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Total phenol content and antioxidant activities of the fungus *Ulocladium botrytis*

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Abstract

The objective of this work was to determine the total phenol content and antioxidant activities of the fungus *Ulocladium botrytis Preuss* isolated from *Draceana* sp. leaves. Extraction of the fungal culture was carried out with ethyl acetate (EtOAc) followed by methanol (MeOH). The different extracts (EtOAc and MeOH) were tested for their in vitro antioxidant activities using two different methods, the 1,1-diphenyl-2-picryl-hydrazyl(DPPH) and the phosphomolybdate complex assays, while their total phenol contents were evaluated by the Folin-Ciocalteu method. The EtOAc extract displayed higher amount of total phenol content as well as higher antioxidant potential than the MeOH extract.

Key words
total phenol content, antioxidant activities, DPPH assay

1. Introduction

Ulocladium Preuss (Family: Pleosporaceae) [1] is a fungal genus comprised of various species that have different ecological roles and biotechnological applications. Some species lead to plant diseases for instance cucumber leaf spot [2] and some are known to cause food spoilage for example destroying nuts [3] while some species are employed as bio-control agents [4]. *Ulocladium* also has been utilized as enzyme producer [5, 6]. Investigation of the secondary metabolites of genus *Ulocladium* led to characterization of several classes of secondary metabolites showing different biological activities. The compound piperine, isolated from the mycelium of *Ulocladium* sp., displayed antifungal activity [7]. Moreover, an antifungal cyclopeptide was isolated from the saprophytic fungus *Ulocladium atrum Preuss* [8] The bioassay guided isolation of secondary metabolites from the sponge-derived fungus *Ulocladium botrytis* led to separation of the tyrosine kinase inhibitor ulocladol together with an antifungal xanthone [9].

In the present study, the EtOAc and MeOH extracts of the fungal strain *Ulocladium botrytis* Preuss were assayed for their total phenol contents in addition to their in vitro antioxidant activities.

2. Experimental Protocol

2.1. General experimental procedures

Absorbances were measured by the UV-Visible Spectrophotometer (SPECTRONIC ® GENESYS 2PC UV, USA), while the rotary evaporator (HAHNSHIN,Korea) was used for evaporation of the solvent of the obtained extracts. All solvents were distilled prior to use. The autoclave (Raypa®, Spain) was used for sterilization of the culture media.

2.2. Fungal material

The fungal strain *Ulocladium botrytis* was isolated from the leaves of *Dracaena* sp. that was collected from Alexandria Governorate, Egypt in 2010. The pure fungal strain was grown on Potato Dextrose Agar medium (fresh potato tubers (200 g); dextrose (10 g); agar (15 g) and distilled water up to 1 L) in Petri dishes and kept at 28 °C for 21 days.

The fungus was identified according to conidial morphology. [10] Subculture of the fungal strain *Ulocladium botrytis* was deposited in the culture collection of Assiut University Mycology Centre (AUMC) under the voucher number 6837.

2.2.1. Large scale cultivation of the fungus

Fermentation of the pure fungal strain was carried out in Erlenmeyer flasks (1 L) on a solid rice medium. Twenty Erlenmeyer flask were utilized. Commercially available rice (100 g) in addition to 100 mL of distilled water was added to each flask and kept overnight before autoclaving.

Small pieces (1 cm × 1 cm) of the fungus growing on agar were inoculated to the autoclaved flasks. The fungus was grown 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 left overnight. The media were 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. The

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obtained EtOAc filtrate was evaporated under vacuum to get rid of the solvent and obtain the crude EtOAc extract (48 g). Following extraction with EtOAc, the cultures were exhaustively extracted with MeOH (3x300 mL). The combined MeOH filtrates were also dried under vacuum to yield the crude methanolic extract (70g).

2.3. Determination of total phenolic content

The obtained EtOAc and methanolic extracts of the fungus were assayed for their total phenolic contents employing the Folin-Ciocalteu method [11]. The reaction was performed by adding 50 µL Folin-Ciocalteu reagent (2N), 300 µL of sodium carbonate (10 %) and 3.5 mL of deionized water to 50 µL of the fungal extract (10 mg/mL). The mixture was kept at room temperature for 30 min in the dark. The absorbance of the developed color was measured at 730 nm by means of a UV-visible spectrophotometer. The same procedure was performed for gallic acid which was used as a standard for preparation of the calibration curve. The blank was prepared by mixing all reagents except for the extract, which was replaced with MeOH. All measurements were carried out in triplicate 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; phosphomolybdate and DPPH assays.

2.4.1. Phosphomolybdate assay (total antioxidant capacity)

The phosphomolybdate method was used to determine the total antioxidant capacity of the extracts employing ascorbic acid as a standard[12]. The reaction was done by mixing 0.3 mL of sample extract (10mg/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 triplicate. Ascorbic acid was used as a standard for plotting the calibration curve. Therefore, the antioxidant activity was expressed relative to that of ascorbic acid.

2.4.2. DPPH radical scavenging activity assay

The free radical scavenging activity of the different fungal extracts was tested using the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) method.[13] A volume of 2 mL of DPPH methanolic solution (0.1 mM) was added to 200 µL of each extract (10 mg/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 triplicate. The 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

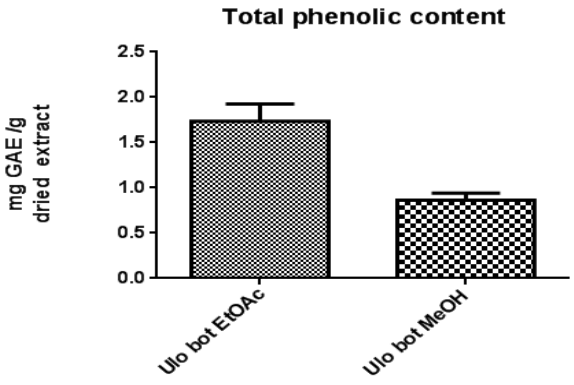
2.5. Statistical analysis

Data were expressed as mean ± SEM, and analyzed by the 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*). Correlation coefficients (R) to determine the relationship between two variables were calculated using MS Excel software (CORREL statistical function).

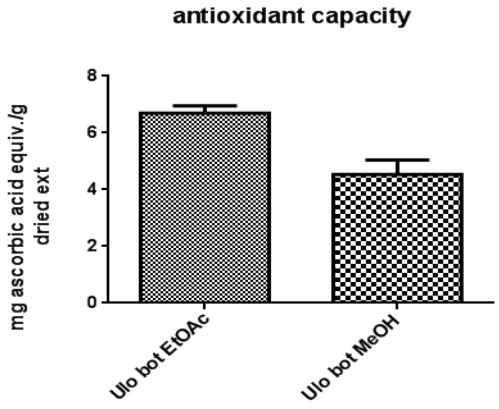
3. Results and Discussion

Determination of the total phenol contents of both the EtOAc and MeOH extracts of the fungus *Ulocladium botrytis* utilizing the Folin-Ciocalteu method displayed that the EtOAc extract had higher amount of total phenols (1.54 mg GAE/g dried extract) than the MeOH extract (0.88 mg GAE/g dried extract) (**Figure 1**). Furthermore, estimation of their in vitro antioxidant potential of both extracts employing the phosphomolybdate assay revealed the higher antioxidant potential of the EtOAc extract (6.17 mg ascorbic acid equivalent/g dried extract) compared to that of the MeOH extract (4.62mg ascorbic acid equivalent/g dried extract) (**Figure 2**). However, only the EtOAc extract showed radical scavenging activity through the DPPH assay (4.41%) (**Figure 3**).

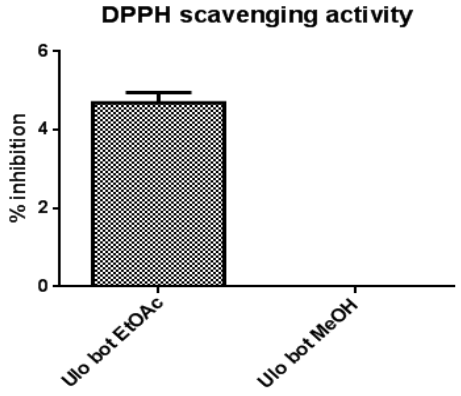
The EtOAc extract of the fungus displayed higher antioxidant activity in both tests carried out in this study, DPPH and phosphomolybedate assays. This may be attributed to the higher phenol content of the EtOAc extract compared to that of the MeOH extract. Further studies are recommended to be done on the EtOAc extract with the aim of finding the lead molecules probably contributing to this antioxidant activity that can be used in the treatment of many degenerative diseases such as aging, arthritis, cancer, inflammatory disorders, neurodegenerative diseases (as Alzheimer) and atherosclerosis. All these disorders have increased tremendously mainly due to the oxidative damage caused by reactive oxygen species ROS [14, 15].



(Figure 1)



(Figure 2)



(Figure 3)

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