Phytochemical prospecting, vitamins, total phenolics and flavonoids, and antioxidant and acetylcholinesterase activities of *Scleroderma citrinum* Persoon (Sc) mushroom extract

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Abstract

Scleroderma is a genus of Gasteromycetes and Basidiomycota mushrooms in tropical and subtropical regions. This study aimed to evaluate the ethanolic extract of the mushroom *Scleroderma citrinum* regarding phytochemical prospecting, vitamin, phenolic, and total flavonoid content, and antioxidant (FRAP and DPPH) and acetylcholinesterase (AChE) inhibition activities. Fruiting bodies of S. citrinum were collected and the ethanolic extract was produced by maceration. Phytochemical prospecting was performed for several phytochemical groups using colorimetric means; the content of vitamins A, B, C, D, and E was obtained qualitatively by colorimetric methods, the content of phenolic and flavonoids by the colorimetric method and quantification by spectrophotometry. The spectrophotometric method performed the antioxidant activity in reducing FRAP and DPPH radicals by spectrophotometry and the acetylcholinesterase inhibition activity. Seventeen positive phytochemical groups were observed, the qualitative presence of vitamins of the A, B, and D complexes, 195.03 mg GAE g^{-1} of total phenolics, 93.10 mg QE g^{-1} of total flavonoids, FRAP reduction of 3.941 µM TE g^{-1}, DPPH reduction of 127.78 µg mL^{-1} and AChE inhibition of 55.6%. The extract of the mushroom *Scleroderma citrinum* proved to be rich in phytocompounds, vitamins, and important biological antioxidant and acetylcholinesterase inhibition effects.

Keywords: Gasteromycetes, *Scleroderma genus*, *Scleroderma citrinum*, DPPH, FRAP.

Prospecção fitoquímica, vitaminas, fenólicos e flavonoides totais e atividades antioxidante e acetilcolinesterase de extrato do cogumelo *Scleroderma citrinum* Persoon (Sc)

Resumo

Scleroderma é um gênero de cogumelos Gasteromicetos, Basidiomicota encontrados em regiões tropicais e subtropicais. Este estudo teve por objetivo avaliar o extrato etanólico do cogumelo *Scleroderma citrinum* quanto a prospecção fitoquímica, conteúdo de vitaminas, fenólicos e flavonoides totais, e atividades antioxidante (FRAP e DPPH) e de inibição da acetilcolinesterase (AChE). Corpos de frutificação de *S. citrinum* foram coletados e o extrato etanólico produzido por maceração. A prospecção fitoquímica foi realizada para diversos grupos fitoquímicos utilizando meios colorimétricos, o conteúdo de vitaminas A, B, C, D e E foram obtidos qualitativamente por métodos colorimétricos, o conteúdo de fenólicos e flavonoides pelo método colorimétrico e a quantificação por espectrofotometria. A atividade antioxidante foi realizada na redução dos radicais FRAP e DPPH por espectrofotometria e a atividade de inibição da acetilcolinesterase pelo método espectrofotométrico. Foram observados 17 grupos fitoquímicos positivos, a presença qualitativa de vitaminas dos complexos A. B e D,

195,03 mg EAG g^{-1} de fenólicos totais, 93,10 mg QE g^{-1} de flavonoides totais, redução do FRAP de 3,941 µM TE g^{-1}, redução do DPPH de 127,78 µg mL^{-1} e inibição da AChE de 55,6%. O extrato do cogumelo *Scleroderma citrinum* demonstrou ser rico em fitocompostos, vitaminas e importantes feitos biológicos antioxidante e de inibição da acetilcolinesterase.

Palavras-chave: Gasteromicetos, Gênero *Scleroderma*, *Scleroderma citrinum*, DPPH, FRAP.

1. Introduction

Scleroderma was first introduced by Persoon (1801) with 11 species. However, several new species of *Scleroderma* were described later and the number now exceeds 25 species (PHOSRI et al., 2009). *Scleroderma* taxa are reported from several continents such as North, Central, and South America, Africa, Australia, and Europe, with a large number of taxa in tropical, temperate, and subtropical countries such as Brazil, Mexico, Indonesia, India, and Thailand (Guzmán et al., 2013; Sufaati et al., 2023).

The *Scleroderma* group belongs to Gasteromycetes, Basidiomycotina (Soytong et al., 2014), where several morphological and molecular studies confirm the systematic position of the genus *Scleroderma*, in the suborder Sclerodermataceae and order Boletales. This genus of fungi with hard-skinned basidiomata can be recognized by its epigenous and single-layered peridium opening by irregular dehiscence and gleba without capillitium (Gurgel et al., 2008; Houhra et al., 2012).

Among the mushrooms belonging to the *Scleroderma* group, *Scleroderma citrinum* Persoon (Sc) (Figure 1) is an ectomycorrhizal mushroom with reticulate to echinulate globose spores generally known as earthballs (Łopusiewicz, 2018). In studies, *S. citrinum* is reported to form an ectomycorrhizal association with some pine species of the genus *Pinus*, *P. abies* (Brunner et al., 1992), *P. patula* (Mohan et al., 1993), *P. menziesii* and *P. pinaster* (Parlade, 1996) and *Eucalyptus* spp. (Kumar et al., 1999) and in *Spondias purpurea* (Anacardiaceae) in this study in Brazil.

Regarding the phytochemical constitution, *S. citrinum* presents two natural dyes, Norbadione A and Sclerocitrin by Steffan and Steglich (1984), in addition to the Xerocomic acid described by Steglich et al. (1968), Kanokmedhakul et al. (2003) reported the presence of a lanostane-type tripernoid (20S,22S,23E)-22-0-acetyl-25hydroxylanosta-8,23(*E*)-dien-3-one, Methyl 4,4'-dimethoxyvulpinate and 4,4'-dimethoxyvulpinic acid, the latter with antibacterial activity. The mushroom *S. citrinum* demonstrated in a study on tolerance and accumulation capacity of heavy metals, mainly Cadmium (Cd) (Carrillo-González and González-Chávez (2011), as a potent antioxidant agent in the ABTS method test, important content of total phenolics (TPC), flavonoids (TFC), tannins (TTC), anti-inflammatory, antiseptic and as an antibacterial agent against *Bacillus cereus*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Łopusiewicz, 2018; Chaudhary et al., 2023).

Although several groups of mushrooms have some type of research, especially edible mushrooms, the other groups have a reduced number of studies, so new research is needed to understand the special metabolites, possible groups of vitamins, phenolic compounds, flavonoids, and antioxidant activities in the reduction of oxidizing agents that attack DNA, as well as possible medications for the treatment of Alzheimer's (Jiang et al., 2020; Assemie; Abaya, 2022; Li et al., 2022).

This study aimed to verify the qualitative phytochemical constitution, phenolic and flavonoid contents antioxidant biological activities, and the inhibition of acetylcholinesterase from the ethanolic extract of the mushroom *Scleroderma citrinum*.

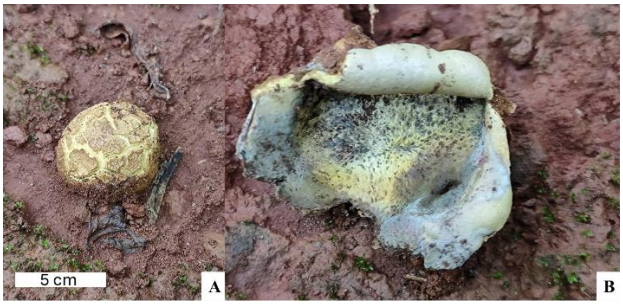

Figure 1. Vegetative body in maturation (**A**) and (**B**) vegetative body after release of *Scleroderma citrinum*

spores. Source: Authors, 2023.

2. Materials and Methods

2.1. Reagents and equipment

Acetic Acid (Neon, 95%, Brazil), AChE enzyme (Sigma-Aldrich, USA), Aluminum chloride (Dinâmica, 96%, Brazil), Ascorbic acid (Synth, 99%, USA), Ethanol alcohol (NEON, 96.5% P.A, Brazil), Folin-Ciocalteu reagent (Sigma-Aldrich, 98%, USA), Gallic acid (Sigma-Aldrich, 99%, India), Iron chloride (Dinâmica, 98%, Brazil), Quercetin (Sigma-Aldrich, 99%, USA), Sodium acrylate (Dinâmica, 99%, Brazil), Sodium carbonate (Isofar, 98.9%, Brazil), Sodium hydroxide (Neon, 99%, Brazil), Sodium nitrate (Synth, 98.9%, Brazil), 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, 99%, USA), 2,4,6-tripyridyl-s-triazine (FRAP) (Sigma-Aldrich, 99%, India), 3,5-di-tert-4- Butylhydroxytoluene (BHT) (Sigma-Aldrich, 99%, India, 6-Hydroxy-2,5,7,8-Tetramethylchroma NE-2-Carboxylic acid, Trolox (ACS Científica, 99%, 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), UV-*Vis* Spectrophotometer (Bel Photonics, Mod. M-51, Italy).

2.2. Collection and identification

Six hundred (600 g) of the vegetative phase of *S. citrinum* were collected in a natural area located in a rural property in the municipality of Rio Verde, Goiás, Brazil, with the following geographic coordinate (17°43’14.5’’S and 50°53’04.8’’W). The collection was carried out in November 2023. Biologist Antonio Carlos P. M. Filho identified the mushroom, and a sample was prepared and maintained at the authors' Mycological Bank in the Technological Chemistry laboratory of the Goiano Federal Institute, Rio Verde, Goiás State, Brazil with the Voucher (SV04/2024).

2.3. Extract production

The extract of *S. citrinum* was obtained from 200 g of mushroom previously cleaned in running water and dried in an oven at 35 °C for 3 h. It was then ground in a processor with 250 mL of 70% ethanol (*v/v*) for 5 min. After this process, the mixture was transferred to an amber flask and kept in a refrigerator at 4 °C for 10 days. After this period, the mixture was filtered, the supernatant was collected, reduced in a rota evaporator, and then lyophilized.

2.4. Phytochemistry prospecting

Phytochemical tests were carried out on the 70% ethanolic mushroom extract of the qualitative determination according to 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. Qualitative analysis of vitamins

Vitamin tests were carried out on the 70% ethanolic mushroom extract of the qualitative determination according to Balamurugan et al. (2019). Vitamins A, C, D, and E were analyzed.

2.6. Total phenolics

The total phenolic content was determined according to the colorimetric Folin-Ciocalteu method as 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 (0-650 mg L^{-1}) prepared in 96% ethanol, and R^2 = 0.9997. 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 solution was added to a test tube containing 1.25 mL of distilled water. Then, the $NaNO_3$ 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*) $AlCl_3$ 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 (0-800 mg L^{-1}) prepared in 96% ethanol and R^2 = 0.9991. 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. Accurately, 25 mL Lacetate buffer (3.1 g $C_3H_3NaO_2$ and 16 mL concentrated CH_3COOH per 1 L of buffer solution) was mixed with 2.5 mL 2,4,6-tripyridyl-*s*-triazine solution (10 mM L^{-1} 2,4,6-tripyridyl-s-triazine in 40 mM L^{-1} hydrochloric acid) and 2.5 mL iron (III) chloride solution (20 mM L^{-1} $FeCl_3.6H_2O$ in distilled water) as the FRAP working solution. Then 0.1 mL sample or standard was mixed with 3 mL FRAP reagent and 3 mL 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 samples was determined against a standard of known FRAP value and was expressed as µM of Trolox equivalent (µMTE) g^{-1} of dried extract. The experiment was performed in triplicate.

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 (5-5.000 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.

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

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.

2.9. 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 fungal extract was diluted in conc. 1 mg mL^{-1} in phosphate buffer solution + 10% (*v/v*) DMSO. Serial dilutions of the fungal 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 extracts 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 samples, and the color of the sample solutions, respectively. The AChE assay was performed in triplicate.

3. Results

In Table 1, several groups of special metabolism with positive results in important groups of pharmacological interest can be observed. The presence of 17 groups in the special metabolism of *S. citrinum* was qualitatively described.

Table 1. Phytochemical prospecting for special metabolism groups in the extract of the mushroom *Scleroderma citrinum.*

Compounds	**Results**
Alkaloids	+
Tannins	+ (Gr)
Carbohydrates	-
Flavonoids	+
Saponins	+
Steroids	+
Reducing sugars	+
Non-reducing sugars	-
Resins	-
Amino acids	-
Coumarins	+
Glycosides	-
Purines	+
Organic acids	+
Aromatic and Aliphatic compounds	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	+

Note: (+) Positive and (-) Negative reaction. (Gr) Green = Condensed or catechetical. Red = Aromatic compounds. Source: Authors, 2024.

The extract of the *S. citrinum* mushroom (Table 2) showed the positive presence of vitamins A, B, and D complexes.

Table 2. Qualitative prospecting of vitamins in the extract of the mushroom *Scleroderma citrinum*.

Vitamins	**Results**
A	+
B	+
C	-
D	+
E	-

Note: (+) Positive and (-) Negative reaction. Source: Authors, 2024.

Important phenolic and flavonoid contents were obtained, in addition to the high antioxidant capacity by the FRAP and DPPH methods, the acetylcholine inhibition capacity is considered moderate for the *S. citrinum* mushroom extract (Table 3).

Table 3. Total phenolic and flavonoids, antioxidants, and acetylcholinesterase inhibition from *Scleroderma citrinum* mushroom extract.

Mushroom extract	**Results**
Total phenolics (mg GAE g TPC^{-1})	195.03 ± 0.32
Total flavonoids (mg QE g TFC^{-1})	93.10 ± 0.44
FRAP (µM TE g^{-1})	3.941 ± 0.09
DPPH (µg mL^{-1})	127.78 ± 0.61
AChE inhibition (%)	55.6 ± 0.07

Note: Averages obtained from repetitions followed by standard deviation. Source: Authors, 2024.

4. Discussion

Scleroderma citrinum, as already mentioned, was first scientifically described in 1801 by Christian Hendrik Persoon, with few reports of toxicity with members of *Scleroderma*. The fruiting bodies of the "Puffball" mushroom are considered the worst poison and the most prominent mushroom poisoning in the United Kingdom (Borthakur; Joshi, 2017); in Brazil, there are no reports of poisoning by *S. citrinum* and *S. verrucosum* found in Brazilian soil.

These possible toxic activities are due to several special metabolic compounds produced by these microorganisms, for protection or even part of the primary metabolism, such as gliotoxin (peptides) and aflotoxins and mycotoxins (polyketides) (Redrado et al., 2022; El-Dawy et al., 2024). However, there are larger quantities of phytochemical groups of medical interest such as alkaloids that are compounds such as morphine (used as an analgesic), quinine (antimalarial), and atropine (used to dilate pupils and as an antispasmodic) (Carvalho et al., 2020; Ferreira et al., 2023); Flavonoids that have antioxidant, anti-inflammatory and anticancer properties, these compounds are explored in the prevention and treatment of various diseases, including cardiovascular diseases (Matos Silva et al., 2020; Skinder et al., 2021); Tannins are a large number of phenolic compounds with astringent and antibacterial properties (Toledo et al., 2021); Terpenes such as taxol that is used in the treatment of various types of cancer due to its antitumor properties (Barbosa et al., 2019); Cardiac glycosides that are used in the treatment of heart diseases (Tiwari; Bae, 2022).

In a comparative study, Borthakur and Joshi (2017) found a large number of phytochemical groups by qualitative analysis for the extract of the mushroom *S. citrinum* collected in forests of the East Khasi Hills of Meghalaya, especially for the groups of alkaloids, flavonoids, terpenes, saponins, cardiac glycosides, phenolics, and carbohydrates. Among species of the genus Scleroderma, Menezes Filho et al. (2022) verified the presence of alkaloids, flavonoids, triterpenoids, steroids, tannins, organic acids, reducing sugars, aromatic compounds, and carboxylic acids in the extract of the mushroom *S. verrucosum*, noting the similarity between the species.

Other fungal groups such as *Colletotrichum yulongense*, *C. cobbittiense*, and *C. alienum* were described with positive results for alkaloids, flavonoids, phenolics, terpenoids, and steroids by Toppo et al. (2024). In the genus *Aspergillus*, *A. nidulans*, *A. fumigatus*, and *A. flavus*, the group of researchers led by Sharaf et al. (2022), elucidated the presence of the following phytochemical group's glycosides, steroids, alkaloids, terpenoids, p-terphenyls, diphenyl ether cytochalasins, xanthones, phenalenones, sterols and anthraquinone for the extracts of these mushrooms.

Complementing the phytochemical analysis of *S. citrinum*, Chaudhary et al. (2023) quantified the tannins content in mushrooms collected in Nepal, where the result obtained was 80 mg GAE g of extract^{-1}.

This study presents the first data on the presence of vitamins A, B, and D in the *Scleroderma genus*. As observed in the qualitative tests for the presence of vitamins, the extract of the mushroom *S. citrinum* has shown to be a possible source of the A, B, and D complexes, and these qualitative data are suitable for a more detailed analysis by HPLC on the quantitative content of these vitamins. Several groups of mycorrhizal mushrooms were studied where the presence of groups of vitamins was found with important levels, Chalyasut and Sivamaruthi (2017) in a review of the mushroom *Hericium erinaceus* describe that this fungal species has significant vitamin contents. Furlani and Godoy (2008) described substantial contents of vitamins B1, B2, B12, C and D, niacin, folates, and ergosterol (provitamin D2) in cultivated mushrooms. Bilski et al. (2000) verified the positive presence of vitamin B6 (Pyridoxine) in the mushroom *Cercospora nicotianae*. In another study, the research group of Strzelczyk et al. (1991) verified the presence of B vitamins (Biotin, Thiamine, and Nicotinic acid) in four fungal species obtained from in vitro cultivation at different pHs. Also in this study, the mushroom *Hebeloma crustuliniforme* was responsible for the highest content of nicotinic acid and the mushroom *Cenococcum graniforme* produced pantothenic acid.

According to Malyskin, (1955); Shemakhanova, (1962); Vedenyapina, (1955); Zhang et al. (2021) vitamins play a special role in the growth and development of mycorrhizal mushrooms and the formation of mycorrhizae.

Regarding the antioxidant activity capable of reducing several groups of oxidizing agents such as Singlet Oxygen ($^3O_2$), superoxide ($O_2^{\cdot-}$), hydroxyl ($HO^{\cdot}$), peroxyl ($ROO^{\cdot}$), alkoxyl ($RO^{\cdot}$) and nitric oxide ($NO^{\cdot}$) (GULCIN, 2020), the extract of the mushroom *S. citrinum* presented good quantitative results (Table 3), these results are due to the phytochemical constitution as discussed later. According to Nowacka et al. (2015), polyphenols are potent antioxidant agents. In our study, the extract of the mushroom *S. citrinum* was demonstrated to be a potential source of phenolic compounds. Menezes Filho et al. (2022) also verified a high content of total phenolics (309.14 mg GAE 100 g^{-1}) and high antioxidant activity in the reduction of the free radical DPPH (5.97 µg mL^{-1}) in the extract of the mushroom *S. verrucosum*. In a large study with 31 mushroom species, Nowacka et al. (2015) researchers presented important data on the total phenolic content for a large group of fungal genera, including *Scleroderma*, where for *S. citrinum* a TPC content of 11.03 mg GAE g^{-1} and DPPH reduction activity = 11.49 mg/mg^{-1} DPPH of lyophilized extract were observed, results lower than those of this study.

The Folin–Ciocalteu's method is commonly used for total phenolic content determination; however, the results obtained by different authors are difficult to compare due to different ways of expression. Our findings

demonstrated that the extract of the mushroom *S. citrinum* presented significant flavonoid contents. Other studies also corroborate our research (Alvarez-Parrilla et al., 2007; Borthakur; Joshi, 2017). In the methanolic extract of *S. citrinum*, Borthakur and Joshi (2017) found a result of 1.57 mg QE g^{-1} of dry mass. The high level of flavonoid attributes *S. citrinum* as a rich source of antioxidants. However, in the study by Menezes Filho et al. (2022), no total flavonoid content was observed for the species S. verrucosum. Studies indicate that a large number of wild mushroom species have a higher content of polyphenols and flavonoids when compared to mushrooms of food interest (Alvarez-Parrilla et al., 2007). Differences in the quantity of natural compounds may vary. This variation is due to the time of collection, period, region, and climate (Ralepele et al., 2021).

As observed, the flavonoid content was significant in the extract of the *S. citrinum* mushroom, however, Menezes Filho et al. (2022) did not find the presence of total flavonoids in both qualitative and quantitative methods for the extract of the *Scleroderma verrucosum* mushroom obtained from individuals collected in Brazil. Saleh et al. (2021) trata desse assunto em seu estudo, onde grupos fitoquímicos foram observados em uma espécie e em outra não no mesmo gênero. Causas possíveis são a genética e a fisiologia das diferentes espécies incluídas em um mesmo gênero.

The DPPH is a free radical which when reacted with an active antioxidant can donate its hydrogen or electron and gets reduced from dark violet to pale yellowish. Our results for the reduction of the DPPH radical were satisfactory and comparable to the results obtained by Borthakur and Joshi (2017) who evaluated the methanolic extract of *S. citrinum* with IC_{50} of 227.38 µg mL^{-1}.

Several special fungal metabolites show important results regarding their ability to inhibit AChE. In our study, it is possible to verify that the extract of the mushroom S. citrinum showed high efficiency in the ability to inhibit AChE. Studies reported by Elawady et al. (2023) for endophytic fungi (*Aspergillus versicolor*) found an AChE inhibition capacity of 79.5%. A promising result was also observed by Laib et al. (2020) for the extract of the endophytic mushroom *Trichoderma* sp. with 80% inhibition of AChE. We aimed to verify the real inhibition capacity of the mushroom extract against AChE. In the case of cholinesterase enzymes, the goal is to identify compounds that can act as inhibitors of these enzymes. This information can then be used to identify potential inhibitors for further study and optimization.

5. Conclusions

The extract of the *Scleroderma citrinum* mushroom proved to be a promising biological agent in this study, with a large number of qualitative phytocompounds groups by phytochemical prospecting, qualitative presence of important vitamins of the A, B, and D complexes, significant quantitative contents of flavonoids and phenolics, high capacity to reduce oxidative agents and important inhibitory action on acetylcholinesterase.

Further studies should be carried out to verify other biological activities and identification by high-performance liquid chromatography (HPLC) of the compounds and contents of the *S. citrinum* extract evaluated.

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7. Authors' Contributions

Paulo Cesar Barbosa Neto: study development, writing, data collection, analyses, and article submission. *Júlio Cesar Candido Nunes*: writing, data collection, analyses, post-review revisions. *Aurélio Ferreira Melo*: translation and post-review revisions. *Antonio Carlos Pereira de Menezes Filho*: article writing, solution preparation, quantitative analyses, article writing, final revisions, and translation. *Matheus Vinícius Abadia Ventura*: supervisor, funding, specimen collection and identification, and article writing.

8. Conflicts of Interest

No conflicts of interest.

9. Ethics Approval

Not applicable.

10. References

Alvarez-Parrilla, E., De La Rosa, L. A., Martínez, N. R., Aguilar González, G. A. (2007). Total phenols and antioxidant activity of commercial and wild mushrooms from Chihuahua, Mexico. *Ciencia Y Tecnologia Alimentaria*, 5(5), 329-334.

Assemie, A., Abaya, G. (2007). The effect of edible mushrooms on health and their biochemistry. *International Journal of Microbiology*, 2022, 1-7. https://doi.org/10.1155/2022/8744788

Azieana, J., Zainon, M. N., Noriham, A., Rohana, M. N. (2017). Total phenolic and flavonoid content and antioxidant activities of ten Malaysian wild mushrooms. *Open Access Library Journal*, 4, e3987. https://doi.org/10.4236/oalib.1103987

Balamurugan, V., Sheerin Fatima, M. A., Velurajan, S. (2019). A guide to phytochemical analysis. *International Journal of Advance Research and Innovative Ideas in Education - IJARIIE*, 5(1), 236-245. https://doi.org/16.0415/IJARIIE-9430

Barbosa, D. A., Lucena, R. F. P., Cruz, D. D. (2019). Traditional knowledge as basis for phytochemical prospecting of *Sideroxylon obtusifolium* (Roem. & Schult.) T. D. Penn. Aiming at conservation in the Brazilian semi-arid zone. *Ethnobotany Research & Applications*, 18(3), 1-10, 2019. http://doi.org/10.32859/era.18.3.1-10

Bilski, P., Li, M. Y., Ehrenshaft, M., Daub, M. E., Chignell, C. F. (2000). Vitamin B6 (Pyridoxine) and its derivatives are efficient singlet oxygen quenchers and potential fungal antioxidants. *Photochemistry and Photobiology*, 71(2), 129-134. https://doi.org/10.1562/0031-8655(2000)0710129SIPVBP2.0.CO2

Borthakur, M., Joshi, S. R. (2017). Pigskin poison earthball mushroom of Meghalaya: An identification paradox. *The NEHU Journal*, 15(1), 65-78.

Brunner, I., Amiet, R., Zollinger, M., Egli, S. (1992). Ectomycorrhizal synthesis with *Pinus abies* and three fungal species: a case study on the use of an *in vitro* technique to identify naturally occurring ectomycorrhizae. *Mycorrhiza*, 2(2), 89-96.

Carrillo-González, R., González-Chávez, M. C. A. (2011). Tolerance to and accumulation of Cadmium by the mycelium of the fungi Scleroderma citrinum and Pisolithus tinctorius. *Biological Trace Element Research*, 146, 388-395. https://doi.org/10.1007/s12011-011-9267-7

Carvalho, G. G., Peres, G. C., Mendonça, R. M. C., Santos Filho, E. X. (2020). Phytochemical prospection and antibacterial activity of native plants from the cerrado of goiás, Brazil. *Journal of Pharmacognosy and Phytochemistry*, 9(2), 29-37.

Chaiyasut, C., Sivamaruthi, B. S. (2017). Anti-hyperglycemic property of *Hericium erinaceus* - a mini review. *Asian Pacific Journal of Tropical Biology*, 7(11), 1036-1040. https://doi.org/10.1016/j.apjtb.2017.09.024

Chaudhary, P., Panth, N., Raut, M., Shrestha, N. P. N., Shakya, S., Mishra, B. T. A. D., Parajuli, N. (2023). Biochemical, antimicrobial, and antioxidant activities of some wild mushrooms from Nepal. *Bibechana*, 20(2), 161-175. https://doi.org/10.3126/bibechana.v20i2.54887

Elawady, M., Hamed, A. A., Alsallami, W. M., Gabr, E. Z., Abdel-Monem, M. O., Hassan, M. G. (2023). Bioactive metabolite from endophytic *Aspergillus versicolor* SB5 with anti-acetylcholinesterase, anti-inflammatory and antioxidant activities: *In vitro* and *in silico* studies. *Microorganisms*, 11(4), 1062. https://doi.org/10.3390/microorganisms11041062

El-Dawy, E. G. A., Gherbawy, Y. A., Hussein, M. A. (2024). Characterization of *Aspergillus* section Flavi associated with stored grains. *Mycotoxin Research*, 40, 187-202. https://doi.org/10.1007/s12550-023-00514-1

Ferreira, W. U., Menezes Filho, A. C. P., Sharma, P., Castro, C. F. S., Ferreira, L. U., Ventura, M. V. A. (2023). Phytochemical prospecting and biological activities of the floral extract from [*Impatiens walleriana* (Hook.)] (Balsaminaceae). *Brazilian Journal of Science*, 2(11), 43-50. https://doi.org/10.14295/bjs.v2i11.398

Furlani, R. P. Z., Godoy, H. T. (2008). Vitamins B1 and B2 contents in cultivated mushrooms. *Food Chemistry*, 106, 816-819. http://doi.org/10.1016/j.foodchem.2007.06.007

Gulcin, I. (2020). Antioxidants and antioxidant methods: an updated overview. *Archives of Toxicology*, 94, 651-715. https://doi.org/10.1007/s00204-020-02689-3

Gurgel, F. E., Silva, B. D. B., Baseia, I. G. (2008). New records of Scleroderma from Northeastern Brazil. *Mycotaxon*, 105, 399-405.

Guzmán, G., Cortés-Pérez, A., Guzmán-Dávalos, L., Ramírez-Guillén, F., Sánchez-Jácome, M. R. (2013). An emendation of *Scleroderma*, new records, and review of the known species in Mexico. *Revista Mexicana de Biodiversidad*, 84, S173-S191. https://doi.org/10.7550/rmb.31979

Jiang, Q., Zhang, M., Mujumdar, A. S. (2020). UV induced conversion during drying of ergosterol to vitamin D in various mushrooms: Effect of different drying conditions. *Trends in Food Science & Technology*, 105, 200-210. https://doi.org/10.1016/j.tifs.2020.09.011

Kumar, R. V., Reddy, B. V. P., Mohan, V. (1999). Distribution of ectomycorrhizal fungi in forest tree species of Andhra Pradesh, southern India - a new record. *Indian-Forester*, 125(5), 496-502.

Laib, D. E., Benzara, A., Akkal, S., Bensouici, C. (2020). The anti-acetylcholinesterase, insecticidal and antifungal activities of the entophytic fungus *Trichoderma* sp. isolated from *Ricinus communis* L. against *Locusta migratoria* L. and *Botrytis cinerea* Pers.: Fr. *Acta Scientifica Naturalis*, 7(1), 112-125. https://doi.org/10.2478/asn-2020-0011

Li, Y., Liang, H., Zhou, D., Xing, Y., Chen, J. (2022). Phenolics, flavonoids content and antioxidant activities of *Tuber indicum* at different maturity stages. *Chemistry & Biodiversity*, 19(3), e202100830. https://doi.org/10.1002/cbdv.202100830

Łopusiewicz, Ł. (2018). Scleroderma citrinum melanin: isolation, purification, spectroscopic studies with characterization of antioxidant, antibacterial and light barrier properties. *World Scientific News – An International Scientific Journal*, 94(2), 115-130.

Malyskin, P. E. (1955). Stimulation of tree growth by microorganisms. *In*: Mycotrophy in Plants. Ed. A. A. Imshenetski. Acad. Sci. USSR, Trans Israel Program Sci. Trans., Jerusalem.

Matos Silva, S. A. N., Barros, A. B., Souza, J, M, T, Moura, A. F., Araújo, A. R., Mendes, M. G. A., Daboit, T. C., Silva, D. A., Araújo, A. J., Filho, J. D. B. M. (2020). Phytochemical and biological prospection of *Mimosa* genus plants extracts from Brazilian northeast. *Phytochemistry Letters*, 39, 173-181. https://doi.org/10.1016/j.phytol.2020.08.010

Menezes Filho, A. C. P., Ventura, M. V. A., Alves, I., Castro, C. F. S., Soares, F. A. L., Teixeira, M. B. (2023). Extratos florais do dominio Cerrado e inibição da acetilcolinesterase. *Nativa*, 11(2), 207-211. https://doi.org/10.31413/nativa.v11i2.14555

Menezes Filho, A. C. P., Ventura, M. V. A., Alves, I., Taques, A. S., Batista-Ventura, H. R. F., Castro, C. F. S., Teixeira, M. B., Soares, F. A. L. (2022). Phytochemical prospection, total flavonoids and total phenolics and antioxidant activity of the mushroom extract *Scleroderma verrucosum* (Bull.) Pers. *Brazilian Journal of Science*, 1(1), 1-7. https://doi.org/10.14295/bjs.v1i1.2

Mohan, V., Natarajan, K., Ingleby, K. (1993). Anatomical studies on ectomycorrhizas III. The ectomycorrhizas produced by *Rhizopogon luteolus* and *Scleroderma citrinum* on *Pinus patula*. *Mycorrhiza*, 3, 51-56.

Nouhra, E. R., Caffot, M. L. H., Pastor, N., Crespo, E. M. (2012). The species of *Scleroderma* from Argentina, including a new species from the Nothofagus forest. *Mycologia*, 104, 488-495. https://doi.org/10.3852/11-082

Nowacka, N., Nowak, R., Drozd, M., Olech, M., Los, R., Malm, A. (2015). Antibacterial, antiradical potential and phenolic compounds of thirty-one polish mushrooms. *PloS ONE*, 10(10), e0140355. https://doi.org/10.1371/journal.pone.0140355

Parlade, J., Pera, J., Alvarez, I. F. (1996). Inoculation of containerized *Pseudotsuga menziesii* and *Pinus pinaster* seedlings with spores of five species of ectomycorrhizal fungi. *Mycorrhiza*, 6, 237-245.

Phosri, C., Martín, M. P., Watling, R., Jeppson, M., Sihanonth, P. (2009). Molecular phylogeny and re-assessment of some *Scleroderma* spp. (Gasteromycetes). *Anales del Jardín Botánico de Madrid*, 66, 83-91. http://doi.org/10.3989/ajbm.2199

Ralepele, F. M., Chimuka, L., Nuapia, Y., Risenga, I. (2021). UPLC-DAD-QTOF-MS/MS analysis of targeted poly-phenolic compounds from Moringa oleifera leaves as function of seasonal responses. *South African Journal of Plant*, 143, 107-115. https://doi.org/10.1016/j.sajb.2021.07.032

Redrado, S., Esteban, P., Domingo, M. P., Lopez, C., Rezusta, A., Ramirez-Labrada, A., Arias, M., Pardo, J., Galvez, E. M. (2022). Integration of *In Silico* and *In Vitro* analysis of gliotoxin production reveals a narrow range of producing fungal species. *Journal of Fungi*, 8(4), 361. https://doi.org/10.3390/jof8040361

Saleh, M. S. M., Jalil, J., Zainalabidin, S., Asmadi, A. Y., Mustafa, N. H., Kamisah, Y. (2021). Genus Parkia: Phytochemical, medicinal uses, and pharmaceutical properties. *International Journal of Molecular Sciences*, 22(2), 618. https://doi.org/10.3390/ijms22020618

Sharaf, M. H., Abdelaziz, A. M., Kalaba, M. H., Radwan, A. A., Hashem, A. H. (2022). Antimicrobial, antioxidant, cytotoxic activities and phytochemical analysis of fungal endophytes isolated from *Ocimum basilicum*. *Applied Biochemistry and Biotechnology*, 194, 1271-1289. https://doi.org/10.1007/s12010-021-03702-w

Shemakhanova, N. M. (1962). *Mycotrophy of woody plants*, p. 329. Acad. Sci., USSR, Trans. Israel Program Sci. Trans. Jerusalem.

Skinder, B. M., Ganai, B. A., Wani, A. H. (2021). Chapter 16 – Bioprospecting of endophytic fungi for antibacterial and antifungal activities. *Phytomedicine*, 427-460. https://doi.org/10.1016/B978-0-12-824109-7.00025-X

Steffan, B., Steglich, W. (1984). *Angewandte Chemie Internation Edition*, 96, 435-437.

Steglich, W., Furtner, W. Prox, A. (1968). *Naturforsch B*, 23, 1044-1045.

Strzelczyk, E., Dahm, H., Pachlewski, R. (1991). B-group vitamins production by mycorrhizal fungi in response to pH (*in vitro* studies). *Plant and Soil*, 137, 237-241, 1991. https://doi.org/10.1007/BF00011202

Sufaati, S., Suharno, Agustini, V., Suwannasai, N. (2023). New records of the diversity of *Scleroderma* spp. from Papua, Indonesia. *Biodiversitas*, 24(8), 4269-4276. https://doi.org/10.13057/biodiv/d240808

Sytong, K., Sibounnavong, P., Kanokmedhakul, K., Kanokmedhakul, S. (2014). Biological active compounds of *Scleroderma citrinum* that inhibit plant pathogenic fungi. *Journal of Agricultural Technology*, 10(1), 79-86.

Tiwari, P., Bae, H. (2022). Endophytic fungi: key insights, emerging prospects, and challenges in natural products drug discovery. *Microorganisms*, 10(2), 360. https://doi.org/10.3390/microorganisms10020360

Toledo, A. G., Souza, J. G. L., Santana, C. B., Mallamann, A. P., Santos, C. V., Corrêa, J. M., Pinto, F. G. (2021). Antimicrobial, antioxidant activity and phytochemical prospection of *Eugenia involucrata* DC. leaf extracts. *Brazilian Journal of Biology*, 83, e245753. https://doi.org/10.1590/1519-6984.245753

Toppo, P., Jangir, P., Mehra, N., Kapoor, R., Mathur, P. (2024). Bioprospecting of endophytic fungi from medicinal plant *Anisomeles indica* L. for their diverse role in agricultural and industrial sectors. *Scientific Reports*, 14, 588. https://doi.org/10.1038/s41598-023-51057-5

Vedenyapina, N. S. (1955). *Effect of Azotobacter on growth of oak seedlings*. In Mycotrophy in Plants. Ed. A. A. Im- shenetski, p. 253. Acad. Sci. USSR, Trans. Israel Program Sci. Trans Jerusalem.

Zhang, Y., Wang, D., Chen, Y., Liu, T., Zhang, S., Fan, H., Liu, H., Li, Y. (2021). Healthy function and high valued utilization of edible fungi. *Food Science and Human Wellness*, 10(4), 408-420. https://doi.org/10.1016/j.fshw.2021.04.003

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