First report, phytochemical screening, and antioxidant and acetylcholinesterase inhibition activities of *Macrolepiota mastoidea* (Fr.) Singer mushroom extract in the Southwest region of Brazil

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Abstract

The Agaricales family is rich in species of edible macrofungi. *Macrolepiota mastoidea* is a representative of this group, found in various regions of the world, including Brazil. It is an edible species with several recorded biological activities. This study aimed to report the first findings on *M. mastoidea* and its phytochemical screening, as well as its antioxidant activity and acetylcholinesterase inhibition in a 70% (*v/v*) hydroethanolic extract from fresh mushrooms. *M. mastoidea* was collected from a Cerrado area in the Southwest of Goiás, Brazil. The hydroethanolic extract was produced, and the phytochemical screening was conducted for various phytochemical groups. Antioxidant activity was assessed using the DPPH free radical model, and acetylcholinesterase inhibition activity was determined using *Electrophorus electricus* type VI. The results showed the presence of various phytochemical groups such as alkaloids, sugars, flavonoids, and tannins. The DPPH reduction activity was IC_{50} 305.16 µg mL^{-1}, and acetylcholinesterase inhibition was 72%. *Macrolepiota mastoidea* demonstrated potential for further pharmacological studies with promising biological activities.

Keywords: alkaloids, flavonoids, *Macrolepiota* genus, DPPH, acetylcholinesterase, edible mushrooms.

Primeiro relato, prospecção fitoquímica e atividades antioxidante e unibição da acetilcolinesterase de extrato do cogumelo *Macrolepiota mastoidea* (Fr.) Singer na região Sudoeste do Brasil

Resumo

A família Agaricales é repleta de espécies de cogumelos superiores (macrocogumelos) comestíveis. *Macrolepiota mastoidea* é um representante desse grupo, encontrado em diversas regiões do mundo, inclusive no Brasil. É uma espécie comestíveil, com diversas atividades biológicas registradas. Este estudo teve por objetivo relatar o primeiro relato de *M. mastoidea* e sua prospecção fitoquímica bem como, atividade antioxidante e na inibição da acetilcolinesterase do extrato hidroetanólico 70% (*v/v*) em cogumelos *in natura*. *M. mastoidea* foi coletado em área de Cerrado no Sudoeste de Goiás, Brasil. O extrato hidroetanólico foi produzido. A prospecção fitoquímica foi realizada para diversos grupos fitoquímicos. A atividade antioxidante foi realizada para o modelo de radical livre DPPH e a atividade de inibição da acetilcolinesterase determinada utilizando *Electrophorus electricus* tipo VI. Os resultados demonstraram diversos grupos fitoquímicos como alcaloides, açúcares, flavonoides e taninos. A atividade redutora do DPPH foi de CI_{50} 305.16µg mL^{-1} e para AChE = 72%. *Macrolepiota mastoidea* demonstrou aptidão para novos estudos farmacológicos com promissoras atividades biológicas.

Palavras-chave: alcaloides, flavonoides, gênero *Macrolepiota*, DPPH, acetilcolinesterase, cogumelos comestíveis.

1. Introduction

The family Agaricaceae of Agaricales (Basidiomycota) was established by Singer (1948) and includes various groups and species of mushrooms, some of which are of commercial interest for culinary purposes (Shim et al., 2005). Currently, there are about 30 recognized species of *Macrolepiota* worldwide (Kirk et al. 2008). *Macrolepiota mastoidea* is a species of higher fungi belonging to the Agaricaceae family. Its fruiting body grows solitary or gregariously in open forests, under shrubs, on the ground among grasses and herbs, and in agricultural areas. The growing season for this mushroom is between summer and autumn in Turkey (Kolcuoğlu et al., 2007). In Brazil, *M. mastoidea* is mainly found in spring, particularly from September to December, and can be abundant in the Cerrado region, specifically in gallery forests.

The cap of the fruiting body measures between 70-150 mm in diameter, ranging from light ochre to brown, oval when young, and later becoming bell-shaped or flat with age, often featuring a nipple-like protrusion. The flesh of the mushroom is white, thick at the center of the cap, and thin towards the edge, with a weak odor and mild flavor. The stipe measures between 80-150 *x* 8-15 mm, cylindrical, solid when young and hollow when old, light ochre, and smooth (Kolcuoğlu et al., 2007).

Various authors discuss the benefits of the genus *Macrolepiota*, particularly its use in treating stomach and heart diseases by the local population (Ćirić et al., 2019). Furthermore, *M. mastoidea* is regarded as a wild mushroom consumed in cuisine due to its significant nutritional values, as reported by Kolcuoğlu et al. (2007) (33%) protein, (55%) total carbohydrates, and (4.5%) crude fat. Various studies present mushrooms as emerging sources of numerous pharmacological properties, including antimicrobial, anticancer, antioxidant, antiviral, immunomodulatory, immunosuppressive, anti-allergic, anti-inflammatory, and cholesterol-lowering activities. Specific groups of mushrooms have been used as food since ancient times, and their biological functions are continually being explored today, with edible species gaining importance as functional foods or functional food ingredients (Wasser, 2014; Soković et al., 2017; Glamočlija; Soković, 2017).

In the study by Ćirić et al. (2019), low levels of caloric values were found for the extract of *M. mastoidea* collected in Serbia. Free sugars such as mannitol and trehalose, along with oxalic and malic acids, were the predominant organic acids, while *p*-hydroxybenzoic and *p*-coumaric acids were identified as the main phenolic compounds. Three isoforms of tocopherols were identified and quantified: α-, β-, and δ-tocopherol. Additionally, biological activities with antioxidant potential, antimicrobial potential, and cytotoxic activity were observed in different tested tumor cell lines.

This study aimed to report the first encounter and evaluate the phytochemical profile, as well as the antioxidant activity and acetylcholinesterase inhibition of the hydroethanolic extract from the fruiting body of the mushroom *Macrolepiota mastoidea*.

Figure 1. Individuals of *Macrolepiota mastoidea* collected in a Cerrado area in the state of Goiás, Brazil, in 2024. Source: Authors, 2024.

2. Materials and Methods

2.1 Collection and identification of Macrolepiota mastoidea

Fruit bodies of *M. mastoidea* (300 g) were collected in a rural area in Rio Verde, Goiás, Brazil, on October 1, 2024, at the geographic coordinates (and). The collection site features a permanent preservation area of the Gallery Forest type. The specimen *M. mastoidea* was identified by Biologist and Master Tullyo and deposited in the mycological bank of the Technological Chemistry Laboratory at the Goiano Federal Institute (IF Goiano) in Rio Verde, Goiás State, Brazil, acquiring the voucher number MP03.

2.2 Preparation of the Extract

The *M. mastoidea* extract was obtained using 500 g of mushroom, previously cleaned with running water. Next, fresh mushrooms were ground in a food processor with 250 mL of 70% ethanol (*v/v*) for 5 min. The resulting mixture was transferred to an amber flask and stored in a refrigerator at 4 °C for 10 days. After this period, the mixture was filtered, the supernatant was collected, concentrated using a rotary evaporator, and subsequently lyophilized. The extract was stored in a freezer at -10 °C until analysis.

2.3 Qualitative Screening of Phytochemicals

Qualitative screening of the following phytochemicals was performed according to Sharaf et al. (2022) using the following standard methods. Test for alkaloids, flavonoids, glycosides, steroids, terpenoids, tannins, saponins and reducing sugars,

2.4 Antioxidant assay

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 (5-7.000 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. 70% ethanol was used as blank. The scavenging ability (%) was calculated according to (Equation 1), and ascorbic acid and 3,5-di-tert-4- Butylhydroxytoluene (BHT) were used as positive standards.

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

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).

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

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

3.1 Morphological description of Macrolepiota mastoidea

Macrolepiota mastoidea (Fr. : Fr.) Singer in Lilloa 22:417. 1951 (‘1949’).

Agaricus mastoideus Fr. : Fr., Syst. mycol. 1: 20. 1821.

Lepiota mastoidea (Fr. : Fr.) P. Kumm., Führ. Pilzk.: 135. 1871.

Lepiotophyllum mastoideum (Fr. : Fr.) Locq. in Bull. mens. Soc. linn. Lyon 11: 40. 1942.

Leucocoprinus mastoideus (Fr. : Fr.) Locq. in Bull. mens. Soc. linn. Lyon 14: 46. 1945.

Simplified key for *Macrolepiota* recognized in Brazil

1 Basidiomata with a volva at the base of the stipe…….*M. velosa*

1* Basidiomata without a volva at the base of the stipe

2 Pileus surface with brown plate-like squamules; annulus complex; clamp connections common at the base of the basidia

3 Stipe surface with conspicuous fine brown squamules on a whitish background; pileus squamules made up of yellowish-brown walled long hyphal segments, mainly 25-90 *x* 7-11 (14) μm…...……*M. procera*

3* Stipe surface with fine brown squamules on a whitish background; pileus squamules made up of yellowish-brown walled short hyphal segments, mainly 15-25 *x* 7-11 μm…….*M. detersa*

2* Pileus surface with pale ochraceous to brown fine squamules; annulus simple, or only slightly thicker near the edge; clamp connections absent or present

4 Stipe surface with brown squamules; usually without clamps at the base of basidia

4* Stipe surface smooth; usually with clamps at the base of basidia……..……*M. mastoidea*

Basidiomata are medium-sized to large. Pileus 5-11 cm in diam., fleshy, ovoid when young, becoming convex to plano-convex when mature, with a distinct umbo at the disc, white to off-white, covered with grey-brownish furfuraceous squamules, which are at first smooth and continuous, then gradually break up into irregular patches, and become minute and sparse toward margin; margin slightly appendiculate. Lamellae are free, crowded, white to greyish white, with 2-3 lengths lamellulae.

The stipe was subcylindrical, 6-15 *x* 0.5–1.0 cm, attenuating upwards, whitish, covered with tiny furfuraceous brownish squamules, especially above the annulus; base slightly enlarged. Annulus ascending, simple, whitish, membranous. Context whitish, not changing color when cut. Taste mild. Basidiospores 12.0-14.0 *x* 8.0-9.5 μm, ellipsoid to ovoid in side view, ellipsoid in front view, thick-walled, smooth, hyaline, dextrinoid, congophilous, metachromatic in cresyl blue, with a germ pore caused by an interruption in the episporium on the rounded apex, covered with a hyalinous cap in KOH; apiculus 1-1.5 μm long. Basidia 32-44 x 12.0-14.0 μm, clavate thin-walled, hyaline, 4-spored. Cheilocystidia 15-20 *x* 7-10 μm, clavate, hyaline, thin-walled, in bunches forming a sterile edge. Pleurocystidia absent. Squamules on pileus a palisade of subcylindric, clampless hyphae (6-12 μm in diam.), with terminal elements slightly attenuate toward the tip, with yellowish to brownish vacuolar pigment, slightly thick-walled. Clamp connections are occasionally observed at the base of basidia.

3.2 Phytochemical prospecting

In Table 1, the phytochemical results for *M. mastoidea* are presented. Positive results were observed for alkaloids, flavonoids, terpenoids, and reducing sugars. The tannin groups identified were hydrolyzable and gallic tannins.

Table 1. Phytochemical prospecting of the hydroethanolic extract of the mushroom *Macrolepiota mastoidea*.

Special metabolites	Results
Alkaloids	+
Glycosides	-
Flavonoids	+
Steroids	-
Terpenoids	+
Tannins	Blue
Reducing sugars	+
Saponins	-

Note: (-) no reagent. (+) reagent. Analyses performed in triplicate. Source: Authors, 2024.

The hydroethanolic extract of *M. mastoidea in natura* demonstrated, in our study, an antioxidant capacity against the DPPH free radical with an IC_{50} = 305 µg mL^{-1}, and an acetylcholinesterase inhibition assay result of 72% (Table 2).

Table 2. Antioxidant activity and acetylcholinesterase inhibition of the hydroethanolic extract of the mushroom *Macrolepiota mastoidea*.

Mushroom extract	**Results**
DPPH (µg mL^{-1})	305.16 ± 0.74
AChE inhibition (%)	72.1 ± 0.49

Note: Mean followed by standard deviation. Analyses performed in triplicate. Source: Authors, 2024.

4. Discussion

Our collections were conducted in a gallery forest area near rural fields of soybean and corn. M. mastoidea grew on the ground and was saprotrophic, occurring solitary. Ge et al. (2010) described *M. mastoidea* collected in China as inhabiting terrestrial areas, saprotrophic, and either solitary or dispersed in open meadows or mixed forests. It is distributed from the Northeast to the Southwest of Chinese territory. The genus *Macrolepiota* was also described in Australia with three species: *M. clelandii*, *M. eucharis*, and *M. dolichaula*, by Vellinga (2003).

Several studies have shown that higher fungi, or macrofungi, contain various phytochemical groups in their specialized metabolism. A significant number of potential biological activities are attributed to these phytochemical groups. Moreover, potent neurotoxins have also been identified, which are being studied for medical and recreational purposes. Ćirić et al. (2019) described that *M. mastoidea* exhibits a wide range of biological activities, including antimicrobial, anticancer, antioxidant, antiviral, immunomodulatory, immunosuppressive, anti-allergic, anti-inflammatory, and anti-cholesterol properties.

In our results, the presence of the main phytochemical groups described for a large proportion of mushrooms was observed, many of which are edible. Alkaloids, flavonoids, terpenoids, tannins, and sugars are groups extensively studied in medicine. Reducing sugars was also quantitatively described by Barros et al. (2007) in three samples of *M. mastoidea* mushrooms. The levels of reducing sugars reported were 0.30, 0.46, and 0.67 g/100 g of fresh $weight^{-1}$. Ćirić et al. (2019) reported the presence of sugars such as mannitol and trehalose, organic acids including oxalic acid, malic acid, citric acid, and fumaric acid, as well as phenolic acids like *p*-hydroxybenzoic acid, *p*-coumaric acid, and cinnamic acid in their study.

Macrolepiota mastoidea in our study demonstrated significant antioxidant activity in reducing the DPPH free

radical. Ćirić et al. (2019) reported antioxidant activity with an EC_{50} of 5.4 mg mL^{-1} for DPPH scavenging, 1.85 mg mL^{-1} for β-carotene/linoleate inhibition, and 0.47 mg mL^{-1} for TBARS, highlighting *M. mastoidea* as a mushroom species with substantial free radical reducing capabilities. Similarly, Barros et al. (2007) described remarkable antioxidant activities in three samples of *M. mastoidea* collected in Portugal, with results for DPPH reduction, reducing power, β-carotene bleaching inhibition, and lipid peroxidation inhibition showing reductions of 8.18, 8.49, and 25.60 mg mL^{-1}; 4.35, 4.44, and 4.79 mg mL^{-1}; 6.48, 8.92, and 8.10 mg mL^{-1}; and 24.20, 34.42, and > 50 mg mL^{-1}, respectively.

These findings further emphasize the potent antioxidant properties of *M. mastoidea*. Alzheimer's disease is a neurodegenerative condition characterized by memory and cognitive impairment, and it is responsible for 50 to 75% of all dementia cases worldwide (Niu et al., 2017). In this context, it is of great interest to evaluate fungal species for their neuroprotective potential. Exceptional activity in acetylcholinesterase inhibition was described in this study, with an inhibition rate of 72%. In the study proposed by Akata et al. (2019), the researchers confirmed the potential acetylcholinesterase (AChE) inhibition activity of the extracts from *M. mastoidea* and *M. procera*, with results of 0.94 and 0.83 mg GALAE g $extract^{-1}$, respectively. Cholinesterase inhibitors enhance synaptic plasticity, thereby facilitating learning and memory (Parsons et al., 2013). Interestingly, mushrooms have been identified as a revolutionary agent in the fight against neurodegenerative disorders (Sabaratnam et al., 2013). Indeed, several lines of evidence highlight the neuroprotective properties of mushrooms in improving neuronal health (Phan et al., 2013, 2015; Seow et al., 2013).

5. Conclusions

Macrolepiota mastoidea demonstrated various phytochemical groups of medicinal interest. The specialized metabolic phytochemical groups of *M. mastoidea* showed that the hydroethanolic extract of the mushroom exhibits remarkable antioxidant activity and acetylcholinesterase inhibition. Future studies should evaluate other phytochemical groups, as well as explore various biological activities, to expand the knowledge of this edible mushroom.

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7. Authors' Contributions

Hugner Vicente da Silva: collection of biological material, writing of the article, phytochemical analysis, and article writing. *Marcelo Augusto Martins de Freitas*: phytochemical analysis, antioxidant activity, and acetylcholinesterase inhibition, writing of the article, and revision. *Tullyo Henrique Lima Machado*: identification of the fungal specimen, final corrections, and publication. *Antonio Carlos Pereira de Menezes Filho*: laboratory analysis, writing of the article, final corrections, and translation. Matheus Vinícius Abadia Ventura: translation and final corrections. *Elizabete Nunes da Rocha*: supervisor, final corrections, and publication.

8. Conflicts of Interest

No conflicts of interest.

9. Ethics Approval

Not applicable.

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