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In vitro antioxidant activities and total phenol content of different extracts of the fungus *Ulocladium chartarum* isolated from Draceana leaf

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Abstract

The objective of this study is to investigate the total phenol content and *in vitro* antioxidant potential of the different extracts of the fungal strain *Ulocladium chartarum*, isolated from *Draceana* leaf. The fungal culture was extracted with ethyl acetate (EtOAc) followed by methanol (MeOH). The antioxidant activities of the extracts were tested using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and phosphomolybdate complex assays, while total phenol content was evaluated by Folin-Ciocalteu method. The MeOH extract of *Ulocladium chartarum* displayed higher free radical scavenging activity (46.98% in DPPH assay) and antioxidant activity (28.86 mg ascorbic acid equivalent/g dried extract employing phosphomolybdate assay) in comparison to the EtOAc extract that displayed (11.63%) in DPPH assay and (9.5 mg ascorbic acid equivalent/g dried extract) in phosphomolybdate assay. It is noteworthy that a direct correlation between the total phenol content and the antioxidant activity was observed.

Key words
antioxidant activities, total phenol content, DPPH assay

1. Introduction

Phytochemical investigation of the metabolites of the fungal genus *Ulocladium* led to isolation of several classes of secondary metabolites exhibiting different biological activities [1, 2]. The endolichenic fungus *Ulocladium sp*. isolated from the lichen *Everniastrum sp.* was reported to produce ophiobolane sesterterpenes [3], polyketides [1] and tricycloalternarenes [4] upon cultivation on different culture media according to OSMAC (One Strain Many Compounds) approach. The soil-derived fungal strain *Ulocladium sp.* HKI 0226 produced (–)-terpestacin (a compound that inhibited syncytium formation by cells infected with respiratory syncytial virus (RSV)) and the phytotoxin L-tenuazonic acid. [5] The antifungal alkaloid piperine was recovered from the mycelium of *Ulocladium sp.*[6] while infectopyrone was detected in *Ulocladium consortiale*. [7] An antifungal cyclopeptolide was isolated from saprophytic fungus *Ulocladium atrum* Preuss.[2] The fungus *Ulocladium chartarum* yielded the compounds ulocladol A and B that have a mixed sesquiterpenoid-polyketide nature. [8]

Endophytes are microorganisms, either fungi or bacteria, that live inside healthy plant tissues without causing any infectious symptoms [9]. Endophytic fungi are considered a promising source of natural antioxidants [10]. The isolation of phenolic compounds from the endolichenic fungus *Ulocladium sp*. [1] provoked us to investigate the total phenol content of the EtOAc and methanolic extracts of the fungus *Ulocladium chartarum* belonging to the same genus and isolated from *Draceana* leaf. In addition, the antioxidant activity of the extracts were investigated using two different methods, DPPH and phosphomolybdate complex assays.

2. Experimental Protocol

2.1. General experimental procedures

The UV-Visible Spectrophotometer (SPECTRONIC ® GENESYS 2PC UV, USA) was used to measure the absorbance, while the rotary evaporator (HAHNSHIN, Korea) was used for evaporation of the solvent off the obtained extracts. All solvents were distilled before use. Sterilization of the culture media was performed in the autoclave (Raypa®, Spain).

2.2. Isolation of fungal material

The fungal strain *Ulocladium chartarum* (Preuss) E.G. Simmons was isolated from the leaves of *Dracaena sp*. collected from Alexandria Governorate, Egypt in 2010. The plant material was rinsed with sterilized water then surface sterilized with 70% ethanol for 1-2 minutes and ultimately air dried under a laminar flow hood. Dissection of the plant material was done with a sterile scalpel under sterile conditions. The obtained small pieces were placed onto Potato Dextrose agar plates(200 g fresh potato tubers; dextrose 10 g; agar 15 g and distilled water up to 1 L) [11]. The plates were left for 3 weeks at 28 °C to allow the growth of the endophyte, and then hyphal tips of the fungus were removed and transferred to fresh

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Potato Dextrose agar medium for purification of the culture. The pure endophyte was obtained by repeated inoculation. The pure fungal strain was grown on Potato Dextrose Agar medium in Petri dishes and kept at 28 °C for 21 days. The fungus was identified according to conidial morphology [12]. A Subculture of the fungal strain *Ulocladium chartarum* was deposited in the culture collection of the Assiut University Mycology Centre (AUMC) under voucher number 6866.

2.2.1. Fermentation of the fungus

Cultivation of the pure fungal strain was carried out in Erlenmeyer flasks (1 L) on solid rice medium. Commercially available rice (100 g) in addition to 100 mL of distilled water were added to each flask and kept overnight before autoclaving. Inside the laminar flow cabinet, small pieces (1 cm × 1 cm) of the fungus growing on agar were inoculated to the autoclaved flasks. The fungus was grown aseptically under static conditions at room temperature for four weeks.

2.2.2. Extraction of fungal cultures

The growth of the fungal culture in each flask was stopped by adding 300 mL of EtOAc and was left overnight. The media was then cut into pieces to facilitate extraction of the produced fungal metabolites. After that, the flasks were filtered and fresh EtOAc was added to repeat extraction till exhaustion (3 x 300 mL). The obtained EtOAc filterate was evaporated under vacuum at 45 °C to get rid of the solventand obtain the crude EtOAc extract (4 g). Following extraction with EtOAc, the cultures undergone exhaustive extraction with 70% MeOH (3 x 300 mL). The combined MeOH filterates were also dried under vacuum to yield the crude methanolic extract (7 g).

2.3. Determination of total phenolic content

The obtained EtOAc and methanolic extract of the fungus were assayed for their total phenolic content employing Folin-Ciocalteu method. [13] The reaction was performed by adding 50 µl Folin-Ciocalteu reagents (2N), 300 µl of sodium carbonate (10 %) and 3.5 ml of deionized water to 50 µl of fungal extract (10 mg/ml). The mixture was kept at room temperature for 30 minutes in the dark. The absorbance of the developed color was measured at 730 nm by means of a UV-vis spectrophotometer. The same procedure was performed for gallic acid which was used as a standard for calibration curve. The blank was prepared by mixing all reagents except for the extract which was replaced with methanol. All measurements were carried out in triplicates and the results were expressed in gallic acid equivalent in mg/g dried extract.

2.4. Antioxidant assays

Antioxidant activities of the fungal extracts were evaluated using two different methods; DPPH and phosphomolybdate assays.

2.4.1. DPPH radical scavenging activity assay

Free radical scavenging activity of the different fungal extracts was tested using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) method. [14] A volume of 2 ml of DPPH methanolic solution (0.1 mM) was added to 200 µl of each extract (10mg/ml). The mixture was then shaken well and left to stand for 15 min at room temperature in the dark. For preparation of the control solution, methanol was added instead of the tested extract. The absorbance of each solution was measured at 517 nm. All determinations were carried out in triplicates. Free radical scavenging activity was expressed as a percentage according to the following equation:

DPPH scavenging effect (%) = $[(A_0 - A_1/A_0)] \times 100$
where A_0= the absorbance of the control
and A_1= the absorbance in the presence of the extract

2.4.2. Phosphomolybdate assay (total antioxidant capacity)

Phosphomolybdate method was used to determine the total antioxidant capacity of the extracts employing ascorbic acid as a standard. [15] The reaction was done by mixing 0.3 ml of sample extract (10 mg/ml) solution with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were sealed and incubated in a water bath at 95 °C for 90 min. After that, the tubes were left to cool to room temperature and the absorbance was measured at 695 nm against a blank. The blank, (3 ml of reagent solution in addition to 0.3 ml of methanol), was incubated under the same conditions. All measurements were done in triplicates. Ascorbic acid was used as a standard for plotting the calibration curve. Therefore, the antioxidant activity was expressed relative to that of ascorbic acid.

2.5. Statistical analysis

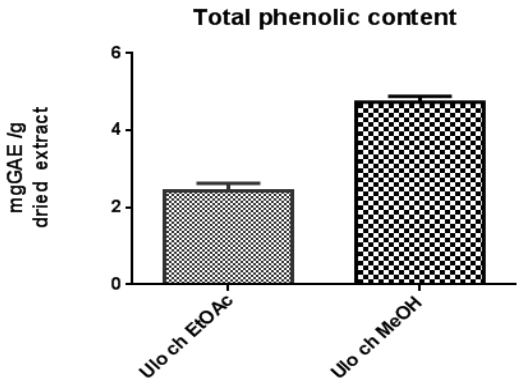
Data are expressed as mean ± SEM and analyzed by one-way analysis of variance (ANOVA) test using the Graph Pad Prism 6 software (Version 6.00 for Windows, GraphPad Software, San Diego California USA, *www.graphpad.com*).

3. Results and Discussion

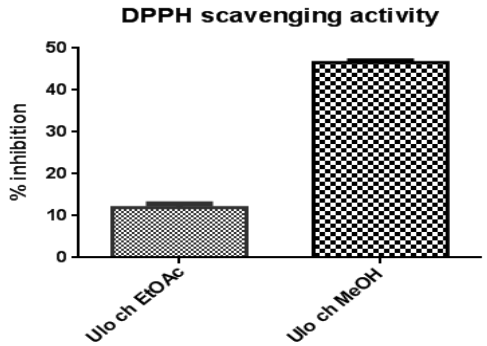
Investigation of their total phenolic content of the EtOAc and methanolic extract of the fungus *Ulocladium chartarum* employing Folin-Ciocalteu method revealed that the methanolic extract had higher total phenolic content (4.74 mg GAE/g dried extract) than the EtOAc extract (2.28 mg GAE/g dried extract) (**Figure 1**). Moreover, estimation of in vitro antioxidant potential of both extracts by DPPH radical scavenging activity assay displayed the higher free radical scavenging activity of the methanolic extract (46.98%) than the EtOAc extract (11.63%) (**Figure 2**). The finding that was further confirmed by the phosphomolybdate assay where the methanolic extract showed stronger reducing power (28.86 mg ascorbic acid equivalent/g dried extract) than the EtOAc extract (9.5 mg ascorbic acid equivalent/g dried extract) (**Figure 3**). A direct correlation between the total phenol content and the antioxidant activity

was observed. Therefore, this antioxidant activity may be attributed to the phenolic constituents of the fungal extract.

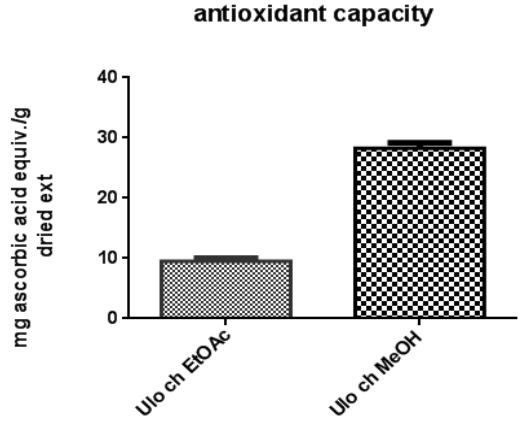
It is well known that oxidative damage caused by reactive oxygen species is the main cause of many degenerative diseases such as aging, arthritis, cancer, inflammatory disorders, neurodegenerative diseases (as Alzheimer) and atherosclerosis [16, 17]. Hence, further investigation of the methanolic extract is suggested to be done in search for natural antioxidant compounds that may act as candidates in treatment of such diseases.



(Figure 1)



(Figure 2)



(Figure 3)

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