Antioxidant activity of melanin extracted from *Aspergillus niger*

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Abstract

Background: Melanin is heterogeneous pigment widely distributed in diverse species. The pigment exhibits a diverse spectrum of biological activities, encompassing antioxidant, anticancer, antivenin, and radiation resistant activities. **Aim of the study:** The present study aimed to isolation and identification of *Aspergillus niger* from soil, then Extraction of melanin from the isolated fungus and finally, to study antioxidant activity. **Materials and methods.** The fungus Aspergillus niger was extracted from the soil of a residential garden in Tikrit Governorate and identified using several culture media. The melanin pigment was isolated from the Aspergillus niger strain via the Potato Dextrose Agar (PDA) culture technique. The antioxidant properties of melanin in relation to erythrocytes were also assessed. **Result:** As the melanin concentration increased, the antioxidant activity of Radical Scavenging Activity (RSA) increased. Melanin had antioxidant activity of 37.5%, 43.75%, 56.25%, 62.5%, and 65% at doses of 10, 20, 40, 80, and 100 µg/ml for T1. At doses of 10, 20, 40, 80, and 100 µg/ml, melanin showed antioxidant activity of 40%, 47.5%, 52.5%, 60%, and 61.37%) for T2. While for T3, the antioxidant activity at doses of 10, 20, 40, 80, and 100 µg/ml were (35%, 40%, 50%, 56.25%, and 60%). Finally, T4 showed antioxidant activity against melanin (31.25%, 40%, 43.75%, 50%, and 56.25%) at doses of 10, 20, 40, 80, and 100 µg/ml.

Conclusion: The present study concluded that *A. niger* contains a high percentage of melanin, making it an important source of this pigment. In addition melanin is considered a powerful antioxidant, and showed greater antioxidant effect.

Keywords: *Aspergillus niger*, melanin, UV-Vis spectroscopy, antioxidant activity, DPPH assay.

النشاط المضاد للأكسدة للميلانين المستخلص من *Aspergillus niger*

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الخلاصة

الخلفية: يُعدّ الميلانين صبغة غير متجانسة واسعة الانتشار بين مختلف الكائنات الحية، ويتميّز بطيف واسع من الأنشطة الحيوية، من بينها النشاط المضاد للأكسدة، والمضاد للسرطان، والمضاد للسموم، إضافة إلى مقاومته للإشعاع.

هدف الدراسة : هدفت هذه الدراسة إلى عزل وتشخيص فطر *Aspergillus niger* من التربة، ثم استخلاص صبغة الميلانين من الفطر المعزول، وتقييم نشاطها المضاد للأكسدة .**المواد وطرائق العمل:** تم عزل فطر *Aspergillus niger* من عينات تربة جُمعت من حديقة سكنية في محافظة تكريت، وتم تشخيصه باستخدام عدة أوساط زرعية مختلفة. كما جرى استخلاص صبغة الميلانين من العزلات الفطرية باستخدام تقنية الزرع على وسط Potato Dextrose Agar (PDA). وتم تقييم الفعالية المضادة للأكسدة للميلانين المستخلص باستخدام اختبار نشاط Radical Scavenging Activity. **النتائج :** أظهرت النتائج وجود علاقة طردية بين تركيز الميلانين ونشاطه المضاد للأكسدة. إذ سجلت العزلة T1 نشاطًا مضادًا للأكسدة بلغ ٣٧,٥%، ٤٣,٧٥%، ٥٦,٢٥%، ٦٢,٥%، و٦٥% عند التراكيز ١٠، ٢٠، ٤٠، ٨٠، و١٠٠ ميكروغرام/مل على التوالي. أما العزلة T2 فقد أظهرت نشاطًا مضادًا للأكسدة بنسبة ٤٠%، ٤٧,٥%، ٥٢,٥%، ٦٠%، و٦١,٣٧% عند التراكيز نفسها. في حين سجلت العزلة T3 نشاطًا مضادًا للأكسدة بلغ ٣٥%، ٤٠%، ٥٠%، ٥٦,٢٥%، و٦٠% عند التراكيز ذاتها. وأخيرًا، أظهرت العزلة T4 نشاطًا مضادًا للأكسدة بنسبة ٣١,٢٥%، ٤٠%، ٤٣,٧٥%، ٥٠%، و٥٦,٢٥% عند التراكيز ١٠، ٢٠، ٤٠، ٨٠، و١٠٠ ميكروغرام/مل على التوالي. **الاستنتاج :** تشير نتائج هذه الدراسة إلى أن فطر Aspergillus niger يحتوي على نسبة مرتفعة من صبغة الميلانين، مما يجعله مصدرًا مهمًا لهذه الصبغة. كما أظهر الميلانين المستخلص فعالية عالية كمضاد للأكسدة، حيث تزداد هذه الفعالية بزيادة التركيز.

الكلمات المفتاحية : Aspergillus niger، الميلانين، مطيافية الأشعة فوق البنفسجية–المرئية ، النشاط المضاد للأكسدة، اختبار DPPH .

Introduction

Fungi are among the important microorganisms in dye production, which can produce large quantities of metabolites, making them "microbial cell factories" and contributing to the commercial viability of the bioprocess [1]. Fungi are responsible for the synthesis of a number of pigments, one of the most important of which is melanin, the production of which has been known for more than 150 years [2]. fungus are responsible for the production of a secondary metabolite known as melanin, which plays a function in the survival strategy of fungus in settings that are not suitable [3].

The name "melanin" comes from the Greek word "melanos," meaning "black." Melanins are hydrophobic, negative-charged, high-molecular-weight dark or black pigments. Oxidative polymerization of phenolic or indole molecules produces these colors [4]. Melanins are abundant in plants, animals, fungi, protists, pathogenic bacteria, and parasitic worms[5].

Melanin has a number of unique physical, chemical, and biological characteristics. It is black, organic solvent insoluble, acid hydrolyzed resistant, can reduce silver nitrate and ammonia, interacts well with polyphenols, and can survive in extreme environments [6].

Fungal melanin is frequently extracellular (released into the environment) or intracellular (intracell forestry wall) in location. Melanin is associated with the fibrous stroma and is protruded to the outside of the cell wall in some fungi, but in other fungi is presented as a separate and discrete layer [7]. Additionally, melanin serves diverse biological and physiological functions such as protection of fungus against oxidative stress, ultraviolet light, enzymatic degradation, and extreme temperatures. Here, it acts as a physiological redox buffer, providing structural rigidity to the cell walls and protecting fungi from antimicrobial agents [8]. Melanin is also known as the "fungal armour" for the properties that it provides in order to ensure fungal survival in a wide range of extreme environments, thus increasing the virulence of many fungal pathogens in plants and humans [9]. Melanin has attracted considerable scientific attention, as recent studies have shown that it has different biological and functional characteristics. It is essential for a systematic review to understand the significance of fungal melanin and the current status of its research.

Materials and methods

Isolation of *A. niger* from the soil

The top layer of soil was removed from the home garden soil in Tikrit, the fungus was cultivated on Malt extract agar and Potato Dextrose Agar (PDA), the genus of the fungus was identified based on the shape and color of the colony, while the species was identified based on the sources of fungal diagnosis[10]

Melanin extraction

The fungus was cultivated in Potato Dextrose Agar (PDA) for ten days at 25°C. Following the incubation period, KOH was utilized at a ratio of 8 grammes to

200 millilitres and maintained in a water bath at 100°C for 2 hours. Subsequently, centrifugation was performed at 4000 RPM for 20 minutes, and repeated twice. The liquid was extracted from the top into a specialized beaker, and 9 ml of HCl was added for every 90 ml. It was allowed to sediment overnight, after which it was transferred to specialized tubes and washed three times with distilled water for 15 minutes each. Subsequently, it was rinsed with 5 ml of ethanol simultaneously. Following the completion of the washing procedure, the drying phase commenced utilizing Na_2SO_4 and was allowed to proceed for four days until fully dried [10,11]. A UV spectrophotometric technique was used to examine the absorption features of the melanin suspension.

Antioxidant activity measurement using the DPPH method

Radical scavenging activity (RSA) was measured using the DPPH assay method, according to [12].

Statistical Analysis

Using SPSS statistical software, version 20.0, one-way analysis of variance (ANOVA) was used for statistical analysis. Three replicates of each experiment were performed, and results are presented as mean ± standard deviation (SD).

Result

The results of isolation and identification of A. niger from soil samples taken from different regions after incubation at 25°C on Potato Dextrose Agar (PDA) medium. Colonies appeared on the culture medium as soft white at first and then turned black due to dark colored conidia with a velvety or cottony texture. The conidia heads were large and brown and were observed under the microscope as spherical, while the conidia heads were double brown. The phialids were spherical, dark brown, and rough-walled. Figure.(١)

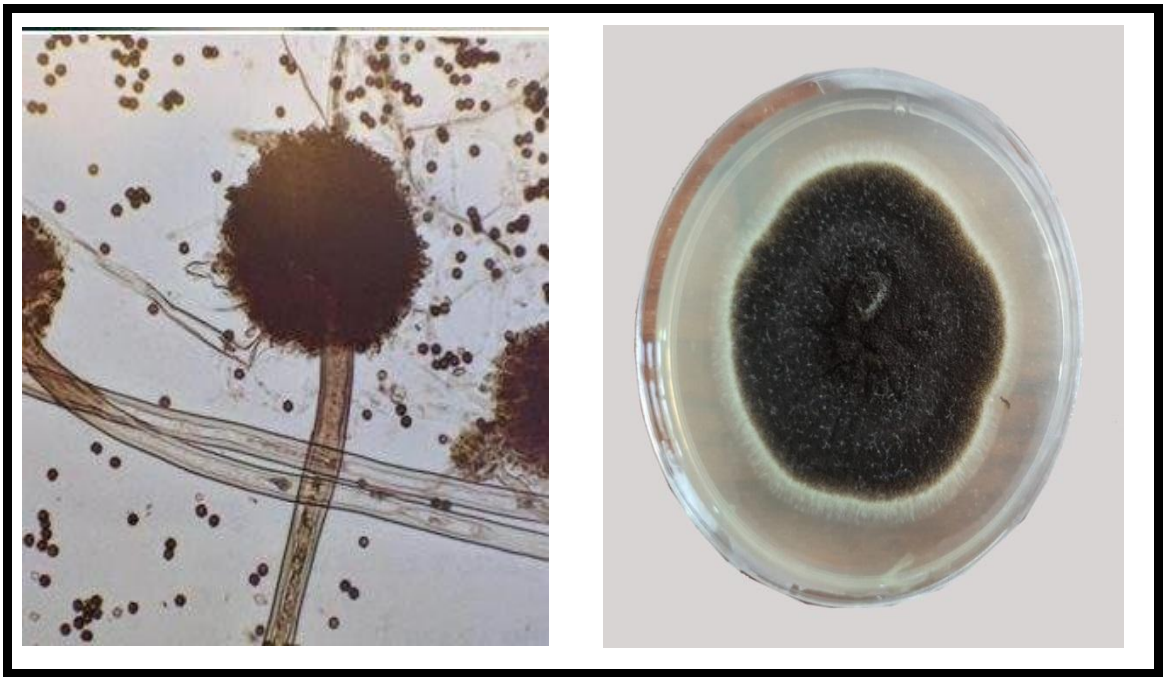


Figure (1) *Aspergillus niger* colony on PDA medium (40X).

Only four of the twenty-five fungal isolates were able to produce a diffusible brown-black pigment (melanin) on nutritional agar that included one percent tyrosine, according to the findings of the study. These four isolates were T1, T2, T3, and T4.

To select the isolate with the highest melanin production, the four isolates were cultured under identical conditions. The melanin concentration of each isolate was estimated based on the standard curve for synthetic melanin. The results showed that the four isolates produced the highest melanin concentration (1.112 µg/ml) and were therefore selected for further steps of this study, as shown in Table (1) and Figure (2).

Table (1): Concentration of melanin pigment produced by *A. niger* isolates

melanin concentration (µg/mL)	*A.nigar*
1.112	T1
1.032	T2
1.093	T3
1.072	T4

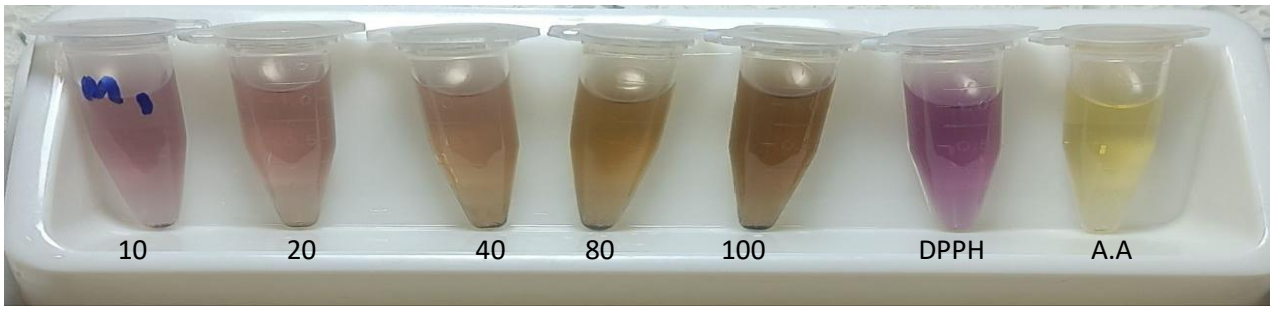
Figure (2) Dye extract powder

The results shown in Table (2) and Figure (3) show the absorbance and antioxidant activity values of the RSA formula for the prepared compounds at different concentrations. As the melanin concentration increased, the antioxidant activity of Radical Scavenging Activity (RSA) increased. Melanin had antioxidant activity of 37.5%, 43.75%, 56.25%, 62.5%, and 65% at doses of 10, 20, 40, 80, and 100 µg/ml for T1. At doses of 10, 20, 40, 80, and 100 µg/ml, melanin showed antioxidant activity of 40%, 47.5%, 52.5%, 60%, and 61.37%) for T2. While for T3, the antioxidant activity at doses of 10, 20, 40, 80, and 100 µg/ml were (35%, 40%, 50%, 56.25%, and 60%). Finally, T4 showed antioxidant activity against melanin (31.25%, 40%, 43.75%, 50%, and 56.25%) at doses of 10, 20, 40, 80, and 100 µg/ml.

Table (2): Absorbance and antioxidant activity values in the RSA formula for the prepared compounds at different concentrations.

Conc	**T1**		**T2**		**T3**		**T4**		**Ascorbic acid**	
	Abs(As)	RSA %	Abs	RSA %	Abs	RSA %	Abs	RSA %	Abs	RSA %
100	0.28nm	65%	0.309	61.375	0.32	60	0.35	56.25	0.3	62.5 %
80	0.3	62.5	0.32	60	0.35	56.25	0.4	50	0.33	58.75
40	0.35	56.25	0.38	52.5	0.4	50	0.45	43.75	0.34	57.5
20	0.45	43.75	0.42	47.5	0.48	40	0.48	40	0.4	50
10	0.5	37.5	0.48	40	0.52	35	0.55	31.25	0.52	35

DPPH(Ac)	0.8	-	-	-					-	-



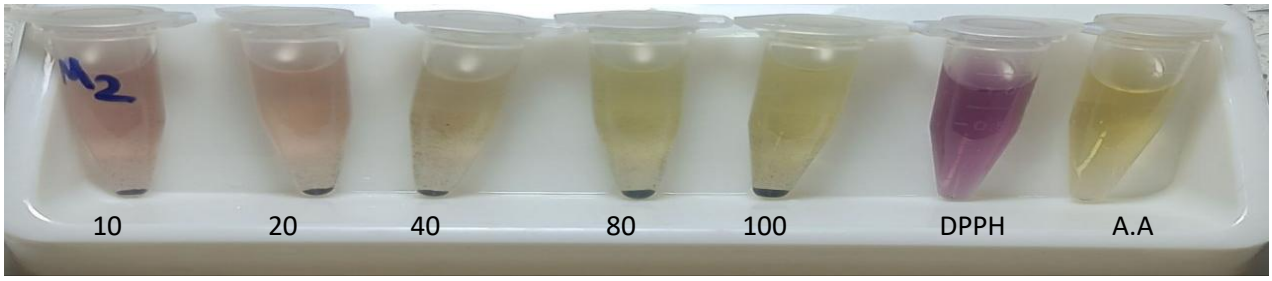
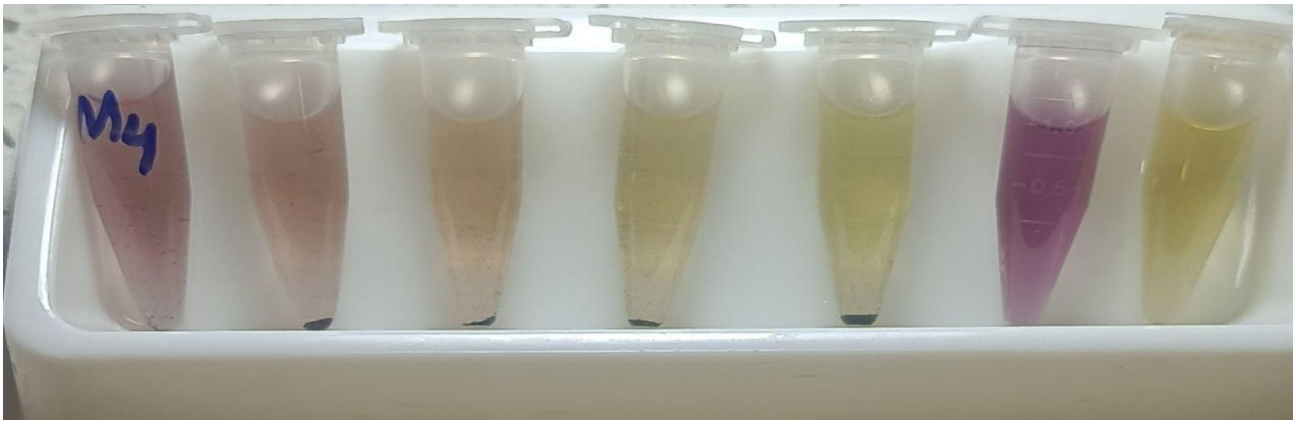


Figure (3): Shows the result of the antioxidant activity test using the DPPH assay for melanin extracts. The effect is evident through the change in the color of the reagent from purple to yellow and the comparison with the effectiveness of the reference ascorbic acid compound AA. When melanin with antioxidant activity is added to the DPPH solution, the dark purple color begins to change to light yellow or transparent, depending on the strength and concentration of the antioxidant substance. The higher the antioxidant activity, the more obvious the color change, which indicates the



efficiency of the extracted melanin in reducing free radicals.

Discussion

Melanin is the most important ones are free radical scavenging, and drug delivery capability [13]. The DPPH method was used to verify melanin's free radical scavenging ability to assess its antioxidant activity in a study by [14]. Melanin exhibited a robust antioxidant activity of 89.01 ± 0.02% at a concentration of 100 µg/ml, in contrast to 96.16 ± 0.01% observed with an equivalent dosage of ascorbic acid. Prior research has demonstrated that elevated melanin levels enhance antioxidant activity. Despite this, the amount of free radical scavenging activity ranged from 65.58 percent to 68.91 percent [15] and from 87 percent to 96 percent [16]. Because of the presence of valence electrons in its molecules, melanin is capable of rapidly absorbing free radicals and other compounds that are reactive. Because of their antioxidant properties, these chemicals are used in cosmetics to reduce the amount of tissue damage caused by toxins [17].

DPPH is a stable organic radical and has been widely used as a simple, rapid, and sensitive method for assessing the free radical scavenging capabilities of natural

antioxidants. Melanin can be in different states of oxidation, and its antioxidant activity depends on its oxidation state. The phenolic groups in melanin are converted to quinone groups during oxidation, reducing melanin's antioxidant capacity. Therefore, phenolic content has been used as an indicator to assess the degree of melanin oxidation [18].

In [15] study Schizophyllum commune produced melanin which having the higher free radical scavenging activity of fungus melanin at different concentrations (10 to 50%) as the concentration of the melanin increased, antioxidant activity also increased and reached up to 96%. According to [19], melanin synthesized by Aspergillus has been mainly applied as free radical scavenger for human health and also as an environmentally friendly, non-artificial dye.

The standard for assessing antioxidant activity is the IC50, which identifies the IC50 value. The IC50 is the concentration necessary to reduce the initial DPPH concentration by 50%; hence, a lower IC50 value indicates greater antioxidant activity [20]. Melanin derived from H. werneckii exhibited an IC50 value of 61.38 µg/mL in a DPPH free radical scavenging experiment. This aligns with the recorded findings for the isolated melanin pigment from Aspergillus bridgeri, which exhibits a free radical scavenging activity IC50 of 54.12 µg/mL [21].
According to the study, melanin can scavenge free radicals at many levels. Melanin's complex structure, with hydroxyl and aromatic groups, stabilizes hydrogen donation to DPPH radicals, leading to significant antioxidant activity[21].

Conclusion

The present study concluded that *A. niger* mushroom contains a high percentage of melanin, making it an important source of this pigment. Inaddition melanin is considered a powerful antioxidant, and shown its greater antioxidant effect compared to ascorbic acid.

Recommendation

The present study recommended extraction melanin from other types of fungi and bacteria. Inaddition comparing the inhibitory effect of melanin with medicinal plant extracts against pathogenic microbes.

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