# Phenolics and Antioxidant Activity of Mushroom Leucopaxillus giganteus Mycelium at Different Carbon Sources

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# Phenolics and Antioxidant Activity of Mushroom *Leucopaxillus giganteus* Mycelium at Different Carbon Sources

L. Barros, I.C.F.R. Ferreira* and P. Baptista

*CIMO- Escola Superior Agrária, Instituto Politécnico de Bragança*
*Campus de Sta. Apolónia, Apartado 1172, 5301-855 Bragança, Portugal*

This article reports the first approach to the antioxidant potential evaluation of the edible mushroom *Leucopaxillus giganteus* mycelium obtained in the presence of four different carbon sources: glucose, sucrose, fructose and mannitol. Despite the use of *Leucopaxillus* mushroom species in chemical industry for extraction of clitocybin antibiotic, the production of its mycelium for pharmacological applications has not been explored. The concentration of antioxidant compounds increased along the growth time as a response to the oxidative stress and therefore free radicals production. The aldohexose glucose proved to be the most appropriate carbon source to increase antioxidant activity, leading to the highest phenols content and lowest $EC_{50}$ values. Significant negative linear regressions were established between phenols and flavonoids contents, and antioxidant activity, which support that the extracts mechanism of action for the different antioxidant activity assays may be identical, being related with the content in those compounds and their free radical scavenging activity.

*Key Words:* mushrooms, mycelium, phenols, flavonoids, antioxidant activity, carbon source

## INTRODUCTION

Reactive oxygen species (ROS) such as hydroxyl and superoxide radicals produced by sunlight, ultraviolet, chemical reactions, and metabolic processes have a wide variety of pathological effects on cellular processes (Aust and Sringen, 1982; Pryor et al., 1982; Torel et al., 1986). Superoxide radical is one of the strongest free radicals in cellular oxidation reactions because, once it forms, it further produces various kinds of cell-damaging free radicals and oxidizing agents (Maccarrone et al., 1997). There are many diseases such as heart disease, cancer, arthritis, and the aging process itself, in which free radicals are implicated. To combat these free radicals the body needs antioxidants (Harman, 1997) and in this respect, flavonoids and other phenols have shown to posses an important antioxidant activity towards these radicals, which is principally based on the redox properties of their phenolic hydroxyl groups and the structural relationships between different parts of their chemical structure (Bors and Saran, 1987). Antioxidant compounds can increase shelf life by retarding the process of lipid peroxidation, which is also one of the major reasons for deterioration of food products during processing and storage (Halliwell, 1997; Halliwell and Gutteridge, 1999). Thus a need for identifying sources of antioxidants has been created, and the search for natural antioxidants, especially of plant origin, has notably increased in recent years (Skerget et al., 2005).

Mushrooms have become attractive as a functional food and as source for the development of drugs and nutraceuticals (Chang, 1996; Chang, 1999), namely for antioxidant compounds (Yen and Hung, 2000; Mau et al., 2002; Yang et al., 2002; Cheung et al., 2003; Cheung and Cheung, 2005; Lo and Cheung, 2005). In addition to dried mushrooms, alternative or substitute mushroom products are their mycelia, mainly prepared from submerged culture. This mycelium could be used as food and food-flavoring material, or in the formulation of nutraceuticals and functional foods. The nutritional value and taste components of some mushroom mycelia have been studied (Weng, 2003). The growth of an edible mushroom is a lengthy and complex process involving the use of solid compost or lignocellulosic bed, such as straw or cotton, followed by a long cultivation period (Vedder, 1978). Growing mushroom mycelium in liquid culture on a defined nutrient medium has long been a simple and fast alternative method to produce fungal biomass (Cirillo et al., 1960; Litchfield, 1967; Cheung, 1995). The fruiting bodies of edible mushrooms are commonly used in human diets as a source of

**To whom correspondence should be sent (e-mail: iferreira@ipb.pt).*
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protein (Gray, 1973) and mycelia have already showed comparable nutritional values to mushroom and fruiting bodies (Hadar and Cohen-Arazi, 1986). Also some mycelia antioxidant properties were described (Mau et al., 2004), but little information is available about the influence of the culture medium on mycelium production and antioxidant activity.

In this study, we evaluated the influence of four different carbon sources in the production and antioxidant activity of *Leucopaxillus giganteus* (Sowerby) Singer mycelium, a *Basidiomycete* fungus belonging to the order *Agaricales*, and family *Tricholomataceae*. *Leucopaxillus* species have been reported to have medicinal activity, being used in chemical industry for extraction of clitocybin antibiotic (Breitenbach and Kränzlin, 1991).

The mycelium growth was followed along the time (15, 30, 45, and 60 days) and the antioxidant activity was evaluated for each carbon source and for each growth day, using several assays. We studied the free-radical scavenging capacity and reducing power of the extracts (Ferreira et al., 2007), and also the inhibition of oxidative hemolysis in erythrocytes induced by 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), which has been extensively studied as model for the peroxidative damage in biomembrane. The presence of different antioxidants in the mycelium extracts that can hinder the extent of $\beta$-carotene bleaching by neutralizing the linoleate free radical and other free radicals formed in the system was also tested. All these antioxidant activity parameters were correlated to the total content of phenolic compounds and flavonoids present in the samples.

# MATERIALS AND METHODS

## Material

### *Standards and Reagents*

Standards BHA (2-*tert*-butyl-4-methoxyphenol), TBHQ (*tert*-butylhydroquinone), L-ascorbic acid, $\alpha$-tocopherol, gallic acid, and (+)-catechin were purchased from Sigma (St Louis, MO, USA). 2,2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). All other chemicals were purchased from Sigma Chemical Co. (St Louis, MO, USA) and methanol from Pronalab (Lisbon, Portugal). Water was treated in a Mili-Q water purification system (TGI Pure Water Systems, USA).

### *Samples*

Mycelia of *L. giganteus* (Sowerby) Singer was isolated from sporocarps collected under grassland in Bragança (NE of Portugal, Herbarium of Agrarian School – Instituto Politécnico Bragança) on solid Melin-Norkans (MMN) medium at pH 6.6 (NaCl 0.025 g/L; $(NH_4)_2HPO_4$ 0.25 g/L; $KH_2PO_4$ 0.50 g/L; $FeCl_3$ 0.050 g/L; $CaCl_2$ 0.50 g/L; $MgSO_4.7H_2O$ 0.15 g/L; thiamine 0.10 g/L; casamino acids 1.0 g/L; malt extract 10 g/L; glucose 10 g/L; agar and 20 g/L). The strain was maintained in the same medium at 25 °C in the dark and sub-cultured every month.

## Methods

### *Effect of Carbon Source on Mycelia Growth Rate*

For aseptic establishment of assay, 10 hyphal plugs (5 mm diameter) of 1-week-old *L. giganteus* mycelia were transferred into flasks (700 mL) containing 250 mL of MMN liquid medium performed with four different carbon sources: glucose, sucrose, fructose, and mannitol. Inoculated flasks were shaken and maintained in the dark at 25 °C. After 15, 30, 45, and 60 days of growth the mycelium was recovered from the liquid medium by filtration, washed with distilled water, weighted (fw) and dried at 50 °C, during 24 h, to obtain the yield of biomass (dw). Three replicate flasks of each combination were performed (12 flasks per carbon source).

### *Effect of Carbon Source on Antioxidant Activity*

The dried mycelium (~1 g) obtained after different growth times (15, 30, 45, and 60 days) and in the presence of each carbon source (glucose, sucrose, fructose, and mannitol) was extracted by stirring with 100 mL of methanol at 25 °C at 150 rpm for 24 h and filtered through Whatman no 4 paper. The residue was then extracted with one additional 100 mL portion of methanol, as described earlier. The combined methanolic extracts were evaporated at 40 °C to dryness and redissolved in methanol at a concentration of 10 mg/mL, and stored at 4 °C for further use.

### *Determination of Antioxidant Components*

Phenolic compounds in the mycelium extracts were estimated by a colorimetric assay, based on procedures described by Singleton and Rossi (1965) with some modifications. Briefly, 1 mL of sample was mixed with 1 mL of Folin and Ciocalteu's phenol reagent. After 3 min, 1 mL of saturated sodium carbonate solution was added to the mixture and adjusted to 10 mL with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm (Analytikijena 200-2004 spectrophotometer). Gallic acid was used to calculate the standard curve (0.01–0.4 mM) and the results were expressed as mg of gallic acid equivalents (GAEs) per gram of extract.

Flavonoid contents in the extracts were determined by a colorimetric method described by Jia et al. (1999) with some modifications. The mycelium extract (250 µL) was mixed with 1.25 mL of distilled water and 75 µL of a

5% $NaNO_2$ solution. After 5 min, 150 µL of a 10% $AlCl_3 \cdot H_2O$ solution was added. After 6 min, 500 µL of 1 M NaOH and 275 µL of distilled water were added to the mixture. The solution was mixed well and the intensity of pink colour was measured at 510 nm. (+)-Catechin was used to calculate the standard curve (0.022–0.34 mM) and the results were expressed as mg of (+)-catechin equivalents (CEs) per gram of extract.

### *DPPH Radical-scavenging Assay*

The capacity to scavenge the 'stable' free-radical DPPH was monitored according to the method of Hatano et al. (1988). Various concentrations of mycelium extracts (0.3 mL) were mixed with 2.7 mL of methanolic solution containing DPPH radicals ($6 \times 10^{-5}$ mol/L). The mixture was shaken vigorously and left to stand for 60 min in the dark (until stable absorption values were obtained). The reduction of the DPPH radical was determined by measuring the absorption at 517 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discolouration using the equation:% RSA $= [(A_{DPPH} - A_S)/A_{DPPH}] \times 100$, where $A_S$ is the absorbance of the solution when the sample extract has been added at a particular level, and $A_{DPPH}$ is the absorbance of the DPPH solution. The extract concentration providing 50% inhibition ($EC_{50}$) was calculated from the graph of RSA percentage against extract concentration. BHA and $\alpha$-tocopherol were used as standards.

### *Reducing Power Assay*

The reducing power was determined according to the method of Oyaizu (1986). Several concentrations of mycelium methanolic extracts (2.5 mL) were mixed with 2.5 mL of 200 m mol/L sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. After 2.5 mL of 10% trichloroacetic acid (w/v) was added, the mixture was centrifuged at 1000 rpm for 8 min (Centorion K24OR- 2003 refrigerated centrifuge). The upper layer (5 mL) was mixed with 5 mL of deionised water and 1 mL of 0.1% of ferric chloride, and the absorbance was measured spectrophotometrically at 700 nm. The extract concentration providing 0.5 of absorbance ($EC_{50}$) was calculated from the graph of absorbance at 700 nm against extract concentration. BHA and $\alpha$-tocopherol were used as standards.

### *Assay for Erythrocyte Hemolysis Mediated by Peroxyl Free Radicals*

The antioxidant activity of the mycelium extracts was measured as the inhibition of erythrocyte hemolysis (Miki et al., 1987; Zhang et al., 1997). Blood was obtained from male ram (churra galega transmontana) of body weight~67 kg. Erythrocytes separated from the plasma and the buffy coat were washed three times with 10 mL of 10 mM phosphate buffer saline (PBS) at pH 7.4 (prepared by mixing 10 mM of $NaH_2PO_4$ and $Na_2HPO_4$, and 125 mM of NaCl in 1L of distilled water) and centrifuged at 1500 *g* for 5 min. During the last washing, the erythrocytes were obtained by centrifugation at 1500 × *g* for 10 min. A 0.1 mL of a 20% suspension of erythrocytes in PBS was added to 0.2 mL of 200 mM AAPH solution (in PBS) and 0.1 mL of mycelium methanolic extracts of different concentrations. The reaction mixture was shaken gently (30 rpm) while being incubated at 37 °C for 3 h. The reaction mixture was diluted with 8 mL of PBS and centrifuged at 3000 × *g* for 10 min; the absorbance of its supernatant was then read at 540 nm by a spectrophotometer, after filtration with a syringe filter (cellulose membrane 30 mm, 0.20 µm, Titan). The percentage hemolysis inhibition was calculated by the equation% hemolysis inhibition $= [(A_{AAPH} - A_S)/A_{AAPH}] \times 100$, where $A_S$ is the absorbance of the sample containing the mycelium extract, and $A_{AAPH}$ is the absorbance of the control sample containing no mushroom extract. The extract concentration providing 50% inhibition ($EC_{50}$) was calculated from the graph of hemolysis inhibition percentage against extract concentration. L-Ascorbic acid was used as standard.

### *Antioxidant Assay Using the β-Carotene Linoleate Model System*

The antioxidant activity of mushroom extracts was evaluated by the β-carotene linoleate model system (Mi-Yae et al., 2003). A solution of β-carotene was prepared by dissolving 2 mg of β-carotene in 10 mL of chloroform. Two milliliters of this solution were pipetted into a 100 mL round-bottom flask. After the chloroform was removed at 40 °C under vacuum, 40 mg of linoleic acid, 400 mg of Tween 80 emulsifier, and 100 mL of distilled water were added to the flask with vigorous shaking. Aliquots (4.8 mL) of this emulsion were transferred into different test tubes containing 0.2 mL of different concentrations of the mycelium extracts. The tubes were shaken and incubated at 50 °C in a water bath. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm using a spectrophotometer. Absorbance readings were then recorded at 20 min intervals until the control sample had changed color. A blank, devoid of β-carotene, was prepared for background subtraction. Antioxidant activity was calculated using the following equation: Antioxidant activity = (β-carotene content after 2 h of assay/initial β-carotene content) × 100. The extract concentration providing 50% antioxidant activity ($EC_{50}$) was calculated from the graph of antioxidant activity percentage against extract concentration. TBHQ was used as standard.

*Statistical Analysis*

All the assays were carried out in triplicate and the results are expressed as mean values and standard error or standard deviation. Differences among means were done by analysis of variance (ANOVA), using SAS v.9.1.3, and averages were compared using Tukey test ($p<0.05$). A regression analysis, using Excel for Windows, was established between phenolic and flavonoid contents and $EC_{50}$ values obtained by different antioxidant assays.

# RESULTS AND DISCUSSION

### Effect of Carbon Source on Mycelia Growth Rate

The *L. giganteus* mycelium growth varied with the sugar, the disaccharide sucrose being the best carbon source (Figure 1). Glucose led to a lower mycelium yield although it has been referred to as the most suitable carbon source for mycelium growth of a majority of mushrooms (Chang, 1989; Wang, 1993; Yang, 1996). The mycelium growth in the presence of sucrose significantly increased until 30 days of incubation ($0.367 \pm 0.0175$ g) decreasing after 45 days of growth ($p<0.05$). Although in the presence of glucose the mycelium growth increased significantly until 30 days ($0.255 \pm 0.1215$ g), the dry weight obtained after 45 and 60 days did not varied significantly ($p>0.05$). Using fructose as carbon source the mycelium yield significantly increased from $0.024 \pm 0.0019$ to $0.270 \pm 0.0531$ g (after 45 days), remaining with a similar dry weight ($p>0.05$) until 60 days of growth. The presence of mannitol significantly increased the mycelium growth along the incubation time ($p<0.05$).

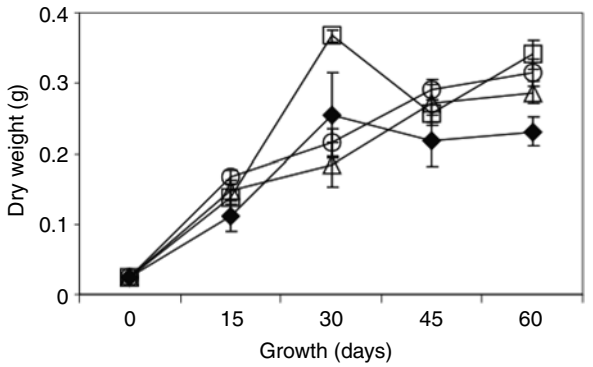


**Figure 1.** Mycelium growth (dw) of *Leucopaxillus giganteus* in the presence of different carbon sources: (◆) glucose; (Δ) sucrose; (□) fructose; and (○) manithol. Each value is expressed as mean ± standard error ($n=3$).

The maximum yield of the mycelium for sucrose (disaccharide) and glucose (aldohexose) was obtained after 30 days of incubation, while for fructose (ketohexose) and mannitol (hydroxilated monosaccharide) was obtained only after 60 days. This result implies that the mycelium growth not only varies with the carbohydrate source, but also changes with the time period of growth.

### Effect of Carbon Source on Antioxidant Activity

Sucrose was more efficient for the mycelium growth (Table 1), however glucose proved to be the best carbon source for the synthesis of phenols and flavonoids compounds, showing the highest content at all growth times ($12.7 \pm 1.09$ and $1.9 \pm 0.55$ mg/g, respectively after 60 days of growth). This phenols amount was even higher than the value found in the fresh mushroom as we had already described in a previous study (Barros et al., 2007). The amount found in the other extracts (2.9 to 5.7 mg/g) was significantly ($p<0.05$) lower than the content found in the first case. In general, monosaccharides (glucose, fructose) seemed to be better than disaccharides (sucrose) to the antioxidants synthesis. In fact, the presence of

**Table 1.** Flavonoids and phenols contents in the mycelium extracts obtained using different carbon sources after 15, 30, 45, and 60 days of growth.

| Growth | Glucose (mg/g) | Sucrose (mg/g) | Fructose (mg/g) | Mannitol (mg/g) |
|---|---|---|---|---|
| 15 days | | | | |
| Flavonoids | 1.3 ± 0.29 a | 0.8 ± 0.08 b | 1.0 ± 0.11 ab | 0.5 ± 0.09 b |
| Phenols | 6.9 ± 0.71 a | 2.1 ± 0.71 b | 2.8 ± 0.62 b | 2.3 ± 0.71 b |
| 30 days | | | | |
| Flavonoids | 1.4 ± 0.26 a | 0.8 ± 0.05 bc | 1.1 ± 0.18 ab | 0.7 ± 0.11 c |
| Phenols | 8.0 ± 0.83 a | 2.3 ± 0.70 c | 4.6 ± 0.90 b | 2.2 ± 0.68 c |
| 45 days | | | | |
| Flavonoids | 1.7 ± 0.57 a | 1.0 ± 0.07 ab | 1.1 ± 0.12 ab | 0.9 ± 0.11 b |
| Phenols | 9.6 ± 1.10 a | 2.6 ± 1.21 bc | 5.2 ± 0.69 b | 2.5 ± 0.89 c |
| 60 days | | | | |
| Flavonoids | 1.9 ± 0.55 a | 1.0 ± 0.17 b | 1.4 ± 0.11 ab | 1.0 ± 0.13 b |
| Phenols | 12.7 ± 1.09 a | 3.5 ± 0.94 b | 5.7 ± 0.97 b | 2.9 ± 0.94 b |

Each value is expressed as mean ± SD ($n=3$). Values in the the same row followed by different letters are significantly by different ($p<0.05$).

monosaccharides significantly increased ($p<0.05$) the phenols content along the time. Mannitol (hydroxylated monosaccharide) led to lower phenols and flavonoids concentrations ($2.9\pm0.94$ and $1.0\pm0.13$ mg/g, respectively). For all the carbon sources, the antioxidant components content increased along the growth time (15 to 60 days). This is probably a response to the oxidative stress, and therefore free radicals production (Anderson, 1996), related to the mycelium growth.

Polyphenols include other subclasses besides flavonoids, such as phenolic acids, stilbenes, lignans, tannins, and oxidized polyphenols. Many of these compounds display a large diversity of structures and escape quantification, usually carried out by High Performance Liquid Chromatography (HPLC) and diode array detection, because (i) there is lack of commercial standards and (ii) numerous structures are not yet elucidated (Georgé et al., 2005). The method of Folin-Ciocalteu is, therefore, largely used to evaluate total phenols despite all the interferences present in this method since the reagent (mixture of phosphotungstic acid and phosphomolibdic acid) reacts with other no phenolic reducing compounds leading to an overvaluation of the phenolic content. It is known that ascorbic acid is the main reducing agent, which can interfere in the Folin-Ciocalteu reaction (Georgé et al., 2005), but ascorbic acid content in *L. giganteus* is very low as we reported in a previous work (Barros et al., 2007). In this study, the assays were performed in the whole extract, since it could be more beneficial than isolated constituents due to additive and synergistic effects; also, a bioactive individual component can change its properties in the presence of other compounds present in the extracts (Liu, 2003).

The highest content of these antioxidant compounds in the mycelium extract grown in the presence of glucose might account for the better results found in their antioxidant activity (Velioglu et al., 1998; Ferreira et al., 2007).

Figure 2 shows the scavenging activity on DPPH radicals, reducing power, hemolysis inhibition, and antioxidant activity by $\beta$-carotene bleaching method of the mycelium extracts after 30 days of growth (Table 2). The RSA values of mycelium methanolic extracts were expressed as the ratio percentage of sample absorbance decrease and the absorbance of DPPH solution in the absence of extract at 517 nm. From the analysis of Figure 2, we can conclude that the scavenging effects of mycelium extracts on DPPH radicals increased with the concentration increase and were very good for the extract from the glucose-medium (79.5% at 10 mg/mL), but lower than the scavenging effects of BHA (96% at 3.6 mg/mL) and $\alpha$-tocopherol (95% at 8.6 mg/mL). The RSA values at 10 mg/mL were moderate for the other carbon sources (fructose 57.4%, sucrose 49.1%, and mannitol 48.7%). The extracts from the sucrose and mannitol medium showed a very similar scavenging activity, after 30 days of growth.

The reducing power of the mycelium extracts increased with concentration. Reducing powers obtained for all the extracts were excellent (Figure 2); at 10 mg/mL they were higher than 1.60 and in the

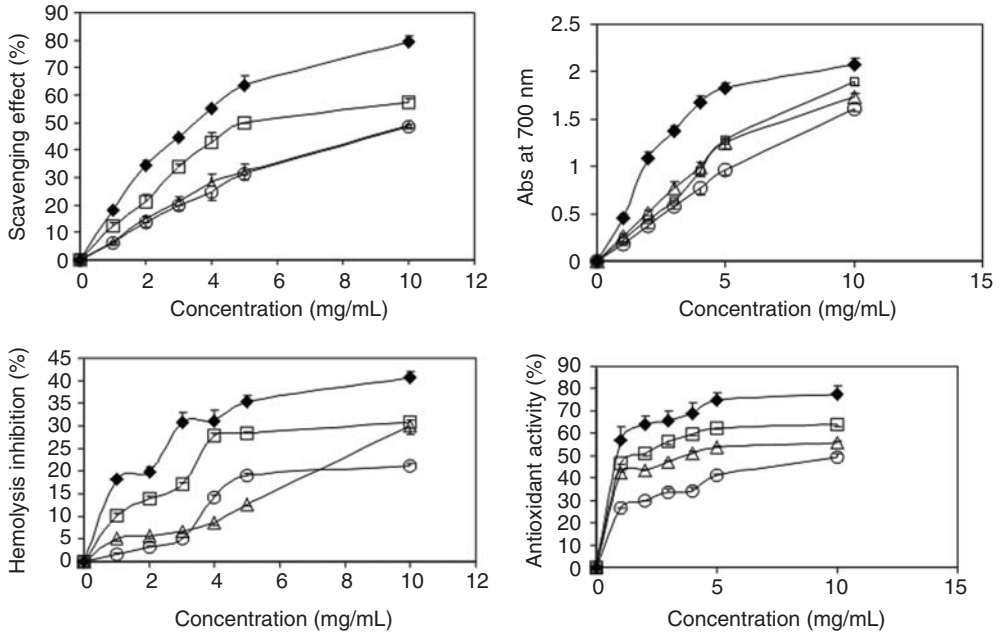


**Figure 2.** Scavenging activity on DPPH radicals (%), reducing power, hemolysis inhibition (%), and antioxidant activity by $\beta$-carotene bleaching method (%) of the mycelium extracts after 30 days of growth in the presence of different carbon sources: (△) sucrose; (□) fructose; (○) manithol; and (◆) glucose. Each value is expressed as mean ± standard error ($n=3$).

following order: glucose > fructose > sucrose > mannitol. Reducing power of BHA at 3.6 mg/mL and $\alpha$-tocopherol at 8.6 mg/mL was only 0.12 and 0.13, respectively. Methanolic extracts obtained in the presence of sucrose and fructose showed similar reducing power values. It was reported that the reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Shimada et al., 1992). Accordingly, the extract from the glucose-medium might contribute for the synthesis of higher amounts of reductone, which could react with free radicals to stabilize and block radical chain reactions.

In this study, the protective effect of the mycelium extracts on hemolysis by peroxyl RSA was also investigated. AAPH is a peroxyl radical initiator that generates free radicals by its thermal decomposition and attack the erythrocytes to induce the chain oxidation of lipids and proteins, disturbing the membrane organization and eventually leading to hemolysis.

The mycelium extracts inhibited hemolysis, as a result of protection against the oxidative damage of cell membranes of erythrocytes from ram induced by AAPH, in a concentration-dependent manner (Figure 2). Once more, the extract from the glucose-medium showed higher protective effect against erythrocytes hemolysis (40.7% at 10 mg/mL) than the other carbon sources (fructose 30.8%, sucrose 29.9%, mannitol 21.1%). However, the inhibition percentage of the standard L-ascorbic acid on hemolysis of red blood cell was much higher (94.6% at 1 mg/mL) than those of mycelium extracts.

The antioxidant activity of the mycelium extracts measured by the bleaching of $\beta$-carotene is displayed in Figure 2. The linoleic acid free radical attacks the highly unsaturated $\beta$-carotene models. The presence of different antioxidants can hinder the extent of $\beta$-carotene bleaching by neutralizing the linoleate free radical and other free radicals formed in the system (Jayaprakasha et al., 2001). Accordingly, the absorbance decreased rapidly in samples without antioxidant, whereas in the presence of an antioxidant, they retained their color, and thus absorbance, for a longer time. Antioxidant activity of mycelium extracts growth in the presence of glucose, fructose, glucose, and mannitol increased with their increasing concentration. Their antioxidant activities were 77.7, 64.1, 55.9, and 49.1% at 10 mg/mL, but antioxidant activity of TBHQ standard reached 82.2% at 2 mg/mL. It is likely that the antioxidative components in the mycelium extracts can reduce the extent of $\beta$-carotene destruction by neutralizing the linoleate free radical and other free radicals formed in the system. Again, the extract from the glucose-medium was the most effective for antioxidant activity.

$EC_{50}$ values for the antioxidant activity assays obtained from each mycelium-methanolic extract, after 15, 30, 45, and 60 days of growth (Table 2) revealed that the extract from the glucose-medium had better antioxidant properties (significantly lower $EC_{50}$ values; $p < 0.05$) than the other carbon sources, in agreement with the higher content of phenols found in the first case. The $EC_{50}$ values obtained for reducing power and antioxidant activity using the linoleate-$\beta$-carotene system were better than for scavenging effects on

**Table 2.** Scavenging effect, reducing power, and antioxidant activity $EC_{50}$ values of mycelium extracts grown in the presence of different carbon sources (glucose, sucrose, fructose, and mannitol) after 15, 30, 45, and 60 days of growth.

| | $EC_{50}$ (mg/mL) | | | |
|---|---|---|---|---|
| Growth | Glucose | Sucrose | Fructose | Mannitol |
| 15 days | | | | |
| Scavenging effect | 3.6 ± 0.121 c | >10.0 a | 6.2 ± 0.040 b | >10.0 a |
| Reducing power | 1.7 ± 0.253 c | 2.3 ± 0.129 ab | 2.1 ± 0.098 bc | 2.9 ± 0.320 a |
| Antioxidant activity | 1.5 ± 0.097 c | >10.0 a | 9.8 ± 0.057 b | >10.0 a |
| 30 days | | | | |
| Scavenging effect | 3.5 ± 0.031 c | >10.0 a | 5.1 ± 0.160 b | >10.0 a |
| Reducing power | 1.1 ± 0.154 c | 2.3 ± 0.166 ab | 2.0 ± 0.195 b | 2.6 ± 0.361 a |
| Antioxidant activity | 0.9 ± 0.153 d | 3.7 ± 0.401 b | 1.8 ± 0.342 c | >10.0 a |
| 45 days | | | | |
| Scavenging effect | 3.4 ± 0.126 c | >10.0 a | 4.7 ± 0.176 b | >10.0 a |
| Reducing power | 1.0 ± 0.086 b | 2.1 ± 0.273 a | 1.8 ± 0.265 a | 2.5 ± 0.387 a |
| Antioxidant activity | 0.8 ± 0.087 c | 2.3 ± 0.212 b | 0.9 ± 0.132 c | 4.5 ± 0.542 a |
| 60 days | | | | |
| Scavenging effect | 3.2 ± 0.155 c | >10.0 a | 4.3 ± 0.203 b | >10.0 a |
| Reducing power | 0.8 ± 0.133 c | 1.9 ± 0.140 a | 1.5 ± 0.197 b | 2.3 ± 0.187 a |
| Antioxidant activity | 0.7 ± 0.126 b | 1.0 ± 0.129 b | 0.9 ± 0.117 b | 1.6 ± 0.238 a |

Each value is expressed as mean ± SD ($n = 3$). Values in the the same row followed by different letters are significantly different ($p < 0.05$).

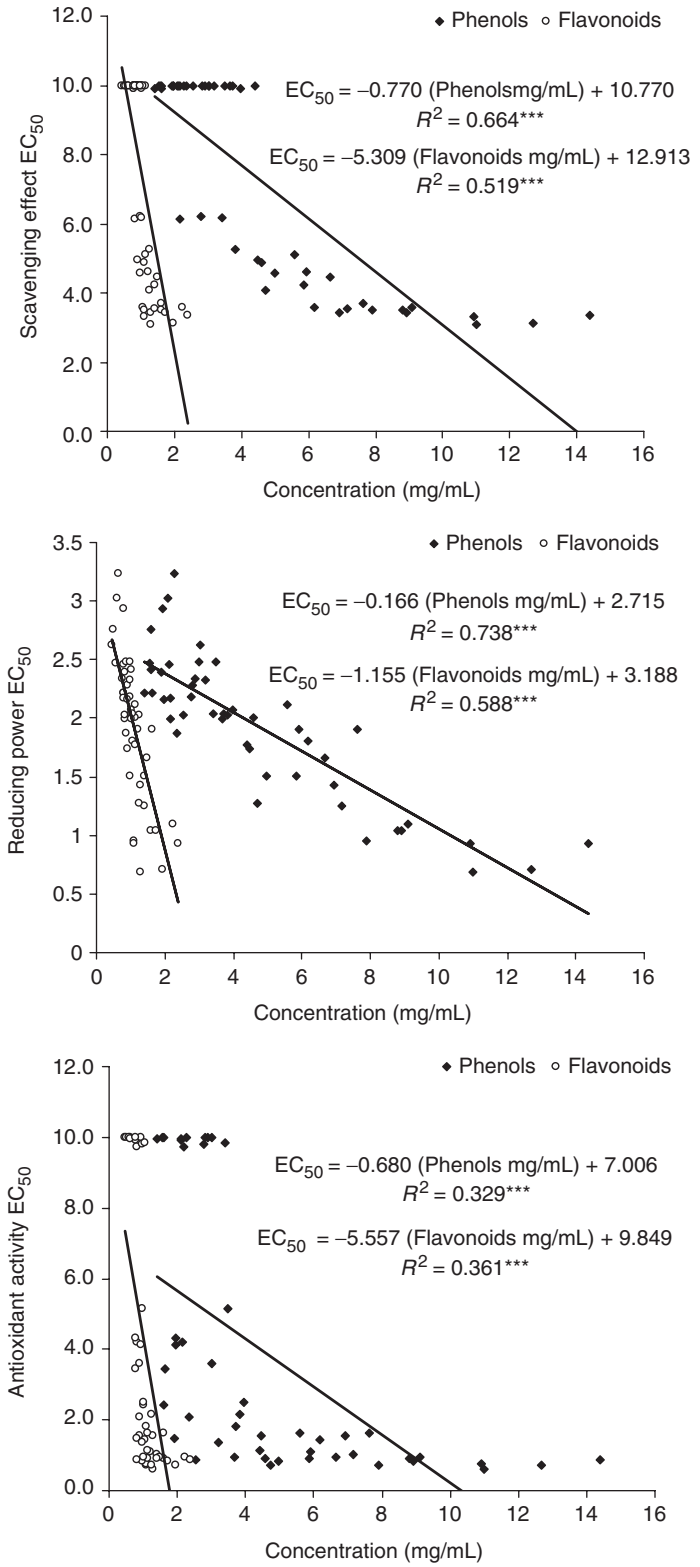


**Figure 3.** Correlation established between phenols (◆) and flavonoids content (◯) and scavenging effect on DPPH radicals [$EC_{50} = -0.680$(Phenols mg/mL) $+ 7.006$ $R^2 = 0.329$*; $EC_{50} = -5.557$(Flavonoids mg/mL) $+ 9.849$ $R^2 = 0.361$***], reducing power [$EC_{50} = -0.166$(Phenols mg/mL) $+ 2.715$ $R^2 = 0.738$***; $EC_{50} = -1.155$(Flavonoids mg/mL) $+ 3.188$ $R^2 = 0.588$***], and antioxidant activity [$EC_{50} = -0.680$(Phenols mg/mL) $+ 7.006$ $R^2 = 0.329$***; $EC_{50} = -5.557$(Flavonoids mg/mL) $+ 9.849$ $R^2 = 0.361$***]. *** means strong statistical significance.

DPPH radicals and for hemolysis inhibition mediated by peroxyl free radicals. In the last case, $EC_{50}$ values were always higher than 10 mg/mL. $EC_{50}$ values for all the antioxidant activity assays decreased along the growth time.

Significant negative linear correlations (Figure 3) were established between the phenols and flavonoids content, and $EC_{50}$ values of DPPH scavenging activity (determination coefficient 0.664 for phenols and 0.519 for flavonoids; $p < 0.001$), reducing power (determination coefficient 0.738 for phenols and 0.588 for flavonoids; $p < 0.001$) and antioxidant activity (determination coefficient 0.329 for phenols; $p < 0.001$ and 0.361 for flavonoids: $p < 0.001$).

In conclusion, the negative linear correlations obtained in this study prove that the sample with the highest phenols content shows higher antioxidant activity and lower $EC_{50}$ values (glucose as carbon source), while the sample with the lowest phenols content presents lower antioxidant activity and higher $EC_{50}$ values (mannitol as carbon source). The correlations also support that the mechanism of action of the extracts for the antioxidant activity may be identical, being related with the content in phenols and flavonoid compounds, and their free-radical scavenging activity. To our best knowledge, the present study was the first report to demonstrate that the antioxidative components in the extracts depends on the carbon source used for the mycelium growth, increasing along the growth time as a response to the oxidative stress and therefore free radicals production.

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