Oliveira *et al.*
Cerrado: Agricultural and Biological Research, 2(1), 1-6, 2025
https://doi.org/10.14295/cerrado.v2i1.707

ORIGINAL RESEARCH PAPER

First report on the phytochemical profiling, antioxidant activities, and acetylcholinesterase inhibition of the hydroethanolic extract of the mushroom *Dacryopinax spathularia* (Schwein.), collected in the southwest region of Goiás, Brazil

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Article history
Received: July 03, 2024
Revised: September 14, 2024
Accepted: November 25, 2024

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Abstract: *Dacryopinax spathularia* (Schwein) is an edible mushroom rich in specialized metabolism molecules. This study aimed to report the occurrence of this species in a Brazilian Cerrado area, evaluate its hydroethanolic extract, and determine its antioxidant and acetylcholinesterase inhibitory activities. *D. spathularia* mushrooms (150 g) were collected, and the hydroethanolic extract was prepared. Phytochemical screening was performed using colorimetric tests. Antioxidant activity was assessed by the reduction of the DPPH free radical, and acetylcholine inhibition was evaluated using *Electrophorus electricus* type VI enzyme. Twelve groups of phytocompounds were identified. The antioxidant activity, measured by DPPH reduction, showed an IC_{50} of 10.95 µg mL^{-1}, and acetylcholinesterase inhibition demonstrated a result of 68.37%.

Keywords: DPPH; *Dacryopinax* genus; phytochemical prospecting; Basidiomycota; Edible mushrooms.

1. Introduction

Dacryopinax spathularia (Schwein) is part of a large number of edible and medicinal mushrooms belonging to the Basidiomycota group, which includes nearly 10,000 species, about 700 of which have been reported for their pharmacological properties (Karaman et al., 2012; Dandapat et al., 2015; Sharma et al., 2024). Traditionally, *D. spathularia* (Schwein) has been used to treat various diseases and disorders, such as exhibiting antiviral, antitumor, antibacterial, immunomodulatory, anti-inflammatory, antidiabetic, nephroprotective, and hepatoprotective activities (Mitko et al., 2008; Adebayo et al., 2012). However, there is a scarcity of scientific validation regarding the pharmacological and medicinal properties and the efficacy of this species.

Several phytochemical compounds have been described for *D. spathularia*, including carotenoids (Goldstrohm & Lilly, 1965), total phenolics, total flavonoids, alkaloids, tannins, and saponins (Kumar et al., 2018, 2019), and glycolipids (Bitzer et al., 2019). Considering the potential of these phytocompounds, it is evident that they serve as excellent agents for enhancing the reduction of various free oxidant forms, such as singlet oxygen, among others (Shahidi; Zhong, 2015). An antioxidant molecule inhibits the oxidation of other oxidizing agents. Oxidation is a chemical reaction that transfers electrons or hydrogen atoms from substances to an oxidizing agent. These oxidation reactions can generate free radicals, which can initiate chain reactions. When such reactions occur within a cell, they can lead to cell damage or death. Antioxidants prevent these chain reactions by neutralizing free radical intermediates and inhibiting further oxidative reactions, as described by Moharram

& Youssef (2014), Shenoy & Shirwaiker (2002), and Ames et al. (1993).

Additionally, numerous neurodegenerative diseases, including Alzheimer's disease (AD), are being studied using natural molecules as alternatives to synthetic ones, which are often highly toxic for treating this condition. The principal role of acetylcholinesterase (AChE) is the termination of nerve impulse transmission at the cholinergic synapses by rapid hydrolysis of acetylcholine (ACh) (Mukherjee et al., 2007). Inhibition of AChE serves as a strategy for the treatment of AD, senile dementia, ataxia, myasthenia gravis, and Parkinson's disease (Brenner, 2000; Rahman; Choudhary, 2001). There are a few synthetic medicines, e.g. tacrine, donepezil, and the natural product-based rivastigmine for the treatment of cognitive dysfunction and memory loss associated with AD (Oh et al., 2004).

This study aimed to report on and evaluate the phytochemical composition, biological antioxidant activities, and acetylcholinesterase inhibition properties of the hydroethanolic extract of *Dacryopinax spathularia*.

Figure 1. Various individuals of *Dacryopinax spathularia* developing on a tree trunk in leaf litter within a gallery forest area in the Cerrado domain, Goiás, Brazil. Source: Authors, 2024.

2. Material and Methods

2.1. Collection and identification

A total of 150 grams of the vegetative phase of *D. spathularia* was collected in a natural area located on a rural property in the municipality of Rio Verde, Goiás, Brazil, at the following geographic coordinates: 17°36'47.3"S and 50°47'02.5"W. The collection was conducted in October 2023. The mushroom was identified by the biologist, Master Tullyo Henrique Lima Machado, and a sample was prepared and preserved in the authors' Mycological Bank at the Technological Chemistry Laboratory of the Federal Institute of Goiás, Rio Verde, Goiás State, Brazil, with the voucher number DS01/2024.

2.2. Extraction process

The 70% hydroethanolic extract of the mushroom *D. spathularia* was prepared using 100 g of mushrooms previously cleaned with running water. The fresh mushrooms were then ground in a food processor for 1 minute. The processed material was transferred to an amber glass flask, to which 100 mL of 70% hydroethanolic solution (*v/v*) was added. The flask was stored in a refrigerator at 10 °C for 48 h. Subsequently, the extract was concentrated under a vacuum using a rotary evaporator. The concentrated extract was then frozen and lyophilized. The lyophilized extract was stored at 8 °C until analysis.

2.3. Phytochemistry prospecting

Phytochemical colorimetric assays were performed on the hydroethanolic extract of mushroom by qualitative determination as proposed by Madike et al. (2017), Sembiring et al. (2018), Mehdi et al. (2019) and Balamurugan et al. (2019). Alkaloids, carbohydrates, flavonoids, tannins, saponins, quinones, terpenoids, steroids, reducing sugars and non-reducing sugars, resins, amino acids, coumarins, glycosides, purines, organic acids, aromatic and aliphatic compounds, fatty acids, resins, gums and mucilage, anthraquinones, cardiac glycosides, polyphenols, phenolics, phytosterols, saponins, steroids, xanthoproteins, chalcones, triterpenoids, anthocyanins, leucoanthocyanins, emodins, polysaccharides, phlobatannins, carboxylic acids, and oxylates.

2.4. Antioxidant activity (DPPH)

The DPPH assay was carried out according to Menezes Filho et al. (2022). The 2,2-Diphenyl-1-picrylhydrazyl scavenging ability assay was used to evaluate the antioxidant activity of the mushroom extract. The test was conducted on a 96-well plate. Twenty (20 µL) stock solution of algae extracts in different concentrations (5-6.000 ppm) and 180 µL of DPPH solution conc. 0.147 mMol mL^{-1} was added to each well. After 60 min incubation at room temperature in a dark room, absorbance was read at 517 nm using a micro-plate reader of UV-*Vis* spectrophotometer. The 70% hydroethanolic solution was used as the instrumental blank. The elimination capacity (%) was calculated

according to Equation (1), and ascorbic acid was used as the standard antioxidant (positive control).

$$\%DPPH = (Abs\ sd - Abs\ ce)/Abs\ sd \times 100 \quad Eq.\ (1)$$

Where: sd = (Abs standard); ce = (Abs crude extract).

All tests were performed in triplicate. The concentration of mushroom extract samples resulting in 50% inhibition on DPPH (IC_{50} value) expressed in μg mL^{-1} was calculated. An assay for DPPH free radical reduction was performed in triplicate.

2.5. Acetylcholinesterase inhibition

The AChE inhibition method was colorimetric as described by Menezes Filho et al. (2023) and proposed by Miloševića et al. (2020). The AChE enzyme conc. (0.09 U mL^{-1}), acetylcholine iodide conc. (0.014 M) and DTNB (0.01 M) were dissolved in conc. phosphate buffer solution (0.1 M, pH = 8), the mushroom extract was diluted in conc. 1 mg mL^{-1} in phosphate buffer solution + 10% (v/v) DMSO. Serial dilutions of the mushroom extract (40 μL) were prepared directly in a 96-well microplate so that the concentration range in the final volume was between 0.4-400 μM. The solutions were adjusted to 160 μL with phosphate buffer working solution and then enzyme (20 μL) was added. After 15 min of incubation in D.B.O. without photoperiod at 25 ºC, aliquots of DTNB (10 μL) and AChE (10 μL) were added to the microplate wells.

Then, the plate was homogenized and incubated for another 40 min. The absorbance (Abs) at 405 nm was performed in a UV-*Vis* microplate reader. As blank, the phosphate buffer (180 μL) DTNB (10 μL), and AChE (10 μL) solutions were used. The maximum enzymatic activity was obtained by replacing the extracted sample with 10% DMSO phosphate buffer solution and the Abs of the mushroom extract by replacing the enzyme solution with phosphate buffer. A conc. eserine solution (10 μM) was used as a positive control (standard inhibitor). The percentage (%) inhibition of the enzymatic reaction was calculated as follows (Equation 2).

$$AChE\% = [(A-B)-(C-D)]/(A-B) \times 100 \quad Eq.\ (2)$$

Where: A, B, C, and D are the absorbances of the maximum enzymatic activity, reaction blank, enzymatic activity in the presence of the sample, and the color of the sample solutions, respectively. The AChE assay was performed in triplicate.

3. Results

Dacryopinax spathularia (Schwein.) G.W. Martin, Lloy-dia 11(2), 116, 1948. ≡ *Guepinia spathularia* (Schwein.) Fr., Elenchus Fungorum 2: 32. 1828.

Basidiomata scattered or gregarious, spathulate, stipitate bearing sinuate flabellate to petaloid pileus, orange, white-yellow at the sterile surface of basidiomata, soft cartilaginous, 1.1–1.7 cm high, 0.8–3.4 cm diameter at stipe. Internal hyphae branched, thin-walled, septate, pale yellow, 1–2.8 μm diam, without clamp connections. Hymenium unilateral. Probasidia cylindrical to clavate, pale yellow, 21–45 × 2–7 μm, becoming bifurcate. Basidiospores subglobose to reniform, with an apiculum at the base, thin-walled, pale yellow, 8.3–11.2 × 2.7–5.3 μm, 0–1.5 septate. The gelatinous fruiting bodies are typically orange to yellowish-orange in colour. This includes microscopic structures such as basidia (spore-bearing cells), basidiospores (reproductive spores), and septate hyphae (gelatinous filamentous structures), also described by Lopez & Garcia (2002) and Thachunglura et al. (2023).

The hydroethanolic extract of *D. spathularia* showed the positive presence of several phytochemical groups, particularly alkaloids, tannins, flavonoids, saponins, carotenoids, reducing sugars, amino acids, aliphatic compounds, phenolics, and carbohydrates (Table 1).

Table 1. Phytochemical prospecting of the main groups belonging to the secondary metabolism of the mushroom *Dacryopinax spathularia*.

Compounds	Results
Alkaloids	+
Tannins	+
Flavonoids	+
Saponins	+
Steroids	-
Carotenoids	+
Reducing sugars	+
Non-reducing sugars	-
Resins	-
Amino acids	+
Coumarins	-
Glycosides	-
Purines	-
Organic acids	+
Aromatic and Aliphatic compounds	Aliphatic
Fatty acids	-
Gums and Mucilage	+
Anthraquinones	-
Cardiac Glycosides	-
Polyphenols	-
Phenolics	+
Phytosterols	-
Xanthoproteins	-
Chalcones	-
Triterpenoids	-
Anthocyanins	-
Leucoanthocyanins	-
Emodins	-
Polysaccharides	-
Phlobatannins	-
Carboxylic acids	-
Oxylates	-
Terpenes	-
Carbohydrates	+
Psilocyn and Psilocybin	-

Note: Analyses performed in triplicate. Source: Authors, 2024.

The extract of the mushroom *D. spathularia* showed an IC_{50} reduction rate of 10.95 ± 0.25 µg mL^{-1} compared to the standard BHT (IC_{50} = 3.17 ± 0.10 µg mL^{-1}). The acetylcholinesterase inhibition rate was 68.37%.

4. Discussion

Dacryopinax spathularia can be naturally found in Asia, Africa, America, Australia, and other parts of the Pacific region (Hui et al., 2024). In Brazil, there are records of *D. spathularia* (Bauermann; Guerrero, 1989; Alvarenga; Xavier-Santos, 2017). For the state of Goiás, the only prior report is by Alvarenga and Xavier-Santos (2017) for the Central-West region. Our study presents the first data on this edible mushroom species inhabiting gallery forest areas in the Cerrado biome of the southwestern region.

These authors described the following characteristics of this species: Basidiomata scattered or gregarious, spathulate, stipitate, bearing a sinuate, flabellate to petaloid pileus, orange with a white-yellow sterile surface on the basidiomata. The basidiomata are soft-cartilaginous, 5–16 mm in height, with a stipe diameter of 0.6–2 mm. Internal hyphae are branched, thin-walled, septate, pale yellow, 1–2.5 µm in diameter, and lack clamp connections. The hymenium is unilateral. Probasidia are cylindrical to clavate, pale yellow, 20–38 × 2–4 µm, becoming bifurcate. Basidiospores are subglobose to reniform, with an apiculum at the base, thin-walled, pale yellow, 9–10 × 3–4 µm, 0–1 septate (Alvarenga; Xavier-Santos, 2017).

Several groups of edible mushrooms exhibit a remarkable content of phytocompounds with medical, pharmaceutical, and agricultural interests. In our study, we observed that the hydroethanolic extract of *D. spathularia* contained 12 phytochemical groups. Similar studies have reported findings that corroborate ours, although conducted in different regions of the world with *D. spathularia*. Kumar et al. (2018) identified significant levels of total phenolics (4.82 mg/g^{-1}), flavonoids (2.89 mg/g^{-1}), alkaloids (11.64 mg/g^{-1}), tannins (5.81 mg/g^{-1}), and saponins (28.56 mg/g^{-1}) in the aqueous extract of this mushroom.

Regarding antioxidant activity in reducing the DPPH free radical, our study demonstrated that the hydroethanolic extract showed greater reduction compared to the literature. Kumar et al. (2018) reported a maximum DPPH reduction for the aqueous extract of *D. spathularia* with an IC_{50} of 14.75 µg/mL^{-1}. Çayan et al. (2021) investigated the potential AChE inhibitory activity of various mushroom extracts. Mushrooms such as *Omphalotus olearius* and *Schizophyllum commune* were able to reduce AChE activity by 71.58% and 60.04%, respectively. In a previous report, the AChE activities of methanolic extracts from the mushrooms *Agaricus campestris*, *Agaricus arvensis*, *Fomes fomentarius*, *Armillaria mellea*, *Coriolus versicolor*, *Coprinus micaceus*, and *Lactarius deliciosus* were studied by Dundar et al. (2016). In this study, while the mushroom extracts were considered inactive against AChE, they demonstrated inhibition against BChE ranging from 23.26 ± 1.1% to 67.54 ± 0.1% at a concentration of 200 µg/mL^{-1}. The inhibitory activities of AChE (3.91 ± 0.15%–61.78 ± 0.42%) and BChE (5.60 ± 0.93%–61.78 ± 1.09%) for methanol, ethyl acetate, and hexane extracts of *Funalia*

trogii, *Ganoderma lucidum*, *Pleurotus ostreatus*, *Lyophyllum decastes*, and *Gyromitra esculenta* were reported by Tel et al. (2015).

5. Conclusion

Dacryopinax spathularia demonstrated a rich variety of phytochemical groups. Furthermore, it exhibited a high capacity to scavenge DPPH free radicals and showed potential acetylcholinesterase inhibition. Future studies should focus on evaluating the hydroethanolic extract of *D. spathularia* for additional biological activities, thereby expanding knowledge about this edible macrofungus.

6. Acknowledgement

The authors would like to thank the Research Foundation of Brazil (National Council for Scientific and Technological Development (CNPq), the Coordination for Upgrading Higher Institution Personnel (CAPES)); the Research Support Foundation of the State of Goiás (FAPEG); the Financier of Studies and Projects (FINEP); Center of Excellence in Bioinputs (CEBIO) and the Federal Institute Goiano for their financial and structural support to conduct this study.

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Funding Information

No funds were used in this study.

Author's Contributions

Guilherme Henrique Marques de Oliveira: project writing, mushroom collection, sample preparation, laboratory analyses, article writing, and publication. *Antonio Carlos Pereira de Menezes Filho*: species identification, laboratory analyses, article writing, translation, and publication. *Matheus Vinícius Abadia Ventura*: article writing, revisions, and publication. *Elizabete Nunes da Rocha*: advisor, project writing, mushroom morphological analysis, and final article writing.

Ethics

No conflicts of interest.