Hydnopolyporus fimbriatus (Fr.) D.A. Reid mushroom: phytochemical screening, antioxidant activity, total flavonoid and total phenolic compounds

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Abstract

Hydnopolyporus fimbriatus is a species of mushroom of the Meripilaceae Jülich family found in some regions of Central and South America. This study aimed to evaluate the qualitative phytochemical composition, the antioxidant activity in the reduction of DPPH and hydroxyl free radicals and to determine the flavonoid contents and total phenolics in the ethanolic extract of the mushroom *H. fimbriatus*. The ethanolic extract was prepared and lyophilized. Qualitative phytochemical prospection assays were performed for different groups of organic compounds. The antioxidant assay was carried out in the reduction of the 2,2-diphenyl-1-picrylhy-drazyl free radical and in the reduction of the hydroxyl free radical by colorimetric methods. The ethanolic extract of *H. fimbriatus* positively exhibited several phytochemical groups, moderate antioxidant activity in reducing free radical DPPH 101.09 µ mL^{-1}, high hydroxyl radical reducing activity 87.51% and potential total phenolic content 20.15 mg GAE 100 g^{-1} of dry extract. Flavonoids content was not observed possibly due to the colorimetric method using aluminum chloride. With these results, it was possible to characterize the ethanolic extract of the vegetative part of *Hydnopolyporus fimbriatus*. New studies should be carried out in an attempt to develop a quantitative method of total flavonoids as well as test the extract in other biological models, thus being able to develop new drugs or products for agricultural use.

Keywords: Meripilaceae, *Hydnopolypors* genus, Phytochemistry, Total phenolics, Antioxidant activity

Resumo

Hydnopolyporus fimbriatus é uma espécie de cogumelo da família Meripilaceae Jülich encontrada em algumas regiões da América Central e do Sul. Este estudo teve por objetivo avaliar a composição fitoquímica qualitativa, a atividade antioxidante na redução dos radicais livres DPPH e hidroxila e determinar os conteúdos de flavonoides e fenólicos totais no extrato etanólico do cogumelho H. fimbriatus. O extrato etanólico foi preparado e liofilizado. Os ensaios qualitativos de prospecção fitoquímica foram realizados para diferentes grupos de compostos orgânicos. O ensaio antioxidante foi realizado na redução do radical livre 2,2-difenil-1-picril-hidrazila e na redução do radical livre hidroxila por métodos colorimétricos. O extrato etanólico de H. fimbriatus exibiu positivamente diversos grupos fitoquímicos, moderada atividade antioxidante na redução do radical livre DPPH 101.09 µ mL^{-1}, alta atividade de redução do radical hidroxila 87,51% e potencial conteúdo de fenólicos totais 20.15 mg EAG 100 g^{-1} de extrato seco. Não foi observado conteúdo de flavonoides, possivelmente devido ao método colorimétrico utilizando cloreto de alumínio. Com esses resultados, foi possível caracterizar o extrato etanólico da parte vegetative de *Hydnopolyporus fimbriatus*. Novos estudos deveram ser realizados na tentativa

de desenvolver um método quantitativ de flavonoids totais bem como, testar o extrato em outros modelos biológicos podendo assim, desenvolver novos fármacos ou produtos de uso agrícola.

Palavras-chave: Meripilaceae, Gênero *Hydnopolyporus*, Fitoquímica, Fenólicos totais, Atividade antioxidante

Resumen

Hydnopolyporus fimbriatus es una especie de champiñón de la familia Meripilaceae Jülich que se encuentra en algunas regiones de América Central y del Sur. Este estudio tuvo como objetivo evaluar la composición fitoquímica cualitativa, la actividad antioxidante en la reducción de radicales libres DPPH e hidroxilo y determinar el contenido de flavonoides y fenoles totales en el extracto etanólico del champiñón *H. fimbriatus*. El extracto etanólico fue preparado y liofilizado. Se realizaron ensayos cualitativos de prospección fitoquímica para diferentes grupos de compuestos orgánicos. El ensayo antioxidante se realizó en la reducción del radical libre 2,2-difenil-1-picrilhidrazilo y en la reducción del radical libre hidroxilo por métodos colorimétricos. El extracto etanólico de *H. fimbriatus* exhibió positivamente varios grupos fitoquímicos, moderada actividad antioxidante en la reducción del radical libre DPPH 101,09 μmL^{-1}, alta actividad reductora de radicales hidroxilo 87,51 % y potencial contenido fenólico total 20,15 mg EAG 100 g^{-1} de extracto seco. No se observó contenido de flavonoides posiblemente debido al método colorimétrico con cloruro de aluminio. Con estos resultados se logró caracterizar el extracto etanólico de la parte vegetativa de *Hydnopolyporus fimbriatus*. Se deberían realizar nuevos estudios en un intento de desarrollar un método cuantitativo de flavonoides totales así como probar el extracto en otros modelos biológicos, pudiendo así desarrollar nuevos fármacos o productos para uso agrícola.

Palabras clave: Meripilaceae, Género *Hydnopolymorus*, Tamizaje fitoquímico, Fenólicos totales, Actividad antioxidante

1. Introduction

The Meripilaceae Jülich family is composed of macroscopic, lignicolous and polyporoid mushrooms with wide distribution among biomes in the world, including the Cerrado domain in South America, Brazil (Leal; Gugliotta, 2008). Microorganisms belonging to this family have recognized ecological articulation with great expression within forest ecosystems, actively participating in the cycling of nutrients and in the decomposition of humic material, cellulose and lignin (Gilbertson; Ryvarden, 1986, 1987; Ryvarden, 1991; Rayner, 1995; Ruiz; Varela, 2006).

According to Kirk et al. (2001) and Castiblanco-Z et al. (2017) Meripilaceae is represented by nine genera: *Antrodia* P. Karst, *Grifola* Gray, *Henningsia* Möller, *Meripilus* Karst, *Physisporinus* Karst, *Diachanthodes* Singer, *Abortiporus* Murrill, *Rigidoporus* Murrill, and *Hydnopolyporus* D. A. Reid. *Hydnopolyporus fimbriatus* (Figure 1) has a heterogeneous distribution in Brazil (São Paulo register), and in 2017 Castiblanco-Z and collaborators recorded the first data in the literature of this species in Colombia, in addition to records of three other genera with discrete distribution, with 10 cataloged species. *H. fimbriatus* has annual, sessile, solitary to cespitose basidiomas, with flabelliform, irregular to spatulate hairs that form rosettes, has a variable hymenial surface and a monomitic hyphae system (Gilbertson; Ryvarde, 1986).

It is known that numerous mushrooms have important phytochemical compounds of great relevance for the production of medicines, enzymes, biofuels and also among the flora of the rhizosphere in plants and fungi (symbionts). *H. fimbriatus* still has few studies evaluating the extracts produced by different solvents. Contato et al. (2020) found total amounts of phenolics, cytotoxicity, effects on mitochondrial respiration, antimicrobial and antioxidant activities in different models using the aqueous extract of *H. fimbriatus*. In addition, mushrooms, whether edible or not, generally have nutritional characteristics that make them functional foods, that is, they bring health benefits and are rich in proteins, vitamins, minerals, essential amino acids, fiber, among other nutrients (Corrêa et al., 2016).

Although there are studies with *H. fimbriatus*, they have not yet explored the possible uses of this mushroom. Therefore, this study aimed to evaluate the hydroethanolic extract of *Hydnopolyporus fimbriatus* regarding the main phytochemical groups, biological activities (antioxidant) and the total flavonoid and phenolic contents of the vegetative organ.

Figure 1. *Hydnopolyporus fimbriatus* individual. Red bar corresponds to 15 cm. Source: Authors, 2022.

2. Materials and Methods

Reagents and equipment

Ethyl alcohol (LS Chemical), magnesium ribbon (Vetec), sodium potassium tartrate (Neon), potassium thiocyanate (Neon), iron sulfate (Neon), copper sulfate (Exodus), lead nitrate (Exodus) , potassium permanganate (Synth), potassium ferrocyanide (Vetec), sodium hydroxide (Neon), potassium iodate (Neon), iron chloride (Exodus), hydroxylamine hydrochloride (Neon), activated carbon (Neon), carbonate calcium (Aphatec) aluminum chloride (Neon), ascorbic acid (Vetec), salicylic acid (Vetec), hydrogen peroxide (Neon), 3,5-dinitrobenzoic acid (Neon), 2,6-Di-tert-butyl-4-methylphenol (Sigma Aldrich), galic acid (Vetec), Folin-Ciocalteu (Sigma Aldrich), 2,2-diphenyl-1-pikryl-hydrazyl (Sigma Aldrich) and copper acetate (Proquímicos).

UV-*Vis*-spectrophotometer (Belphotonic, Mod. M-51, Italy), Analytical balance (Shimadzu, Mod., AY220, Brazil), oven with air circulation (Thoth, Mod. Th-510-480, Brazil), orbital shaker table (Solab, Mod. SL-180/DT, Brazil), magnetic stirrer with temperature (Solab, Mod. SL 91, Brazil), centrifuge (Sieger, Mod. Sirius 4000, Brazil), lyophilizer (Solab, Mod. SL 404/B, Brazil), elisa microplate reader (Kasuaki, Mod. DR-200BS-NM-BI, Brazil) and UV camara (Solab, Mod. SL 204, Brazil).

Material collection and identification

The mushroom, were collected in February 2022, in the municipality of Rio Verde, located in the state of Goiás, Brazil. Part of the material was destined to the preparation of exsiccate, which were deposited and registered by the Micology Banck in Technological Chemistry laboratory, Instituto Federal Goiano, Rio Verde, Goiás, Brazil. The specie *H. fimbriatus* received the following registration number. BMLQT: 14.017.

Identification key for the species of Meripilaceae

1. Basidioma always cespitose, with numerous caps united forming rosettes…….*Hydnopolyporus fimbriatus*

Production of hydroethanolic extract

The extract of *H. fimbriatus* was obtained from 300 g of mushroom previously cleaned in running water and dried in an oven at 35 °C for 4 h, where it was then groundin a processor with 150 mL of 70% ethanol (*v/v*) for 5 min. After this process, the mixture was transferred to an amber flask and kept in a regrigerator at 4 °C for 10 days. After this period, the mixture was filtered and the supernatant collected and reduced in a rotaevaporator and then lyophilized.

Phytochemical screening

Phytochemical screening was performed based on the methods described by Menezes Filho et al. (2022) for phenols, tannins, alkaloids, flavonoids, saponins, quinones, terpenoids and steroids, reducing sugars,

non-reducing sugars, resins, amino acids, coumarins, glycosides, purines, organic acids, aromatic and aliphatic compounds, polysasscharides, carboxylic acids, catechins and oxylates.

Antioxidant activities

DPPH free radical reduction assay

The DPPH scavenging ability assay was used to evaluate the antioxidant activity of the mushroom extract. Test was conducted in a 98-well plate according to Sembiring et al. (2018) adapted. 20 µL stock solution of mushroom extract in different concentrations (5-5.000 ppm) and 180 µL of DPPH solution concentration 0.147 mMol mL^{-1} were added to each well. After 60 min incubation at room temperature in dark room, absorbance was read at (Abs 517 nm) using micro-plate reader of UV-*Vis* spectrophotometer. 70% ethanol was used as blank. The scavenging ability (%) was calculated according to equation [1] and Ascorbic acid and BHT was used as positive standards.

$$\%DPPH = (Abs\ standard - Abs\ crude\ extract)/(Abs\ standard)*100 \quad Eq.\ (1)$$

All tests were performed in triplicate. Concentration of mushroom extract samples resulting in 50% inhibition on DPPH (IC_{50} value) expressed in µg mL^{-1} was calculated.

Hydroxyl radical scavenging assay (HRS)

This was determined according to the literature by Mu et al. (2012). 0.5 mL of $C_7H_6O_3$ ethanol solution conc. 9.1 mMol L^{-1}, 0.5 mL of sample solution at different concentrations, 0.5 mL of $FeSO_4$ solution conc. 9.1 mMol L^{-1} and 3.0 mL of distilled water were successively mixed in a tube. The reaction was initiated by the addition of 3.0 mL H_2O_2 conc. 8.8 mMol L^{-1} to the mixture above, and the absorbance at 510 nm was read. The hydroxyl radical scavenging activity percentage (P) was calculated as follows equation [2]:

$$P(\%) = [1 - Abs(sample) - Abs(control)Abs(blank)]*100 \quad Eq.\ (2)$$

Where: the distilled water instead of H_2O_2 was used for the control, while distilled water instead of sample was used for the blank. All tests were performed in triplicate and the mean of Abs was used in the equation above.

Determination of total flavonoid content

Total flavonoid content was determined by $AlCl_3$ colorimetric assay the according Simbiring et al. (2018) modified. Standard solution of quercetin in conc. (1-500 µg mL^{-1}) was prepared in 98% ethanol. 50 µL of mushroom extract 1 mg mL^{-1} or standard solution was added to 10 µL of 10% the $AlCl_3$ solution and followed by 150 µL of 98% ethanol. 10 µL of 1 Mol L^{-1} $C_2H_3NaO_2$ was added to the mixture in a 96-well plate. 98% ethanol was used as reagent blank. All reagents were mixed and incubated for 60 min at room temperature protected from light. The Abs was measured at 415 nm with a 96-well plate reader UV-*Vis* spectrophotometer microplate. The total flavonoid contents were expressed as mg Quercetin equivalents (mg QE 100 g^{-1}) of mushroom extract.

Determinarion of total phenolic content

The total phenolic content was determined by Folin-Ciocalteu colorimetric method described by Simbering et al. (2018) modified. A total of 25 µL of the diluted mushroom extract were mixed with 100 µL of 1:9 diluted Folin-Ciocalteu reagent and shaken for 5 min in a flat-bottom 96-well microplate. The mixture was left for 15 min and then 75 µL of Na_2CO_3 solution conc. 100 g L^{-1} were added and the mixture was shaken at medium speed for 60 sec. After 2 h at room temperature, the Abs was measured at 765 nm UV-*Vis* spectrophotometer microplate. The Abs of the same reaction with ethanol instead of the mushroom extract or standard was subtracted from the Abs of the reaction with the sample. Gallic acid dilutions conc. (1-500 mg L^{-1}) was used as standards for calibration. Total phenolic contets were expressed as mg Gallic acid equivalents (mg GAE 100 g^{-1}) of mushroom extract.

Statistical analysis

Analyses were carried out in triplicate ± SD were calculated. The data was statistically analysed by ANOVA and means were compared by the Duncan multiple range test significance with the use of the IBM SPSS Statistics 26 software program. The *P* level of < 5% was supposed to be significant in determining the variations among mean values.

3. Results and Discussion

This is the first report of *H. fimbriatus* recorded in the state of Goiás, Brazil. Phytochemical prospection of mushroom *H. fimbriatus* hydroethanolic extract indicated the presence of different special metabolites classes

(Table 1). Many of them are known to have different therapeutic applications. Campi et al. (2019) evaluated the ethanolic extract of *H. fimbriatus* collected in Paraguay where they describe the presence of alkaloids, triterpenes and steroids, coumarins and reducing sugars. The phytochemical compounds of the species metabolite groups may vary in terms of qualitative and quantitative contents, as well as the presence or absence of groups. This action is due to biotic and abiotic factors to which the mushroom is present.

Thus, it is important to highlight the main biological and pharmacological properties described in the literature for alkaloids, with antimalarial, anti-inflammatory, anti-diabetic, anti-rheumatic and antipyretic activities (Marmitt and Rempel, 2016; Afewerki et al., 2019; Novanna et al., 2019; Ur Rashid et al., 2019; Carvalho et al., 2020); phenolic compounds have an antioxidant capacity to neutralize the activity of free radicals (EROs) generated in the body, with associations with several chronic-degenerative diseases such as neoplasms, diabetes and inflammatory processes, and reducing the prevalence of cardiovascular diseases (Rocha et al., 2011; Carvalho et al., 2020); foamy and hemolytic saponins have surfactant, cholesterol-lowering and anti-hemolytic actions (Duarte et al., 2014); organic acids exhibit gram-negative bactericidal action, *in vitro* as long as the acid molecules are ionized and there is contact with the bacteria for an adequate time (Reynolds, 1977; Duarte et al., 2014); aliphatic compounds have nematicidal, adjuvant, antimicrobial and larvicidal actions and inhibition of vegetable germination (Gall, 1966; Özbek et al., 2007; Seo et al., 2010); and carbocylic acids have antitumor, photoinduced biochemical activities (Tokuyama et al., 1993; Gielen et al., 2000). These chemical constituents can mainly account for the possible biological activity (antioxidants and flavonoids and phenolic contents).

Table 1. Phytochemical screening of mushroom *Hydnopolyporus fimbriatus* extract.

Special metabolites	**Results**
Phenols	+
Tannins	-
Alkaloids	+
Flavonoids	+
Foamy saponins	+
Hemolytic saponins	+
Quinones	-
Terpenoids & Steroids	-
Reducing sugars	+
Non-reducing sugars	+
Resins	-
Amino acids	-
Coumarins	-
Cardiac glycosides	+
Purines	-
Organic acids	+
Aromatic and Aliphatic compounds	Aliphatic
Polysasscharides	-
Carbocylic acids	+
Catechins	-
Oxylates	-

Note: (+) Positive. (-) Negative. Source: Authors, 2022.

According Matthäus, (2002) and Mu et al. (2012), DPPH is a well-known radical which demonstrates a strong absorption band at about Abs 520 nm, and it becomes colorless or light yellow or pale yellow when neutralized. DPPH radical is reduced by antioxidants through the donation of proton forming the reduced DPPH, and is

commonly used to evaluate the radical scavenging capacity of antioxidants.

The scavenging activity of *H. fimbriatus* mushroom extract against the DPPH radical were shown in (Table 1). Although the mushroom extract showed moderate DPPH radical-reducing activity, it was statistically inferior to ascorbic acid and BHT antioxidant standards. Campi et al. (2019) found for the ethanolic extract of *H. fimbriatus* a reducing activity of 4.95 mg g^{-1} on the DPPH radical. Accorging to Mu et al. (2012) the hydroxyl radical, more likely to be produced *in vivo*, is considered to be the most reactive and poisonous free radical in living organisms. The hydroxyl radical in the biomolecules and in type's diversity cells can easily cross cell membranes at specific sites and cause tissue damage and cell death. Thus, removing hydroxyl radical is very important for the protection of living systems. As shown in Table 2, the sample exhibited hight scavenging activity on hydroxyl radical. The hydroxyl radical reduction reaction by the extract, however, was inferior when compared to ascorbic acid and BHT, where they presented statistical difference according to the test applied.

According Gil-Ramírez et al. (2016) and Campi et al. (2019) mushrooms do not contain flavonoids. Although several researchers confirm the presence of "total flavonoids" in fungal extracts, others sustain the absence of this metabolite within the Fungi Kingdom (Ruíz-Rodríguez et al., 2009; Gil-Ramírez et al., 2016). Christ and Müller (1960), Gil-Ramírez et al. (2016) and Campi et al. (2019) discusses in his study, where most researchers used an unspecific colorimetric method developed to determine them in plants or plant products; the colorimetric method used is aluminum chloride as a selective reaction agent with certain flavonoids molecules depending on the reaction medium used, this technique is nonspecific for fungi, since they contain chlorogenic acid, hydroxycinnamic acid, *O*-diphenols and molecules that include catechol residues as pigments; and can give false positives in the aluminum chloride colorimetric assays.

Our methodology is based on the colorimetric method using aluminum chloride, our results did show absence of absorption by the flavonoid groups. Possibly, a negative reaction may occur due to very low concentrations of this group in the extract. Although they still use specific tests for plant extracts, today we know that fungi are organisms with a metabolism independent from plants and therefore we must establish specific tests taking into account the metabolites produced by them. The flavonoids showed an important result in the qualitative test, and this mushroom is an important source of this group of biomolecules with important biological activities as mentioned above (Table 1). The reaction between concentrated hydrochloric acid and magnesium shavings demonstrated the qualitative presence. Although this is a qualitative test and based on the studies cited, new quantitative tests should be performed.

The total phenolic content showed a satisfactory result. Demonstrating that this mushroom presents as a potential source of this type of compound (Table 2). Campi et al. (2019) found a quantitative content of 6.66 mg GAE 100 g^{-1} of total phenolics in the ethanolic extract of *H. fimbriatus* collected in Paraguay, this value is well below when observed in our study and by Contact et al. (2020) where also obtained a similar result to this study evaluating the extract of *H. fimbriatus* with phenolic content 25.85 (EF41) and 14.58 (EF44) mg GAE 100 g^{-1}.

Table 2. Antioxidant activity in the reduction of DPPH and hydroxyl radical and total phenolic and flavonoid contents in the ethanolic extract of the mushroom *Hydnopolyporus fimbriatus*.

Mushroom extact	**DPPH***	**HRS****	**Total flavonoids**	**Total phenolics**
	(µg mL^{-1})	**(%)**	**(mg QE 100 g^{-1})**	**(mg GAE 100 g^{-1})**
H. fimbriatus	101.09 ± 0.08c	87,51 ± 1.07b	nd	20.15 ± 0.65

Note: nd = not determined. *Ascorbic acid (1.37a ± 0.06 µg mL^{-1}) and BHT (2.15b ± 0.03 µg mL^{-1}) DPPH. **Ascorbic acid (99,70a ± 1.77 %) and BHT (99.11a ± 1.24 %) HRS. Source: Authors, 2022.

With respect to the DPPH and hydroxyl radical assay (Table 2), the mushorrom extract showed positive correlation between the concentrations of phenolic and flavonoid compounds and the antioxidant activity: low concentrations of these compounds (Table 1) indicated moderate antioxidant activity (Table 2), consistent with Campi et al. (2019), Olennikov (2011) and Keleş et al. (2011) who explain that these are the main antioxidant compounds of the special metabolism of mushrooms, mainly emphasizing phenolic and flavonoid compounds.

4. Conclusions

The phytochemical prospection of mushroom *Hydnopolyporus fimbriatus* extract detected the presence of

phenols, alkaloids, foamy and hemolytic saponins, reducing sugars and no-reducing sugars, cardiac glycosides, flavonols, organic acids, aliphatic compounds and caboxylic acids. Moderate DPPH free radical scavenging activity, high hydroxyl free radical scavenging activity and potential total phenolic content.

It is noteworthy that the colorimetric method using aluminum chloride is not an effective method for the determination of flavonoids, requiring another method for quantification.

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