ANTIOXIDANT, \( \alpha \)-GLUCOSIDASE INHIBITORY AND ANTIFUNGAL ACTIVITIES OF EXTRACTS FROM LICHENS COLLECTED IN VIET NAM

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ABSTRACT

Lichens are one of the earliest colonizers of terrestrial habitats on the earth. They are a unique life form of symbiosis between fungi (mycobionts) and algae and/or cyanobacteria (photobionts). Lichens have been used as a cure for diabetes, coughs, pulmonary tuberculosis, wound curing, and dermatological diseases. The aim of this study is to evaluate the antioxidant, \( \alpha \)-glucosidase inhibitory and antifungal activities of methanol extracts of lichens from eleven lichen species isolated in Viet Nam including Usnea, Lobaria, and Parmotrema were evaluated. The antioxidant activity was determined by ABTS radical scavenging activities. The results indicated that eleven species of lichens extracts possessed relatively high antioxidant activity with IC\(_{50}\) values ranging from 21.59 to 570.85 \( \mu \)g/ml for 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay. The extracts of U101 was more efficient for \( \alpha \)-glucosidase inhibitory activity, with the IC\(_{50}\) value of 75.23 ppm. In addition, all extracts also exhibited inhibitory activity on the growth of Aspergillus fumigatus (17.5 – 32.7 %) and Penicillium sp. (16.7 – 33.4 %).

Keywords: antifungal, \( \alpha \)-glucosidase inhibitory, antioxidant, lichen, methanol extract.

1. INTRODUCTION

Lichens which can inhabit in some of the most extreme environments on the earth are symbiotic organisms of fungi and algae or cyanobacteria [1]. For hundreds of years, lichens have
Antioxidant, α-glucosidase inhibitory, and antifungal activities of lichen extracts …

been used as a traditional medicine to treat several diseases such as diabetes, coughs, pulmonary tuberculosis, wound curing, and dermatological diseases in many European countries [2]. They are also used for human nutrition, animal nutrition, production of colors, perfumes, alcohols and in the medical industry [3]. The morphology, physiology, and biochemistry of lichens are very different from those of the isolated fungus and alga in culture.

There are many unique biological compounds such as usnic, acid lichesterinic and atranorin, which have been isolated from lichens. Developments in analytical techniques and experimental methods have detected the identification of about 1050 lichen substances (including those found in cultures) [4]. Lichens and their metabolites have a manifold biological activity such as antiviral, antibiotic, antitumor, allergic, plant growth inhibitory, antiherbivore, and enzyme inhibitory effects. A report of Manojlović N. et al. [5] identified protocetraric acid, usnic acid, depsidone salazinic acid, atranorin and chloroatranorin in Parmelia sp. by HPLC-UV method. Besides, this report indicated that tested lichen compounds possessed a strong antioxidant, antimicrobial, and anticancer activities [5]. The Ramalina genus determined by Antonio SNM. et al. [6] possess more than 153 compounds, and can be considered as excellent antimicrobial, antitumor and anti-inflammatory agents. According to a report of Marijana MK. et al. [1], Parmelia caperata, Parmelia sulcata, and Parmelia saxatilis exhibited a high anticancer activity with IC₅₀ values ranging from 9.55 to 22.95 µg/ml. Lichens are an untapped source of bioactivities because their potential applications are not yet fully explored and utilized. Studies for lichens in Viet Nam had only limited to isolation, diversity or morphology. Research of bioactivities, biological constituents and applications of lichens have not still been interested, although Viet Nam is a tropical nation with a great lichen biodiversity. The aim of this study was to antioxidant, α-glucosidase inhibitory and antifungal activities of eleven species of Vietnam lichens extracts selected from Bidoup-Nui Ba National Park, Lam Dong province, Vietnam.

2. MATERIALS AND METHODS

2.1. Materials

Eleven lichen samples were collected from Bidoup-Nui Ba National Park, Lam Dong province, Vietnam. The lichens were identified and classified by morphological method using a stereomicroscope, light microscope, and chemically with the help of color reactions, UV light and standardized thin-layer chromatography (TLC) [7, 8]. Candida albicans (Ca), Microsporum gypseum (Mg), Trichophyton rubrum (Tr), Trichophyton mentagrophytes (Tm), Aspergillus niger (An), Aspergillus fumigatus (Af), Penicillium sp. (Pe), and Fusarium sp. (Fs) used in antifungal assay were obtained from Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Medicine and Pharmacy HCMC, Viet Nam, ABTS (2,2-azinobis-3-ethylbenzothiazolin-6-sulfonic acid) (Sigma), p-nitrophenyl-α-D-glucopyranoside (pNPG) (sigma). Other chemicals and reagents were of analytical grade.

2.2. Methods

2.2.1. Extract preparation

The lichen samples were dried at 37 °C and then stored in paper bags at room temperature with active ventilation. After that, dry ground thalli of the investigated lichens were powdered and extracted for 24 h with methanol at a ratio of thallus/methanol (1:10 w/v). The extracts were
filtered and then concentrated under reduced pressure in a rotary evaporator (IKA, Germany). The concentrated extracts were stored at −18 °C until use.

2.2.2. Determination of the antioxidant activity

The measurement of ABTS radical scavenging activity was used with some modifications [9]. Seven micromolar solution of ABTS was mixed with 2,45 mM potassium persulphate solution. The reaction mixture was left to settle at room temperature for 12-16 h in the dark before using. ABTS solution was diluted with phosphate buffer to adjust its absorbance to within 0.70 ± 0.02 at 734 nm. Then, three milliliters of ABTS solution was mixed with 100 µl of various concentrations of samples. Finally, the absorbance was measured at 734 nm after reaction at room temperature for 30 minutes. The free radicals scavenging activity was calculated by the following equation: (1 − A/A0) × 100 %. Where A and A0 are the absorbance values in the presence and absence of the test samples, respectively. Each experiment was carried out in triplicates and consequences were exhibited as mean % antioxidant activity ± SD. The concentration of 50 % inhibition (IC$_{50}$ value) based on the percentage of ABTS radical scavenging activity was calculated from the plotted graph of the means at the concentrations of the samples. Vitamin C was considered as a positive control.

2.2.3. Determination of the α-glucosidase inhibitory activity

Assay for α-glucosidase inhibition was performed by slight modification of a previously published method [10]. Briefly, solutions of α-glucosidase (from lichen extracts) and its substrate (p-nitrophenyl α-D-glucopyranoside pNPG) were prepared in phosphate buffer (100 mM, pH 6.9). 5% DMSO was used as a preferred solvent for preparation of inhibitor solutions. The inhibition assays were conducted by adding inhibitor solution (50 µL) to 40 µl of enzyme solution (0.2 unit/ml) in 100 mM phosphate buffer (pH 6.8) followed by room temperature for 20 min. After pre incubation, 40 µl of 3 mM substrate (pNPG) prepared in phosphate buffer was added to the mixture to initiate enzymatic reaction. The reaction mixture was incubated at room temperature for 30 min, and the reaction was stopped by addition of 130 µl of 0.2M Na$_2$CO$_3$. Acarbose was used as a positive control. The α-glucosidase activity was determined by measuring the p-nitrophenol released from pNPG at 405 nm using an Elx 800 Micro plate reader. The % inhibition was calculated using the following equation: Inhibition (%) = [1 − (A$_{sample}$/A$_{control}$)] × 100 %. IC$_{50}$ values of potent inhibitors were determined by testing 5 serial dilutions of inhibitors and were calculated by using the program Microsoft Excel.

2.2.4. Determination of the antifungal activity

The antifungal activity of the extract was screened by disc diffusion method [11]. Here, 20 ml quantities of Muller Hinton Agar (for Candida albicans) and Sabouraud dextrose (for other fungi) were plated in petri dishes. Extracts were dissolved in 5 % DMSO (Prolabo, France) to a concentration of 10 mg/ml. Each well had been previously impregnated with 50 µl of 10 mg/ml extract solutions were laid on the inoculated media. Fluconazole (25 µg/disc) and Terbinafine (30 µg/disc) were considered as a positive control for Candida albicans and other fungi respectively. The 5 % DMSO was considered as a negative control. Each sample was used in triplicate for the determination of antifungal activity. The inhibitory rate was calculated by the following equation: I (%) = 100 - (R1/R2) × 100 %. Where I is the inhibitory rate of fungal...
growth, R1 and R2 are radii of inhibition zones when are presence and absence of the test samples, respectively.

3. RESULTS AND DISCUSSION

3.1. Morpho-anatomical features of the identified lichen species

The collected lichen specimens from Bidoup Nui Ba National Park were identified using standardized analytical techniques, significant lichen identification keys and monographs [7, 8, 12, 13, 14, 15]. Wide variations in the parameters were observed among the investigated lichen species. Eleven lichen species were identified, including L5: Parmotrema tinctorum (Delise ex Nyl.) Hale; L100: Lobaria pulmonaria (L.) Hoffm; U2: Usnea rubicunda Stirt.; U15: Usnea glabrescens (Nyl. ex Vain.) Vain; U18.6: Usnea baileyi (Stirton) Zahlbr.; U29: Usnea fulvoreagens (Rasanen) Rasanen; U31: Usnea nipparensis Asahina; U38.1: Usnea flammea Stirt.; U41.3: Usnea rubrotincta Stirt.; U100: Usnea pectinata Stirt.; U101: Usnea schadenbergiana Göpp. & Stein.

3.2. Determination of the antioxidant activity

The results of ABTS inhibition assay using different extracts are shown in Table 1. It revealed that the methanol lichen extracts possessed ABTS radical scavenging activities. The extracts from U29 and U100 showed a wide range of ABTS radical scavenging activity (IC\(_{50}\) = 21.59 ± 3.64 and 21.59 ± 0.33 µg/ml, respectively) which was lower than the standard antioxidants, ascorbic acid (IC\(_{50}\) = 30.86 ± 0.05 µg/ml).

Table 1. IC\(_{50}\) values of extracts in ABTS free radical scavenging assays of the examined extracts.

| Samples | IC\(_{50}\) values (µg/ml) |
|---------|--------------------------|
| U2      | 221.11 ± 2.47            |
| U15     | 464.04 ± 3.25            |
| U18.6   | 482.23 ± 87.99           |
| U29     | 21.59 ± 3.64             |
| U31     | 349.47 ± 7.13            |
| U38.1   | 211.99 ± 3.34            |
| U41.3   | 570.85 ± 8.87            |
| U100    | 21.59 ± 0.33             |
| U101    | 351.83 ± 20.20           |
| L5      | 249.23 ± 17.69           |
| L100    | 221.91 ± 2.54            |

Lichens have been widely used as traditional medicines for a great many years. There are some reports which have shown that lichens possess antioxidant and antifungal activity [1, 2]. Lichen components have been deemed as important natural antioxidants agents presenting in
plants and foods. The results in the current study showed that the methanol extracts containing high antioxidant activities (Table 1). Branislav R Ranković et al. [16] indicated a contrary consequence that tested extracts exhibited the highest radical scavenging activity with the greatest amount of phenolic and flavonoid contents. According to the report of Nedeljko TM et al. [17], antioxidant property of the lichen could be attributed to the significant amount of depsidones, especially salazinic acid. Other minor phenolic compounds should not be neglected since synergy of the different chemicals with each other should be taken into consideration for the biological activity.

3.3. Determination of the α-glucosidase inhibitory activity

The in vitro α-glucosidase inhibitory activity of methanol extracts of lichen was tested. Varying concentration of the extract 400, 500, 750 and 1000 ppm were taken and assayed for identifying the IC$_{50}$. Results indicated that the extract of U101 was the most efficient for α-glucosidase inhibitory activity, with the IC$_{50}$ value of 75.23 ppm. All methanol lichen extracts showed the IC$_{50}$ values from 75.23 ppm (U101) to 712.51 ppm (L5) (Figure 1). The extract of lichen sample U43.1, U31 and U29 also showed significant inhibitory activity of 104.84, 140.66 and 150.21 ppm in comparison with the commercial drug acarbose (0.2 unit/ml) which showed the IC$_{50}$ value of 3597.12 ppm. From the result, it is evident that methanol extracts of lichen own the significant α-glucosidase inhibitory activity comparable to the commercial drug acarbose. α-glucosidase is one of the numbers of glucosidases located in the brush border surface membrane of intestinal cells and is a key enzyme of carbohydrate metabolism [18]. From the results, it can be concluded that methanol extracts of lichen be excellent alternative of α-glucosidase inhibiting drug and can thus reduce the rate of digestion and absorption of carbohydrates.

Figure 1. The values of IC$_{50}$ for lichen extracts against α-glucosidase.

3.4. Determination of the antifungal activity

The antifungal activity of extracts was quantitatively assessed by the presence or absence of clear zones indicating inhibition zones as given in Table 2. No activity was recorded against Candida albicans (Ca), Trichophyton rubrum (Tr), Trichophyton mentagrophytes (Tm), Aspergillus niger (An), and Fusarium sp. (Fs). Methanol extracts of the lichen had the greatest effect on plates inoculated with Penicillium sp. (Pe) with a percent of inhibition ranging from 16.7 % to 35.6 %. Most of methanol lichen extracts were showing activity against Aspergillus fumigatus (Af), especially the extracts of U101. The inhibitory percentage of this extract was
32.7 % for Aspergillus fumigatus. Besides, the extracts of U29, U31, and U100 showed a weak inhibition against Microsporum gypseum (Mg) with the inhibitory percentage from 4.2 to 10.4 %. This result indicated that the extracts of Usnea species showed strong activity against Aspergillus fumigatus (Af), Penicillium sp. (Pe), Microsporum gypseum (Mg) compared to other species extracts. Additionally, the growth of both Penicillium sp. (Pe) and Aspergillus fumigatus (Af) were significantly inhibited by U15, U29 and U101 extracts. The results in the current study showed that the methanolic extracts from U29 and U101 which were demonstrated strong antioxidant activity inhibited dramatically the growth of both Penicillium sp. (33.4 and 25.6 % for percent of inhibition) and Aspergillus fumigatus (25.0 and 32.7 % for percent of inhibition).

Table 2. Fungal-growing inhibitory rate (%) of extracts.

| Samples | Af  | Pe  | Mg  | Ca  | Tm  | Tr  | An  | Fs  |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|
| U2      | 24.5| 23.3| -   | -   | -   | -   | -   | -   |
| U15     | 25.0| 33.4| -   | -   | -   | -   | -   | -   |
| U18.6   | 28.9| 25.6| -   | -   | -   | -   | -   | -   |
| U29     | 25.0| 33.4| 4.2 | -   | -   | -   | -   | -   |
| U31     | 22.5| 31.2| 4.2 | -   | -   | -   | -   | -   |
| U38.1   | 23.1| 25.6| -   | -   | -   | -   | -   | -   |
| U41.3   | 20.0| -   | -   | -   | -   | -   | -   | -   |
| U100    | 17.5| 31.1| 10.4| -   | -   | -   | -   | -   |
| U101    | 32.7| 25.6| -   | -   | -   | -   | -   | -   |
| L5      | 26.7| 16.7| -   | -   | -   | -   | -   | -   |
| L100    | 22.2| 19.1| -   | -   | -   | -   | -   | -   |

It was observed that extracts behaved differently in their antibacterial effectiveness depending on species. Take some reports to illustrate, the methanolic extract from Umbilicaria cylindrica, Cladonia furcata, Tonia candida, Usnea barbata and Lecanora atra showed a relatively strong inhibitory activity in Candida albicans (MIC- Minimum inhibitory concentration = 31.25, 6.26, 12.5, 6.25, and 12.5 µg/ml, respectively) [2, 17]. Branislav Ranković et al. [2] suggested that differences in antimicrobial activity are probably a consequence of the presence of diverse components with varying antimicrobial activity. However, it is necessary to understand that extracts are mixtures of natural compounds, and their antimicrobial activity is not only a result of the different activities of the individual components but may also be the result of their interactions, which can cause different effects on the overall activity of the extracts [2].

4. CONCLUSION

The present study provides results of supporting the use of lichen extracts as natural antioxidant, α-glucosidase inhibitory and antifungal agents. Based on these results, lichens appear to be good natural antioxidant and antifungal resources and also, could be of significance in the food industry and in the control of various human, animal diseases. Future investigation will be focused on determination of their biological activities in vitro and in vivo. Moreover,
there need to be further studies on relationships between biological activities and biological constituents.

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TÓM TÁT

HOẠT TÍNH KHẢNG OXY HÓA, ỨC CHẾ α-GLUCOSIDASE, KHÁNG NĂM CỦA CAO CHIẾT ĐỊA Y THU THẤP TẠI VIỆT NAM

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Địa y là một trong số những sinh vật tiền phong ở can trên trái đất. Chúng là dạng sống cổ sinh giữa nam và tạo/học vi khuẩn lam. Từ lâu, địa y đã được sử dụng trong thành phần thuốc để điều trị tiêu đường, ho, lao phổi, chữa các vết thương và bệnh ngoài da. Mục tiêu của nghiên cứu này là đánh giá hoạt tính kháng oxy hóa, ức chế enzyme α-glucosidase, kháng nấm của 11 cao chiết methanol địa y thuộc chi Usnea, Lobaria, Parmotrema. Hoạt tính kháng oxy hóa được xác định bằng phương pháp bắt gốc tự do ABTS. Kết quả chỉ ra 11 cao chiết địa y có tiềm năng kháng oxy hóa với giá trị IC50 từ 21.59 đến 570.85 µg/ml. Cao chiết U101 ức chế mạnh enzyme α-glucosidase, với giá trị IC50 75.23 ppm. Bên cạnh đó, tất cả các cao chiết ức chế sự phát triển của nấm Aspergillus fumigatus (17.5 – 32.7 %) và Penicillium sp. (16.7 – 33.4 %).

Từ khóa: địa y, kháng oxy hóa, ức chế enzyme α-glucosidase, kháng nấm, cao chiết methanol.