TẠP CHÍ KHOA HỌC
TRƯỜNG ĐẠI HỌC SƯ PHẠM TP HỒ CHÍ MINH
Tập 21, Số 3 (2024): 381-390
Website: https://journal.hcmue.edu.vn
ISSN: 2734-9918

HO CHI MINH CITY UNIVERSITY OF EDUCATION
JOURNAL OF SCIENCE
Vol. 21, No. 3 (2024): 381-390
https://doi.org/10.54607/hcmue.js.21.3.4063(2024)

Research Article

ALPHA-GLUCOSIDASE INHIBITORY AND ANTIOXIDANT ACTIVITIES OF ETHYL ACETATE EXTRACT FROM *Graphis* sp. MYCOBIONT

***Bui Anh Tuyet*[1], *Vo Thi Phi Giao*[2], *Tran Thi Minh Dinh*[1*]**

[1]*Ho Chi Minh City University of Education, Vietnam*

[2]*University of Science, Vietnam National University Ho Chi Minh City, Vietnam*

[*]*Corresponding author: Tran Thi Minh Dinh – Email: dinhttm@hcmue.edu.vn*

Received: December 16, 2023; Revised: January 16, 2024; Accepted: January 18, 2024

ABSTRACT

Lichens, formed by the symbiosis of fungi and algae or cyanobacteria, are increasingly recognized for their potential medicinal properties, including their potential to treat type 2 diabetes. Many studies have shown that the bioactive metabolites of lichens originate from their fungal partners. This study isolated the mycobiont from a Graphis lichen sample and investigated the antioxidant and α-glucosidase inhibitory activity of its ethyl acetate extract. The extract exhibited weak DPPH radical scavenging activity but a significant α-glucosidase inhibitory activity, with an IC50 value of 43.3 ± 1.9 μg/mL. The results suggest that lichens have the potential to control postprandial hyperglycemia and highlight the potential of Graphis sp. mycobionts as a promising source for the management of type 2 diabetes.

Keywords: alpha-glucosidase inhibitiom; antioxidant activity; *Graphis* sp.; mycobiont

1. Introduction

Diabetes mellitus is an endocrine metabolic disorder characterized by high blood glucose levels. The global prevalence of diabetes is rising at an alarming rate. By 2030, the number of diabetes cases will reach 578 million cases. Type 2 diabetes is the most common form of diabetes, accounting for 90 to 95% of all cases. This type of diabetes is caused by a combination of insulin resistance and impaired insulin secretion. This combination leads to hyperglycemia, a major risk factor for various health complications (Dirir et al., 2022).

α-Glucosidase inhibitors offer a noninvasive approach to suppress the elevation of postprandial blood glucose levels by temporarily delaying the intestinal absorption of carbohydrates. However, only three alpha-glucosidase inhibitory drugs, including acarbose, miglitol, and voglibose, are used in clinical practice. This has prompted the need for research

Cite this article as: Bui Anh Tuyet, Vo Thi Phi Giao, & Tran Thi Minh Dinh (2024). Alpha-glucosidase inhibitory and antioxidant activities of ethyl acetate extract from *Graphis* sp. mycobiont. *Ho Chi Minh City University of Education Journal of Science, 21*(3), 381-390.

focused on new and more efficient inhibitors (Dirir et al., 2022).

Studies and clinical evidence have revealed that the generation of reactive oxygen species increases in both types of diabetes. The elevation of reactive oxygen species, particularly mitochondrial superoxide in endothelial cells, drives the development and progression of insulin resistance and hyperglycaemia. Additionally, both hyperglycaemia and insulin resistance can also contribute to the generation of oxidative stress, which can also produce impaired insulin action, thus creating a vicious cycle. Fortunately, several studies have demonstrated that antioxidants are able to improve insulin sensitivity and offer protection against the deleterious effects of hyperglycemia. Therefore, antioxidants hold promise as a potential therapy in diabetes mellitus treatment (Sarian et al., 2017).

A lichen is a symbiotic association between a fungus and algae and/or cyanobacteria. Lichens are characterized by the production of a variety of specialized metabolites with various and significant bioactivities. Research has extensively investigated the antioxidant potential of lichens, using various assays, including DPPH radical scavenging, reducing power, superoxide anion radical scavenging, nitric oxide radical scavenging, and lipid peroxidation inhibition. Besides, several studies have highlighted the positive potential of lichens as natural antidiabetic agents (Thadhani & Karunaratne, 2017). Notably, Vietnamese lichens remain remarkably unexplored from a chemical perspective, despite some recent discoveries of new metabolites from this untapped source (Duong et al., 2018).

Although the exact process of how lichen substances are produced in the symbiotic relationship is not fully understood, it is believed that mycobiont is responsible for the production of lichen substances. Furthermore, some studies showed that cultured mycobionts are capable of producing new compounds, which are not found in the natural lichen thalli (Tanahashi & Takenaka, 2015). The α-glucosidase inhibitory and antioxidant activity of *Graphis* sp. lichen have been reported in previous studies, but research focused on the mycobionts is limited. Therefore, investigation of the antioxidant and α-glucosidase inhibitory activity of ethyl acetate extract from *Graphis* sp. mycobiont may lead to the discovery of new potential natural antidiabetic agents. Moreover, this research also paves the way for further research and development of antidiabetic therapy derived from lichen mycobionts.

2. Materials and methods

2.1. Materials

A crustose lichen sample was collected on the rosewood bark in Duc Trong District, Lam Dong Province (11°41′ 50″N, 108°18′58″E), Vietnam (600-1000 m alt.), in March 2022 (Fig 1). It was identified to be *Graphis* sp. by Vo Thi Phi Giao, Faculty of Biology and Biotechnology, University of Science, Vietnam National University-Ho Chi Minh City. The lichen sample was deposited at the University of Sciences, Ho Chi Minh City, Vietnam (registration No. UE-L010).

Figure 1. *The lichen sample used in this study and its ascospores*

2.2. Methods

2.2.1. Mycobiont isolation

The lichen mycobiont is isolated as described in previous studies (Do et al., 2022). First, the lichen sample was rinsed thoroughly with tap water for 15 minutes to remove dirt and surface debris. Next, the sample was surface disinfected by soaking in 70% ethanol for 90 seconds, followed by 4% NaOCl for 5 minutes. The sample was then rinsed three times with sterile distilled water. After that, the sample was left to dry naturally on a clean bench. Lichen specimens were thinly sliced with a sterilized razor and transferred onto a sterilized slide. Fungal spores were observed at 40X and 100X magnification. The fungal spores were then aspirated with a micropipette and transferred to a Petri dish containing MY10 medium (malt extract 10 g, yeast extract 4 g, sucrose 100 g, agar 15 g). Finally, the plates were incubated in the dark at 18^{o}C for 1 month.

2.2.2. Mycobiont culture

Lichen mycobiont was cultured in Petri dishes containing MY10 medium, at a temperature of 22 – 23^{o}C, in the dark. Mycobiont biomass was collected at 6 weeks, 8 weeks, 10 weeks, and 12 weeks after incubation.

2.2.3. Ethyl acetate extract

Mycobiont biomass was dried in an oven at 45^{o}C. Next, the fungal biomass was soaked with an equal volume of ethyl acetate solvent (AR, China). The mixture was filtered using Whatman filter paper and concentrated under low pressure in a rotary evaporator (Heidolph, Germany) to gain crude ethyl acetate extracts.

2.2.4. DPPH scavenging assay

The ethyl acetate crude extracts were diluted in methanol to the concentration of 1,000 µg/mL. Then, 200 µL of sample solution was added to each test tube, followed by 1 mL of 80 µM DPPH (TCI, Japan) solution in methanol (VWR Chemicals BDH, France). The mixture was incubated for 30 minutes in the dark at room temperature. Then, the mixture was measured at the optical density OD value at $\lambda = 517$ nm. Methanol was used as a negative control, and ascorbic acid (Merck, Germany) was used as a positive control. The antioxidant activity at different concentrations was calculated according to the formula. DPPH

scavenging rate (%) = (ODc - ODs)/ODc × 100%, with ODs: optical density value of the sample. ODc: optical density value of negative control.

2.2.5. Alpha-glucosidase inhibitory assay

The method of investigating α-glucosidase enzyme inhibitory activity was based on the literature (Do et al., 2022). Ethyl acetate crude extracts was diluted to the concentration of 200 µg/mL with 5% dimethyl sulfoxide (DMSO, Sigma-Aldrich, Germany) in 1 mM phosphate-buffered solution (pH 6.8). After that, 40 µl of enzyme (Sigma-Aldrich, Germany) and 50 µl of sample were added to each well of the 96-well plate. Then, the plate was incubated for 20 minutes at 37°C. The reaction was initiated by the addition of 40 µl of 5 mM p-nitrophenyl-D-glucopyranoside (ps-NPG, Sigma-Aldrich, Germany) followed by an additional 20 minute incubation at 37°C. Finally, 130 µl of 1 M Na_2CO_3 was added to the mixture. The enzyme activity was quantified by measuring the absorbance OD at 405 nm. DMSO 5% solution served as negative control. All experiments were carried out in triplicates. The percentage inhibition was assessed using the following formula: Inhibition percentage (%) = (ODc - ODs)/ODc × 100%, with ODs: optical density value of the sample. ODc: optical density value of negative control.

3. Results and discussion

3.1. Isolation and culture of mycobiont from Graphis sp. lichen

Mycobionts can be isolated from lichens by using ascospores, conidia, soredia, or thallus fragments. However, the isolation from ascospores was reported to be the most successful method (Zakeri et al., 2022). So, in this study, *Graphis* sp. lichen mycobiont was isolated by cultivating ascospores on MY10 medium. After incubation for 2 weeks at 18^{o}C, white fungal colonies with a diameter of about 1.4 mm appeared on the surface of the medium (Figure 2A). Since fungal colonies were grown from typical ascospores of *Graphis* lichen, and only one type of colony was obtained on all plates with 12 replicates, the fungal colony that appeared was the mycobiont of the lichen *Graphis* sp.

The fungal colonies grew slowly. The diameter of colonies increased by about 3 mm per month. After three months of culture, the colony diameter reached 10 - 12 mm. Initially, the fungal colonies were white, after one month they turned pale pink (Figure 2B), and finally turned red (Figure 2C and 2D). The surface of the colonies was rough and raised, the middle part of the colonies was concave. The lichen mycobiont grew at a very slow rate, as described in previous studies (Pichler et al., 2021).

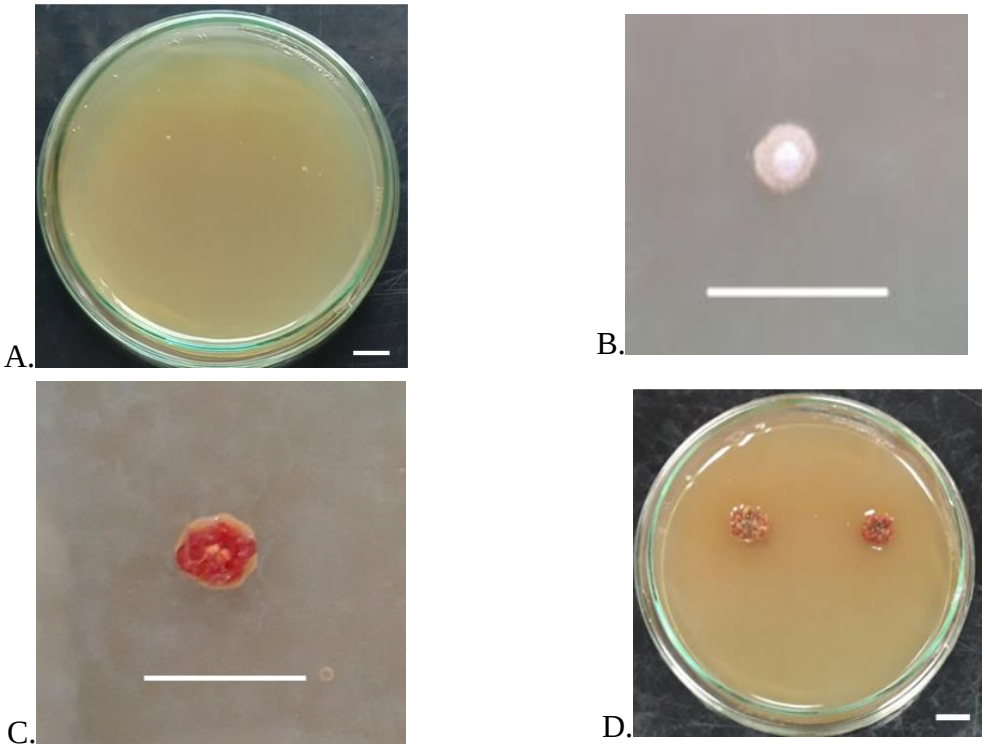

Figure 2. *Graphis sp. mycobiont colonies on MY10*
A: 2 weeks, B: 1 month, C: 2 months, D: 3 months, white bar: 1 cm

Ethyl acetate is widely used to extract compounds from lichen or lichen mycobiont. So, crude extract of mycobiont was created using ethyl acetate solvent in this study.

Figure 3. *Dried mycobiont biomass*

The dried mycobiont biomass (Figure 3) was ground and soaked in ethyl acetate. Then, the filtrate was concentrated by a rotary evaporator (Heidolph, Germany), and the sample was collected. The weight of mycobiont biomass and the corresponding ethyl acetate extract is presented in Table 1. These ethyl acetate extracts were used for biological activity assays.

Table 1. *Weigh of biomass and ethyl acetate extract from biomass of Graphis sp. mycobiont*

Culture period (week)	Biomass (g)	Weigh of ethyl acetate extract (g)
6	20.2	2.3
8	23.3	4.4
10	21.1	5.1
12	25.2	6.9

3.2. DPPH scavenging of ethyl acetate extract from the mycobiont biomass

Lichens appear to be a promising source of unique phenolic compounds, which possess significant antioxidant activity (Thadhani & Karunaratne, 2017). However, limited research has been done on the antioxidant properties of *Graphis* spp. lichen and their mycobionts were found in the extant literature. Therefore, we examined the antioxidant properties of ethyl acetate extract from *Graphis* sp. lichen mycobiont by *in vitro* DPPH assay. In the DPPH assay, the purple color solution is changed to yellow color because of the reduction of hydrogen or electron donation by the antioxidants present in the ethyl acetate extract. The antioxidant activity of ethyl acetate extracts is presented in Table 2.

Table 2. *DPPH scavenging percentage of ethyl acetate extract from biomass of Graphis* sp. *mycobiont*

Culture period (week)	DPPH scavenging (%)
6	1.9 ± 0.4
8	6.0 ± 0.6
10	14.1 ± 0.9
12	33.1 ± 1.0

As can be seen in Table 2, the DPPH scavenging activity of *Graphis* sp. mycobiont depended upon the length of the culture period. The activity gradually increased with increasing culture time. However, at the final concentration of 200 µg/mL, the highest activity of ethyl acetate extracts of *Graphis* sp. mycobiont only reached 33.1%, equivalent to DPPH scavenging activity of ascorbic acid at the concentration of 9.8 µg/mL. This result showed that the ethyl acetate extracts of *Graphis* sp. mycobiont exhibited weak antioxidant activity.

A study on *G. ajarekarii* lichen showed that the IC_{50} value of its ethanol extract in DPPH radical scavening was 109.0 µg/mL (Haritha et al., 2019). Another study showed that methanol extracts of mycobionts of *G. guimarana, G. nakanishiana,* and *G. schizograpta* exhibited weak antioxidant activity when cultured on Malt-yeast medium. However, they showed high antioxidant activity when cultured on modified Bold's basal medium. The IC_{50} values of methanol extracts of *G. guimarana, G. nakanishiana and G. schizograpta* were 19.7, 11.2, and 12.3 µg/mL respectively (Behera et al., 2006). In this study, the mycobiont was cultured on malt-yeast medium supplemented with 10% sucrose, which may be the reason why the antioxidant activity of the mycobiont was quite weak. It is necessary to search for the optimal medium for the antioxidant activity of this *Graphis* mycobiont in future studies.

3.3. Alpha-glucosidase inhibition

α-Glucosidase is a key enzyme involved in the digestion of dietary carbohydrates in humans. It hydrolyzes oligosaccharides and disaccharides into glucose, which is absorbed through the gut wall to become blood glucose. Thus, inhibition of α-glucosidase activity is viewed as one of the most effective therapeutic approaches in the reduction of glucose levels in plasma and, as a consequence, the suppression of postprandial hyperglycemia (Thadhani & Karunaratne, 2017). Some studies showed that lichen was an abundant source of α-glucosidase inhibitors. However, there has been no research on the α-glucosidase inhibitory activity of *Graphis* mycobionts. So, ethyl acetate extracts were screened for α-glucosidase inhibitory activity at the final concentration of 200 µg/mL. The results are shown in Table 3.

Table 3. *α-Glucosidase inhibition percentage of ethyl acetate extracts*

Culture period (week)	% inhibition
6	18.5 ± 1.9
8	95.7 ± 0.5
10	1.2 ± 0.6
12	51.8 ± 3.7

As can be seen in Table 3, the α-glucosidase inhibitory activity of *Graphis* sp. mycobiont fluctuated at different sampling times. Ethyl acetate extract of *Graphis* sp. mycobiont showed the highest activity at eight weeks of culture, which inhibited 95.7% of α-glucosidase activity. So, it was diluted into various concentrations by 2-fold dilution to determine α-glucosidase inhibition. IC_{50} value was determined to be 43.3 ± 1.9 µg/mL (R^2 = 0.9948) by plotting between α-glucosidase inhibition percentage and the logarithm of ethyl acetate extract concentrations using GraphPad Prism 8 software (Figure 4).

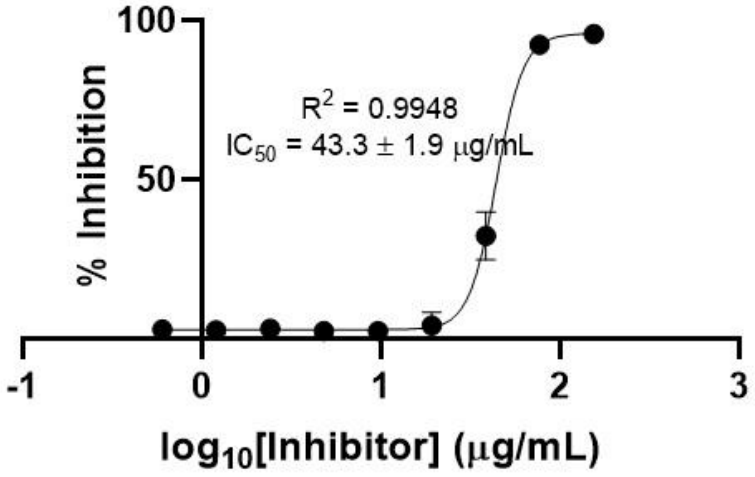

Figure 4. *The relationship between α-glucosidase inhibition percentage and logarithm of ethyl acetate extract concentrations*

The IC_{50} of ethyl acetate extract from *Graphis* sp. mycobiont was comparable with or lower than the IC_{50} of extracts from some other lichen species. Specifically, ethanolic extracts of *Ochrolechia frigida, Placopsis contortuplicata*, and *Umbilicaria antarctica* lichen had the IC_{50} was 16 ± 0.015 μg/mL, 139.56 ± 0.056, and 151.94 ± 0.022 μg/mL, respectively, lower than IC_{50} of the standard acarbose (206.604 ± 0.008 μg/mL) (Torres-Benítez et al., 2023). In another study, IC_{50} values of the ethanolic extracts of lichen species *Cladonia chlorophaea* and *C. gracilis* were 108.590 ± 0.006 and 91.323 ± 0.010 μg/mL, respectively, also lower than that of acarbose 192.8 ± 0.004 μg/mL (Torres-Benítez et al., 2023).

The IC_{50} of the crude ethyl acetate extract of *Graphis* sp. mycobiont showed that the mycobiont extracts possessed significant α-glucosidase inhibitory activity and potential for further antidiabetic research.

4. Conclusion

A *Graphis* sp. mycobiont was isolated from the lichen sample and cultured on MY10 medium. Sufficient mycelia were sampled at different culture periods to create crude ethyl acetate extract for DPPH scavenging and α-glucosidase inhibitory assay. Ethyl acetate extracts from the mycobiont biomass showed weak antioxidant activity. However, ethyl acetate extract of mycobiont biomass cultured on MY10 after eight weeks showed high α-glucosidase inhibition activity, with IC_{50} of 43.3 ± 1.9 μg/mL. Their potential antioxidant and α-glucosidase inhibitory activities suggest their potential applications in the management of type 2 diabetes. Further research efforts should focus on searching for the appropriate medium compositions and culture conditions for this mycobiont strain to produce the highest antioxidant and α-glucosidase inhibitory activities.

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

❖ ***Acknowledgement:*** *This research is funded by Ho Chi Minh City University of Education Foundation for Science and Technology under grant number CS.2022.19.13TĐ.*

REFERENCES

Behera, B. C., Adawadkar, B., & Makhija, U. (2006). Tissue-culture of selected species of the *Graphis* lichen and their biological activities. *Fitoterapia*, *77*(3), 208-215. https://doi.org/10.1016/j.fitote.2006.02.002

Dirir, A. M., Daou, M., Yousef, A. F., & Yousef, L. F. (2022). A review of alpha-glucosidase inhibitors from plants as potential candidates for the treatment of type-2 diabetes. *Phytochemistry Reviews*, *21*(4), 1049-1079. https://doi.org/10.1007/s11101-021-09773-1

Do, T.-H., Aree, T., Nguyen, H.-H., Nguyen, H.-C., Vo, T.-P. G., Nguyen, N.-H., & Duong, T.-H. (2022). Two new guaiane-sesquiterpenes from the cultured lichen mycobiont of *Diorygma pruinosum*. *Phytochemistry Letters*, *52*, 59-62. https://doi.org/10.1016/j.phytol.2022.09.005

Duong, T.-H., Beniddir, M. A., Genta-Jouve, G., Aree, T., Chollet-Krugler, M., Boustie, J., Ferron, S., Sauvager, A., Nguyen, H.-H., Nguyen, K.-P.-P., Chavasiri, W., & Pogam, P. L. (2018). Tsavoenones A–C: Unprecedented polyketides with a 1,7-dioxadispiro[4.0.4.4]tetradecane core from the lichen *Parmotrema tsavoense*. *Organic & Biomolecular Chemistry*, *16*(32), 5913-5919. https://doi.org/10.1039/C8OB01280F

Haritha, P., Patnaik, S. K., & Tatipamula, V. B. (2019). Chemical and pharmacological evaluation of manglicolous lichen *Graphis ajarekarii* PATW. & C. R. KULK. *Vietnam Journal of Science and Technology*, *57*(3), Article 3. https://doi.org/10.15625/2525-2518/57/3/13679

Pichler, G., Candotto Carniel, F., Muggia, L., Holzinger, A., Tretiach, M., & Kranner, I. (2021). Enhanced culturing techniques for the mycobiont isolated from the lichen *Xanthoria parietina*. *Mycological Progress*, *20*(6), 797-808. https://doi.org/10.1007/s11557-021-01707-7

Sarian, M. N., Ahmed, Q. U., Mat So'ad, S. Z., Alhassan, A. M., Murugesu, S., Perumal, V., Syed Mohamad, S. N. A., Khatib, A., & Latip, J. (2017). Antioxidant and antidiabetic effects of flavonoids: A structure-activity relationship based dtudy. *BioMed Research International, 2017*, Article 8386065. https://doi.org/10.1155/2017/8386065

Tanahashi, T., & Takenaka, Y. (2015). Sesquiterpene derivatives from cultured lichen mycobionts of *Diorygma* sp. *Planta Medica*, *81*(16), PM_55. https://doi.org/10.1055/s-0035-1565432

Thadhani, V. M., & Karunaratne, V. (2017). Potential of lichen compounds as antidiabetic agents with antioxidative properties: A Review. *Oxidative Medicine and Cellular Longevity*, *2017*, Article 2079697. https://doi.org/10.1155/2017/2079697

Torres-Benítez, A., Ortega-Valencia, J. E., Jara-Pinuer, N., Sanchez, M., Vargas-Arana, G., Gómez-Serranillos, M. P., & Simirgiotis, M. J. (2023). Antioxidant and antidiabetic activity and phytoconstituents of lichen extracts with temperate and polar distribution. *Frontiers in Pharmacology*, *14*, Article 1251856. http://doi.org/10.3389/fphar.2023.1251856

Torres-Benítez, A., Ortega-Valencia, J. E., Sánchez, M., Hillmann-Eggers, M., Gómez-Serranillos, M. P., Vargas-Arana, G., & Simirgiotis, M. J. (2023). UHPLC-MS chemical fingerprinting and antioxidant, enzyme Inhibition, anti-inflammatory *In silico* and cytoprotective activities of *Cladonia chlorophaea* and *C. gracilis* (Cladoniaceae) from Antarctica. *Antioxidants*, *12*(1), Article 1. https://doi.org/10.3390/antiox12010010

Zakeri, Z., Junne, S., Jäger, F., Dostert, M., Otte, V., & Neubauer, P. (2022). Lichen cell factories: Methods for the isolation of photobiont and mycobiont partners for defined pure and co-cultivation. *Microbial Cell Factories*, *21*(1), Article 80 (2022). https://doi.org/10.1186/s12934-022-01804-6

HOẠT TÍNH ỨC CHẾ ENZYME α-GLUCOSIDASE VÀ HOẠT TÍNH KHÁNG OXY HOÁ CỦA CAO ETHYL ACETATE TỪ NẤM CỘNG SINH ĐỊA Y *Graphis* sp.

Bùi Ánh Tuyết[1], Võ Thị Phi Giao[2], Trần Thị Minh Định[1*]

[1]Trường Đại học Sư phạm Thành phố Hồ Chí Minh, Việt Nam

[2]Trường Đại học Khoa học Tự nhiên, Đại học Quốc gia Thành phố Hồ Chí Minh, Việt Nam

[*]Tác giả liên hệ: Trần Thị Minh Định – Email: dinhttm@hcmue.edu.vn

Ngày nhận bài: 16-12-2023; ngày nhận bài sửa: 16-01-2024; ngày duyệt đăng: 18-01-2024

TÓM TẮT

Địa y, được hình thành do sự cộng sinh của nấm và tảo hoặc vi khuẩn lam, ngày càng được công nhận về các đặc tính chữa bệnh tiềm năng, bao gồm cả tiềm năng của chúng trong điều trị bệnh tiểu đường loại 2. Nhiều nghiên cứu đã chỉ ra rằng các chất chuyển hóa có hoạt tính sinh học của địa y do nấm cộng sinh tạo ra. Nghiên cứu này đã phân lập nấm cộng sinh từ mẫu địa y Graphis, khảo sát hoạt tính chống oxy hóa và ức chế α-glucosidase của cao ethyl acetate của nó. Cao chiết thể hiện hoạt tính loại gốc tự do DPPH yếu. Tuy nhiên, nó thể hiện hoạt tính ức chế α-glucosidase mạnh, với giá trị IC_{50} là 43,3 ± 1,9 µg/mL, cho thấy tiềm năng của nó trong việc kiểm soát tình trạng tăng đường huyết sau bữa ăn. Những phát hiện này cho thấy tiềm năng của nấm cộng sinh địa y Graphis sp. trong kiểm soát bệnh tiểu đường loại 2.

Từ khóa: hoạt tính ức chế alpha glucosidase; hoạt tính kháng oxy hoá; *Graphis* sp.; nấm cộng sinh địa y