Phytochemical prospection, total flavonoids and total phenolics and antioxidant activity of the mushroom extract *Scleroderma verrucosum* (Bull.) Pers

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Abstract

Scleroderma verrucosum is a species of mushroom belonging to the family Sclerodermataceae found in the Americas and Europe. This study aimed to evaluate the 70% ethanol extract of the vegetative part of the mushroom *S. verrucosum* for qualitative phytochemical constituents and total content of flavonoids and phenolics, and the DPPH free radical reduction activity. Mushroom extract was prepared in 70% ethanol solution. Qualitative phytochemical assay was performed for different groups using colorimetric reagents. The contents of total flavonoids and phenolics, and antioxidant activity in reducing the DPPH free radical were quantitatively determined. The positive presence of alkaloids, flavonoids, phenolics, triterpenoids, steroids, tannins, organic acids, reducing sugars, aromatic compounds and carboxylic acids were observed. The extract exhibited total phenolics = 309.14 GAE 100 g^{-1} and free radical reduction of = 5.97 μg mL^{-1}. Mushroom extract *Scleroderma verrucosum* demonstrated the presence of several medicinal important phytochemical groups as well as total flavonoid and phenolic content that exhibit the potential for antioxidant activity.

Keywords: *Scleroderma genus*; Antioxidant activity; Flavonoids content; Total phenols; Sclerodermataceae.

Resumo

Scleroderma verrucosum é uma espécie de cogumelo pertencente à família Sclerodermataceae encontrada nas Américas e na Europa. Este trabalho teve como objetivo avaliar o extrato etanólico 70% da parte vegetativa do cogumelo S. verrucosum quanto aos constituintes fitoquímicos qualitativos e ao conteúdo total de flavonóides e fenólicos, e a atividade redutora do radical livre DPPH. O extrato de cogumelo foi preparado em solução de etanol a 70%. O ensaio fitoquímico qualitativo foi realizado para diferentes grupos usando reagentes colorimétricos. Os conteúdos de flavonóides e fenólicos totais e a atividade antioxidante na redução do radical livre DPPH foram determinados quantitativamente. Foi observada a presença positiva de alcalóides, flavonóides, fenólicos, triterpenóides, esteroides, taninos, ácidos orgânicos, açúcares redutores, compostos aromáticos e ácidos carboxílicos. O extrato apresentou teor de fenólicos totais = 309,14 EAG 100 g^{-1} e redução de radical livre de = 5,97 μg mL^{-1}. O extrato de cogumelo *Scleroderma verrucosum* demonstrou a presença de vários grupos fitoquímicos importantes para fins medicinais, bem como o conteúdo total de flavonóides e fenólicos que apresentam potencial para atividade antioxidante.

Palavras-chave: Gênero *Scleroderma*; Atividade antioxidante; Conteúdo de flavonóides; Fenóis totais; Sclerodermataceae.

Resumen

Scleroderma verrucosum es una especie de hongo perteneciente a la familia Sclerodermataceae que se encuentra en América y Europa. Este estudio tuvo como objetivo evaluar el extracto etanólico al 70% de la parte vegetativa

del hongo *S. verrucosum* en cuanto a constituyentes fitoquímicos cualitativos y contenido total de flavonoides y fenólicos, y la actividad reductora de radicales libres DPPH. Se preparó extracto de hongos en una solución de etanol al 70%. Se realizó un ensayo fitoquímico cualitativo para diferentes grupos utilizando reactivos colorimétricos. Se determinaron cuantitativamente los contenidos de flavonoides y fenólicos totales y la actividad antioxidante en la reducción del radical libre DPPH. Se observó la presencia positiva de alcaloides, flavonoides, fenólicos, triterpenoides, esteroides, taninos, ácidos orgánicos, azúcares reductores, compuestos aromáticos y ácidos carboxílicos. El extracto exhibió un contenido total de fenoles totales = 309,14 EAG 100 g^{-1} y reducción de radicales libres de = 5,97 μg mL^{-1}. El extracto de hongo *Scleroderma verrucosum* demostró la presencia de varios grupos fitoquímicos importantes para la medicina, así como un contenido total de flavonoides y fenólicos que exhiben el potencial de actividad antioxidante.

Palabras clave: Género *Escleroderma*; Actividad antioxidante; Contenido de flavonoides; Fenoles totales; Esclerodermatceae.

1. Introduction

Mushrooms of the genus *Scleroderma* Pers. Fr. emend. Guzmán (1967 and 1970), Guzmán & Ramírez- Guillén (2010) and Gusmán et al. (2013) are classified by their morphostructure and the development of the basidioma and peridium surface, in addition to the type of dehiscence, gleba color and the ornamental pattern of the basidiospores.

Scleroderma is included in the family Sclerodermataceae Corda, order Boletales E,-J-Gilbert, class Agaricomycetes Dowel and Phylum Basidiomycota Whittaker ex Moore. This group of mushrooms is cosmopolitan, developing in all temperate and tropical regions of the world, often forming an ectomucorrhizal association with a wide variety of plants (Montagner, 2014; Pinzón-Osorio; Pinzón-Osorio, 2018). According to Nouhra et al. (2012) *Scleroderma* is one of the most adapted and generalist fungal genera in the Kingdom Fungi, they are closely linked to exotic forest stands.

Thirty species of *Scleroderma* are identified in the world. In Brazil, 13 species are described, especially in the Southern region of the country (Sulzbacher et al., 2013; Montagner, 2014). *S. verrucosum* was identified in the Rio Grande do Sul region, Brazil, by Cortez et al. (2011), together with 11 other species *S. albidum* Pat. and work emendment Gusmán, *S. bovista* Fr., *S. citrinum* Pers., Syn., *S. dictyosporum* Pat., Bull, *S. fuscum* (Corda) E. Fisch., and *S. leave* Lloyd emend. Guzmán, still in this study, the authors add that there are few molecular studies with the genus *Scleroderma* in Brazil.

The mushroom *S. verrucosum* has characteristics similar to other species of the same genus, although according to Guzmán et al. (2013), the *S. verrucosum* has the following characteristics: basidiome (20-) 25-0 (-45) mm diam., globose, shortly pseudostipitate. Peridium thin, membranaceou when mature, yellowish-brown, covered with small dark brown or blackish scales. Pseudostipe short, up to 15 mm long, solid, pale brownish, frequently lacunose, up 0 mm long, whitish to pale brownish. Basidiospores (8-) 9-12 (-14) μm, echinulate, with spines 0.5-2 μm high. Clamp connections absent. Other features as those in *S. nitidum* and *S. areolatum*. Taxonomic summary habitat and distribution. Presents how gregarious, sometimes fasciculated, epigeous on soil, in *Pinus quercus* or cloudy forests. Common in Europa, North America, Central America and South American. *Scleroderma verrucosum* is one of the most reported species in the genus, in part for its abundance, but also because several times specimens of *S. areolatum* and *S. nitidum* are erroneously determined. Smith (1951) mixed *S. verrucosum* with *S. lycoperdoides*, which is a synonym of *S. areolatum*. He described basidiospores (8-) 10-15 (-18) μm diam.

There is little information about the genus *Scleroderma* regarding phytochemical characteristics (Vrkoč et al., 1976; Gonzalez et al., 1983; Jung; Tamai, 2012; Morandini et al., 2016) and biological activities, mainly regarding antioxidant activity.

Thus, this study aimed to evaluate the ethanol extract of the vegetative part of the mushroom *Scleroderma verrucosum* in terms of phytochemical prospecting, total flavonoid and phenolic contents andantioxidant activity in reducing the DPPH free radical.

2. Materials and Methods

Five hundred (500 g) of the vegetative phase of *S. verrucosum* 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 mushroom was identified by Biologist Antonio Carlos P. M. Filho, and a sample was prepared and maintained at the authors' Mycological Bank in the Technological Chemistry laboratory of the Instituto Federal Goiano, Rio Verde, Goiás states, Brazil with the Voucher (SV03/2021).

The extract of *S. verrucosum* was obtained from 150 g of mushrooms previously cleaned in running water and dried in an oven at 35 °C for 3 h, where it was then groundin a processor with 200 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 8 days. After this period, the mixture was filtered and the supernatant collected and reduced in a rotaevaporator and then lyophilized.

Phytochemical tests were carried out on the 70% ethanolic mushroom extract of the qualitative determination according to Sembiring et al. (2018), Madike et al. (2017), De Silva et al. (2017) and Mehdi et al. (2019). Alkaloids, flavonoids, tannins, saponins, quinones, terpenoids and steroids, reducing sugars and non-reducing sugars, resins, amino acids, coumarins, glycosides, purines, organic acids, aromatic and aliphatic compounds, phenolics, polysaccharides, phlobatannins, carboxylic acids and oxylates.

The total phenolic content was determined according to colorimetric *Folin-Ciocalteu* method as described by Labiad et al. (1996) modified. 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 the addition of 5 mL of sodium carbonate conc. (7.5%) (*w/v*). The solution was stored in a dark room for 60 min., and 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}) were 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).

Total flavonoid contents were measured using a modified colorimetric method described by Labiad et al. (1996). Aliquot containing 0.25 mL of mushroom extract solution was added to a test tube containing 1.25 mL of distilled water. Then, the sodium nitrite 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*) aluminum chloride solution was added and 1 min. homogenized. After 6 min., 0.5 mL of conc. 1 Mol L^{-1} (*w/v*) of sodium hydroxide 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 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}) were 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 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging ability assay was used to evaluate the antioxidant activity of the mushroom extract. Test was conducted in a 96-well plate according to Sembiring et al. (2018) modification. 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} were added to each well. After 60 min incubation at room temperature in dark room, absorbance was read at 517 nm using micro-plate reader of UV-*Vis* spectrophotometer. 70% ethanol was used as blank. The scavenging ability (%) was calculated according to equation (1), and Ascorbic acid and 3,5-Di-tert-4- butylhydroxytoluene (BHT) was used as positive standards.

$$\% \text{ reduction} = (\text{Abs standard} - \text{Abs crude extract})/\text{Abs standard}*100 \quad \text{Eq. [1]}$$

All tests were performed in triplicate. Concentration of mushroom extract samples resulting in 50% inhibition on DPPH (IC50 value) expressed in µg mL^{-1} was calculated. Assay for DPPH free radical reduction was performed in triplicate followed by ± SD. When significant differences were observed, they were analyzed using the Duncan's test ($p < 5\%$) using the Statistics IBM SPSS software.

3. Results and Discussion

In this study, it should be clarified that the mushroom *S. verrucosum* was collected in the soil close to individuals of *Spondias purpurea* (L.).

The mushroom *S. verrucosum* extract showed a positive presence for the phytocompounds class's alkaloids, flavonoids, triterpenoids, tannins, organic acids, reducing sugars, aromatic complexes and carboxylic acids (Table 1).

There are few reports on the phytochemical constitution from the special metabolites *of Scleroderma*, the reports

found in the literature refer to steroidal compounds. As noted earlier, the 70% ethanol extract exhibited the presence of this class of compounds, the same is observed by other authors. The presence of steroid molecules is also described for *S. aurantium* by Vrkoč et al. (1976) ester diol, ergosterol, ergosterol peroxide, 9(11)-dehydroergosterol peroxide, lanosta-8,23-dien-3β,25-diol, lanosta-8,24-diene-3β,23-diol, and mannitol, and fatty acid compounds palmitic acid and linoleic acid. In *S. polyrhizum* por Gonzalez et al. (1983) isolated two steroidal compounds ergosta-4,6,8(14),22-tetraen-3-on and 5α,8α- epidoxyergosta-6,22-dien-3β-ol, in addition to fatty acid compounds, palmitic acid and oleic acid. Yayli et al. (2007) described the presence of essential oil in *S. verrucosum*, Turkey, with the following major compounds 3-Octone (49.1%), 3-Octanol (26.8%) and *n*-Octanol (5.1%).

In medicine, several phytochemical groups are used in the treatment of several pathologies, alkaloids, terpenes, flavonoids, glycosides and proteins with antiviral activities (Ben-Shabat et al., 2020), (monoterpene oxide, monoterpene alcohol, sesquiterpene, steroid and cardiac glycoside) antimicrobial, anti oxidant, anti-inflammation, anticancer, hepato protective, diuretic, antiasthma activities (Pakkirisamy et al., 2017). Although plants are the living organisms with the greatest number of studies, fungi also present a good share of therapeutic knowledge, in addition to having many species of wide culinary and nutritional use (Furlani & Godoy, 2007; Orsine et al., 2012; Pazza et al., 2012; Pazza et al., 2012). al., 2019) and also with species with hallucinogenic and toxic chemical groups (Erguven et al., 2007; White et al., 2019).

These phytochemical compounds are known to support bioactive activities in medicinal fungi and thus responsible for the antioxidant activities of this mushroom extract used in this study.

Table 1. Phytochemical screening of the mushroom of *S. verrucosum* extract.

Phytochemical	Results
Alkaloids	+
Flavonoids	+
Saponnins	-
Triterpenoids	+
Steroids	+
Tannins	Green
Quinones	-
Organic acids	+
Purines	-
Reducing sugars	+
No-reducing sugars	-
resins	-
Amino acids	-
Glycosides	-
Aromatic and aliphatic	Red
Phlobatannins	-
Carboxylic acids	+
Oxylates	-
Polysaccharides	-

Note: (-) absent. (+) present. Green = Condensed or catechetical. Red = Aromatic compounds.

The extract of the mushroom *S. verrucosum* showed no reaction for the determination of total flavonoids. But it had a high content of total phenolics, in addition to showing an important capacity to reduce the free radical DPPH (Table 2). When compared to standards of antioxidants ascorbic acid and BHT, the extract of *S. verrucosum* showed statistical difference according to Duncan's test. In the study by Łopusiewicz (2018), the researcher found potential free radical reducing activity for the *S. citrinum* extract.

Mushroom extracts that have a high capacity for reducing free radicals are influenced both by the content of flavonoids and phenolic compounds and are an attractive option in industrial production as a means of reducing the oxidizing action of reactive oxygen species, hydroxyl radical, peroxidase, alkyl, peroxyl, alkoxyl, phenolxyl, phenyl, superoxide among others (Selim et al., 2018; Jia et al., 2020).

Table 2. Total flavonoid and phenolic, and antioxidant DPPH scavenging activity of the mushroom extract of *Scleroderma verrucosum* extract.

Extract	Total flavonoids	Total phenolics	DPPH
	nr	309.14 ± 1.16	5.97 ± 0.91b

Note: *Expressed in mg QE 100 g^{-1}. ** Expressed in mg GAE 100 g^{-1}. Expressed in µg mL^{-1}. ***Acid ascorbic 1.96 ± 0.53a and BHT 3.77 ± 0.90a. nr = non-reactive. Different letters in Table 2 and Note show statistical difference according to Duncan's test $p < 5\%$ of probability.

4. Conclusions

The mushroom extract Scleroderma verrucosum demonstrated a wide range of special metabolites of medical, biological and agricultural importance, as well as expressive content of total phenolics that influence the ability to reduced the free radical DPPH, making this mushroom species attractive for future tests biological products of interest in the production of medicines, lotions, topical emulsions, as well as in biotechnological use due to the presence of reducing sugars.

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