida	+	-	-	-	+

Description: – = Not detected; + = Weak; ++ = Moderate; +++ = Strong; and ++++ = Very Strong

Figure 1. Morphology of lichens: (a) Parmotrema hypotropum; (b) Cladonia portentosa; (c) Letharia vulpina; (d) Usnea trichodea; and (e) Cladonia rapii

Table 2. Antioxidant Activity of Methanol Extract Lichen in Sicike-cike Nature Park

Lichens	IC50 (µg/Ml)
Cladonia portentosa	82.2710
Cladonia rapii	63.5240
Letharia vulpina	59.9691
Parmotrema hypotropum	42.3244
Usnea trichodea	86.7479

The findings of Atni et al. (2025) indicate that species from the genus *Parmotrema* possess moderate antioxidant activity, with *P. clavuliferum* (IC_{50} = 250.30 µg/mL) and *P. tinctorum* (IC_{50} = 159.70 µg/mL) showing measurable effects. Fahmi et al. (2024) further reported that *P. perlatum* has a stronger antioxidant activity (IC_{50} = 70 µg/mL), while *P. hypotropum* demonstrated even greater potency. This variation underscores the significant intra-genus diversity in antioxidant capacity. Such activity is closely linked to the phytochemical composition of lichens, particularly the presence of alkaloids, flavonoids, and tannins. Notably, *P. hypotropum*, which contains high levels of alkaloids and flavonoids, exhibited the strongest antioxidant activity.

A positive correlation between alkaloid content and antioxidant activity has been supported by earlier studies (Dalimunthe et al., 2018; Fahmy et al., 2021). However, *Usnea trichodea*, despite its high alkaloid content, showed weaker activity, suggesting that the type of alkaloid and its interactions with other compounds may influence bioactivity (Khan & Chaudhary, 2024; Zandavar & Babazad, 2023). These findings highlight the importance of detailed phytochemical profiling to better understand the antioxidant potential of lichen species.

The IC_{50} values obtained in this study reflect the antioxidant activity of the tested specimens. Lower IC_{50} values correspond to higher antioxidant potential; however, contextual interpretation remains essential. According to the classification proposed by Jun et al. (2003) and supported by other references (Shahidi, 1997), antioxidant activity is categorized as very strong (IC_{50} < 50 µg/mL), strong (50–100 µg/mL), moderate (100–150 µg/mL), weak (150–250 µg/mL), and inactive when IC_{50} exceeds 250 µg/mL. Hassanpour & Doroudi (2023) further emphasize that antioxidant activity largely depends on the phytochemical composition, particularly the presence of polyphenols and flavonoids, which are known to enhance antioxidant capacity. Accordingly, flavonoids and/or phenolic compounds have been reported to show a positive correlation with the antioxidant activity of extracts, as previously demonstrated by Cai et al. (2004) & Kähkönen et al. (1999).

The IC_{50} value reflects the antioxidant capacity of a compound, particularly its ability to donate electrons or hydrogen atoms to neutralize DPPH free radicals. This capacity is closely related to the presence of specific functional groups, especially hydroxyl (-OH) groups in phenolic structures, which are known to effectively donate hydrogen atoms and stabilize free radicals through resonance mechanisms (Chen et al., 2024; Parcheta et al., 2021). The higher the number of electrons or hydrogen atoms transferred to DPPH, the greater the reduction in absorbance at 517 nm, resulting in a higher percentage of inhibition and a lower IC_{50} value—indicative of stronger antioxidant activity (Ramadhanty et al., 2023). Nevertheless, the antioxidant activity of phenolic compounds can vary considerably, depending on their molecular structure, the number and position of hydroxyl groups, and their degree of polymerization. Some phenolics demonstrate potent antioxidant effects, while others are moderate or weak. In addition, phenolic compounds can interact synergistically or antagonistically with other phenolics or with biomolecules such as carbohydrates, lipids, and proteins, which may further influence the overall antioxidant performance of an extract or formulation (Lobiuc et al., 2023; Rice-Evans, 1999).

Conclusion

This study revealed notable differences in the composition of secondary metabolites and antioxidant activity among five lichen species, with *Parmotrema hypotropum* exhibiting the highest antioxidant potential. The detection of bioactive compounds such as flavonoids, alkaloids, and tannins highlight the potential of these lichens as sources of natural antioxidants with possible pharmaceutical applications, particularly as cell-protective or anti-inflammatory agents. These findings provide a basis for future research aimed at isolating and characterizing the specific active compounds and evaluating their practical uses in pharmaceutical and industrial contexts.

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Author Contributions

P.A.L. contributed to the research, product development, data analysis, and manuscript writing; E.I. supervised the research activities through to manuscript completion; I.W. contributed to the conceptualization of the study.

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Conflicts of Interest

The authors declare no conflict of interest.

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