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## ***Scleroderma citrinum* melanin: isolation, purification, spectroscopic studies with characterization of antioxidant, antibacterial and light barrier properties**

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### **ABSTRACT**

The aim of this study was to isolate and evaluate biological properties of raw and purified melanins isolated from *Scleroderma citrinum*. Native melanin was isolated from the gleba of fresh *S. citrinum* fruiting bodies by alkaline extraction. Obtained pigment was purified by acid hydrolysis and washed by organic solvents. Chemical tests, FT-IR and Raman spectroscopy analysis were conducted to determine the melanin nature of the isolated pigments. UV-Vis, transmittance, total polyphenolic content and colour properties were evaluated. Antioxidant activity was determined using ABTS and antibacterial activity by a well diffusion method. The results of the study demonstrated that melanins isolated from *S. citrinum* had antioxidant, light barrier and antibacterial properties. A purified form of melanin offered better light properties and higher antioxidant activity than the raw form. Both melanins inhibited the growth of *E. faecalis* and *P. aeruginosa*. This study revealed that *S. citrinum* may be considered as a promising source of natural melanin. Isolated pigments presented all the physical and chemical properties common to natural and synthetic melanins. Raw and purified melanins showed differences in chemical composition, antioxidant activity and light barrier properties. Results suggest that, melanins from *S. citrinum* possess remarkable therapeutic action and could be applied in the food, cosmetics and pharmaceutical industries.

**Keywords:** melanin, *Scleroderma citrinum*, earthball, antioxidant, antibacterial, light barrier

## 1. INTRODUCTION

The genus *Scleroderma* comprises of gesteroid ectomycorrhizal (ECM) basidiomycetes with reticulate to echinulate globose spores, usually known as earthballs [1,2]. *Scleroderma* taxa are distributed worldwide in tropical, temperate and subtropical ecosystems [3], and have been reported from Asia [4-9], Africa [10], Australia [11], Europe [12-15], North America [13] and South America [2,3,16]. Several morphological and molecular studies have confirmed the systematic position of the *Scleroderma* genus, placing it in the suborder *Sclerodermataceae* within the *Boletales* order. This genus of fungi with hard-skinned basidiomata can be recognized by its epigenous and single-layered peridium opening by irregular dehiscence and gleba without capillitium [1,16].

*Scleroderma citrinum* Pers., occurs as a saprotroph on soil or humus with mosses, sometimes on rotting wood but has also been demonstrated to form typical ECM and is common in coniferous and mixed forest [13]. When mature, brown, leathery, rounded structures become dry and crack at maturity, and the dry, powdery, dark basidiospores are dispersed by the wind blowing through fissures in the upper surface of the basidiome. The dark colour of basidiospores is caused by melanin. Several studies describe the biological activities of *S. citrinum* including insecticidal [17], antibacterial [4], antifungal [18,19] and antiviral [20]. The known chemical compounds from *S. citrinum* have been reported including lanostane-type steroids [18], lanostane-type terpenoids [20], pulvinic acid derivatives [4,21], carbonyl reductases [22] and pigments such as sclerocitrin and norbadione A [23].

Melanins have been isolated from a variety of phylogenetic sources: animals [24], plants [25], bacteria [26,27] and fungi [28,29]. Melanins are commonly represented as black and brown pigments, high molecular weight heterogenous polymers derived from the oxidation of monophenols and the subsequent polymerization of intermediate o-diphenols and their resulting quinones [30]. Melanins are types of pigments, possessing broad biological activities including; antioxidant, radioprotective, thermoregulative, chemoprotective, antitumor, antiviral, antimicrobial, immunostimulating and anti-inflammatory properties [24-30]. Based on these features, natural melanin has the potential to be of great value and application in the fields of pharmacology, cosmetics, and functional foods [28,31]. However, knowledge relating to pharmacological and the biological activities of melanins from *S. citrinum* is highly limited. In recent years there has been a revival of interest in the development of natural colorants as food additives, and also in the cosmetic and pharmaceutical industries. This has been encouraged by strong consumer demand, as synthetic colorants are frequently perceived as undesirable or harmful [28,32]. Owing to the high toxicity of synthetic compounds, the search for new natural colorants with antiradical, light barrier as well as antimicrobial properties still remains a challenge for modern science.

The aim of present study was to isolate, characterize and investigate the antioxidant, antimicrobial and light barrier properties of raw and purified melanins form *S. citrinum*. This represents a first report on the isolation and biological activities of melanins from *S. citrinum*.

## 2. MATERIALS AND METHODS

### 2. 1. Test fungus

The tests were made up of fresh mature fruiting bodies of *S. citrinum* within this study.

The fruiting bodies were collected in September 2017 in a mixed forest near Szczecin, Poland (53° 20' N, 14° 49' E).

## 2. 2. Chemicals

NaOH, HCl, FeCl<sub>3</sub>, H<sub>2</sub>O<sub>2</sub>, AgNO<sub>3</sub>, acetone, ethanol, ethyl acetate, chloroform, DMSO and methanol (Chempur, Poland) were used to extract, purify and offer up a characterisation of the active substances from the *S. citrinum* fruiting bodies. ABTS and KBr (Sigma Aldrich) were also used in this study. To verify the antimicrobial properties of any melanin, Mueller-Hinton broth and Mueller-Hinton agar media (Merck, Germany) were used. All media were prepared according to the Merck protocol.

## 2. 3. Extraction and purification

The isolation and purification of melanin was performed as described by Łopusiewicz [28]. External layers of peridium were removed, and only gleba was used for melanin extraction. To summarise, 5 g of gleba were homogenised in 50 ml of 1 M NaOH, extracted in orbital shaker (150 rpm, 50 °C, 24 h) and centrifuged at 6000 rpm for 10 min to remove fungal tissue. Alkaline SC-RM (Scleroderma citrinum raw melanin) mixture was first adjusted to pH 2.0 with 1 M HCl to precipitate melanin, followed by centrifugation at 6000 rpm for 10 min and a pellet was collected. Then, the pellet was hydrolyzed in 6 M HCl (90 °C, 2 h), centrifuged (6000 rpm, 10 min) and washed by distilled water five times to remove acid. The pellet was washed with chloroform, ethyl acetate and ethanol three times to wash away lipids and other residues. Finally, the purified melanin (SC-PM – Scleroderma citrinum pure melanin) was dried, ground to a fine powder in a mortar and stored at -20°C until testing.

## 2. 4. Chemical tests

Different diagnostic tests, as described by Selvakumar et al. [33], were conducted on the SC-RM and SC-PM isolated melanins in comparison with L-DOPA melanin used as a melanin standard. The testing organic solvents included ethanol, methanol, chloroform, ethyl acetate, acetone and DMSO.

## 2. 5. Ultraviolet-visible absorption and transmittance spectra

Melanin solutions were prepared at concentration 0.1 mg/mL and UV-Vis absorption spectra were measured between 200 and 800 nm. The absorbance ratio (A<sub>300</sub>/A<sub>600</sub>) values and plots of optical densities against wavelenghts of melanins were also calculated [25,28]. Transmittance values were measured between 200 and 800 nm at 0.01; 0.05; 0.1; 0.5 and 1 mg/mL for SC-RM and SC-PM; for L-DOPA melanin 0.01; 0.05; 0.1; 1 mg/mL concentrations were measured. All spectrophotometric assays were conducted in a Thermo Scientific Evolution 220 spectrophotometer.

## 2. 6. IR spectroscopy

The IR spectra of melanins solid samples were obtained at room temperature by attenuated total reflection with a Fourier transform infrared spectrometer (Perkin Elmer). The samples were evenly mixed with KBr, and pressed into tablets, then scanned at a range

between  $650\text{ cm}^{-1}$  and  $4000\text{ cm}^{-1}$  (64 scans and  $1\text{ cm}^{-1}$  resolution) [28]. Obtained spectra have been normalized, baseline corrected and analysed using SPECTRUM software.

## 2. 7. Raman spectroscopy

Melanin samples were analysed using a Raman station (RamanStation 400F, Perkin Elemer) with point-and-shot capability using an excitation laser source at 785 nm, 100 micron spot size, 25% laser power, 4 shots, 8 second exposition time. Obtained spectra have been normalized, baseline corrected and analysed using SPECTRUM software.

## 2. 8. The antioxidant activity (ABTS assay)

An ABTS assay was performed according to Łopusiewicz [28]. Radical 2,2'-azino-bis(3-ethylbenzothiazoline)-6-sulphonic acid (ABTS $\cdot$ +) was produced by mixing 7 mM ABTS with 2.45 mM potassium persulfate (5 mL of ABTS + 5 mL of potassium persulphate 4.9 mM). The mixture was incubated in darkness at room temperature for 16 h, diluted with 7 mM phosphate buffer (pH 7.4) to reach an absorbance of between 1.0 and 1.2 at 734 nm. For the ABTS assay, 50  $\mu$ L of melanin (SC-RM, SC-PM, L-DOPA melanin; 0.0625; 0.125; 0.25, 0.5; 1 mg/mL), or dissolvent as control, were mixed with 1.95 mL of ABTS $\cdot$ + solution, incubated in darkness for 10 min at 37 °C, and then the absorbance was measured at 734 nm and antioxidant activity (%AA) was calculated as  $\%AA = [(A_m * 100) / A_c]$ ; where  $A_c$  and  $A_m$  are absorbances for the control and melanin sample, respectively.

## 2. 9. Determination of the total phenolics content (TPC) of melanins

The total phenolics content (TPC) of the melanins were determined by the Folin-Ciocalteu reaction according to Cuevas-Juárez et al. [16]. Melanin dissolved in DMSO (20  $\mu$ l) was mixed with 1.58 ml of deionized water and 100  $\mu$ l of the Folin-Ciocalteu reagent, stirred gently for 5 min and added with 300  $\mu$ l of saturated solution of  $\text{Na}_2\text{CO}_3$ . The mixture was allowed to stand in darkness for 30 min at 40 °C, the absorbance was then measured at 765 nm. A calibration curve of gallic acid in water-methanol (1:1, v/v) (0, 50, 100, 200, 400 and 500  $\mu$ g/mL) was prepared and TPC was calculated as miligrams of gallic acid equivalents (GAE)/gram of melanin (mg GAE/g). The results were presented as an average of three samples with standard deviation.

## 2. 10. The visual colour of melanins

The visual colour of melanin solution (0.1 mg/mL) values were measured by a Konica Minolta CR-5 colorimeter with the Hunter LAB colour system. The colour values were expressed as  $L^*$  (brightness/darkness),  $a^*$  (redness/greenness) and  $b^*$  (yellowness/blueness) as an averages of five measurements.

## 2. 11. The antibacterial activity of isolated melanins

Test microorganisms, including *Bacillus cereus* ATCC14579, *Enterococcus faecalis* ATCC29212, *Escherichia coli* DSMZ1576, *Pseudomonas aeruginosa* ATCC2753 and *Staphylococcus aureus* DSMZ346 were separately cultivated in Mueller-Hinton broth. The antibacterial activity was tested through a well diffusion method. 50 mL of Mueller-Hinton broth was inoculated by a single bacterial strain and incubated at 37 °C for 24 h. Mueller-

Hinton agar was autoclaved and on reaching approx. 45°C, 200 µL of bacterial suspension was added to 20 mL of the medium, vigorously vortexed and poured on 90 mm Petri dishes. Wells were cut out by sterile tips (9 mm diameter) in triplicate on each plate and 100 µL of melanin solutions at 0.1 mg/mL in DMSO were placed in the wells. DMSO served as a control. Plates were incubated at 37°C for 24 h. The inhibition zones were measured after incubation. The results were presented as an average of three samples with standard deviation.

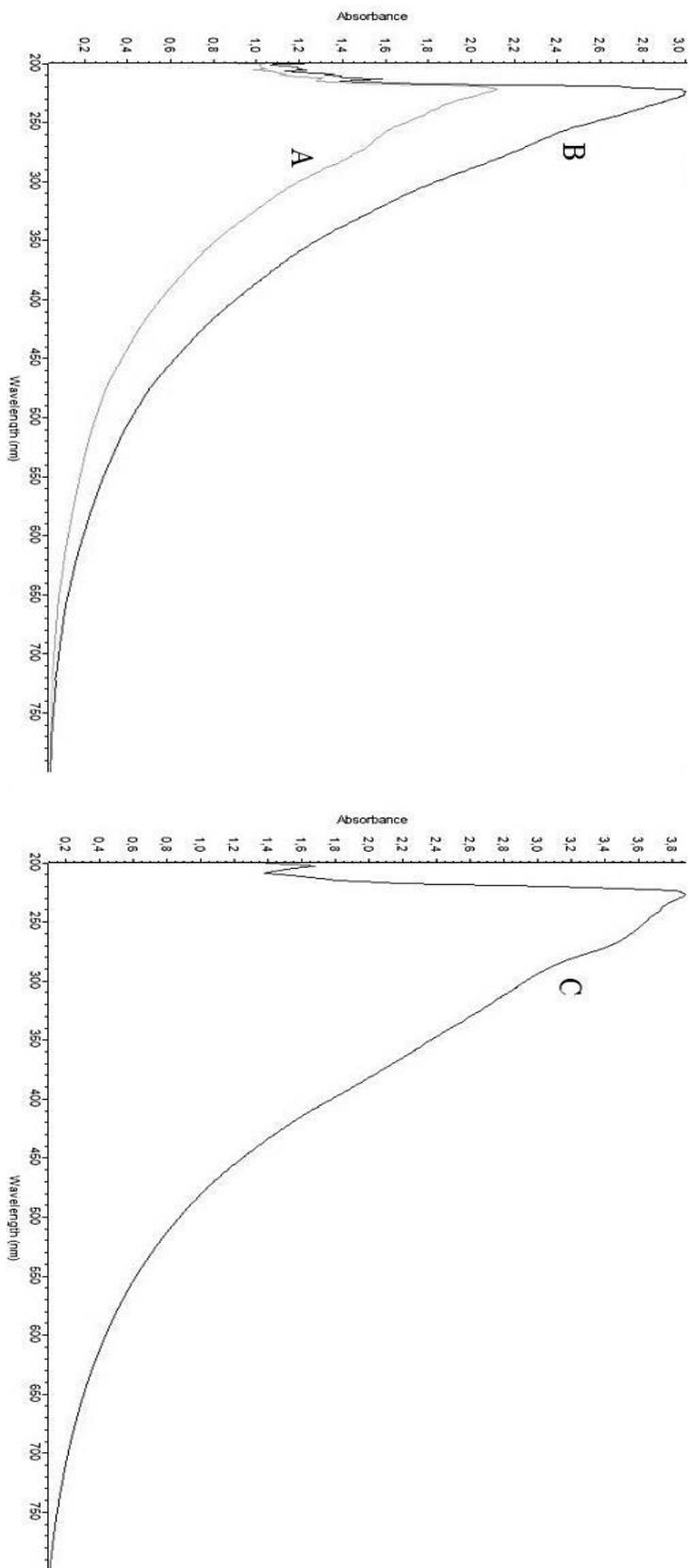
### 3. RESULTS

The results of the study demonstrated that raw and purified black pigments from *S. citrinum* had antioxidant, antibacterial and light barrier properties. The SC-RM and SC-PM pigments presented all of the physical and chemical properties common to natural melanins and the experimental data within this work were found to be comparable to those reported in literature. The results are summarized in Table 1, which also shows the properties of the L-DOPA melanin sample used for calibration.

**Table 1.** Diagnostic tests for melanins.

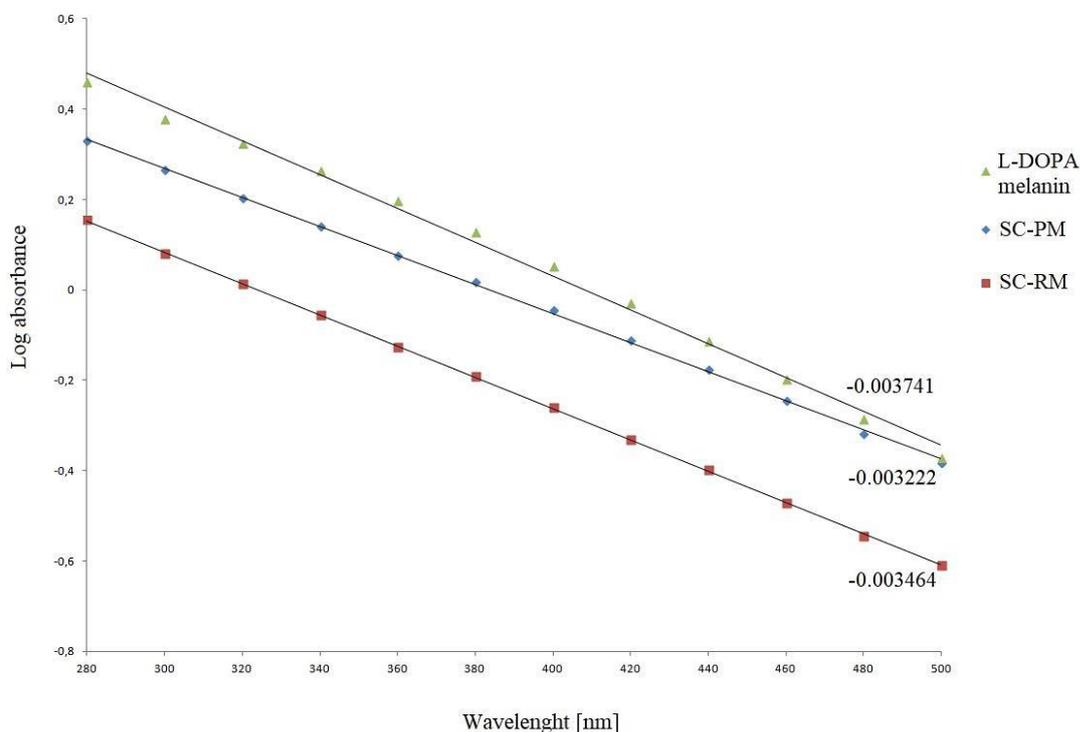
No	Test	Result		
		SC-RM	SC-PM	L-DOPA melanin
1.	Solubility in water	Insoluble		
2.	Solubility in organic solvents (acetone, chloroform, ethanol, ethyl acetate, methanol)	Insoluble		
3.	Solubility in 1 M NaOH	Soluble		
4.	Precipitation in acidic conditions	Readily precipitation		
5.	Reaction with oxidizing agents (H <sub>2</sub> O <sub>2</sub> )	Decolorized		
6.	Reaction with ammoniacal AgNO <sub>3</sub> solution	Gray coloured silver precipitate on tube side		
7.	Reaction for polyphenols (FeCl <sub>3</sub> test)	Brown precipitate		
8.	Colour	Black		

Figure 1 shows SC-RM and SC-PM have maximum absorption peaks at 222 nm and 225 nm, respectively, and exhibited an exponential decrease in the visible region. This behaviour in SC-RM and SC-PM were similar to the melanin synthesized from L-DOPA, which is used as a melanin standard.



**Fig. 1.** The absorbance of SC-RM (A), SC-PM (B) and L-DOPA melamin (C)

Figure 2 shows the log of optical density of a melanin solutions when plotted against the wavelength of SC-RM, SC-PM and L-DOPA melanin. SC-RM, SC-PM and L-DOPA melanin had straight lines with negative slopes of -0.003464, -0.003222 and -0.003741, respectively. The light barrier properties of SC-RM, SC-PM and L-DOPA melanin are shown in Figure 3. It was noted that in all analysed concentrations, the SC-RM transmittance values were higher than those of the corresponding SC-PM, which suggests that in purified form, melanin had better light barrier properties, even when the transmittance values of SC-PM were smaller than the synthetic melanin.

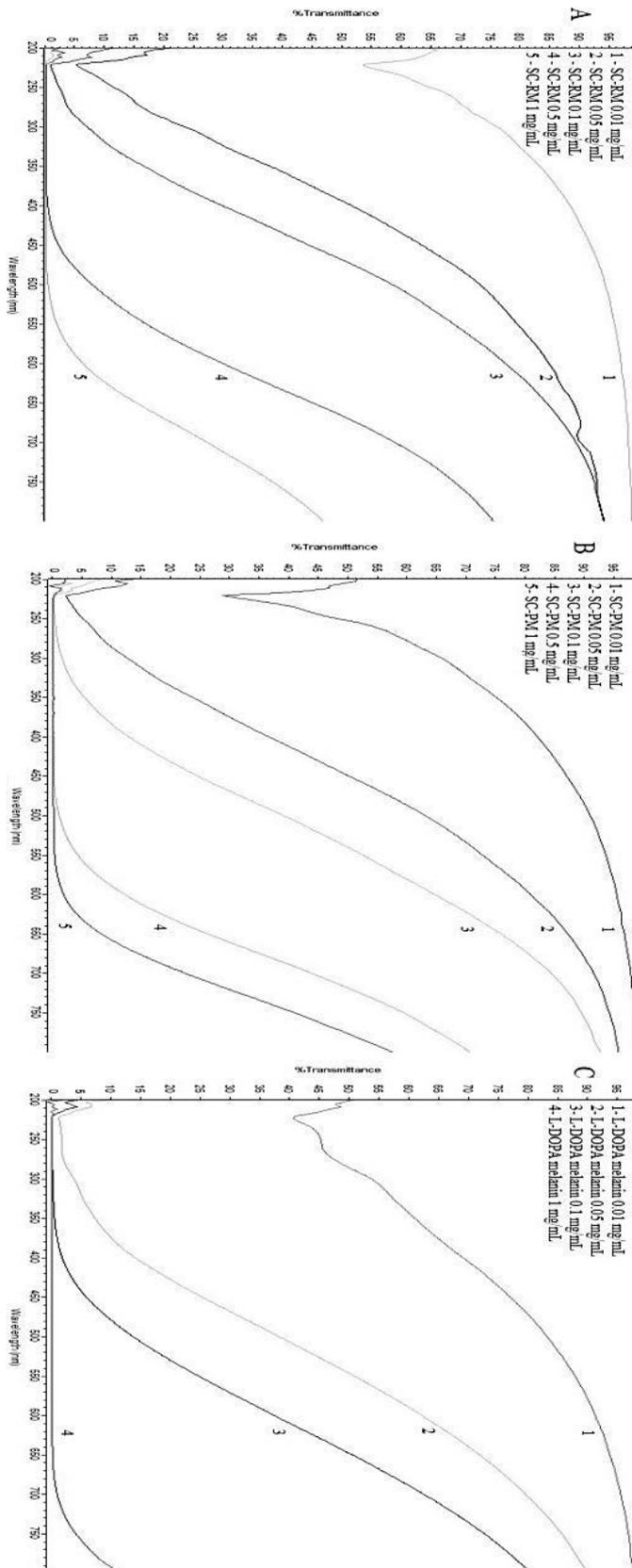


**Fig. 2.** A plots of optical densities against wavelength for L-DOPA melanin, SC-PM and SC-RM

The colour values of SC-RM, SC-PM and synthetic melanin are shown in Table 2. Results from the colorimeter indicated that SC-PM presented lower L\* value, and higher a\* and b\* values than SC-RM in Hunter Lab colour system.

**Table 2.** The visual colour values of SC-RM, SC-PM and L-DOPA melanin (mean±SD, n=5)

	L*	a*	b*
SC-RM	88.17±0.01	2.58±0.00	22.23±0.01
SC-PM	78.34±0.00	7.14±0.02	34.94±0.00
L-DOPA melanin	74.87±0.00	10.56±0.00	47.74±0.02

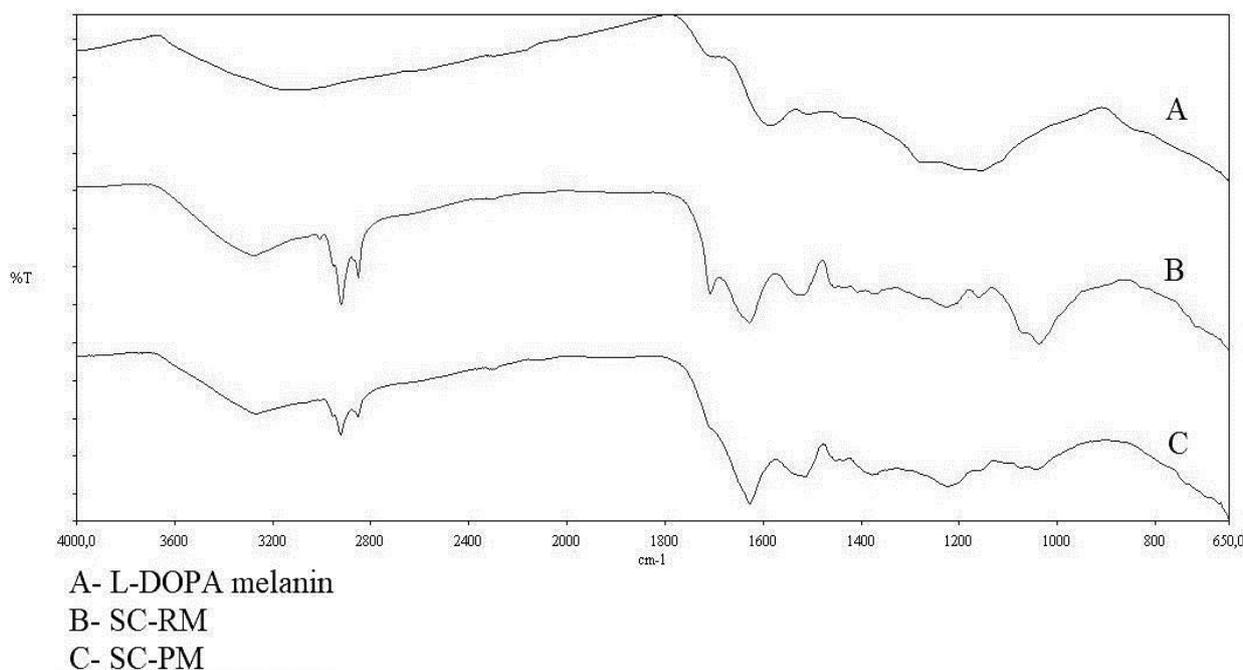


**Fig. 3.** Transmittance values of SC-RM (A), SC-PM (B) and L-DOPA melanin (C)

The TPC values for melanins were  $0.18 \pm 0.01$ ;  $0.26 \pm 0.03$ ;  $0.29 \pm 0.05$  mg GAE/g for SC-RM, SC-PM and L-DOPA melanin, respectively. In general, the %AA values of SC-PM were higher than those of SC-RM, and the %AA of both melanins were lower than the corresponding concentrations of L-DOPA melanin, as shown in Table 3.

**Table 3.** The antioxidant activity (%AA values) of SC-RM, SC-PM and L-DOPA melanin (mean $\pm$ SD, n = 3)

	%AA	SC-PM	%AA	L-DOPA melanin	%AA
0.0625 mg/mL	12.68 $\pm$ 0.57	0.0625 mg/mL	13.99 $\pm$ 0.31	0.0625 mg/mL	20.31 $\pm$ 0.26
0.125 mg/mL	21.05 $\pm$ 1.11	0.125 mg/mL	30.77 $\pm$ 0.08	0.125 mg/mL	31.51 $\pm$ 1.04
0.25 mg/mL	23.76 $\pm$ 0.25	0.25 mg/mL	40.98 $\pm$ 0.44	0.25 mg/mL	50.75 $\pm$ 0.18
0.5 mg/mL	40.27 $\pm$ 0.23	0.5 mg/mL	50.37 $\pm$ 0.19	0.5 mg/mL	95.91 $\pm$ 0.33
1 mg/mL	75.66 $\pm$ 0.13	1 mg/mL	86.71 $\pm$ 1.23	1 mg/mL	97.16 $\pm$ 0.05

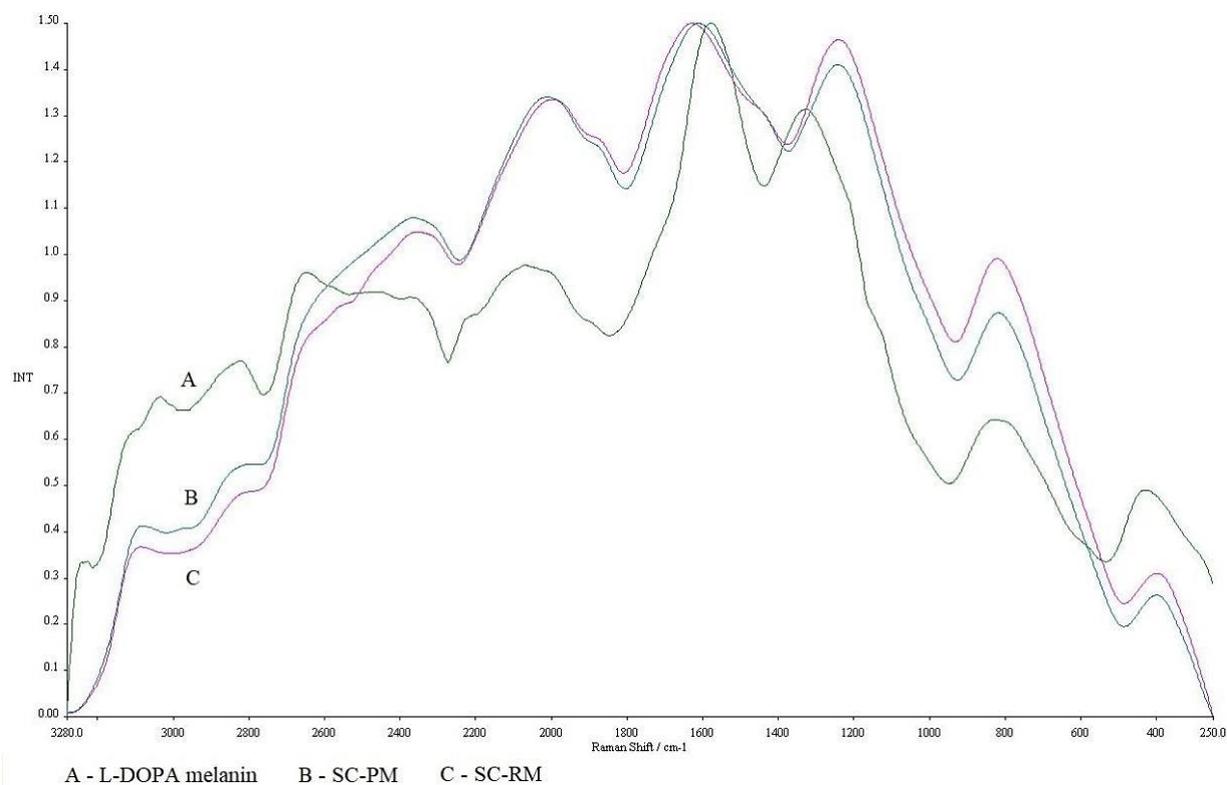


**Fig. 4.** Infrared spectra of L-DOPA melanin, SC-RM and SC-PM

Figure 4 shows the IR-spectra of SC-RM, SC-PM and L-DOPA melanin. Extra display broad absorption bands at  $3600-3000 \text{ cm}^{-1}$  were noted, attributed to stretching vibrations of C-

H, N-H and/or O-H groups. The C-H could be due to the presence of aromatic rings, with strong bands at  $1630\text{ cm}^{-1}$  and  $1627\text{ cm}^{-1}$ , for SC-RM and SC-PM, respectively, which corresponds to the vibration of aromatic C=C, and more intense in SC-PM. Two peaks at  $2921\text{ cm}^{-1}$  to  $2851\text{ cm}^{-1}$  in both melanins may result from the oscillation of aliphatic  $\text{CH}_2$  and  $\text{CH}_3$  groups. The bands at  $1228\text{ cm}^{-1}$  and  $1224\text{ cm}^{-1}$  due to C-N and C-O, would support the presence of phenols and aromatic amines. It is difficult to state whether there is an amide group, as the C=O group that it complements might be joined in the band corresponding to the aromatic C=C. There are differences between the SC-RM and SC-PM spectra which may be a result of the purification process.

Figure 5 shows the Raman spectra of synthetic melanin (A), SC-PM (B) and SC-RM (C). SC-RM and SC-PM Raman spectra were similar to spectrum of L-DOPA melanin. The Raman spectrum of SC-RM is dominated by two intense and broad peaks at about  $1612\text{ cm}^{-1}$  and  $1238\text{ cm}^{-1}$ , while at SC-PM spectrum peaks at  $1625\text{ cm}^{-1}$  and  $1245\text{ cm}^{-1}$  were observed. A peak at  $2000\text{ cm}^{-1}$  from both melanins is noticeable. Peaks  $395\text{ cm}^{-1}$  and  $400\text{ cm}^{-1}$  for SC-RM and SC-PM, respectively, are present.



**Fig. 5.** The Raman spectra of L-DOPA melanin (A), SC-PM (B) and SC-RM (C)

The results of an antibacterial activity assessment of SC-RM and SC-PM are illustrated in Table 4. The zones of growth inhibition of *E. faecalis* and *P. aeruginosa* were  $12.6 \pm 0.2$  mm and  $13.5 \pm 0.1$  mm for SC-RM, respectively, while SC-PM, were  $10.3 \pm 0.3$  mm and  $11.7 \pm 0.2$  mm. No inhibition on *B. cereus*, *E. coli* and *S. aureus* was observed.

**Table 4.** The antibacterial activity of SC-RM, SC-PM and L-DOPA melanin (zones of growth inhibition, mm), (mean±SD, n = 3)

	BC	EC	EF	PA	SA
SC-RM	-	-	12.6±0.2	13.5±0.1	-
SC-PM	-	-	10.3±0.1	11.7±0.2	-
L-DOPA melanin	-	-	11.4±0.2	13.1±0.1	-

“-“ – no inhibition zone, “BC” - *B. cereus* ATCC14579, “EC” - *E. coli* DSMZ1576, “EF” - *E. faecalis* ATCC29212, “PA” - *P. aeruginosa* ATCC2753, “SA” - *S. aureus* DSMZ346

#### 4. DISCUSSION & CONCLUSIONS

Through the work of this study, it is clear that the melanins isolated from *S. citrinum* possess promising antioxidant, light barrier and antibacterial properties.

Chemical tests and FT-IR conducted on isolated pigments in comparison to the synthetic L-DOPA melanin clearly demonstrated that they are melanins. Purified melanin (SC-PM) was obtained by acid hydrolysis, repeated precipitation and purification through the use of organic solvents. The structure of melanin polymers is poorly understood and an accurate definition of melanin is still required. However, the following criteria indicate melanin is: black/brown in colour, insoluble in water and most other organic solvents, resistant to degradation by hot or cold acids, bleached by oxidizing agents and solubilised by alkali solutions [28,31].

Infrared spectroscopy has been used in the chemical structure study of many melanins. It has been suggested that identical melanin structures do not exist in nature and their chemical characterization is a complicated task. Their composition depends not only on their different monomeric units, but also on environmental conditions during polymerization. Infrared spectrometric techniques offer information on the main functional groups in the melanin structure [24,25,28]. A detailed comparative analysis of the infrared spectra of the melanins studied may supply valuable information on the effect of each treatment step used to purify the melanin and the distinct functional groups prevailing in the various samples.

The Raman spectrum of SC-RM is dominated by two intense and broad peaks at about 1612  $\text{cm}^{-1}$  and 1238  $\text{cm}^{-1}$ , while SC-PM spectrum peaks at 1625  $\text{cm}^{-1}$  and 1245  $\text{cm}^{-1}$  were noted. The peaks can be interrelated as originating from the in-plane stretching of the aromatic rings and the linear stretching of the C-C bonds within the rings, along with some contributions from the C-H vibrations in the methyl and methylene groups [34]. The peaks at about 2000  $\text{cm}^{-1}$  from both melanins are similar to those obtained by Galvan et al. [35] from eumelanin and may be caused by the stretching of three of the six C-C bonds within the melanin aromatic rings. It was noted, that on both melanin Raman spectra peaks (395  $\text{cm}^{-1}$  and 400  $\text{cm}^{-1}$ , for SC-RM and SC-PM, respectively) are present, which are thought to correspond to peaks obtained from pheomelanin and eumelanin and are caused by an out-of-

plane deformation of phenyl rings. Obtained spectra of *S. citrinum* melanins are similar to *Exidia nigricans* melanins obtained in previous study [28].

There was no absorption peak between 260-280 nm in the UV-Vis spectra, indicating, that melanins do not contain proteins and nucleic acids [28]. The UV-Vis absorption spectra of the impure (RM) and purified (PM) melanins were similar to those reported in other literature. Also light barrier properties are in accordance with previous studies, showed that purified melanin form from *E. nigricans* offered better light properties than the raw form [28].

In general, melanins are dark because they do not re-radiate the absorbed visible or invisible light, but transform the energy into rotational and vibrational activity within the molecule and then dissipate it as heat. This phenomenon protects melanised tissues against light-induced damage [28,34]. In previous study it has been also observed that purified melanin showed lower L\* and higher a\* and b\* values [28]. The high antioxidant activity of the melanins was expected due to the protection against UV-radiation and free radical scavenging being their main functions [36-39]. The ability of melanin to scavenge reactive oxygen species (ROS), such as singlet oxygen, hydroxyl radical and superoxide anion, has been firmly established in model systems, suggesting that melanin could protect pigmented cells against oxidative stress that may accompany the formation of ROS in cells. Even though critical damage to oxidatively stressed cells may result from the reaction of crucial cellular constituents with ROS, an efficient antioxidant may protect the cells by scavenging other oxidizing radicals such as the peroxy radical, and by interacting with molecular oxygen [40]. The high antioxidant activity of melanin isolated from various sources has been reported by other authors [24-40]. Antioxidant activity of *S. citrinum* melanins is higher than those analyzed in previous study, also polyphenolic content is higher, which suggest that, presence of higher polyphenolics in melanin molecules is linked with higher antioxidant activity [28].

The A300/A600 ratios offer information about the oxidation state and the range size of melanin molecules [25,28]. Melanin oxidation induces lower absorbance values at 600 nm (A600), and the A300/A600 absorbance ratio was proposed as a measure of oxidation extent, with high values corresponding to greater oxidized melanin molecules. Also, it was argued that during the melanin oxidation, phenolics are converted to semiquinones or quinones, which produce more oxidized (higher A300/A600 absorbance ratios) and smaller melanins (molecular weight < 1000 Da) [28]. SC-RM showed a higher value (10.23) than its corresponding pure SC-PM (9.72) and L-DOPA melanin (16.00). This data supports the fact that SC-RM are a more complex mixture of melanin molecules than that of SC-PM, with a variability in size and degree of oxidation. In contrast, for *E. nigricans* melanins, opposite observations have been made, showed that pure form of melanin had higher A300/A600 value being more complex than the raw form [28]. These data are also consistent with the results of Cuevaz-Juárez et al. [25] and also with observations made by Hung et al. [41] who noted that oxidized and reduced melanins obtained from black tea have variances in their absorption spectra. Reduced forms of melanin have phenolic form prevalence, which when oxidized, forms show preponderance for quinone forms.

Many fungi contain melanins in vegetative as well reproductive structures (e. g. spores). Melanins such as 1,8-dihydroxynaphthalene and L-3,4-dihydroxyphenylalanine (L-DOPA) types have been mostly associated to ascomycetous and basidiomycetous forms, respectively. Some fungi have the ability to synthesize a type of melanin according to the environmental conditions, as well as at the developmental stage. The presence of melanins in fungi adapted to different environments and the fact that the same melanin is synthesized by a fungus,

indecently of environmental conditions, suggests that this molecule plays different crucial biological roles in fungal physiology [36-38]. Melanins enhance the tolerance of fungi to environmental stresses, improving their survival. Melanins protect fungal structures from UV radiation, temperature, desiccation, oxidizing agents and toxic compounds, such as antibiotics or heavy metals, due to their chelating ability [28,36-39].

Both melanins showed antibacterial activity against *P. aeruginosa* and *E. faecalis*. No antibacterial activity towards *B. cereus*, *E. coli* and *S. aureus* was observed. This data are supported by previous study showed that melanins from *E. nigricans* have been active against *P. aeruginosa* and *E. faecalis* [28]. Antimicrobial assessment results are partially consistent with results found by other authors. Helan Soundra Rani et al. [42] noted the antimicrobial activity of melanin isolated from halophilic black yeast *Hortaea werneckii*. Laxmi et al. [43] observed that growth of *P. aeruginosa* was inhibited on the presence of melanin obtained from *Providencia rettgeri*, but in their study some *Bacillus* species were sensitive to melanin. Xu et al. [44] analysed the antimicrobial activity of melanin from *Lachnum* YM30 and noted that it was active against a wide spectrum of bacteria, including *S. aureus*. The authors suggest that melanin antibacterial activity might result from damage of the cell membrane and affect bacteria membrane function. A discrepancy in melanin antimicrobial activity may result in differences within the molecule structure and composition [46]. From the other hand there are some reports that melanins have antibiofilm activity against pathogenic bacteria including *P. aeruginosa* and could interfere with bacterial quorum-sensing system, regulate its associate functions and prevent bacterial pathogenesis [43,44,46,47].

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