



Research Article

TOTAL PHENOLIC CONTENT, AND BIOLOGICAL POTENTIAL OF THE METHANOLIC EXTRACT FROM THE LICHEN *PYRENULA* SP.

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ABSTRACT

Pyrenula lichens are highly diverse and widely distributed in Vietnam; however, their chemical composition and biological activities remain largely unexplored. In this study, preliminary screening of major groups of secondary metabolites, the phenolic content, antioxidant, antibacterial, and α -glucosidase inhibitory activities of *Pyrenula* lichen collected in Ho Chi Minh City were investigated. The presence of alkaloids, terpenoids, coumarins, quinones, tannins, phenols, and flavonoids in the extract was confirmed by qualitative screening. The total phenolic content (TPC) was determined to be 30.26 ± 0.51 mg GAE/g extract. In terms of bioactivities, the extract exhibited moderate antioxidant activity in the DPPH scavenging assay, with an IC_{50} value of 97.4 ± 8.5 μ g/mL. Besides, the α -glucosidase inhibition of the extract ($IC_{50} = 46.5 \pm 3.6$ μ g/mL) suggests its potential for antidiabetic management. Moreover, the extract showed strong antibacterial activity against *Staphylococcus aureus*, confirmed by an inhibition zone diameter of 20.9 ± 0.1 mm and a MIC value of 15.62 μ g/mL. These results show that *Pyrenula* sp. is a promising source for further investigation of biologically active secondary metabolites and their potential applications.

Keywords: antibacterial; antioxidant; α -glucosidase inhibition; *Pyrenula* sp.; total phenolic content

1. Introduction

The crustose lichen genus *Pyrenula* Ach. belongs to the family Pyrenulaceae Rabenh., order Pyrenulales, class Ascomycetes, phylum Ascomycota, kingdom Fungi. This genus was first established by Acharius, with *Pyrenula nitida* (Weigel) Ach. as the type species (Acharius, 1814). In 2012, Aptroot reviewed the existing literature and recognized 169 valid species within the genus *Pyrenula*. By 2023, numerous new lichen species in this genus had been described, and the genus currently comprises approximately 238 species (Aptroot, 2012; Lücking et al., 2023; Soto-Medina et al., 2023). *Pyrenula* is among the lichen genera currently attracting scientific interest due to its chemical diversity and biological potential.

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Xanthone compounds from the lichen-forming fungus *P. japonica* exhibited antioxidant activity superior to that of the synthetic antioxidants α -tocopherol and BHT (Takenaka et al., 2000). Seven alkylated decalin-type polyketide substances from the lichen-forming fungus *Pyrenula* sp. demonstrated inhibitory activity against mammalian DNA polymerases α and β , with the half-maximal inhibitory concentration (IC₅₀) values ranging from 8.1 to 19.5 μ M (Le et al., 2014). Furthermore, polyphenolic and terpenoid compounds from the species *P. nitida* displayed antioxidant capacity with an IC₅₀ = 125.76 \pm 0.02 μ g/mL (Pradhan et al., 2024). These findings further emphasize the lichen genus *Pyrenula* as a promising source of natural bioactive materials.

Despite the high species diversity and widespread distribution of *Pyrenula* in various ecological regions in Vietnam, research focusing on its chemical constituents and biological activities remains remarkably scarce, as current literature primarily focuses on taxonomy (Dou et al., 2024; Junior et al., 2021). For the above-mentioned reasons, this study was conducted to perform preliminary phytochemical screening, determine total phenolic content, and evaluate the antioxidant, antibacterial, and α -glucosidase inhibitory activities of a methanolic extract from *Pyrenula* sp.. The results of this study will contribute to the understanding of the genus and provide baseline data for further studies on the biological properties of Vietnamese *Pyrenula* species.

2. Materials and methods

2.1. Materials

The *Pyrenula* sp. lichen sample (Figure 1) was collected from Dau Tieng Ward, Ho Chi Minh City (11°16'11.6" N, 106°21'51.5" E) in October 2025 and was used in this research. The species identification was carried out by Dr. Vo Thi Phi Giao, Faculty of Biology and Biotechnology, University of Science, Ho Chi Minh City, Vietnam, based on the thallus morphology, ascospore characteristics, and specific chemical reactions. The sample was stored at the Biochemistry-Microbiology Laboratory, Department of Biology, Ho Chi Minh City University of Education, under the code UE-LF-2025-01 at room temperature.

2.2. Methods

2.2.1. Lichen sampling method

Crustose lichen samples bearing lirellate apothecia were collected from tree bark. After being detached from the tree bark, the samples were photographed, wrapped in newspapers, and labeled with collection details (Zakeri et al., 2022). They were then transported to the Biochemistry – Microbiology Laboratory, Ho Chi Minh City University of Education, for methanolic extraction.

2.2.2. Morphological examination

Identification was conducted based on macroscopic morphological features, including the coloration, surface texture, and growth habit of the thallus, as well as the appearance and distribution of the ascomata. Microscopic analysis focused on ascospore shape and muriform septation patterns (Dou et al., 2024). In this study, morphological and anatomical characters of thalli and apothecia were examined and photographed under an Olympus CX23 microscope.

2.2.3. Methanol extract

The dry powdered biomass from lichen was macerated with methanol until complete extraction was achieved. The resulting filtrate was concentrated under reduced pressure using a rotary evaporator to obtain a semi-solid extract. Finally, the extract was left at room temperature for approximately seven days to ensure complete solvent evaporation. The extraction efficiency was calculated as follows:

$$\text{Extraction efficiency (\%)} = \frac{\text{Weight of methanol extract}}{\text{Dry biomass weight}} \times 100 \text{ (Nguyen et al., 2024)}$$

2.2.4. Quantitative phytochemical screening and quantitative determination of total phenolic contents

- *Qualitative screening of major groups of secondary metabolites*

The extract was dissolved in methanol to a concentration of 10 mg/mL. Color-forming and precipitation reactions were conducted according to the methods described in the published literature (Phuong et al., 2017) as presented in Table 1.

Table 1. Tests for specific natural compounds in the extracts utilized in this study

| Component | Test | Observation |
|-------------------|--|------------------------|
| Alkaloid | Wagner | Yellow precipitate |
| Flavonoid | Pb(CH ₃ COOH) ₂ 10% | Yellow color |
| Terpenoid | Chloroform + concentrated H ₂ SO ₄ | Jade green color |
| Phenol and tannin | H ₂ O + 2 - 3 drops FeCl ₃ 5% | Blue-black precipitate |
| Quinone | A few drops of concentrated HCl | Green color |
| Coumarin | NaOH 10% | Yellow color |

- *Determination of total phenolic content (TPC)*

To determine the total phenolic content in the extracts, a method based on published literature (Vo et al., 2017) was employed. The results were presented as gallic acid equivalent (mg GAE/g). Briefly, the extract and gallic acid were dissolved in methanol to a concentration of 1 mg/mL and a range of 1.56-200 µg/mL, respectively. The Folin-Ciocalteu (FC) reagent (10%) was diluted with distilled water. For the first step, 1 mL of gallic acid or extract solutions was mixed with 2.5 mL of FC 10% reagent and allowed to react for 5 min. After that, 2 mL of Na₂CO₃ 2% solution was added. After a 45-minute reaction period at room temperature, the optical density (OD) value was determined at 765 nm. The experiment was performed in triplicate. The TPC of the extract was calculated based on the linear equation ($y = ax + b$) derived from a linear calibration curve of gallic acid, using the following formula: $P = a \times V/m$

P: total phenolic content (mg GAE/g extract)

a: the x-value derived from the gallic acid calibration curve (mg/mL)

V: volume of the extract solution (mL)

m: weight of the extract contained in volume V (g).

2.2.5. DPPH scavenging assay

The antioxidant activity of the extract was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay as described in a previous publication by Bui et al. (2024). The extract was diluted in methanol to obtain a range of concentrations from 1.56-800 µg/mL by a 2-fold serial dilution method. Initially, a 200 µL aliquot of the

sample solution was mixed with 1 mL of 80 μ M DPPH solution in a test tube. The mixture was incubated in the dark at room temperature for 30 min before measuring its OD at 517 nm. Methanol was used as the negative control, while vitamin C served as the positive control. Both the extract and DPPH were diluted in methanol. Extracts with strong antioxidant activity were further analyzed to determine their IC₅₀ values. The DPPH free radical inhibition ability was calculated using the following formula:

$$\text{DPPH scavenging percentage (\%)} = \frac{\text{OD of control} - \text{OD of sample}}{\text{OD of control}} \times 100$$

2.2.6. Alpha-glucosidase inhibitory assay

The α -glucosidase inhibitory activity assay was conducted based on the method proposed by Bui et al. (2024). The methanolic extract (1 mg/mL) was prepared in phosphate buffer 0.1 M (pH 6.9) containing 5% DMSO. Alpha-glucosidase from *Saccharomyces cerevisiae* EC 3.2.1.21 and 4-nitrophenyl- α -D-glucopyranoside (pNPG) were dissolved in phosphate buffer 0.1 M (pH 6.9). In a 96-well plate, 40 μ L of enzyme solution and 50 μ L of the sample were pre-incubated at 37°C for 20 min. The reaction was initiated by adding 40 μ L of 5 mM pNPG, followed by another 20-minute incubation. Then, 130 μ L of 0.2 M Na₂CO₃ was added to terminate the reaction. The OD values of the resulting mixture were measured at 405 nm. A 5% DMSO solution served as the negative control, and acarbose was used as the positive control. The positive control, acarbose, was obtained in commercial tablet form (50 mg per tablet) manufactured by DOMESCO Medical Import-Export Joint Stock Corporation (Vietnam). The stock solutions were prepared based on the labeled active pharmaceutical ingredient content. All experiments were performed in triplicate, and extracts with strong inhibition were further analyzed for IC₅₀ values. The inhibition percentage was calculated using the following formula:

$$\alpha\text{-glucosidase inhibition percentage (\%)} = \frac{\text{OD of control} - \text{OD of sample}}{\text{OD of control}} \times 100$$

2.2.7. Antibacterial assay

The antibacterial activity of the methanol extract against *Staphylococcus aureus* ATCC 6538 was assessed using the well diffusion method. The bacterial strain was pre-activated at 37°C for 24 h in Mueller-Hinton Agar (MHA), then suspended in sterile 0.9% saline, vortexed, and adjusted to an OD₆₀₀ of 0.1 (10⁸ CFU/mL). A 100 μ L bacterial suspension was spread onto MHA plates, followed by the addition of 100 μ L of each extract into pre-drilled wells. After incubation at 37°C for 24 hours, antibacterial activity was determined by measuring the inhibition zone diameter, averaged from three replicates (Do et al., 2022).

2.2.8. Minimum inhibitory concentration (MIC) determination

Minimum inhibitory concentration (MIC) was determined using the broth microdilution method in Mueller-Hinton broth (MHB). Bacterial cultures were adjusted to an appropriate density equivalent to OD₆₀₀ or the McFarland standard. The tested sample and antibiotic solution were prepared at twice the highest test concentration and subjected to two-fold serial dilutions in a 96-well microtiter plate containing MHB. Next, 100 μ L of MHB was dispensed into all wells of the microplate. Then 100 μ L of the tested sample and antibiotic were withdrawn from column 1 and added to column 2. The solution in column 2

was mixed by aspiration and transferred to column 3. The procedure of dilution was repeated for column 10. Bacterial inoculum (10^4 - 10^5 CFU/mL) was added 5 μ L to each well in columns 1 to 11. Column 11 was used as the growth control, containing only bacteria and culture medium. Column 12 was used as the negative control for the sterilization of the procedure, so no bacteria were introduced into column 12. Plates were incubated at 37°C for 12–18 h. After that, 10 μ L of 0.1% resazurin solution was dispensed into each well. The microplates were subsequently incubated for an additional 30 min for colorimetric evaluation. The MIC value was determined as the concentration in the last well that exhibited no color change (remaining blue) following the incubation period (Elshikh et al., 2016).

2.2.9. Data analysis

The experiments were carried out in triplicate. The obtained results were analyzed using MS Excel 2016. Data were presented as mean \pm standard deviation. The IC₅₀ values were generated by non-linear regression of dose-response inhibition (variable slope) using GraphPad Prism (version 8.0.1, Insightful Science LLC, USA).

3. Results and discussion

3.1. Morphology of the lichen *Pyrenula* sp. and its spores

The collected lichen specimen exhibits a crustose morphology, characterized by its firm attachment to the substrate. The thallus ranges in color from grayish-white to pale yellow, with a relatively smooth surface. The perithecia are spherical, protruding from the thallus surface, and exhibit a brown to dark brown coloration. These structures are relatively uniform in size and are unevenly distributed across the thallus. Microscopic observation reveals submuriform ascospores featuring multiple transverse and longitudinal septa, which divide the spore into numerous small compartments, each containing 1–2 cells. The ascospores are ovoid to ellipsoid in shape, greenish in color, and have an average dimension of approximately 31.88 μ m \times 20.62 μ m (Figure 1).

These morphological and microscopic characteristics are consistent with the descriptions of species within the genus *Pyrenula* sp. reported in the literature (Miranda-González et al., 2022). According to Miranda-González et al. (2022), species in the *Pyrenula ochraceoflava* group typically exhibit protruding spherical perithecia on the thallus surface with a carbonized surface layer and muriform ascospores. The specimen examined in this study demonstrates high congruence with the genus *Pyrenula*. Based on the observed morphological and ascospore characteristics, the lichen specimen in this research was identified and confirmed as *Pyrenula* sp. by Dr. Vo Thi Phi Giao.

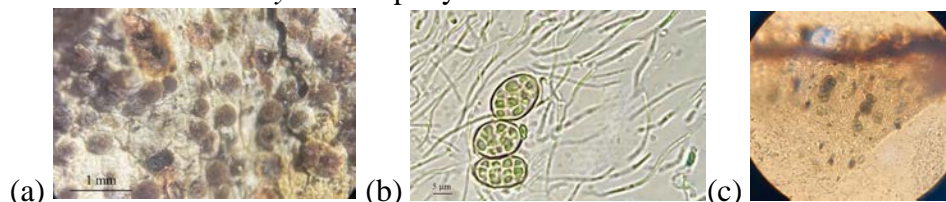


Figure 1. Microscopic characteristics of *Pyrenula* sp.: lichen thallus under the microscope (a), ascospores (b), longitudinal section of an ascoma (c)

3.2. Biological activities of *Pyrenula* sp. methanolic extract

3.2.1. Methanol extract of lichen

The study focused on *Pyrenula* sp. lichen collected directly from tree bark. After collection, the lichen thalli were separated from the bark substrate and dried, yielding 1.06 g of dry biomass. To optimize the recovery of secondary metabolites, methanol was selected as the extraction solvent due to its high polarity (Sargsyan et al., 2021). Extraction of the dry biomass resulted in 147.3 mg of crude extract, corresponding to a yield of 13.9%. This extract was subsequently utilized as the starting material for qualitative and quantitative analyses of specific compounds, as well as for the evaluation of biological activities.

3.2.2. Preliminary phytochemical screening of the methanol extract from *Pyrenula* sp.

To evaluate the biological potential of the lichen *Pyrenula* sp., this study conducted a qualitative analysis of secondary metabolite groups based on standard chemical reactions. The procedure was performed efficiently by observing color changes and precipitation characteristic of each chemical group. The results regarding the compounds present in the methanol extract are summarized in Table 2.

Table 2. Preliminary phytochemical constituents of lichens with methanol extracts

| | Alkaloid | Terpenoid | Coumarin | Quinone | Tannin | Phenol | Flavonoid |
|-----------------------------|----------|-----------|----------|---------|--------|--------|-----------|
| <i>Pyrenula</i> sp. extract | + | + | + | + | + | + | + |

Note: + indicates presence, - indicates absence

The qualitative phytochemical screening of the methanol extract from *Pyrenula* sp. (Table 2) indicates the presence of seven important groups: alkaloids, terpenoids, coumarins, quinones, tannins, phenols, and flavonoids. The presence of all these groups in the extract suggests that it contains a diverse secondary metabolite profile. These compounds have been widely documented to exhibit antimicrobial and antioxidant activities, as well as α -glucosidase inhibition (Sharma et al., 2025; White et al., 2014).

In comparison with a study of nine lichen species in the Kodagu region (India), the *Pyrenula* sp. extract showed similarities in tannins and phenolic compounds, but was distinguished by the simultaneous presence of both alkaloids and flavonoids. This represents a significant difference because none of the methanol extracts from the nine lichen species, including *Parmotrema tinctorum*, *P. grayanum*, *P. reticulatum*, *Usnea subforida*, *Flavoparmelia caperata*, *Heterodermia dissecta*, and *Leptogium burnetiae*, contained both groups (Rashmi & Rajkumar, 2014). The presence of all the aforementioned groups suggested that the *Pyrenula* sp. extract exhibits potential biological activities such as antimicrobial, antioxidant, and α -glucosidase inhibitory activities.

3.2.3. Determination of total phenolic content (TPC)

Phenolic compounds are a group of substances known for their diverse biological activities; therefore, the quantification of TPC served as a crucial indicator for evaluating the biological potential of the methanol extract from the lichen *Pyrenula* sp. To determine the TPC, gallic acid was utilized as the standard and diluted into a concentration range of 1.56-200 $\mu\text{g/mL}$ to construct a linear calibration line. The resulting linear regression equation was $y = 0.0123x + 0.0988$ ($R^2 = 0.9765$) (Figure 2).

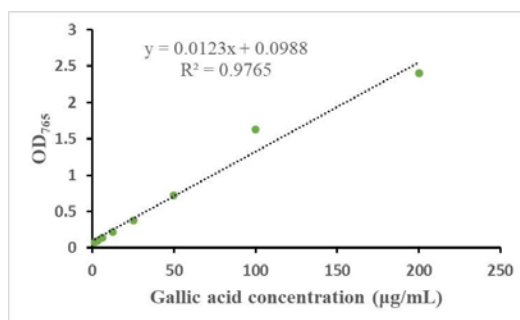


Figure 2. Gallic acid standard curve

Based on this calibration model, the TPC of the methanol extract from *Pyrenula* sp. was determined to be 30.26 ± 0.51 mg GAE/g of extract. This result is consistent with the previous qualitative screening, confirming the presence of phenolic compounds in the sample. Compared to other species, the TPC of *Pyrenula* sp. is significantly higher than that of species such as *Trypethellium virens* (0.63 mg GAE/g) or *Phaeographis dendritica* (0.46 mg GAE/g) (Pradhan et al., 2023). The high phenolic content suggests that *Pyrenula* sp. is a promising candidate for studies on antioxidant and antimicrobial activities.

3.2.4. DPPH scavenging activity of lichen extracts

The antioxidant activity was evaluated via the DPPH assay. Initial screening at 200 µg/mL yielded the radical scavenging percentage of $75.82 \pm 0.88\%$. Therefore, a concentration range of 800–1.56 µg/mL was analyzed to determine the IC_{50} value, with data modeling performed using GraphPad Prism 8.0.1 (Figure 3).

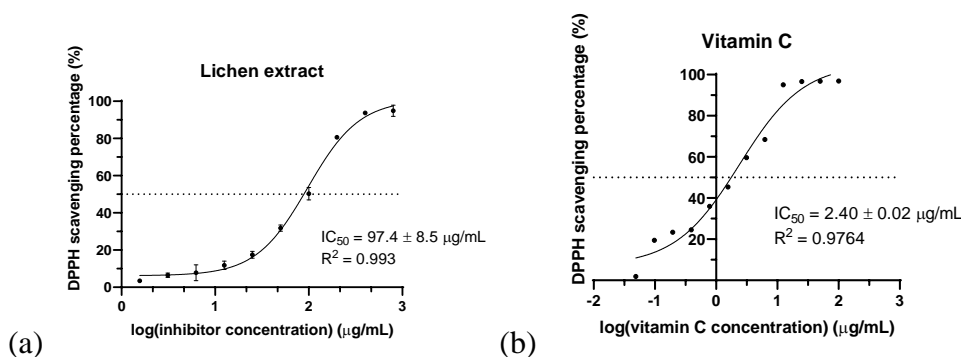


Figure 3. Non-linear regression of DPPH scavenging percentage versus log concentration for *Pyrenula* sp. extract (a), vitamin C (b)

The correlation graph was constructed with a coefficient of determination $R^2 = 0.993$. The correlation equation between the concentration and the DPPH radical scavenging rate was $y = 6.186 + \frac{94.614}{1 + 10^{(1.989 - x) \times 1.570}}$. The IC_{50} value was determined to be 97.4 ± 8.5 µg/mL, approximately 40-fold higher than vitamin C ($IC_{50} = 2.40 \pm 0.02$ µg/mL). The difference between the IC_{50} of the extract and vitamin C reflects the contrast between a crude extract and a pure compound. Despite moderate TPC, the high DPPH scavenging activity suggests the influence of other secondary metabolites, such as flavonoids. Remarkably, its antioxidant potency was significantly higher than reported values for *Myelochroa irrugans* ($IC_{50} = 384$ µg/mL) and six times higher than that of *Flavoparmelia euplecta* ($IC_{50} = 582$

$\mu\text{g/mL}$) (Fernández-Moriano et al., 2016). These results show that this species is a good source of antioxidants, suggesting it is worthy of further investigation into its broader biological applications.

3.2.5. Alpha-glucosidase inhibition of lichen extracts

The methanolic extract of the lichen was further evaluated for its α -glucosidase inhibitory activity at $200 \mu\text{g/mL}$, resulting in an inhibition rate of $99.76 \pm 0.12 \%$. Given this near-complete inhibition, the extract was subjected to a two-fold serial dilution across a concentration range of $800 - 0.78 \mu\text{g/mL}$ to determine IC_{50} . The inhibition percentages were plotted against the logarithm of the extract concentrations, and the data were analyzed using GraphPad Prism 8.0.1 (Figure 4). Commercial acarbose tablets (Domesco, Vietnam) served as the positive control.

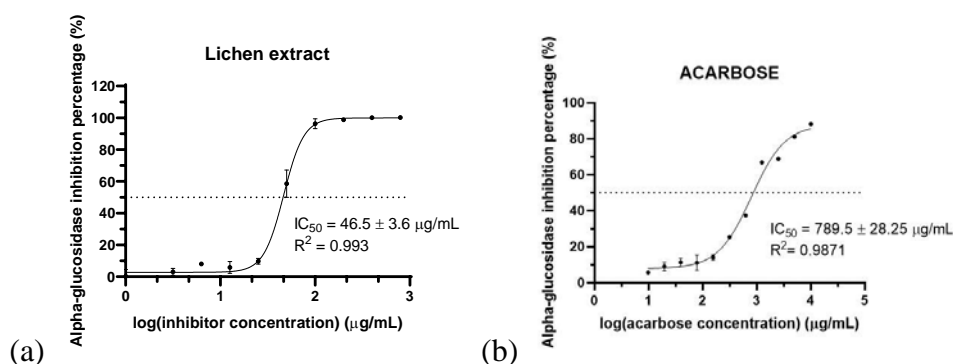


Figure 4. Non-linear regression of α -glucosidase inhibition percentage versus log concentration for *Pyrenula sp.* extract (a), and acarbose (b)

The dose-response curve was constructed with a coefficient of determination $R^2 = 0.993$. The correlation equation between the extract concentration and the α -glucosidase inhibition rate was $y = 2.784 + \frac{97.156}{1 + 10^{(1.667 - x) \times 4.084}}$. The IC_{50} value was determined to be $46.5 \pm 3.6 \mu\text{g/mL}$, indicating a significantly higher potency than acarbose ($\text{IC}_{50} = 789.5 \pm 28.25 \mu\text{g/mL}$). In this study, acarbose was used in a commercial tablet form, so the IC_{50} value recorded was relatively high due to the influence of other components present in the formulation. However, when compared with previously published IC_{50} values of pure acarbose, such as $208.53 \mu\text{g/mL}$ (Jeyakumaran et al., 2015) and $206.614 \pm 0.008 \mu\text{g/mL}$ (Torres-Benítez et al., 2023), the IC_{50} of *Pyrenula* extract in this study is still remarkably lower. The high inhibition against α -glucosidase could be explained by the presence of flavonoids in the extract. Flavonoids are a group of polyphenolic compounds known to inhibit α -glucosidase, a key enzyme involved in the hydrolysis of carbohydrates into glucose in the small intestine (Proença et al., 2017). These compounds are capable of interacting with the active site of the enzyme through hydrogen bonding and hydrophobic interactions, thereby reducing its catalytic activity (Chan & Khachigian, 2013).

The methanolic extract of *Pyrenula sp.* demonstrated α -glucosidase inhibitory activity comparable to that reported for other lichen species. For instance, the ethyl acetate (EtOAc) extract from the lichen *Usnea baileyi* exhibited α -glucosidase inhibitory activity with an IC_{50} value of $34.5 \pm 1.2 \mu\text{g/mL}$ (Do et al., 2024). In addition, the lichen extract in the present

study showed stronger inhibitory activity than the ethanol extracts from *P. contortuplicata* ($IC_{50} = 139.56 \pm 0.06 \mu\text{g/mL}$) and *U. antarctica* ($IC_{50} = 151.94 \pm 0.02 \mu\text{g/mL}$) (Torres-Benítez et al., 2023). These findings indicate that the methanol extract of *Pyrenula* sp. possess significant α -glucosidase inhibitory activity.

3.2.6. Antibacterial activity of lichen extracts

The antibacterial activity of the methanolic extract from *Pyrenula* sp. was evaluated against *Staphylococcus aureus* ATCC 6538 using the agar well diffusion method at a concentration of 10 mg/mL. The recorded inhibition zone diameter was 20.9 ± 0.1 mm (Figure 5). The presence of a distinct inhibition zone without any visible isolated colonies within the cleared area indicates that the active compounds in the extract diffused effectively through the medium and exerted a strong inhibitory effect on the growth of the target bacteria. Under identical experimental conditions, using methanol as the extraction solvent and *Staphylococcus aureus* as the test strain, the inhibition zone of *Pyrenula* sp. significantly surpassed that of *Trypethellium virens* (19 ± 0.2 mm) and *Phaeographis dendritica* (14 mm) (Pradhan et al., 2023).

The obtained results indicate that the *Pyrenula* lichen extract represents a potential natural source for the discovery of antimicrobial compounds. The superior antimicrobial capacity of the genus *Pyrenula* may be due to the presence of specific secondary metabolites, such as phenolics and terpenoids. These classes of compounds are widely recognized for their role in enhancing antimicrobial activity (Sharma et al., 2025) and were qualitatively identified in the *Pyrenula* extract.



Figure 5. Inhibition zones against *S. aureus* for *Pyrenula* sp. extract

Based on the promising antimicrobial activity observed in the agar well diffusion method, the MIC was subsequently determined to accurately evaluate the quantitative antimicrobial efficacy of the test sample. The extract was prepared in DMSO 100% and serially diluted from 1000 $\mu\text{g/mL}$ to 1.95 $\mu\text{g/mL}$ to determine the MIC. The MIC value of the extract against the *S. aureus* strain was 15.62 $\mu\text{g/mL}$ (Figure 6), indicating relatively strong antimicrobial activity.

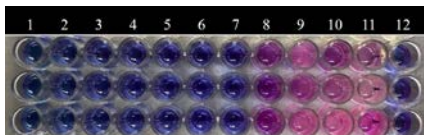


Figure 6. Colorimetric evaluation of MIC on a 96-well microplate

Columns 1-10: respectively concentrations ranging from 1000 $\mu\text{g/mL}$ down to 1.95 $\mu\text{g/mL}$;

Column 11: growth control, and Column 12: negative control

According to established criteria for natural products, an MIC < 100 $\mu\text{g/mL}$ is considered to represent significant antimicrobial activity (Kuetze, 2010). The observed activity of the

extract may be explained by the presence of the compounds identified qualitatively above, particularly phenolic and flavonoid compounds, which are known for their ability to disrupt bacterial cell membranes, denature proteins, or inhibit essential bacterial biosynthetic processes, thereby displaying a direct inhibitory effect on the target pathogen.

Besides, the extract exhibited significantly lower MIC values against the *S. aureus* strain compared to the methanolic extracts from other lichens, such as *Trypethelium virens* (MIC = 250 µg/mL), *Phaeographis dendritica* (MIC = 125 µg/mL) (Pradhan et al., 2023) and *Pamotrema reticulatum* (MIC = 125 µg/mL) (Pant et al., 2025).

4. Conclusion

The specimen was taxonomically identified as *Pyrenula* sp. based on morphological and ascospore structure analysis. Phytochemical profiling of the methanolic extract revealed a diverse range of secondary metabolites, including alkaloids, terpenoids, flavonoids, and phenolics, which likely support its observed bioactive values. The results of the biological activity assessment confirm the superior potential of *Pyrenula* sp. in inhibiting the α -glucosidase enzyme, with an IC₅₀ value of 46.5 ± 3.6 µg/mL, approximately 16 times lower than that of the commercial acarbose. Additionally, the lichen extract exhibited moderate antioxidant capacity with an IC₅₀ of 97.4 ± 8.5 µg/mL and demonstrated markedly strong antimicrobial activity against *S. aureus*, recording an inhibition zone diameter of 20.9 ± 0.1 mm and a MIC of 15.62 µg/mL. While these *in vitro* results provide a fundamental basis for the medicinal potential of *Pyrenula* sp., further cytotoxicity assays and *in vivo* models are required to confirm its safety and efficacy. Additionally, since slow natural growth leads to a challenge for sustainable collection, cultivation of the lichen mycobiont ensures a consistent supply of bioactive metabolites for future pharmaceutical research.

❖ **Conflict of Interest:** Authors have no conflict of interest to declare.

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HÀM LƯỢNG PHENOLIC TỔNG SỐ VÀ TIỀM NĂNG SINH HỌC CỦA CAO CHIẾT METHANOL TỪ ĐỊA Y *PYRENULA* SP.

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TÓM TẮT

Địa y thuộc chi *Pyrenula* có tính đa dạng cao và phân bố rộng rãi tại Việt Nam, nhưng chúng còn nhận được ít sự quan tâm trong các nghiên cứu về hóa học và hoạt tính sinh học. Do đó, sàng lọc hóa học sơ bộ cùng các hoạt tính chống oxy hóa, kháng khuẩn và ức chế enzyme α -glucosidase của địa y *Pyrenula* sp. thu tại Thành phố Hồ Chí Minh đã được khảo sát. Kết quả sàng lọc hóa học đã xác nhận sự hiện diện của alkaloid, terpenoid, coumarin, quinone, tannin, phenol và flavonoid trong cao chiết. Hàm lượng phenolic tổng số (TPC) được xác định là $30,26 \pm 0,51$ mg GAE/g cao chiết. Về hoạt tính sinh học, cao chiết thể hiện khả năng chống oxy hóa ở mức trung bình trong thử nghiệm loại gốc tự do DPPH, với giá trị IC_{50} là $97,4 \pm 8,5$ μ g/mL. Bên cạnh đó, khả năng ức chế α -glucosidase của cao chiết ($IC_{50} = 46,5 \pm 3,6$ μ g/mL) cho thấy tiềm năng trong việc hỗ trợ điều trị bệnh đái tháo đường. Thêm vào đó, cao chiết cho thấy hoạt tính kháng khuẩn mạnh đối với *Staphylococcus aureus*, được xác nhận qua đường kính kháng khuẩn đạt $20,9 \pm 0,1$ mm và giá trị MIC là 15,62 μ g/mL. Những kết quả này cho thấy địa y *Pyrenula* sp. là đối tượng đáng được tiếp tục nghiên cứu về các chất chuyển hóa thứ cấp và hoạt tính sinh học.

Từ khóa: kháng khuẩn; kháng oxy hóa; ức chế α -glucosidase; *Pyrenula* sp.; hàm lượng phenolic tổng