

Mineral Composition and Total Polyphenol Content of Polysaccharide-Rich Extracts from the Edible Mushroom *Auricularia delicata*

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Abstract

This study aimed to evaluate the biopharmaceutical potential of polysaccharides from *A. delicata*, commonly consumed as an edible mushroom by the populations of Kinshasa and its surroundings. This biopharmaceutical aspect makes the species a functional food and a source for the production of new drugs against several human diseases. The objective of the study is to measure the mineral composition and total polyphenol content present in extracts rich in polysaccharides from *A. delicata*. These compounds are reported in the literature as having antioxidant and other biological activities. The results obtained confirmed a moderate fraction of the total polyphenol content, detected at an absorbance of 10.256 ± 0.363 mg GAE/g, and mineral elements, of which iron is dominant in higher quantities than the 19 other minerals measured from polysaccharide-rich extracts. The mineral composition and total polyphenol content in polysaccharide-rich extracts of *Auricularia delicata* show how much the species is a source of functional food (alicament), with medicinal and therapeutic properties. The presence of these compounds (mineral elements and polyphenols) offers hope for the use of *A. delicata* in the biopharmaceutical field by the populations of Kinshasa and its surroundings, but also provides a basis for the development of biologically active molecules.

Keywords: Edible mushrooms; *Auricularia delicata*; total polyphenols; minerals; functional food.

INTRODUCTION

Wild fungal species are an integral part of the human diet, where they are commonly used because of their specific characteristics, particularly their taste, chemical composition, and nutritional value. Apart from sporophores, mycelial filaments were already being used in the development of natural medicines. Biopharmaceutical technologies use fungal species to develop new products because of their richness in secondary metabolites, which are often considered a source of biotherapeutics. Fungi are rich in several biologically active compounds, both nutritious and non-nutritious, such as polysaccharides, including β -glucans, peptides, proteins, terpenoids, fatty acid esters, organic acids, sterols, alkaloids, and phenolic species (Golak-Siwulska and *al.*, 2018). These compounds, which are little known to large consumers, especially those living in developing countries, make these species a functional food capable of interacting with the proper functioning of human beings.

The concentration of biological properties in cis compounds varies significantly depending on the composition of the substrate or harvesting environment

of each species (Hola and *al.*, 2023). Among the fundamental and often environmentally variable compounds are phenolic species, which play a crucial role in destroying free radicals by reducing oxidative stress and preventing several chronic diseases (Ferreira and *al.*, 2009), this could open up promising avenues for the potential use of these organisms in biopharmaceutical, food, and cosmetic technologies (Ahmad et al., 2014; Chowdhury and *al.*, 2015), with a particular focus on species commonly consumed in developing countries, such as the Democratic Republic of Congo.

In the Democratic Republic of Congo, particularly in Kinshasa and its surroundings, species of the genus *Auricularia* are the most commonly consumed, with *Auricularia delicata* occupying a prominent place. Among the populations living in this region, *A. delicata* is one of the dietary supplements necessary for human nutrition (Boa, 2004).

The populations concerned resort to this species during periods when meat is difficult or even rare to obtain, unaware of the biological activities it may contain. Furthermore, pharmacological analyses

conducted among African populations in general and Asian populations, particularly in China and India, have confirmed that *Auricularia delicata* is not only rich in nutrients, but also contains substances with traditional therapeutic properties (Devi and Singh, 2008; Gargano and *al.*, 2017) whose phenolic and polysaccharide compounds are known to lower cholesterol levels and promote immunomodulatory, hepatoprotective, anticancer, antioxidant, anticoagulant, and antimicrobial activities (Saura, 2010; Varsha and *al.*, 2018; Kong and *al.*, 2020).

This purely biopharmaceutical approach could encourage people in Kinshasa and the surrounding area to consider *Auricularia delicata* not only as a dietary supplement to replace animal products such as meat, but also as a functional food (alicament) and a source for the production of new natural medicines to treat several human diseases (Ulziijargal and Mau, 2011).

Due to a lack of precise information on *Auricularia delicata* regarding its non-use in the biopharmaceutical field by consumers in Kinshasa and its surroundings, we undertook to analyze the polysaccharides derived from the sporophores of this species in order to provide convincing information necessary from a medicinal and therapeutic point of view, thereby promoting the species among consumers in Kinshasa and its surroundings.

The overall objective of this study is to measure the mineral composition and total polyphenol content of polysaccharide-rich extracts from the sporophores of the species *A. delicata*, given that polysaccharides, a group of biologically active molecules, perform many functions that are essential to the proper functioning of the human body.

MATERIALS AND METHODS

Biological Material

The biological material used in this study consisted of sporophores of *Auricularia delicata*, collected on the Bateke Plateau, located east of the city-province of Kinshasa, in the Democratic Republic of Congo.

Sample procurement

The sporophores of *A. delicata* were harvested with great care and kept cool until they were transported to the laboratory, where they were rinsed in distilled water before being spread out in a clean area of the laboratory (at room temperature) for approximately 6 hours. Once thoroughly drained, the sporophores were dried in an oven (Memmert brand) at 40°C for 24 hours. After drying, we used an electric grinder to obtain a fine powder from these sporophores, which was used for the extraction and purification of the different families of polysaccharides.

Extraction and purification of water-soluble polysaccharides from *A. delicata* sporophores

Using an analytical balance, we collected ninety grams (90 g) of powder from the sporophores. This powder was decolorized by treatment with acetone (1000 mL) for 5 hours under agitation (500 rpm) at a temperature of 28 °C. After centrifugation (5000g, 20 minutes, 20°C), the pellet was recovered and treated with pure ethanol (750 mL) for 3 hours under agitation (500 rpm) at 75°C. After centrifugation (5000g, 20 minutes, 20°C), the recovered pellet was dried for 24 hours in a ventilated oven at 40°C. The depigmented powder sample was then stored in a cool, dry place, protected from light, until it was used as material from which different families of polysaccharides were extracted and purified.

Extraction with distilled water

Polysaccharide extraction and purification were performed according to the adapted protocol of Chouana (2017). To do this, 30 g of depigmented powder were placed in a flask containing 750 mL of distilled water. The suspension was stirred (500 rpm) for 5 hours at 100°C under reflux. After stirring, the solution was cooled to 4°C in a refrigerator and then filtered using filter paper to retain insoluble macroscopic debris. This filtration process was repeated three times. The filtrate obtained, called filtrate 1, was kept in a refrigerator set at 4°C, and the insoluble debris was subsequently reprocessed as before under reflux in distilled water (750 mL) at 100°C for 5 hours to obtain the maximum amount of polysaccharides. The solution was then cooled to 4°C in a refrigerator and filtered using filter paper to retain insoluble debris as before. The filtrate obtained, called filtrate 2, was mixed with filtrate 1 before the mixture was centrifuged (5000 g, 20 minutes, 20°C) using a centrifuge (REMI R-8C). The supernatant obtained was then filtered using filter paper. The filtrate recovered was concentrated to one third of its initial volume under reduced pressure using a rotary evaporator at a temperature of 60°C for 5 hours. The polysaccharides in the concentrate were precipitated using three volumes of ethanol (96%) at a temperature of -20°C with agitation (500 rpm) for 45 minutes, then recovered by centrifugation (5000 g, 20 minutes, 4°C). The collected sediment was then washed successively with ethanol (750 mL) and then acetone (750 mL). Finally, the washed sediment was dried in an oven at 45°C for 24 hours before being ground into powder using a mortar. The polysaccharides obtained during this extraction were named PS A.

Extraction with soda

For this extraction, we followed the protocol described by Chouana (2017). Thirty grams of depigmented powder were placed in a flask containing 750 mL of sodium hydroxide (NaOH 50 mM). The suspension was stirred (500 rpm) for 5 hours at 100°C under reflux. After

stirring, the solution was cooled to 4°C in a refrigerator and then filtered using filter paper to retain insoluble macroscopic debris. This filtration process was repeated three times. The filtrate obtained, called filtrate 1, was neutralized with a hydrochloric acid solution (1 M) and stored in a refrigerator at 4°C. The insoluble debris was then reprocessed as before under reflux in soda (750 mL) at 100°C for 5 hours in order to obtain the maximum amount of polysaccharides (exhaustion). The solution was then cooled to 4°C in a refrigerator and filtered using filter paper to retain the insoluble debris. The filtrate obtained, called filtrate 2, was mixed with filtrate 1 before the mixture was centrifuged (5000 g, 20 minutes, 20°C) using a centrifuge (REMI R-8C). The supernatant obtained was then filtered using filter paper as before. The filtrate recovered was concentrated to one-third of its initial volume under reduced pressure using a rotary evaporator at 60°C for 5 hours. The polysaccharides in the concentrate were precipitated using three volumes of 96% ethanol at a temperature of -20°C with stirring (500 rpm) for 45 minutes, then recovered by centrifugation (5000 g, 20 minutes, 4°C). The washed pellet was then dissolved in distilled water (300 mL) and precipitated with ethanol (750 mL) to remove the salts formed (NaCl) and finally centrifuged (5000 g, 20 minutes, 4°C). This step of dissolving the pellet in water and precipitating it with ethanol was repeated four times. The final pellet was washed successively with ethanol (750 mL) and then acetone (750 mL). Finally, the purified pellet was dried in an oven set at 45°C for 24 hours before being ground into powder using a mortar. The polysaccharides obtained from this extraction were named PS B.

Extraction with hydrochloric acid

This extraction process is carried out according to the protocol adapted from extraction under alkaline conditions, this time replacing the sodium hydroxide solution (50 mM) with a hydrochloric acid solution (50 mM). The filtrates were then neutralized using a sodium hydroxide solution (1 M). The polysaccharides were subsequently purified by alcoholic precipitation and washing with acetone as described above. The polysaccharides obtained during acid extraction were named PS C.

During this study, all experiments were performed in triplicate (n = 3), each using 30 g of *Auricularia delicata* powder depending on the solvents used. The results obtained are given as mean ± standard deviation, and the extracted polysaccharides were stored at 4°C in airtight containers.

Assay of mineral compounds in Auricularia delicata polysaccharides

The detection of mineral elements present in the polysaccharide sample of *A. delicata* was carried out using the method developed by Tadeu et al., 2019. The

powdered polysaccharide sample (4.2155 g) was homogenized by vigorous stirring in a plastic container and then mixed with 0.8 g of Fluxana and Cereox from Höchstwax as a binder (i.e., 20% of the sample mass) before the mixture was compressed using a Carver pellet press. Meanwhile, IPE-10-31 and BCR-62 pellets were used as reference material to determine errors. The pellets used were placed in one of the positions on the sample changer plate and the wavelength dispersive X-ray fluorescence (WD-XRF) spectrum was measured. The instrument conditions consisted of an X-ray tube with a Rh anode, a voltage of 20–60 kV, and a current of 30–50 mA. Lightweight elements were measured under vacuum. Calibration was performed using certified reference materials (CRMs) with matrix effects corrected using the fundamental parameters method.

Determination of total polyphenols from polysaccharides of A. delicata.

The total polyphenol content of polysaccharide-rich extracts from *A. delicata* was measured using the Folin-Ciocalteu method described by Kim and al., 2003. According to the method, 200 µL of the methanolic extract was mixed with 1 mL of freshly prepared Folin-Ciocalteu reagent (preferably diluted 10 times with distilled water) and 0.8 mL of 7.5% Na₂CO₃ (sodium carbonate). The resulting solution was incubated at room temperature for 90 minutes and the absorbance was measured against a blank using a JENWAY 7315 UV-Visible spectrophotometer at 760 nm. The total polyphenol content was quantified using a calibration curve constructed using gallic acid as a reference standard, in a concentration range between 50 µg/mL and 350 µg/mL in 80% methanol.

Next, spectrometric measurements were performed using a UV-visible spectrophotometer, with each sample processed in five (5) replicates (n = 5) in order to obtain accurate and reproducible results. The absorbances recorded for the sample were 0.105, 0.104, 0.102, 0.102, and 0.106, the average of which was used to determine the total polyphenol content using the gallic acid calibration curve $y = 2.7614x + 0.0293$; $R^2 = 0.997$) with a final volume estimated at 100 mL.

The limits of detection and quantification, symbolized by LOD and LOQ respectively, were determined from the standard deviation of the blank ($\sigma = 0.002$) and the calibration slope ($S = 2.7614$) using the formulas $LOD = 3\sigma/S$ and $LOQ = 10\sigma/S$.

The results obtained were expressed in milligrams of gallic acid equivalent per gram of dry polysaccharide material. The relationship obtained follows the following linear equation: $y = ax + b$, where a is the slope of the line, b is the y-intercept, and R^2 is the coefficient of determination. Figure 1 below shows the dilution diagram for gallic acid in different concentrations.

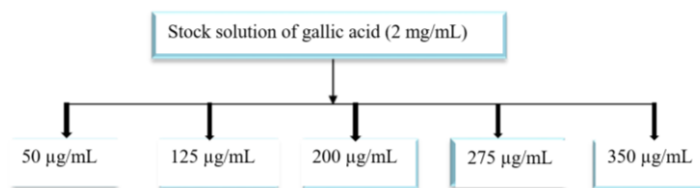


Figure 1. Schematic diagram of gallic acid dilution.

RESULTS AND DISCUSSION

RESULTS

Polysaccharide extraction yield

After extractions using solvents such as distilled water, soda (NaOH 50mM), and hydrochloric acid (HCl), we obtained values of 5.1 g, 3.8 g and 3 g, respectively, which in terms of yields resulted in $17 \pm 0.9\%$, $13 \pm 0.3\%$, and $10 \pm 0.3\%$, respectively, giving an overall average yield of $13.3 \pm 3.5\%$ polysaccharide concentration from *Auricularia delicata*. Figure 2 illustrates the polysaccharide yields from the fungal species *A. delicata* obtained depending on the solvents used.

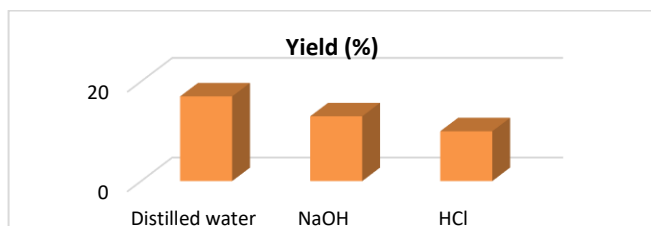


Figure 2. Polysaccharide yields from *A. delicata* as a function of solvents.

Analysis of Figure 2 shows that among the three solvents used in the extraction of different families of polysaccharides, distilled water yielded a 17% yield,

followed by sodium hydroxide (NaOH) with a yield of 13%, while hydrochloric acid (HCl) yielded only 10%, which was significantly lower than the yields obtained using the other solvents.

Comparison of this result with the one-way ANOVA test revealed a statistically significant difference between the yields of extracted polysaccharides and the solvents used, as $p\text{-value} = 0.0117 < 0.05$. After verifying this analysis using Tukey's post-hoc test (HSD), the significant difference observed is mainly due to the high yield of polysaccharides A, extracted with a solvent consisting of distilled water, compared to the low yield of polysaccharides in group C, extracted with hydrochloric acid as a solvent.

Mineral composition and total polyphenol content of polysaccharide-rich extracts from *Auricularia delicata*

Mineral composition of polysaccharides

Using wavelength dispersion X-ray fluorescence (WD-XRF) with an X-ray tube and Rh anode, operating at a voltage of 20–60 kV and a current of 30–50 mA, 20 mineral elements were detected in the sample of polysaccharide-rich extracts from *A. delicata*. Table 1 shows the X-ray fluorescence (XRF) spectrum of all the mineral elements present in the polysaccharides of the fungal species thus analyzed.

Table 1. Mineral composition of polysaccharides determined by XRF.

ELEMENTS	SYMBOL	CONCENTRATION (mg/kg)
Magnesium	Mg	870.4
Aluminum	Al	97.9
Silicon	Si	83.2
Phosphorus	P	96.6
Sulfur	S	26.7
Chlorine	Cl	103.7
Potassium	K	1900.7
Calcium	Ca	1678.6
Vanadium	V	0.16
Manganese	Mn	73.7
Iron	Fe	4000.9
Nickel	Ni	0.80
Copper	Cu	4.30
Zinc	Zn	29.9
Arsenic	As	0.02
Selenium	Se	0.09
Rubidium	Rb	14.8
Strontium	Sr	17.9
Niobium	Nb	0.11
Molybdenum	Mo	0.05
Total	—	9000.0

Analysis of Table 1 shows that the mineral composition of polysaccharide-rich extracts is 9000 mg/kg, or 0.9%, demonstrating a higher degree of processing and a low proportion of inorganic residues. Based on this value of 9000 mg/kg, it can be confirmed that the fraction submitted for analysis contains a large amount of organic compounds and that its mineral composition represents only a minimal fraction of the dry matter. The mineralogical profile observed in this table is largely dominated by iron (Fe) with 4000.9 mg/kg, demonstrating the complexing capacity of *Auricularia delicata* polysaccharides with respect to ferric ions.

Total polyphenols from *Auricularia delicata* polysaccharides

The total polyphenol content of *Auricularia delicata* polysaccharides was 10.256 ± 0.363 mg GAE/g dry weight, determined using the Folin-Ciocalteu calibration curve, with gallic acid as the reference standard. After establishing the calibration curve and before analyzing the actual samples, the LOD and LOQ obtained were 0.00214 mg/mL and 0.00648 mg/mL, respectively. The content of 10.256 mg GAE/g corresponds to 1.0256% of the total polyphenol composition of the polysaccharide-rich extracts of *A. delicata*. Figure 3 shows the calibration curve for gallic acid, used as a reference standard in the evaluation of the total polyphenol content of polysaccharide-rich extracts of *Auricularia delicata*.

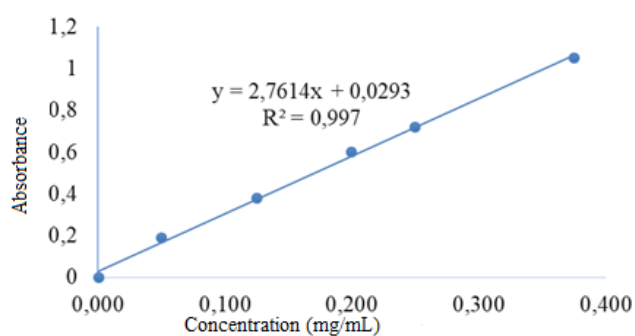


Figure 3. Calibration curve and gallic acid concentrations (mg/mL)

The analysis in Figure 3 illustrates the calibration curve, showing the relationship between measured absorbance and gallic acid concentration in the range 0.000 to 0.400 mg/mL with a linear regression: $y = 2.7614x + 0.0293$ ($R^2 = 0.997$). Given this high linearity and the improved sensitivity of the technique, the recorded content of 10.256 ± 0.363 mg GAE/g could be considered reliable and indicative of the total polyphenol content of the polysaccharides analyzed. This content, in percentage terms, represents 1.0256%, showing that the polysaccharides measured were partially pure.

DISCUSSION

The different families of polysaccharides were obtained from the sporophores of *Auricularia delicata* using solvents such as distilled water, soda (NaOH), and

hydrochloric acid, while solvents such as acetone and ethanol were used to purify the different families of extracted polysaccharides.

After extraction and purification, distilled water used as a solvent yielded a 17% yield, followed by soda (NaOH) with a 13% yield, while hydrochloric acid as a solvent yielded the lowest yield of 10%.

The overall average yield obtained was $13.3 \pm 3.5\%$, which is consistent with the 14% value found by Ruiz (2005) using the algae species *Porphyridium cruentum* as dry matter. During extraction, Ruiz (2005) used hot water as a solvent, unlike in this study, where the solvents used to obtain the total average yield were distilled water, caustic soda (NaOH), and hydrochloric acid. As for hydrochloric acid, the average yield obtained was 10%, considered the lowest recorded in different extraction series compared to the yields of 17% and 13% recorded with distilled water and caustic soda (NaOH), respectively. Compared to this low yield, it is higher than the 4% yield obtained by Ruiz (2005) during the extraction of extracellular polysaccharide from *Rhodella reticulata*. The difference observed in extraction is thought to be due either to the nature of the substrates used or to the methodology and solvents employed. Ruiz (2005) carried out his extractions using an algae species as a substrate and hot water as a solvent, whereas in this study, the substrate used consisted of sporophores of the species *Auricularia delicata*, using hydrochloric acid (HCl) as a solvent.

Regardless of their origin, polysaccharides extracted and purified from the sporophores of the species *Auricularia delicata* are a source of antioxidant activity and other bioactive compounds. After spectrometric analysis of the total polyphenol content, the absorbance obtained of 10.256 ± 0.363 mg GAE/g shows that the extracted polysaccharides contained 1.0256% total polyphenol content. This apparently moderate fraction reveals that the polysaccharide-rich extracts of *Auricularia delicata* underwent fairly good purification. Comparing this purification to that of other authors, Petraglia and al (2023) achieved very good purification, resulting in 0% total polyphenol content from *Pleurotus eryngii* polysaccharides in their work on the antioxidant activity of polysaccharides from the edible mushroom *Pleurotus eryngii*. This difference observed in relation to these authors could be attributed to the methods used, the working conditions, and the use of equipment during the process.

However, the moderate fraction obtained may promote the antioxidant activity and other biological activities of the polysaccharide extract studied (Johnson and Giulivi, 2005; Everette et al., 2010).

When comparing this absorbance to that of previous studies using fungal sporophores as dry matter, it is lower than the values obtained by Karime and Ikram (2022) in their study on the biological activities of the edible mushroom *Pleurotus eryngii*.

This difference observed in phenolic compounds could be due to the nature of the material used in the assay. Karima and Ikram (2022) used an extract derived directly from the sporophores of the species *Pleurotus eryngii*, whereas in this study, the extract used to determine the total polyphenol content was derived from the polysaccharides of the sporophores of *Auricularia delicata*.

Mineralogical analysis revealed that dry matter derived from polysaccharides extracted from *A. delicata* sporophores contains 20 minerals, including high levels of iron, potassium, calcium, and magnesium. The presence of other elements such as silicon, phosphorus, manganese, and zinc proves the nutritional and pharmaceutical properties of the species analyzed and corroborates the work of Ulzizjargal and Mau (2011), according to which the nutritional value of *A. delicata* includes minerals and a low dose of cholesterol, thus offering a variety of beneficial effects for the proper functioning of the human body.

Given the mineral composition and total polyphenol content of the measured polysaccharides in dry matter, *A. delicata* could be a promising source of functional food ingredients for the development of therapeutic remedies for several human diseases.

CONCLUSION

The aim of this study was to evaluate the mineral composition and total polyphenol content of the dry extract of polysaccharides from *Auricularia delicata*. The results confirmed the presence of several trace minerals in the dry matter analyzed, highlighting the potential nutritional value of this species.

Furthermore, the analysis revealed a moderate amount of total polyphenols, essential compounds known for their antioxidant properties. This moderate amount of polyphenols suggests that *A. delicata* could contribute to the neutralization of free radicals and, consequently, play a role in preventing oxidative stress involved in various diseases. This is one of the reasons why *A. delicata* is considered a source of antioxidants and natural medicines, benefiting the populations of Kinshasa and its surroundings who use it as an edible mushroom.

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