Phytochemical screening, phenolic and flavonoid contents, psilocybin, antioxidant, and acetylcholinesterase inhibition activities of the aqueous extract from the fungi *Cyathus striatus*, *Laternea dringii*, and *Marasmius haematocephalus*

Douglas Ferreira da Silva[1], Lucas Sousa Cunha[1], Antonio Carlos Pereira de Menezes Filho[1,2], Aurélio Ferreira Melo[3], Porshia Sharma[4], Tullyo Henrique Lima Machado[6], Vanêcia Oliveira Cunha Machado[7], Matheus Vinícius Abadia Ventura[1,5] & Elizabete Nunes da Rocha[1]

[1] UniBRAS University Center of Rio Verde, Rio Verde, Goiás State, Brazil

[2] Menezes Agricultural Research, Rio Verde, Goiás State, Brazil

[3] SOBRESP Faculty, Goiânia, Goiás State, Brazil

[4] University of Hohenheim, Stuttgart, Germany

[5] Goiano Federal Institute, Rio Verde, Goiás State, Brazil

[6] Goiano Federal Institute, Ceres, Goiás State, Brazil

[7] Assumption School, Itapaci, Goiás State, Brazil

Correspondence: Douglas Ferreira da Silva, UniBRAS University Center of Rio Verde, Rio Verde, Goiás State, Brazil. E-mail: douglasferreira0897@gmail.com

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Abstract

Various families of mushrooms contain important phytochemicals with significant potential. This study aimed to investigate the phytochemical prospecting, presence of psilocybin, antioxidant activities, and acetylcholinesterase inhibition in aqueous extracts of *Cyathus striatus*, *Laternea dringii*, and *Marasmius haematocephalus*. Aqueous extracts were produced from mushrooms, and phytochemical groups were determined. The total phenolic and flavonoid content, DPPH reduction capacity, and FRAP were quantitatively determined. The acetylcholinesterase inhibition assay was performed, and the results were expressed as AChE inhibition percentages. Phytochemical groups such as flavonoids, phenolics, alkaloids, organic acids, and aliphatic compounds were positively detected. For phenolics, the extracts showed values of 208.44, 134.11, and 100.09 mg GAE g TPC^{-1}; for flavonoids, values of 45.12, 56.06, and 39.71 mg QE g TFC^{-1}. The FRAP reduction capacity showed values of 7.56, 14.43, and 4.15 µM TE g^{-1}, while for DPPH, the values were 100.07, 88.12, and 133.65 µg mL^{-1}. Low, medium, and strong AChE inhibition activity was observed with values of 43.11%, 68.53%, and 77.14%, respectively, for *C. striatus*, *L. dringii*, and *M. haematocephalus*. The aqueous extracts of *Cyathus striatus*, *Laternea dringii*, and *Marasmius haematocephalus* exhibited various phytomolecules groups with potential biological activities observed in this study.

Keywords: *Cyathus* Genus, *Laternea* Genus, *Marasmius* Genus, Fungal extract, Mushrooms.

Triagem fitoquímica, teores fenólicos e flavonoides, psilocibina, atividades antioxidantes e inibidoras da acetilcolinesterase do extrato aquoso dos fungos *Cyathus striatus*, *Laternea dringii* e *Marasmius haematocephalus*

Resumo

Diversas famílias de cogumelos apresentam importantes fitocompostos com potencial importância. Este estudo teve por objetivo verificar a prospecção fitoquímica, presença de psilocibina e atividades antioxidantes e de inibição da acetilcolinesterase em extratos aquosos de *Cyathus striatus*, *Laternea dringii* e *Marasmius*

haematocephalus. Extratos aquosos foram produzidos de cogumelos. A prospecção fitoquímica foi determinada para grupos fitoquímicos. O conteúdo de fenólicos e flavonoides totais, capacidade de redução do DPPH e FRAP foram determinadas quantitativamente. O ensaio de inibição da acetilcolinesterase foi realizada e os resultados obtidos em porcentagem de inibição da AChE. Foram detectados positivamente grupos de fitocompostos como flavonoides, fenólicos, alcaloides, ácidos orgânicos e compostos alifáticos. Para fenólicos, os extratos exibiram valores de 208,44; 134,11 e 100,09 mg EAG g TPC^{-1}, para flavonoides com valores de 45,12; 56,06 e 39.71 mg QE g TFC^{-1}. A capacidade de redução do FRAP apresentou valores de 7,56; 14.43 e 4.15 µM TE g^{-1} para DPPH 100,07; 88,12 e 133.65 µg mL^{-1} e baixa, média e forte atividade de inibição da AChE com valores de 43,11; 68,53 e 77,14%, respectivamente para *C. striatus*, *L. dringii* e *M. haematocephalus*. Os extratos aquosos dos cogumelos *Cyathus striatus*, *Laternea dringii* e *Marasmius haematocephalus* exibiram diversos grupos de fitomoléculas com potenciais atividades biológicas observadas neste estudo.

Palavras-chave: Gênero *Cyathus*, Gênero *Laternea*, Gênero *Marasmius*, Extrato fúngico, Cogumelos.

1. Introduction

The Fungi Kingdom is composed of a vast number of families, genera, and species of edible mushrooms, producers of toxins, neurotoxins, phenolic compounds, flavonoids, antioxidant activities, and inhibitors of acetylcholinesterase (AChE) (Sommano et al., 2022). They are also capable of promoting bioremediation in environments contaminated by metals, and oil, among other substances. Additionally, various groups of fungi are used in agriculture as solubilizers of non-labile minerals for plants and contribute to improvements in degraded soils. They are also present in the pharmaceutical, bioengineering, and food industries (Zhang et al., 2017; Chang; Wasser, 2018).

Among this large number of mushroom families, genera, and species, *Cyathus striatus*, *Laternea dringii*, and *Marasmius haematocephalus* are species of mushrooms that inhabit regions of the Midwest belonging to the Cerrado Domain of Brazil, particularly in the state of Goiás, which still shows a low number of recorded mushroom species for this region (Forzza et al., 2010; Teixeira-Silva et al., 2024; Oliveira et al., 2024).

Cyathus striatus (Huds. Ex Pers.) Willd. is a species of mushroom belonging to the higher Basidiomycetes and family Nidulariaceae known as “bird’s nest fungi”. Reports of this species present important antibiotic activity for the new group of striatins A, B, and C identified by Anke et al. (1977). In the study by Bai et al. (2015), researchers isolated and identified six highly oxygenated polycyclic cyathane-xylosides, called striatoids A−F (1−6), from isolates of *C. striatus*. Also in this research, the isolated compounds dose-dependently increased neurite outgrowth mediated by nerve growth factor (NGF) in rat pheochromocytoma cells (PC-12), opening the opportunity for potential pharmaceutical molecules in the treatment of Alzheimer's disease. Other phytochemical groups are already known in *C. striatus* such as cyathane diterpenoids, striatoids with rare 15,4'-ether ring system, indolic substances, sesquiterpene compounds such as schizandronols and several triterpene compounds such as glochidone, glochidonol, glochidiol and glochidiol diacetate, sciatic acid, striated acid, cyathadonic acid and epistriated acid (Johri; Brodie, 1971; Allbutt et al., 1971; Ayer; Reffstrup, 1982; Ayer et al., 1984).

Antitumor, anticancer, anti-inflammatory, antioxidant, antiviral, antibacterial, and antifungal activities have been described for *C. striatus* extract (Chudzik et al., 2015), especially in the inhibition of pancreatic cancer cells in rats by Sharvit et al. The order of Phallales E. Fish is well defined within Phallomycetidae Hosaka, Castellano & Spatafora through molecular analysis (Hosaka et al., 2006; Hibbett et al., 2014). It consists of fungi with colorful basidiomata, with some species emitting strong and unpleasant odors, commonly known as "stinkhorns" (Magnago et al., 2013; Lima et al., 2019). In this order, approximately 51 taxa of phalloid fungi are described (Cortez et al. 2011 a,b), with some species recorded inhabiting areas of the Cerrado Domain. This group is little known in terms of its phytochemical compounds and potential biological activities. Species included in this order exhibit expanded basidiomata, which can be free, reticulate, or sequestrate. The genus *Laternea* includes the species *Laternea dringii*, described in gallery forest areas, foraging in regions with moist, sandy soils in the Brazilian Cerrado (Machado et al., 2024).

So far, there are not many documented studies on the specific biological activities of *L. dringii*. Most research related to fungi of the order Phallales focuses primarily on the taxonomy, ecology, and morphology of these species, while investigations into the phytochemical compounds and potential biological activities of *L. dringii* remain limited.

However, some fungal species from the same order are known to produce bioactive compounds, such as enzymes and secondary metabolites, which may exhibit antimicrobial, antioxidant, or anticancer properties. Future research may reveal potential biological activities associated with *L. dringii*.

The group *Marasmius* Fr. (Marasmiaceae, Agaricales) includes an important group of saprotrophic mushroom-forming species found on plant debris (Oliveira et al., 2022). Most of the species are described in tropical and subtropical forests (Antonín; Noordeloos, 2010). The basidiomata vary in size, with a cap that is generally opaque, dry, and often membranous, white gills, and a filiform to cylindrical stipe. The Index Fungorum database lists 1993 *Marasmius* names, of which 558 are now classified in other marasmioid and gymnopoid genera mostly in Omphalotaceae Bresinsky and Physalacriaceae Corner (Moncalvo et al., 2002; Jenkinson et al., 2014).

Few studies have described the phytochemical composition of the specialized metabolism of *M. haematocephalus*. However, some researchers report that *Marasmius* exhibits bioactive activity due to the presence of terpenoids (Liermann et al., 2012), steroids (Fattorusso et al., 1992), piperidones (Zhang et al., 2009), and naphthalene (Yan et al., 2020). Bhambri et al. (2022) reported that the genus *Marasmius* is included among several genera with immunomodulatory and antibacterial properties due to β-glucans. The genus *Marasmius* is considered part of a respected group of mushrooms involved in traditional medicine. Another species, *M. berteroi*, was studied by Yang et al. (2021), where researchers described the presence of 10 phytocompounds in the extract of this species, including two new ones named (S)-3,7-dihydroxy-1-indanone (1) and (S)-3-hydroxy-4-methoxy-3-methyl isobenzofuran-1(3H)-one (2). In the same study, the authors found that the extract of M. berteroi exhibited strong nematicidal activity against Panagrellus redivivus.

In this regard, fundamental knowledge of the phytochemical constituents of mushroom extracts and their biological activities is important to conduct a detailed analysis of each chemical constituent and thus determine the potential bioactivities of these isolated molecules.

This study aimed to evaluate the aqueous extracts of *Cyathus striatus*, *Laternea dringii*, and *Marasmius haematocephalus* for phytochemical screening, the presence of total phenolics and flavonoids, psilocybin, antioxidant activities in DPPH and FRAP reduction, and acetylcholinesterase inhibition.

2. Materials and Methods

2.1. Reagents and Equipment

Acetic Acid (Neon, 95%, Brazil), AChE enzyme (Sigma-Aldrich, USA), Aluminum chloride (Dinâmica, 96%, Brazil), Ammonium hydroxide (Dinâmica, Brazil), Ascorbic acid (Synth, 99%, USA), Chloroform (Neon, Brazil), Ethanol alcohol (Neon, 96.5% P.A, Brazil), Folin-Ciocalteu reagent (Sigma-Aldrich, 98%, USA), Gallic acid (Sigma-Aldrich, 99%, India), hydrochloric acid (Neon, Brazil), Iron chloride (Dinâmica, 98%, Brazil), Methanol (Neon, Brazil) Quercetin (Sigma-Aldrich, 99%, USA), Paradimethylaminobenzaldehyde (Sigma-Aldrich, India), Psilocybin (Cerilliant®, Supelco, Sigma-Aldrich, USA), Psilocin-D10 (Cerilliant, Supelco, Sigma-Aldrich, USA), Sodium acrylate (Dinâmica, 99%, Brazil), Sodium carbonate (Isofar, 98.9%, Brazil), Sodium hydroxide (Neon, 99%, Brazil), Sodium nitrate (Synth, 98.9%, Brazil).

Lyophilizer (Tecnal, Mod. L108, Brazil), Micro-plate Reader of UV-Vis Spectrophotometer (Heales, Mod. MB-580, China), Rota evaporator (Tecnal, Mod. TE-210, Brazil), tube shaker (Fisatom, Model 772, Brazil), UV-Vis Spectrophotometer (Bel Photonics, Mod. M-51, Italy).

2.2. Collection and Identification

Thirty-five grams (35 g) of the vegetative phase of *C. striatus*, *L. dringii*, and *M. haematocephalus* were collected in a natural area located on a rural property in the municipality of Ceres, Goiás, Brazil, with the following geographic coordinates (15°21'12.23"S and 49º35’42.33’’W and 15º21’11.42’’S and 49º35’41.99’’W). The collection was carried out in October-November 2023. The Biologist Tullyo Henrique Lima Machado and Vanêcia Oliveira Cunha Machado identified the mushrooms, and two samples were prepared and kept in the authors' Mycological Bank at the Laboratory of Technological Chemistry of the Instituto Federal Goiano, Rio Verde, State of Goiás, Brazil with the Vouchers (Ld 1/2023, Cs 4/2023 and Mh 3/2023).

2.3. Extract Production

The aqueous extracts of *C. striatus*, *L. dringii*, and *M. haematocephalus* were obtained from 35 g of mushrooms previously cleaned with running water and dried in an oven at 35 °C for 6 h. They were then ground using a knife mill. The resulting powder was mixed with 100 mL of sterilized distilled water and stored in amber glass bottles in a refrigerator at 8 °C for 24 h. After that, the mixtures were filtered, and the supernatants were collected and used in the experiments.

2.4. Phytochemistry Prospecting

Phytochemical colorimetric assays were performed on the aqueous extracts of mushrooms by qualitative determination as proposed by Madike et al. (2017), Silva 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.

2.5. Determination of Psilocyn and Psilocybin by Thin Layer Chromatography

TLC was performed on 10 cm silica gel plates as described by Gross (2000). Standards of Psilocyn and Psilocybin were applied to each plate along with aqueous mushroom extracts. The plates were developed up to 6 cm at room temperature in a covered chromatographic chamber with a chloroform/methanol solution (9:1 *v/v*). A beaker containing 3 mL of 1 mol L^{-1} Ammonium hydroxide (*v/v*) was added to the chromatographic chamber to aid development. The plate was dried in an oven at 35 °C for 20 min and visualized using a 2% Paradimethylaminobenzaldehyde (*p*-DMAB) spray reagent in an acidic solution (HCl) (*w/v*). The relative R*fs* value for Psilocybin was 0.37 and for Psilocyn, 0.85. The lower detection limit was determined by serial dilutions of the Psilocyn standard and detecting/revealing it until the spot associated with the standard was no longer visible. The lower detection limit for the TLC method was determined to be approximately 0.05 mg mL^{-1}.

2.6. Total Phenolics

The total phenolic content was determined according to the colorimetric Folin-Ciocalteu method described by Menezes Filho et al. (2022). An aliquot containing 0.5 mL of sample solution was mixed with 2.5 mL of *Folin-Ciocalteu* reagent diluted with distilled water conc. (1:9) (*v/v*), followed by adding 5 mL of Na_2CO_3 conc. (7.5%) (*w/v*). The solution was stored in a dark room for 60 min. the absorbance (Abs) was measured at 765 nm using a UV-*Vis* spectrophotometer and glass cuvette (5 mL). The standard curve of Gallic acid is obtained under the same conditions as above using a range of concentrations (0-875 mg L^{-1}) prepared in 96% ethanol, and R^2 = 0.9990. The total phenolic content was measured as Gallic acid equivalents (mg GAE 100 g^{-1} dry mushroom extract). The experiment was performed in triplicate.

2.7. Total Flavonoids

Total flavonoid contents were measured using a modified colorimetric method described by Menezes Filho et al. (2022). An aliquot containing 0.25 mL of mushroom extract solutions was added to a test tube containing 1.25 mL of distilled water. Then, the NaNO3 solution conc. (5%) (*w/v*), 0.075 mL was added to the mixture and maintained for 5 min. Then, 0.15 mL of conc. 10% (*w/v*) AlCl3 solution was added and 1 min. homogenized. After 6 min., 0.5 mL of conc. 1 mol L^{-1} (*w/v*) of NaOH was finally added. The solution was diluted with 0.275 mL of distilled water and homogenized for 5 min. The Abs of the solution was measured at 510 nm in a UV-*Vis* spectrophotometer and glass cuvette.

The standard curve of Quercetin was obtained under the same conditions as above using a range of concentrations (0-900 mg L^{-1}) prepared in 96% ethanol and R^2 = 0.9994. The total flavonoid content was expressed as mg Quercetin equivalent (QE 100 g^{-1}) of dry mushroom extract. The experiment was performed in triplicate.

2.8. Antioxidant Activities (FRAP/DPPH)

According to Azieana et al. (2017), the FRAP assay was carried out. The FRAP solution was freshly prepared. Specifically, 25 mL of acetate buffer (3.1 g of $C_3H_3NaO_2$ and 16 mL of concentrated CH3COOH per one L of buffer solution) was mixed with 2.5 mL of 2,4,6-tripyridyl-s-triazine solution (10 mM L^{-1} of 2,4,6-tripyridyl-s-triazine in 40 mM L^{-1} hydrochloric acid) and 2.5 mL of iron (III) chloride solution (20 mM L^{-1} $FeCl_3.6H_2O$ in distilled water) as the FRAP working solution.

Then, 0.1 mL of the sample or standard was mixed with 3 mL of FRAP reagent and 3 mL of distilled water. The mixture was then incubated in the dark at 37 ºC for 8 min. The absorbance at 593 nm was recorded using a UV-Vis spectrophotometer. The total antioxidant capacity of the samples was determined against a standard of known FRAP value and expressed as µM of Trolox equivalent (µMTE) g^{-1} of dried extract. The experiment was performed in triplicate.

The DPPH assay was conducted following Menezes Filho et al. (2022). The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging ability assay was used to evaluate the antioxidant activity of the mushroom extracts. The test was carried out on a 96-well plate. Twenty (20 µL) of stock solution of algae extracts at different concentrations (5-5,000 ppm) and 180 µL of DPPH solution (0.147 mM) were added to each well. After 60 min of incubation at room temperature in the dark, the absorbance was read at 517 nm using a microplate reader of the UV-*Vis* spectrophotometer. Water was used as a 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.

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

Where: Abs standard = absorbance of the standard; Abs extract = absorbance of the crude extract.

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

2.9. Acetylcholinesterase Inhibition

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

The plate was then homogenized and incubated for another 40 min. Absorbance was measured at 405 nm using a UV-*Vis* microplate reader. As a blank, phosphate buffer (180 µL), DTNB (10 µL), and AChE (10 µL) were used. Maximum enzymatic activity was obtained by replacing the extract with 10% DMSO in phosphate buffer, and extract absorbance was obtained by replacing the enzyme with phosphate buffer. A solution of eserine (10 µM) was used as a positive control (standard inhibitor). The AChE assay was performed in triplicate. The percentage (%) of enzymatic inhibition was calculated using equation (2).

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

Where: A = absorbance of maximum enzymatic activity; B = absorbance of reaction blank; C = absorbance of enzymatic activity in the presence of samples; D = absorbance of sample color.

3. Results and **Discussion**

3.1. Phytochemical Prospecting

The study showed the presence of different secondary metabolites in mushroom extracts (Table 1). The presence

of special metabolites indicates the importance of natural products as therapeutic agents. The presence of positive results for alkaloids, flavonoids, organic acids, aromatic compounds, and phenolic compounds was observed in the three aqueous extracts of the mushrooms *C. striatus*, *L. dringii*, and *M. haematocephalus*.

Various phytochemical constituents are of pharmaceutical, chemical, food, and agricultural interest. The extracts from the mushrooms evaluated in our study showed positive results for several groups of phytomolecules of great scientific interest. Various groups of compounds from the special metabolism of mushrooms, such as alkaloids, flavonoids, saponins, steroids, reducing sugars, amino acids, purines, organic acids, aliphatic compounds, fatty acids, anthraquinones, phenolics, chalcones, emodins, terpenes, and carbohydrates, maybe the main contributors to the possible biological activities reported in this study.

The species *C. striatus* presents limitations regarding its phytochemical composition, making it necessary to compare it with other species of the same genus. In this comparative context, we found the study proposed by Han et al. (2013), in which they evaluated the solid culture of *Cyathus africanus*, a species of medicinal mushroom. They described eight cyathane-type diterpenes, namely cyathins D–H, neosarcodonin *O*, ciathatriol, and 11-*O*-acetylciathatriol, which were isolated. Additionally, 11,15-*O*, *O*-diacetylciathatriol, and 11,14,15-*O*, *O*-triacetylciathatriol were described. In a systematic review study, Qi et al. (2023) describe the presence of cyathane diterpenoids in large quantities for the species *Cyathus helenae*, striatoids A–F, cyathinins A–E, 10-hydroxyerinacine *S*, and the hydroxylation product of C-10 of erinacine S for *C. striatus*.

In a study conducted by Chaudhary et al. (2023) on various mushrooms collected in Nepal, researchers observed a range of positive results for *Scleroderma citrinum* (Sclerodermataceae family), *Suillus punctatipes* (Suillaceae family), *Coriolus hirsutus* (Polyporaceae family), *Russula sanguinea* (Russulaceae family), *Heterobasidion annosum* (Bondarzewiaceae family), and *Cavimalum indicum* (Clavicipitaceae family). They detected polyphenols, reducing compounds, quinones, saponins, alkaloids, terpenoids, and flavonoids in all samples. Glycosides were present only in *H. annosum* and *R. sanguinea*.

For alkaloids, numerous biological activities are described, such as antimalarial, anti-inflammatory, anti-diabetic, anti-rheumatic, and antipyretic activities (Afewerki et al., 2019; Novanna et al., 2019; Ur Rashid et al., 2019); anthraquinones exhibit laxative, antibacterial, antifungal, antiviral, and antitumor actions (Malik; Müller, 2016; Li; Jiang, 2018); catechins belong to the polyphenol group and are involved in various biological activities such as anticancer, anti-inflammatory, antioxidant, chemoprotective, and thermogenic (Schmitz et al., 2005); coumarins demonstrate anticoagulant, anti-inflammatory, antifungal, antineoplastic activities, and act against degenerative diseases such as Alzheimer's and Parkinsonism (Jameel et al., 2016; Stefanachi et al., 2018; Prusty; Kumar, 2019); steroids are potent agents with anti-inflammatory and analgesic activities (Awang et al., 2012); triterpenoids exhibit antimicrobial, antioxidant, and photosensitizing activities (Souza et al., 2013); phenolics and tannins are potent antioxidant agents, and they are also involved in treatments for several chronic-degenerative diseases such as neoplasms, diabetes, and anti-inflammatory processes, while phenolic compounds have reducing and preventive properties in heart diseases (Rocha et al., 2011; Carvalho et al., 2020).

Table 1. Phytochemical prospecting for special metabolism groups in the extracts of the mushroom *Cyathus striatus*, *Laternea dringii*, and *Marasmius haematocephalus*.

Compounds	**Cs**	**Ld**	**Mh**
Alkaloids	+	+	+
Tannins	-	-	-
Flavonoids	+	+	+
Saponins	-	+	-
Steroids	-	+	-
Reducing sugars	-	+	-
Non-reducing sugars	-	-	+
Resins	-	-	-
Amino acids	-	+	+
Coumarins	-	-	-
Glycosides	-	-	-
Purines	-	+	+
Organic acids	+	+	+
Aromatic and Aliphatic compounds	Red	Red	Red
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: Cs = Cyathus striatus; Ld = Laternea dringii and Mh = Marasmius haematocephalus. Red = Aliphatic compounds. Analyses performed in triplicate. Source: Authors, 2024.

3.2. Phenolic Compounds and Flavonoids, Antioxidant Activity, and Acetylcholinesterase Inhibition

As observed in the qualitative analysis for the presence of phenolics and flavonoids, the quantitative results are presented in (Table 2). Significant contents were observed for the three extracts with 208, 134, and 100 mg GAE g TPC^{-1}. The total flavonoid content was also significant, with 45, 56, and 39 mg QE g TFC^{-1}. The antioxidant activity showed a strong reducing capacity for both methods: FRAP, 7, 14, and 4 µM TE g^{-1}, and DPPH, 100, 88, and 133 µg mL^{-1}. The AChE inhibition capacity was 43%, 68%, and 77% for the three mushroom extracts *C. striatus*, *L. dringii*, and *M. haematocephalus*.

In our results for the quantitative content of total phenolics and flavonoids, we obtained significant levels of these two major phytochemicals, which are of great scientific interest and have broad applications in food, pharmaceutical, bioengineering, and agricultural fields. However, some studies have shown higher results than ours when a different extraction solvent was used.

In response to these results, the colorimetric assays for phenolic and flavonoid compounds exhibited weak coloration. This is possibly due to various phytochemicals of the polyphenol and flavonoid groups, which are generally solubilized in polar solvents such as ethanol and methanol. Based on the qualitative analyses, it can be inferred that the solvent used likely did not extract a large quantity of these phytochemicals, which also impacted the quantitative analyses of these groups, as it was not the best option for removing these compounds. This is attributed to the polyphenol oxidase enzyme, which degrades polyphenols in aqueous extracts, whereas, in ethanol and methanol, it is inactive (Toledo et al., 2021).

In an important study on the phytochemical composition of mushrooms from different families, Chaudhary et al. (2023) described significant levels of phenolics, reporting values of 102.30, 82.76, 74.71, 63.23, 51.73, and 45.98 mg GAE g TPC^{-1}, and for flavonoids, values of 225, 200, 175, 175, 125, and 100 mg QE g TFC^{-1} for *S. punctatipes*, *C. indicum*, *C. hirsutus*, *H. annosum*, *R. sanguinea*, and *S. citrinum*, respectively. Our results for different mushroom families do not show similarity to those obtained by Chaudhary et al. (2023), which can be attributed to various biotic and abiotic factors, as well as differences in fungal families.

The three aqueous extracts of the mushrooms evaluated in our study demonstrated potential as potent antioxidant agents in the two oxidative models tested for FRAP and DPPH. There is limited comparative data for the species evaluated, which is why it is necessary to compare as much as possible with the genus, family, or even other mushroom families. Our results are partly novel for *C. striatus*, *L. dringii*, and *M. haematocephalus*. In the study by Asatiani et al. (2007), evaluating twenty-four extracts from Basidiomycete mushrooms, strong antioxidant activity was found in the DPPH model with EC_{50} (mg/mL) between 0.7-9.

Regarding AChE inhibition activity, low activity was observed for *C. striatus*, while moderate to high inhibition activity was found for *L. dringii* and *M. haematocephalus*. A comparative study between species of *Cyathus* by Xu et al. (2022) found strong A ChE inhibition activity with an IC_{50} of 4.60 µM for the extract of the mushroom *C. africanus*. DPPH-reducing activity was also demonstrated for mushroom extracts collected in Nepal by Chaudhary et al. (2023). The ability to reduce DPPH showed IC_{50} values of 16.95, 22.50, 35.34, 39.89, 53.40, and 1238.0 µg mL^{-1}, respectively, for *S. punctatipes*, *C. indicum*, *C. hirsutus*, *H. annosum*, *R. sanguinea*, and *S. citrinum*. Yang et al. (2020), verified for Napthrene compounds from the mycelial fermentation of *Marasmius berteroi* important AChE inhibition results for compounds 2-4 (Dipolynaphthalene *B*; Naphthone *C* and Daldinone *C*) and 7 (8-methoxynaphthalen and -1,7-diol) where they exhibited inhibition at the concentration of 50 µg mL^{-1} of 42.74, 44.63 and 39.50%.

Table 2. Parameters on the total phenolic and flavonoid content, antioxidant activities for FRAP and DPPH, and acetylcholinesterase inhibition capacity for the extracts of the mushrooms *Cyathus striatus*, *Laternea dringii*, and *Marasmius haematocephalus*.

Mushroom extracts	**Cs**	**Ld**	**Mh**
Total Phenolics*	208.44	134.11	100.09
Total Flavonoids**	45.12	56.06	39.71
FRAP (µM TE g^{-1})	7.56	14.43	4.15
DPPH (µg mL^{-1})	100.07	88.12	133.65
AChE inhibition (%)	43.11	68.53	77.14

Note: *Total Phenolics (mg GAE g TPC^{-1}); **Total Flavonoids (mg QE g TFC^{-1}). Cs = *Cyathus striatus*; Ld = *Laternea dringii* and Mh = *Marasmius haematocephalus*. Averages obtained from triplicate analysis. Source:

4. Conclusions

The extracts of the mushrooms *Cyathus striatus*, *Laternea dringii*, and *Marasmius haematocephalus* presented a large number of phytochemical groups of medical importance. The presence of various phytochemicals can be attributed to the potential antioxidant and AChE inhibition properties of the examined samples. Phenolic and flavonoid compounds showed significant quantities, and the same was observed for antioxidant activities and acetylcholinesterase inhibition. Future studies can be conducted with extracts of different polarities to determine whether higher or lower levels are available for the analyses performed, as well as their biological activities.

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6. Authors' Contributions

Douglas Ferreira da Silva: writing, study development, phytochemical analyses. *Lucas Sousa Cunha*: collection of fungal samples, preparation of extracts, laboratory analyses, article writing, publication. *Antonio Carlos Pereira de Menezes Filho*: writing, identification of mushroom species, analyses of biological activity. *Aurélio Ferreira Melo*: funding and final corrections. *Porshia Sharma*: corrections and translation. *Tullyo Henrique Lima Machado*: collection, identification, and preparation of mushroom samples. *Vanêcia Oliveira Cunha Machado*: collection, identification, and preparation of mushroom samples. *Matheus Vinícius Abadia Ventura*: supervisor, final corrections, and publication. *Elizabete Nunes da Rocha*: Advisor, initial and final revisions, and publication.

7. Conflicts of Interest

No conflicts of interest.

8. Ethics Approval

Not applicable.

9. References

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