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Article

Evaluation of the nutritional composition and antioxidant activity of *Bjerkandera adusta*

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Bjerkandera adusta, a globally distributed fungus, is commonly used in the nutritional practices of the East Asian population. In this study, we evaluated the nutritional composition of the lyophilized mycelium of *B. adusta* as well as the phenolic composition and antioxidant activity of its extracts. The mycelium exhibited moisture (7.97 %), ash (3.27 %), and fiber (5.31 %) content values similar to the established values reported in the available literature. In addition, a high protein (9.32 %) and carbohydrate (63.45 %) content was shown, with a low lipid (1.36 %) content. The energy value per 100 g sample of mycelium was 1445.85 kJ. The results obtained indicated a statistically significant variation ($p < 0.05$) in the phenolic composition (81.84–110.96 mg gallic acid equivalents (GAE) per g of extract), free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity (IC_{50} 29.05–340.46 $\mu g \cdot mL^{-1}$), phosphomolybdenum antioxidant content (34.89–55.64 %), reduction of ferricyanide ion (66.55–69.4 %), and thiobarbituric acid reactive substance (TBARS) values (44.66–133.03 %). These results are unprecedented for this species and emphasize its nutraceutical potential.

Keywords: *Bjerkandera adusta*. Nutrient content. Functional food. Food analysis. Fungi.

INTRODUCTION

Bjerkandera is a widely distributed genus that consists of only two species of basidiomycetes: *B. adusta* and *B. fumosa* (Field, Verhagen, Jong, 1995). These fungi are classified as phytopathogenic fungi and are known for causing wood decomposition owing to white-rot (Heinfling *et al.*, 1998).

The fungus *B. adusta* is a species of edible mushrooms consumed in China (Wang *et al.*, 2014). The evaluation of the nutritional composition and biological activities of edible mushrooms has garnered scientific interest for two reasons. Firstly, they are useful sources of proteins and carbohydrates with a low lipid content; secondly, they are also useful sources of extracts and secondary metabolites with antitumor, antioxidant, antinociceptive, antihyperglycemic, and immunological activities (Wang, Fu, Han, 2013; Liu *et al.*, 2016; Carrasco-González, Serna-Saldívar, Gutiérrez-Uribe, 2017). Olennikov *et al.* (2014) reported results for some of the isolates of B. adusta, including simple and halogenated phenols, ergosterol, alkylitaconic acids and esters, squalene, triglycerides.

The optimization of mycelium production in an appropriate culture medium under controlled temperature, humidity, and cultivation time conditions would potentially enable the production of secondary metabolites

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of nutraceutical interest. In addition, this would contribute to the biotechnological development and production of supplies relevant to the functional food and medicine industries (Brakhage, 2013).

Thus, the aim of this work was to investigate the nutritional and phenolic composition as well as the antioxidant activity of the mycelium of *B. adusta* produced under ideal growing conditions.

MATERIAL AND METHODS

Sample preparation

Basidiomes from *B. adusta* were collected from the roots of *Pinus taeda* in the city of Jaguariaíva (Paraná, Brazil). After collection, the mycelium was purified and maintained in tubes containing potato dextrose agar (39 g of extract in 100 mL of ultrapure water). The mycelium cultures were preserved in mineral oil in a dark and air-conditioned environment in the Laboratory of Forest Pathology at the Brazilian Agricultural Research Corporation (EMBRAPA) (Colombo/Paraná, Brazil). The fungus was identified using molecular methods at the Laboratory of Basic Pathology, Federal University of Paraná, by Professor Ida Chapaval Pimentel, and its sequence data was submitted to GenBank (nucleotide sequence number MH486977).

To perform the analyses, it was necessary to ensure biotechnological production under ideal conditions using a biological oxygen demand incubator with controlled humidity (80 ± 5 %) and temperature (24 °C) for 28 days, with photoperiod off and a potato dextrose broth for culturing. After mycelial growth was realized, the mycelium and culture media were separated by filtration. The mycelium was lyophilized, milled, and stored in a closed container until use.

Chemical composition

Nutritional value

The approximate physicochemical composition (humidity, ash, lipid, and protein content) of the lyophilized mycelium of *B. adusta* was analyzed in accordance with the methods prescribed by the Association of Official Analytical Chemists (AOAC) (1995).

The humidity content was determined by warming the lyophilized mycelium sample at 105 °C until a constant weight was achieved. The ash content was determined by weighing the residue obtained after incineration in a furnace at 550 °C. The protein content was determined according to the Kjeldahl method, using a protein conversion factor equal to 4.38, thus excluding the non-protein nitrogen from the chitin present in the fungal cell wall (Sales-Campos *et al.*, 2011). The lipid content was determined according to the AOAC (1995) method by extracting the sample in a Soxhlet extractor using petroleum ether as the solvent. The crude fiber content was determined in accordance with the AOAC (1970) method in which strong acids and bases were used.

The carbohydrate content was determined according to the AOAC (2002) method; the following equation (1) was used in the analysis:

$$CT\ (\%) = (100\%) - (U\% + LT\% + PT\% + FB\% + CZ\%), \quad (1)$$

where

CT (%) = carbohydrate content in percentage (*m*/*m*);
U% = moisture content of the lyophilized sample in percentage;
LT% = lipid content of the lyophilized sample in percentage;
PT% = protein content of the lyophilized sample in percentage;
FB% = crude fiber content of the lyophilized sample in percentage; and
CZ% = total ash content of the lyophilized sample in percentage.

Total energy was calculated using the following equation (2):

$$\text{Total energy (kJ)} = 17 \times (\text{g total carbohydrate} + \text{g crude protein}) + 37 \times (\text{g crude lipid}) \quad (2)$$

Extract preparation

The extracts were prepared in a Soxhlet extractor. The sample of lyophilized mycelium was extracted using solvents of increasing polarity and heated to dryness in a water bath at 70 °C to obtain the respective extracts in the hexane (EH), chloroform (EC), ethyl acetate (EAE), and methanolic (EMEOH) solvents.

Total phenolic composition (TPC)

The Folin-Ciocalteu method was used to evaluate the TPC (Singleton, Orthofer, Lamuela-Raventós, 1999) by diluting the extracts in methanol at a concentration of 1 mg·mL^{-1}. The diluted samples were mixed with Folin-Ciocalteu reagent and ultrapure water and incubated for 10 min. Subsequently, an aqueous solution of 10 % sodium carbonate was added. After 30 min, the absorbance was read at 760 nm on a spectrophotometer.

The TPC values were calculated on the basis of a calibration curve prepared using a gallic acid standard (2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5, and 20.0 µg·mL^{-1}). The TPC was determined as milligrams of gallic acid equivalents (GAE) per g of extract (mg GAE·g^{-1}).

Antioxidant activity assays

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH radical scavenging activity of the mycelium extracts was measured spectrophotometrically. The procedure described by Mensor *et al.* (2001) was followed. The extracts were diluted in methanol to obtain final concentrations between 100 and 500 µg·mL^{-1}. They were then allowed to react with a DPPH methanolic solution at 0.03 mmol·mL^{-1} for 30 min in the dark. The absorbance of the mixture was read at 518 nm. We used ascorbic acid (1.6 at 8.0 µg·mL^{-1}) as the positive control and methanol as the negative control. The inhibition percentage IC_{50} for each extract was calculated using the following equation (3):

$$\text{DPPH scavenging effect (\%)} = [(A_{control} - A_{sample})/(A_{control})] \times 100 \quad (3)$$

where $A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance of the test compound.

Antioxidant activity using the phosphomolybdenum method

The phosphomolybdenum method described by Prieto, Pineda, and Aguilar (1999) was used. The extracts and standard were diluted in methanol (200 µg·mL^{-1}), allowed to react with the reagent solution (28 mL of 0.1 M sodium phosphate, 12 mL of 0.03 M ammonium molybdate, 20 mL of 3 M sulfuric acid, and ultrapure water made up to 100 mL), and incubated at 95 °C for 90 min. The absorbance was read at 695 nm, and the antioxidant activity (AA) % was calculated using the following equation (4):

$$\text{AA\% compared to ascorbic acid} = [(Abs_{(a)} - Abs_{(b)})/(Abs_{(c)} - Abs_{(b)})] \times 100, \quad (4)$$

where $Abs_{(a)}$ is the absorbance of the test compound, $Abs_{(b)}$ (sample diluted in water without reagent) is the absorbance of the blank, and $Abs_{(c)}$ is the absorbance of ascorbic acid.

Reducing power

This analysis was conducted according to the method described by Yen and Chen (1995), with some modifications. The extracts were diluted in ultrapure water to a concentration of 250 µg·mL^{-1}. The samples were incubated with 2.5 mL of a 0.2 M potassium phosphate buffer and 2.5 mL of an aqueous solution of 1 % potassium ferricyanide for 20 min at 45 °C. Thereafter, 2.5 mL of an aqueous solution of 10 % trichloroacetic acid was added. From this mixture, 2.5 mL was transferred to another assay tube and 1.4 mL of ultrapure water and 0.5 mL of an aqueous solution of 1.0 % ferric chloride was added. The absorbance was read at 700 nm. As a standard, ascorbic acid was used at the same concentration as the sample. The results were expressed in relation to standard activity (ascorbic acid).

Thiobarbituric acid reactive substances (TBARS)

We performed the TBARS analysis according to the method described by Morais *et al.* (2006), with some modifications. The extracts were diluted in ethanol at a concentration of 3 $mg \cdot mL^{-1}$. Egg yolk (0.5 % *m*/*v*) was used as the lipid source. For the analysis, 0.1 mL of the sample was added to 0.05 mL of 2,2'-azobis(2-amidinopropane) dihydrochloride at 0.035 % to induce lipid peroxidation. Subsequently, 20 % acetic acid solution, 0.4 % thiobarbituric acid (TBA) diluted in sodium dodecyl sulfate solution, and ultrapure water (0.4 mL) were added. The mixture was allowed to react in a water bath at 95 °C for 1 h. After cooling, *n*-butanol (1.5 mL) was added, and the mixture was centrifuged at 3000 rpm for 3 min. The absorbance of the supernatant was read on the spectrophotometer at 532 nm. As a standard, we used butylated hydroxytoluene (BHT) at the same concentration as the samples. To calculate the antioxidant index of the sample in percentage (PI %), we used the following equation (5):

$$PI\ \% = [(1 - Abs_{(a)} - Abs_{(b)}) / (Abs_{(p)} - Abs_{(b)})] \times 100, \quad (5)$$

where PI % = reduction of the lipid peroxidation in percentage, $Abs_{(a)}$ = sample absorbance, $Abs_{(p)}$ = standard absorbance (vitamin C, rutin), and $Abs_{(b)}$ = blank absorbance.

Statistical analysis

The results are presented as the mean ± standard deviation (SD) of three replicates of each experiment. A p value < 0.05 was used to indicate statistically significant differences among the mean values determined by analysis of variance using the IBM SPSS Statistics 20 software (IBM SPSS Statistics 2011; IBM, Armonk, NY, USA). The results of the phenolic compound content and antioxidant activities were compared using univariate and multivariate analysis (ANOVA), followed by Duncan's multiple range test.

RESULTS AND DISCUSSION

Chemical composition

Nutritional value

The results of the nutritional evaluation of the lyophilized mycelium from *B. adusta* are expressed based on dry weight (dw) as shown in Table I. The content determined, in descending order, was total carbohydrates (72.77 ± 0.07 g/100 g dw), crude proteins (9.32 ± 0.03 g/100 g dw), crude fibers (5.31 ± 2.18 g/100 g dw), and lipids (1.36 ± 0.07 g/100 g dw). The moisture content from the lyophilized mycelium was 7.97 ± 0.30 g/100 g dw.

These results are in accordance with those reported in the available research literature, showing that carbohydrates and crude proteins are the predominant compounds in fungi and that the lipid content is low Kalac (2013). Studies show that the nutritional composition of basidiomycetes can vary according to the species studied, with protein values of 8.6 at 38.6 g/100 g, lipid values of 2.0 at 7.9 g/100 g, carbohydrate values at 42.9 at 83.5 g/100 g, and fiber values at 0.6 at 11.5 g/100 g. The moisture (2.8 at 5.7 g/100 g) and total ash (2.7 at 8.6 g/100 g) content can also vary Sadler (2003).

The energy value for edible fungi has shown a high variation value of 86.44 at 1722.08 kJ/100 g of sample (Kalac, 2013; Liu *et al*., 2016) For the lyophilized mycelium from *B. adusta,* we observed an energy value of 1445.85 kJ/100 g of sample, showing that this fungus has energy potential similar to that described in the literature for other fungi.

Edible fungi can be an alternative for consumption in the human diet due to their nutritional composition with a high content of fatty acids, proteins, vitamins, minerals and low calories. The B. adusta lipid composition determined by CG-MS identified 26 compounds, 20 of which are fatty acids, with a higher oleic and linoleic content. These compounds may reduce the risk of cardiovascular disease and cholesterol levels (Kucukaydin, Duru, 2017), presenting potential for nutraceutical use.

TABLE I - Approximate physicochemical composition of the lyophilized mycelium of *B. adusta*

Analysis	Concentration in g/100 g of lyophilized mycelium
Humidity	7.97 ± 0.30
Proteins*	9.32 ± 0.03
Lipids	1.36 ± 0.07
Carbohydrates	72.77 ± 0.07
Crude fibers	5.31 ± 2.18
Total Ash	3.27 ± 0.95
Energy value (kJ)	1445.85

* We used a protein conversation factor of 4.38 (Sales-Campos *et al.*, 2011)

Total phenolic composition

The TPC of the *B. adusta* extracts are shown in Table II. The highest phenolic compound content was observed in the EMEOH and EAE extracts (110.96 ± 0.005 mg GAE·g^{-1} and 107.55 ± 0.01 mg GAE·g^{-1}, respectively). The difference between these results was not statistically significant. The EC extract also showed a high result with a phenolic compound content of 81.84 ± 0.01 mg GAE·g^{-1}. This variation in the phenolic content in the extracts could be attributed to differences in the affinity for the solvents used, structural characteristics, presence of aromatic rings with hydrogens substituted by hydroxyl groups (in different positions and number), and, consequently, differences in polarity among the compounds (Chisté, Benassi, Mercadante, 2014). It was not possible to evaluate the phenolic content of the EH extract owing to turbidity observed in the sample during analysis.

The ethanolic extract of the *B. adusta* mushrooms was previously found to have a phenolic composition of 12.46 ± 0.42 mg GAE·g^{-1} Nowacka *et al*. (2015), which demonstrates that the mycelial mass has a higher amount of TPC in the different extracts tested. This may be attributed to the cultivation or extraction method used. Oliveira *et al*. (2016) demonstrated that the extraction methods could influence the TPC.

In accordance with the Chew *et al*. (2011) classification, we were able to affirm that all the extracts, except the EH extract, showed a high phenolic compound content (> 50 mg GAE·g^{-1}).

Phenolic compounds are a large group of secondary metabolites, including simple phenols, phenolic acids, coumarins, flavonoids, tannins, lignins, lignans, and stilbenes. These substances have the capacity to inhibit lipid peroxidation and lipoxygenase *in vitro*, owing to their chemical structure, which contributes to their ability to be reduced (as hydroxyls bind at the aromatic ring of the phenolic compounds and electrons bind to the free radicals) (Sousa *et al*., 2007; Boonsong, Klaypradit, Wilaipun, 2016).

These compounds exhibit several biological activities, including their antimicrobial, antiviral, anti-inflammatory, antioxidant, antineoplastic, liver-protective, and anti-parasitic activities (Dornas *et al*., 2009).

Antioxidant activity assays

The methodologies for determining antioxidant capacity are classified into two major groups, one based on the capture of free radicals and the other on the oxidation determination of a target molecule. These techniques are subject to interference, such as a greater affinity for hydrophilic compounds or lipophilic compounds, therefore, the use of two or more techniques is recommended, since no method used alone can quantitatively analyze the total antioxidant action of a sample (Alves *et al*., 2010).

DPPH radical scavenging activity: This analysis evaluated the ability of the extracts to reduce 50 % of the free radical DPPH. The extracts that exhibited activity at the tested concentrations were EAE (229.05 ± 1.3 µg·mL^{-1}) and EMEOH (340.46 ± 2.0 µg·mL^{-1}), as shown in Table II.

The reducing capacity of the free radical DPPH is characteristic of basidiomycetes (Boonsong, Klaypradit, Wilaipun, 2016) Studies carried out on the ethanolic extract of the *B. adusta* mushroom have demonstrated low activity against the free radical DPPH with values greater than 1 mg/mL (Macáková *et al*., 2010). Similarly,

we observed that the mycelial extract has better DPPH radical scavenging activity than the mushroom.

The ability of the extracts to scavenge the free radical DPPH depends on their chemical composition. Phenolic compounds are among the secondary metabolites with a high reducing ability. This activity is associated with their chemical structure and reducing properties that enable them to neutralize and sequester free radicals (Sousa *et al.*, 2007; Dornas *et al.*, 2009; Zubair *et al.*, 2017). The extracts of *B. adusta* exhibited a high phenolic compound content; this result correlates with the antioxidant activity observed in our study.

Antioxidant activity using the phosphomolybdenum method: This analysis revealed the antioxidant ability of the lipophilic and hydrophilic compounds present in the extracts and is based on the reduction of phosphomolybdenum VI a V (Prieto, Pineda and Aguilar, 1999).

According to the results shown in Table II, we observed that the EAE (55.64 ± 2.92 %) extract exhibited better antioxidant activity than the ascorbic acid standard, followed by the EH (48.66 ± 2.86 %), EC (42.34 ± 1.31 %), and EMEOH (34.89 ± 1.31 %) extracts. The antioxidant activity evaluated by this method could be related to the complex mixture of secondary metabolites observed in the extracts, occurring as a result of synergy among them (Balestrin *et al.*, 2008). The reducing activity on the phosphomolybdenum complex has been previously described for other basidiomycetes (Okoro, 2012; Prabu, Kumuthakalavalli, 2016); however, this antioxidant activity has not been studied for other species of the *Bjerkandera* genus.

Reducing power: This analysis evaluated the ability of the extracts to reduce the ferricyanide ion to ferrocyanide in the presence of the ferric ion (from $FeCl_3$) (Santos *et al.*, 2007). Table II shows the results obtained for the extracts of *B. adusta*. We observed that all analyzed samples showed reducing activity, which was statistically similar (between 66 and 69 %), compared with that of the ascorbic acid standard. The methanol extract of *B. fumosa* showed moderate reducing activity (± 35 %), demonstrating that the species used in this study had more relevant activity (Kim *et al.* 2012).

These results showed the oxidation-reduction reaction of the extracts and were supported by the findings of the Folin-Ciocalteu method, indicating the presence of phenolic compounds in the studied samples. These phenolic compounds act by breaking the chain of free radicals, by donating a hydrogen atom, or avoiding the formation of peroxides (Loganayaki, Siddhuraju, Manian, 2013).

Antioxidant activity via the TBARS method: The TBARS test quantifies the malondialdehyde produced by the decomposition of hydroperoxides of unsaturated fat acids during the oxidative process (Guimarães *et al.*, 2010). While evaluating the antioxidant activity of the extracts of *B. adusta* using the TBARS method (Table II), we observed that the EC and EH extracts reduced the lipid peroxidation (PI) by 133.03 % and 75.56 %, respectively, showing higher results than the BHT standard (62.95 %). It should also be noted that the EAE extract had a PI of 65.58 %, which was statistically similar to the results of BHT.

The activity observed in the EC and EH extracts was attributed to their lipophilic characteristics, which permit better interaction with the lipid matrix. An additional justification of the expressed results was the presence of phenolic compounds, which showed the inhibition of lipid peroxidation (Merino *et al.*, 2015). The antioxidant activity of the fungal metabolites observed via the TBARS method may be correlated with the hepatoprotective effect as it prevents the accumulation of superoxides in the liver (Soares *et al.*, 2013).

When comparing the antioxidant activities of all extracts, we observe that the most promising ones are EAE and EMEOH, as these extracts have a higher concentration of phenolic compounds, which provides good activity against the DPPH radical and the phosphomolybdenum, reducing power and TBARS methods

TABLE II - Antioxidant activity of the extracts of *B. adusta* determined using different methodologies

Samples	TPC (mg GAE·g^{-1})	DPPH IC_{50} (µg·mL^{-1})	Phosphomolybdenum assay related to (%)	Reducing Power (%)	TBARS PI (%)
EH	-	> 500	48.66 ± 2.86^{c}	66.55 ± 0.01^{b}	75.56 ± 2.34^{b}
EC	81.84 ± 0.01^{b}	> 500	42.34 ± 1.31^{d}	67.13 ± 0.02^{b}	133.03 ± 3.72^{a}
EAE	107.55 ± 0.01^{a}	229.05 ± 1.3^{b}	55.64 ± 2.92^{b}	69.4 ± 0.01^{b}	65.58 ± 3.95^{c}
EMEOH	110.96 ± 0.005^{a}	340.46 ± 2.0^{c}	34.89 ± 1.31^{e}	67.25 ± 0.01^{b}	44.66 ± 3.31^{d}
Ascorbic acid	N/A	4.92 ± 0.06^{a}	100^{a}	100 ^{a}	N/A
BHT	N/A	N/A	N/A	N/A	62.95^{c}

Note: Hexane extract (EH); chloroform extract (EC); ethyl acetate extract (EAE); methanolic extract (EMEOH); all values are expressed as the mean ± SD (n = 3); different superscript letters in the same column indicate significantly different mean values; PI % = reduction of lipid peroxidation in percentage

(-) the lipophilic character of the extract caused turbidity in the analysis, and the results could not be considered; N/A, not applicable

CONCLUSION

The nutritional composition of the mycelium of *B. adusta* produced under ideal biotechnological conditions according to the described methods demonstrated that this species could have potential in nutraceutical applications The mycelium of *B. adusta* exhibited a high protein, carbohydrate, and crude fiber content and a low lipid content. This species also showed significant antioxidant potential that contributed to the inhibition of free radicals, lipid peroxidation, and oxidation-reduction. Considering that these results were easily obtained in optimized cultivation conditions, our study indicates that this fungus can be used as an alternative in the generation of pharmaceutical and nutritional substances, in response to the current demand.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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