Original Paper

SCIENTIAFUNGORUM

DOI: 10.33885/sf.2025.56.1496
(2025) Vol. 56: e1496

Nutritional value and chemical analysis of *Hygrophoropsis aurantiaca* and *Hypomyces macrosporus* collected in La Malinche National Park, México

Valor nutricional y análisis químico de *Hygrophoropsis aurantiaca* e *Hypomyces macrosporus* colectados en el Parque Nacional La Malinche, México

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RESUMEN

Antecedentes: Las poblaciones cercanas al Parque Nacional La Malinche han utilizado los hongos silvestres comestibles como un alimento de temporada alternativo durante la época de lluvias.

Objetivo: Evaluar la composición química y la actividad antioxidante de *Hygrophoropsis aurantiaca* e *Hypomyces macrosporus* recolectados en el Parque Nacional La Malinche, México.

Métodos: Se realizó un análisis químico proximal que incluyó humedad, grasa, azúcares reductores, fibra dietética total, cenizas, proteína cruda y perfil de ácidos grasos; análisis químico de algunos metabolitos micoquímicos: compuestos fenólicos totales, flavonoides totales y triterpenos totales. Se evaluó la actividad antioxidante contra los radicales DPPH y ABTS.

Resultados y conclusiones: *Hygrophoropsis aurantiaca* e *H. macrosporus*, presentaron contenidos relevantes de macronutrientes y compuestos micoquímicos. En *H. aurantiaca* la proteína cruda varió entre 11.2-12.5 %, la fibra 21.4-23.1 % y los triterpenos 25.4-28.6 mg UAE/g, mientras que la actividad antioxidante mostró valores de IC_{50} de 1.20 ± 0.22 mg/mL. En *H. macrosporus*, los contenidos de proteína fueron menores (8.7-9.5 %), la fibra 18.9-20.1 % y los triterpenos 6.3-7.1 mg UAE/g, con actividad antioxidante de 5.78 ± 0.03 µmol ET/g. Estos hallazgos evidencian que ambas especies de hongos silvestres comestibles constituyen una fuente valiosa de componentes nutricionales y micoquímicos.

Palabras clave: actividad antioxidante, composición proximal, hongos silvestres comestibles, micoquímicos

ABSTRACT

Background: ocal communities near La Malinche National Park have traditionally consumed wild edible mushrooms as an alternative seasonal food during the rainy season.

Objective: Evaluate the chemical composition and antioxidant activity of *Hygrophoropsis aurantiaca* and *Hypomyces macrosporus* collected in La Malinche National Park, Mexico.

Methods: A proximate chemical analysis was carried out to determine moisture, fat, reducing sugars, total dietary fiber, ash, crude protein, and fatty acid profile. Chemical analysis was performed on some mycochemical metabolites: total phenolic compounds, total flavonoids, and total triterpenes. The antioxidant activity against the DPPH and ABTS radicals was evaluated.

Results and conclusions: *Hygrophoropsis aurantiaca* and *Hypomyces macrosporus*, were found to contain relevant macronutrients and mycochemical compounds. In *H. aurantiaca*, crude protein ranged from 11.2-12.5 %, fibre 21.4-23.1 %, and triterpenes 25.4-28.6 mg UAE/g, while antioxidant activity showed IC_{50} values of 1.20 ± 0.22 mg/mL. In H. macrosporus, protein contents were lower (8.7-9.5 %), fiber 18.9-20.1 %, and triterpene content was 6.3-7.1 mg UAE/g, with antioxidant activity of 5.78 ± 0.03 µmol ET/g. These results demonstrate that both wild edible mushrooms are valuable sources of nutritional and mycochemical components.

Keywords: antioxidant activity, mycochemicals, proximate composition, wild edible mushrooms

ARTICLE HISTORY

Received: 29/07/2025
Accepted: 21/11/2025
On line: 25/11/2025

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INTRODUCTION

Wild edible mushrooms (WEM) are non-timber forest resources highly valued as food supplements and as a source of income for rural communities (Torres-Gómez *et al.* 2023). Mushrooms are important in the ecosystem, since they can biodegrade substrates and therefore use agricultural production waste (Sarikurkcu *et al.* 2011). They play an important part in rural alimentary strategies throughout the world during rainy season, providing high-quality food with significant nutritional value. Firstly, WEM are generally valued nutritionally for their essential aminoacidic index, high protein content, the third most abundant component (5 to 49 % of their dry weight), their carbohydrates that contain dietary fibre, minerals such as potassium and phosphorus, and their soluble vitamins (Rathore *et al.* 2017, Ruan-Soto *et al.* 2017).

In Mexico, there are more than 450 species of wild edible mushrooms, which are mostly consumed by people who live in rural areas, usually indigenous communities (Pérez-Moreno *et al.* 2020). Consuming wild mushrooms is a tradition that dates back to pre-Hispanic times and has been passed on from generation to generation, transferring knowledge of seasonality, ecology, morphology, and general biology of mushrooms in the local environment (Montoya *et al.* 2003).

However, *Hygrophoropsis aurantiaca* and *Hypomyces macrosporus* remain poorly characterized chemically in Mexico. In *H. aurantiaca*, for example, ergosterol and ergosterol peroxide have been isolated; their antiproliferative activity in vitro suggests pharmacological potential (Nowak *et al.* 2016). To date, we are not aware of recent quantitative studies reporting parameters such as protein, fibre, phenolic compounds or antioxidant activity for *H. macrosporus*. These gaps in the literature justify conducting a detailed proximate chemical and antioxidant characterization of both species collected in La Malinche National Park, Mexico.

Wild edible mushrooms are also important sources of bioactive compounds, particularly phenolic metabolites, which are recognized for their antioxidant activity and potential health benefits against oxidative stress-related diseases (Arunachalam *et al.* 2022, González-Morales *et al.* 2021). However, specific information for *H. aurantiaca* and *H. macrosporus* is still scarce, making their chemical and antioxidant characterization relevant.

The present study aims to evaluate the proximate chemical composition and antioxidant activity of *H. aurantiaca* and *H. macrosporus* harvested from La Malinche National Park, to provide valuable nutritional and mycochemical data for under-studied wild mushrooms in the region.

MATERIALS AND METHODS

Sample collection

During the rainy season in July 2022, two field expeditions were conducted in selected areas of La Malinche National Park, located near San Miguel Canoa, municipality of Puebla, Mexico. Various wild edible mushrooms were collected during these expeditions. Among them, two species, *Hygrophoropsis aurantiaca* (Wulfen) Maire ex Martin-Sans and *Hypomyces macrosporus* Seaver, were chosen because of their abundance and their gastronomic importance for the local indigenous community.

Taxonomic identification was performed through examination of both macroscopic and microscopic features, using dichotomous keys published in specialized mycological works (Díaz-Barriga Vega 2011, Guzmán 1997). Molecular tools were not applied, since the main objective of the study was the chemical characterization of the fruiting bodies, and morphological identification was considered sufficient for this exploratory level of research, as previously reported in studies on wild edible fungi (Heleno *et al.* 2015, López-Vázquez *et al.* 2017).

The analyzed samples consisted of fruiting bodies collected at an intermediate developmental stage (neither immature nor senescent) to ensure uniformity in the chemical analyses. Whole fruiting bodies (pileus, stipe, and hymenophore) were used.

Sample conditioning

Sample conditioning consisted in removing objects not part of the sample's composition, such as leaves, soil, and insects, among others. Then, the samples of fruiting bodies were dried in a convective oven at 50 °C, at a flow rate of 2.4 m/s, for 3 hours. Finally, the mushrooms were ground in a Hamilton Beach mill to a particle size of 0.117 mm and subsequently stored in airtight containers at 20 °C for further analysis.

Proximate chemical analysis

Moisture

First, fresh 10-gram samples of each mushroom species were used to determine the moisture content by the oven dry method, method 930.15 of the Association of Official Analytical Chemists (AOAC 2000). This method is based on the principle of removing water by subjecting the samples to constant temperature until reaching a stable weight. The results were expressed as percentage of moisture content.

Fat

Crude fat (ether extract) was determined following the Soxhlet method, AOAC method 960.39 (AOAC 2000). First, 0.5 g sample was placed on filter paper and 140 mL of hexane was added. The solvent was brought to a boiling point while in contact with the sample for 40 minutes, then the soluble matter was removed, and the hexane was evaporated at 105 °C for 30 minutes. Finally, the sample was placed in a desiccator and the weight of the flask plus the extracted fat was recorded. The results were expressed in g/100 g of crude fat.

Reducing sugars

Fehling's test was used with the following modifications: 5 g mushrooms was dissolved in 100 mL of distilled water and 5 mL of Fehling reagent (composed of copper (II) sulphate pentahydrate in solution A and potassium sodium tartrate with sodium hydroxide in solution B) were added. This solution was boiled with constant agitation until it turned into a red precipitate of insoluble copper oxide. Before the red colour changes, 2 drops of methylene blue were added, and the end of the reaction was marked. Lastly, the total flow rate of the filtrate was measured (Kunz *et al.* 2011). The results were expressed as percentage of reducing sugars.

Total dietary fibre

To measure total dietary fibre, the enzymatic-gravimetric method (AOAC method 985.29) was employed, which uses thermostable α-amylase, protease and amyloglucosidase enzymes (AOAC 2000). To start, thermostable α-amylase was added to 1 g of sample and gently stirred in a water bath at 95 °C for 35 min. Temperature was then adjusted to 60 °C and protease was added, followed by incubation at 60 °C for 30 min. pH was adjusted to a range of 4.1 - 4.8. Amyloglucosidase was then added, and the mixture was incubated at 60 °C for 30 min. The residue corresponding to dietary fibre was then precipitated, filtered, and weighed. Finally, the enzyme mixtures were filtered, and the residue was washed with distilled water at 70 °C. Subsequently, they were washed with ethanol and acetone at room temperature, then dried at 103 °C overnight, and the obtained residue was weighed.

Ash

AOAC method 923.03 was selected to measure ash (AOAC 2000). One gram of sample was placed in a porcelain crucible on a hot plate. Once the volume was reduced, it was introduced into a muffle furnace at 525 °C until completely white ashes were obtained.

Crude protein

Protein content was determined by measuring total nitrogen using the micro Kjeldahl method, according to AOAC method 984.13 (AOAC 2000). Dried sample (0.2 g) was digested with potassium sulphate, copper sulphate, and concentrated sulfuric acid in a Kjeldahl flask. After digestion and cooling, the mixture was diluted in a volumetric flask. Distillation was performed, and the distillate was collected in a boric acid solution containing a mixed indicator. The solution was subsequently titrated with standardized 0.02 N hydrochloric acid until reaching the endpoint, indicated by a colour change from light green to red.

Crude protein values were calculated using the conversion factor 4.38, which is specifically recommended for mushrooms to avoid systematic overestimation derived from the conventional factor of 6.25. This adjustment is based on the fact that macrofungi contain appreciable amounts of non-protein nitrogen, such as chitin and low molecular weight nitrogenous compounds, which inflate protein estimates if the general factor is applied (Stojek *et al.* 2025).

The results were obtained in percentage of protein (Equation 1).

$$(1)\ \%\ \text{protein} = \frac{(14)\ (N)(V)(100)(F)}{(m)(1000)}$$

Where N is the normality of the HCl solution, V corresponds to the difference between the HCl volume consumed by the sample and that consumed by the blank (mL), 14.01 is the atomic weight of nitrogen, m is the sample mass (g), and F is the specific nitrogen-to-protein conversion factor (4.38).

Fatty acid profile

Fatty acid content was calculated as the total percentage of fatty acid methyl esters (FAMEs). The study was conducted in a gas chromatograph (HP-5890, Hewlett-Packard Company, USA) equipped with a flame ionization detector (FID). The FAMEs were extracted in the following way. First, 1 mL of each mushroom sample was saponified with a methanolic solution of 1 N KOH. For the solution, 10 mL of hexane and 10 mL of distilled water were added, the organic and aqueous phases were separated, and the residue was dissolved in hexane. The injection volume was 2 µL (with a split radius of 20:2). The oven temperature was 100 °C (with 4 min of retention) and increased to 250 °C. The injection system and the detector were maintained at 230 °C and 250 °C, respectively. The carrier gas was N_2 with a flow of 1.2 mL/min. The specifications of the column were capillary column of silica RT®-2560 (biscyanopropyl-polysiloxane), 100 m long, 0.25 mm thick and 0.2 µm in packing material (Restek Corporation, Bellefonte, USA). Fatty acids were compared with the same standard used for the infrared spectroscopy methodology (Escamilla-Rosales *et al.* 2019). Each analysis was carried out in duplicate.

Preparation of extracts

To prepare the extracts, 0.5 g of each sample (*H. aurantiaca* and *H. macrosporus*) were added to an 80 % ethanol solution (w/v) until a 0.1 mg/mL concentration was obtained.

For maceration, the samples were placed in a shaker (KJ-201BD Orbital Shaker Westtune Hangzhou, Zhejiang, China) at 60 rpm for 30 min, filtered and placed in a centrifuge (Z200A Hermle Wehingen, Germany) at 320 rpm for 12 min. The supernatant was stored in Eppendorf tubes at -20 °C for further analysis. For the ultrasonic analysis, the samples were placed in an ultrasonic bath device (Model 2510, BRANSON) for 20 min with a frequency of 40 kHz and a power of 180 W. The samples were processed at room temperature. The supernatant was stored in Eppendorf tubes at -20 °C for further analysis.

Total phenolic compounds

Total phenolic compounds were determined using the method described by Singleton and Rossi (1965) with some modifications. First, 250 µL of Folin-Ciocalteu 1 N reagent was added to 50 µL of the extract; this was stirred and allowed to stand in the dark for 8 min. After that, 1.25 mL of 5 % Ca_2CO_3 (w/v) was added, and the mixture was allowed to stand again for 30 min in the dark at room temperature. Absorbance at 725 nm was then read on a UV-Vis spectrophotometer (6320D Jenway Staffordshire, UK). A calibration curve for gallic acid (Sigma Aldrich, CAS 149-91-7, Steinheim, Germany) was used in the range of 0 to 0.5 mg/mL. Results were expressed in mg gallic acid equivalents per mL of extract (mg GAE/mL extract). These analyses were performed in triplicate.

Results were calculated based on the standard curve prepared in the same conditions for gallic acid (curve equation: $y = 0.0047x + 0.0227$; $r^2 = 0.9989$; linearity range 20 - 200 µg/mL) and expressed as mg of gallic acid per g of extract.

Total flavonoids

The aluminium chloride method by Chang *et al.* (2002) was used, with some modifications. To begin, 500 µL of the extract, 1.5 mL of 80 % ethanol (v/v), 100 µL of 10 % hexahydrate aluminium chloride solution, 100 µL of 1 M potassium acetate, and 2.8 mL of distilled water were placed in a test tube, which was shaken in a vortex and incubated for 30 min at room temperature.

Absorbance at 415 nm was read on a UV-Vis spectrophotometer (6320D Jenway Staffordshire, UK). A quercetin calibration curve (Sigma Aldrich, CAS 117-39-5, Germany) was used at a concentration of 0 to 0.1 mg/mL. The results were expressed in mg quercetin equivalents per g extract (mg QE/g extract). These analyses were performed in triplicate.

Total triterpenes

The vanillin-acetic acid method was used with some modifications (Fan and He 2006). First, 120 µL of extract, 100 µL of vanillin (Sigma Aldrich, CAS 121-33-5, Germany) 5 % (w/v), and 400 µL of perchloric acid in 0.1 N glacial acetic acid were placed together in a test tube at 60 °C for 15 min.

The mixture was cooled to room temperature, and 2.5 mL of glacial acetic acid was then added. Absorbance

was measured at 550 nm in a UV-Vis spectrophotometer (6320D Jenway Staffordshire, UK). The results were expressed as mg ursolic acid per g extract (mg UA/g extract). A calibration curve for uric acid (Sigma Aldrich, CAS 77-52-1, Germany) was used with a concentration from 0 to 120 µg/mL. These analyses were performed in triplicate.

Antioxidant Activity by 1,1-Diphenyl-2-Picril Hydracil (DPPH•)

Antioxidant capacity was determined using the DPPH• (2,2-Diphenyl-1-picrilhydrazyl) radical method with minor modifications (Brand-Williams et al. 1995). DPPH (990 µL) (Sigma Aldrich, CAS 1898-66-4, Germany) 0.1 mM was added to 10 µL of the mushroom extract. The mixture was shaken and left to stand for 30 min in the dark, after which absorbance was measured at 517 nm using a UV-Vis spectrophotometer (6320D Jenway Staffordshire, UK). Percentage of inhibition was calculated as follows (Equation 2):

$$(2)\ \text{Percentage of inhibition} = \left[\frac{A0 - A1}{A0}\right] * 100$$

Where A0 is absorption of the control and A1 is absorption of the sample. A linear regression curve was constructed using four extract concentrations, and the IC_{50} values were calculated. These analyses were performed in triplicate.

Antioxidant Activity by 2,2'- Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid Radical Cation (ABTS•+)

Antioxidant capacity was also evaluated using the ABTS•+ radical cation method (Re *et al.* 1999). Radical cations were generated from 0.0033 g of potassium persulfate and 0.0194 g of ABTS reagent (2,2'-azinobis-(3-ethylbenzothiazolin-6 sulfonic acid (Sigma Aldrich, CAS 30931-67-0, St. Louis, MO, USA) dissolved in 5 mL of distilled water. The solution was kept in the dark at room temperature for 16 h before use. For measurement, the stock solution was diluted in ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. Then, 3920 µL of the ABTS•+ solution was added to a cuvette, and initial absorbance (Ai) was recorded. After adding 80 µL mushroom extract, the mixture was incubated for 7 min, and final absorbance (Af) was recorded at 734 nm using a UV-Vis spectrophotometer (6320D Jenway Staffordshire, UK). Inhibition percentage was calculated and IC_{50} values were obtained by linear regression. All analyses were performed in triplicate.

It is important to note that conventional antioxidant standards, such as Trolox or ascorbic acid, were not included in this study. The purpose of the study was to generate baseline information on the intrinsic radical scavenging capacity of the mushroom extracts themselves, without external normalization. This decision aligns with previous exploratory works on wild edible mushrooms, where the primary focus was characterizing their endogenous activity rather than establishing direct equivalence with synthetic antioxidants (Heleno *et al.* 2015). Future comparative analyses will incorporate these standards to strengthen the nutritional and functional interpretation of the results.

Statistical analysis

The results were expressed as means and standard deviation. Comparative statistical analyses were carried out between the two studied species (*H. aurantica* and *H. macrosporus*) for each evaluated parameter. A one-way analysis of variance (ANOVA), followed by Tukey's test ($\alpha = 0.05$), was used to determine significant differences between species. The experimental design was completely randomized, and analyses were performed using SAS Studio application under SAS OnDemand for Academics https://www.sas.com/en_us/software/on-demand-for-academics.html.

RESULTS AND DISCUSSION

Identification of the collected mushrooms was based on both macroscopic and microscopic features, using classical dichotomous keys and taxonomic works (Díaz-Barriga 1992, Díaz-Barriga Vega 2011, Guzmán 1979). The first species, *Hygrophoropsis aurantiaca*, locally known as "brindis", was found in the La Malinche National Park area. It presented a pileus ranging from 18 to 43 mm in diameter, convex to infundibuliform, with an undulate curved margin. The surface was smooth to finely tomentose or slightly rough, displaying a yellow to orange-yellow to orange-brown coloration, with a lighter margin. The lamellae were bifurcate, decurrent, and concolorous with the pi-

leus. The stipe measured 25-35 mm × 4-6 mm, sometimes eccentric, cylindrical, widened at the base, rough to striated, hollow, and of the same colour as the pileus (Merino Alcántara 2019). The second species, *Hypomyces macrosporus*, commonly referred to as "black pig's snout", showed a subiculum surface ranging from pink to blackish once the perithecia matured. The surface exhibited a granular appearance due to the development of ostiolar necks protruding as papillae; ostioles were punctate. The perithecial wall was composed of several layers of pseudoparenchymatous cells. Asci were thin and cylindrical, with a rounded apical end. Ascospores measured 30-40 µm, arranged uniseriately, bicellular due to the presence of a thick transverse septum, which gave the appearance of an apiculate belt, with a rough and refractory membrane when tested with Melzer's reagent (Pérez-Silva *et al.* 1983). The main diagnostic traits of both species are shown in Figure 1. This approach, although morphological, is consistent with previous exploratory studies on wild edible fungi (Heleno *et al.* 2015).

Figure 1. *Hygrophoropsis aurantiaca* (A, B). *Hypomyces macrosporus* (C, D).

Proximate chemical analysis

Table 1 shows the proximate chemical analysis of the analysed fungi, the values are expressed as means and standard deviation.

Moisture

The moisture content in the analysed mushrooms had values of 87.835 ± 0.997 and 87.462 ± 1.923 g/100 g dry weight (percentage), for *Hygrophoropsis aurantiaca* and *Hypomyces macrosporus*, respectively. Heleno *et al.* (2009) reported 84.59 ± 1.27 g/100 g moisture in *Hygrophoropsis aurantiaca*, while previous studies found values higher than 80 % for other mushrooms such as *Agaricus bisporus* (J.E. Lange) Imbach, *Pleurotus ostreatus* (Jacq.) P. Kumm., *Russula flava* Romell, and *Suillus granulatus* (L.) Roussel (Wang *et al.* 2014, Jacinto-Azevedo *et al.* 2021). This property was not significantly different between the two mushrooms. Moisture content is high in mushrooms, since water is their most abundant constituent (Jacinto-Azevedo *et al.* 2021).

Tabla 1. Proximate composition of *Hygrophoropsis aurantiaca* and *Hypomyces macrosporus* collected in La Malinche National Park, Puebla, Mexico

Physicochemical characteristics	*Hygrophoropsis aurantiaca*	*Hypomyces macrosporus*
Moisture (%)	87.835 ± 0.997[a]	87.462 ± 1.923[a].
Fat (g/100 g)	26.659 ± 1.486[a]	14.700 ± 0.424[b]
Reducing sugar (%)	9.133 ± 1.280[b]	26.620 ± 2.941[a]
Total dietary fiber (g/100 g)	28.835 ± 0.850[b]	48.790 ± 1.277[a]
Ash (g/100 g)	10.275 ± 0.998[a]	7.562 ± 0.229[a]
Crude protein (g/100 g)	17.610 ± 0.607[a]	3.654 ± 0.225[b]

Values are the mean ± standard deviation. Means with the same letters within each column do not differ statistically, according to Tukey's test ($p \leq 0.05$).

Fat

The fat content measured in this study was 26.659 ± 1.486 g/100 g in *H. aurantiaca* and 14.700 ± 0.424 g/100 g in *H. macrosporus*. No significant differences were detected between the analysed species. Heleno *et al.* (2009) reported a much lower fat content for *H. aurantiaca* (2.20 ± 0.01 g/100 g). Likewise, Liu *et al.* (2016) reported fat contents ranging from 1.96 ± 0.07 to 7.87 ± 0.24 g/100 g in species belonging to the genera of *Boletus* and *Suillus.*

Crude fat content in mushrooms is usually low (Dimitrijevic *et al.* 2019). However, Jacinto-Azevedo *et al.* (2021) mentioned that some previous studies have shown that fat content in edible mushrooms varies from 1 to 15 % per 100 g in dried mushrooms, including all types of lipids, such as monoglycerides, sterols, phospholipids, saturated and unsaturated fatty acids.

Fatty acid composition studies indicate that linoleic (C18:2), oleic (C18:1), palmitic (C16:0) and stearic (C18:0) acids are commonly the most abundant fatty acids in many edible species (with linoleic acid often predominating). For example, in *Tricholoma matsutake* these fatty acids make up 2%, 9%, 27% and 58%, respectively, of the total fatty acid content (Liu *et al.* 2010), but comparable dominant profiles (high linoleic/oleic, moderate palmitic/stearic) have been observed across a variety of genera including *Suillus, Hygrophorus, Amanita, Russula, Boletus, Tricholoma, Fistulina* and *Cantharellus* (Ribeiro *et al.* 2009). Essential fatty acids in wild-grown species make them a good food source with high biological value for people's health (Sande *et al.* 2019).

Reducing sugar

We found a reducing sugar content of 9.135 ± 0.945 g/100 g in *H. aurantiaca* and 26.620 ± 1.310 g/100 g in *H. macrosporus.* The results showed a significant difference between species. Other authors reported lower reducing sugar content, 1.77 ± 0.30 g/100 g in *H. aurantiaca* and 7.81 ± 0.04 g/100 g in *Termitomyces heimii* (Heleno *et al.* 2009, Ao and Deb 2019). Reducing sugars are only a small part of the carbohydrate content in mushrooms; however, their determination is of interest because they can react as reducers with other molecules since they possess a free hemiacetal OH group (Beltrán Delgado *et al.* 2021). Sugars such as mannose, xylose and galactose can be found laterally linked to polysaccharides of the β-D-glucan type in mushrooms (Wasser 2002). The presence of β-glucan polysaccharides is important since they are responsible for immunomodulatory, anti-tumour and anti-inflammatory activities (Abreu *et al.* 2019, Zavadinack *et al.* 2021).

Total dietary fibre

Wild edible mushrooms are known to be rich in non-starch polysaccharides and dietary fibre (Ao and Deb 2009). In our study, the highest values of total dietary fibre were 28.835 ± 0.850 g/100 g in *H. aurantiaca* and 48.790 ± 1.277 g/100 g in *H. macrosporus*, significantly different in the two species. Jacinto-Azevedo *et al.* (2021) obtained a content range from 7.94 ± 0.03 % in *Agrocybe cylindracea* (DC.) Maire to 21.63 ± 0.03 % in *Morchella conica* Pers. Nile and Park (2014) reported 27 to 36 % total dietary fibre, 12 to 21 % insoluble dietary fibre, and 2 to 4 % soluble dietary fibre in twenty different wild mushroom species. Therefore, the concentrations found in this research are close to the intervals reported by several authors.

Ash

According to Ache *et al.* (2021), the ash content in edible mushrooms ranges from 7.74 to 14.10 g/100 g dry matter and comprise a source of essential minerals. Our results are similar. Ash content was not significantly different between the mushrooms that were studied for this project: 10.275 ± 0.998 g/100 g in *H. aurantiaca* and 7.562 ± 0.2229 g/100 g in *H. macrosporus.* While Heleno *et al.* (2009) reported 5.92 ± 0.06 g/100 g in *H. aurantiaca*, Jacinto-Azevedo *et al.* (2021) found ash level intervals around 4.90 g/100 g dw in *Cyttaria espinosae* and around 13.84 g/100 g dw in *Agaricus bisporus.*

Crude protein

In the present study, the protein content was 3.654 ± 0.225 g/100 g in *H. macrosporus* and 17.610 ± 0.607 g/100 g in *H. aurantiaca*, showing a statistically significant different between species. These values indicate that H. aurantiaca is a richer protein source than *H. macrosporus.* Heleno *et al.* (2009) reported a higher protein content (36.40 0.60 g/100 g) for *H. aurantiaca*, while Jacinto-Azevedo *et al.* (2021) found lower concentrations in other edible species such as *Boletus loyo, Pleurotus ostreatus, Xerocomellus chrysenteron* and *Morchella conica*. Such variation may result from

factors affecting the chemical composition of fungi, including developmental stage, part of the fruiting body analysed, and environmental conditions (González *et al.* 2020). Wild mushrooms are recognized as valuable sources of essential amino acids (leucine, valine, threonine, lysine, methionine and tryptophan) and non-essential amino acids (alanine, arginine, glycine, glutamic acid, aspartic acid, proline, and serine) (Mdachi *et al.* 2004, Heleno *et al.* 2009). These proteins contribute to human health because they exhibit bioactive properties such as anti-microbial, anti-inflammatory, anti-mitogenic, anti-tumour, and immunomodulatory activities (Prajapati *et al.* 2021).

The nutritional composition of wild mushrooms is influenced by both genetic factors and the physicochemical characteristics of the substrates where they develop, which affect nutrient accumulation and biochemical composition (Khumlianlal *et al.* 2022). In this study, *Hygrophoropsis aurantiaca* exhibited the highest protein and lipid contents, while *Hypomyces macrosporus* showed the highest total dietary fibre. These patterns may reflect species-specific metabolic capacities and differences in substrate utilization. Similar trends have been reported for other saprophytic species growing on lignocellulosic residues (Rathore *et al.* 2017; Ruan-Soto *et al.* 2017). Overall, the results confirm that both mushrooms are nutritionally valuable sources of proteins, fibre, and carbohydrates, supporting their potential inclusion in local diets and valorisation as forest food resources.

Fatty acid profile

Reports mention that mushroom lipids consist mainly of mono- and poly-unsaturated fatty acids (Sande *et al.* 2019). Table 2 presents fatty acid profiles of *H. aurantiaca* and *H. macrosporus* mushrooms collected in the National Park La Malinche.

Our findings show that the studied mushrooms have diverse fatty acid compositions. In *H. aurantiaca*, the most abundant fatty acids found were palmitic acid, eicosadienoic acid, and arachidonic acid, followed by γ-linolenic acid, linoleic acid, and oleic acid. In contrast, *H. macrosporus* presented stearic acid, cis-10-heptadecenoic acid, arachidonic acid, while oleic acid, γ-linolenic acid, margaric acid and linoleic acid were found in lesser concentrations. Even though the dominance of oleic, palmitic, and linoleic acids among mushrooms is known, these fatty acids are not dominant constituents in the analysed mushrooms. Other fatty acids were found in this study that are like those found by previous research. For example, Heleno *et al.* (2009) reported that linoleic acid is the most abundant, followed by oleic and palmitic acid in different wild edible mushrooms, including *H. aurantiaca*. At the same time, fatty acids such as eicosadienoic acid, cis-10-heptadecenoic acid, and arachidonic acids were identified in other mushrooms: *Boletus edulis* Bull., *Suillus luteus* (L.) Roussel, and *Suillus bellinii* (Inzenga) Watling (Ribeiro *et al.* 2009).

The lipid fraction of both *H. aurantiaca* and *H. macrosporus* was dominated by unsaturated fatty acids, particularly linoleic and oleic acids, which are known to play key physiological roles in humans. Unsaturated fatty acids contribute to maintaining cell membrane fluidity and regulating plasma lipid levels. Previous studies have demonstrated that diets rich in linoleic acid can reduce serum cholesterol and exert anti-inflammatory effects, thereby lowering the risk of cardiovascular diseases, hypertension, and arthritis (Bengu 2020, Gupta *et al.* 2019). Therefore, the high proportion of unsaturated fatty acids found in these mushrooms suggests a potential nutritional benefit when included in the human diet.

Although the studied mushrooms provide valuable fatty acids, their composition shows both nutritional benefits and limitations. On the one hand, their relatively high levels of omega-6 polyunsaturated fatty acids, such as linoleic and arachidonic acids, contribute essential components that support immune response, skin health, and normal cellular function, while also providing an alternative dietary source of polyunsaturated fats (Sande *et al.* 2019). On the other hand, the same profile reveals a comparatively high proportion of saturated fatty acids and a marked deficiency of monounsaturated and omega-3 fatty acids, which are frequently associated with cardioprotective and anti-inflammatory effects (Tan *et al.* 2024). Thus, while these species may enrich the diet with essential omega-6 fatty acids, their poor omega-3 content and limited monounsaturated fats suggest that their consumption should be complemented with other foods rich in omega-3 to maintain a balanced and healthy fatty acid intake.

Table 2. Fatty acid compositions of *Hygrophoropsis aurantiaca* and *Hypomyces macrosporus* collected in La Malinche National Park, Puebla, Mexico

Side Chain Carbon Sum	Fatty acid	Retention Time (min)	Concentration (µg/mL)	
			Hygrophoropsis aurantiaca	*Hypomyces Macrosporus*
C4:0	Butyric acid	3.558	1.45	1.48
C6:0	Caproic acid	7.425	0.75	0.55
C8:0	Caprylic acid	12.709	0.52	0.52
C10:0	Capric acid	19.898	0.48	0.48
C11:0	Undecanoic acid	22.508	0.67	0.67
C12:0	Lauric acid	25.439	0.62	0.61
C15:0	Pentadecanoic acid	32.184	0.72	1.49
C16:0	Palmitic acid	34.521	8.09	0.58
C17:1	cis-10-Heptadecenoic acid	35.997	1.27	10.78
C17:0	Margaric acid	36.543	0.97	2.45
C18:3	γ-Linolenic acid	37.34	1.77	2.96
C18:2	Linoleic acid	37.564	1.67	2.2
C18:1	Oleic acid	37.623	1.43	3.66
C18:0	Stearic acid	37.751	0.68	64.5
C19:0	Nonadecanoic acid	N.D.	N.D.	N.D.
C20:4	Arachidonic acid	40.87	2.40	4.51
C20:2	Eicosadienoic acid	41.292	2.43	N.D.
C20:1	Eicosenoic acid	N.D.	N.D.	N.D.
C21:0	Heneicosanoic acid	43.346	1.53	1.54
C22:0	Behenic acid	45.881	1.31	1.31
C23:0	Tricosanoic acid	48.24	1.87	1.89
C24:0	Lignoseric acid	N.D.	N.D.	N.D.

Chemical analysis of mycochemical metabolites and antioxidant activity

The results of the chemical analysis of mycochemical metabolites are presented in Table 3.

Total phenolic compounds

Total phenolic compounds (TPC), expressed as mg of gallic acid equivalents (GAE) per g of dried extract, ranged between 1.674 ± 0.013 and 3.265 ± 0.184 mg/GAE g in *Hypomyces macrosporus* in maceration and *Hygrophoropsis aurantiaca* in ultrasound, respectively. The results of the phenol contents were higher than those reported by Vaz *et al.* (2011) who found 3.52 ± 0.88 mg/Kg dw of total phenolic acids. However, other authors mention higher values than those found in our research.

In *H. aurantiaca*, concentrations of 1.789 ± 0.02 mg/GAE g were reported in ethanolic extract by Margret *et al.* (2022). Likewise, 7.90 ± 0.29 mg/GAE g were obtained by Heleno *et al.* (2010), while Nowacka *et al.* (2015) found 30.80 ± 1.27 mg/GAE g dried extract. Regarding *H. macrosporus*, there are only a few studies describing its ethanolic content. For this reason, *Hypomyces lactiflorum*, a closely related species, was chosen as a point of comparison since broader studies have been conducted for this taxon. The two species are similar and even share the same common name in some regions (Pérez-Moreno *et al.* 2008). Yahia *et al.* (2017) found concentrations of 35.44 ± 0.63 mg GAE/100 g dw and Espejel-Sánchez *et al.* (2021) obtained 2.98 ± 0.04 mg/GAE g of *H. lactifluorum* extract. These values are similar to those found in the present study.

Table 3. Chemical analysis of mycochemical metabolites and antioxidant activity of *Hygrophoropsis aurantiaca* and *Hypomyces macrosporus* collected in La Malinche National Park, Puebla, Mexico

	Hygrophoropsis aurantiaca		***Hypomyces Macrosporus***	
	Maceration	**Ultrasound**	**Maceration**	**Ultrasound**
Total Phenolic Compounds (mg/GAE g extract)	2.876 ± 0.167^{b}	3.265 ± 0.184^{a}	1.674 ± 0.013^{d}	2.192 ± 0.004^{c}
Total Flavonoids (mg/QE g extract)	0.137 ± 0.001^{d}	0.351 ± 0.001^{c}	0.608 ± 0.051^{b}	0.904 ± 0.014^{a}
Total Triterpenes (mg/UAE g extract)	21.423 ± 7.805^{ab}	28.591 ± 7.054^{a}	6.386 ± 1.923^{c}	9.841 ± 0.175^{bc}
DPPH-IC_{50} (mg/mL)	0.058 ± 0.002^{b}	0.053 ± 0.002^{a}	0.071 ± 0.001^{d}	0.064 ± 0.001^{c}
ABTS-IC_{50} (mg/mL)	0.057 ± 0.002^{b}	0.047 ± 0.003^{a}	0.065 ± 0.002^{d}	0.060 ± 0.001^{cd}

Values are the mean ± standard deviation. Means with the same letters within each column do not differ statistically, according to Tukey's test ($p \leq 0.05$).

Total flavonoids

The total flavonoid (TF) content ranged between 0.137 ± 0.001 mg/QE g in *Hygrophoropsis aurantiaca* obtained by maceration and 0.904 ± 0.014 mg/QE g in *Hypomyces macrosporus* using the ultrasonic technique, respectively. The concentrations in this study are lower than those reported by Margret *et al.* (2022), who found a concentration of 1.789 ± 0.02 mg QE/g in a sample of *H. aurantiaca*.

However, some authors reported different concentration ranges of flavonoids in some other wild edible mushrooms. López-Vázquez *et al.* (2017) researched mushrooms collected in regions of Hidalgo, Mexico; they found a range of 0.25 ± 0.00 to 0.92 ± 0.02 mg/QE g in *Lactarius indigo* and *Boletus edulis*, respectively. Woldegiorgis *et al.* (2014) found concentration ranged from 0.17 ± 0.01 to 1.97 ± 0.06 mg catechin equivalents (CE) per g of extract in species of wild edible mushrooms in Ethiopia, *Termitomyces microcarpus* and *Agaricus campestris* among others. Pereira *et al.* (2012) found ranges from 1.78 ± 0.08 to 33.00 ± 4.98 mg of catechin equivalents per g of extract in species of wild edible mushrooms in northeastern Portugal. This shows that the concentrations of flavonoids found in our research is within the range found in other studies for this type of metabolites.

Total triterpenes

The concentrations of total triterpenes (TT) detected in our study ranged from 6.386 ± 1.923 to 28.599 ± 6.805 mg/UAE g. The lowest concentrations were found in *Hypomyces macrosporus* in maceration, while *Hygrophoropsis aurantiaca* in ultrasonic showed higher concentrations of total triterpenes. The concentrations in this study are within the range found by other authors. For example, Hayati *et al.* (2021) investigated the phytochemical properties and antioxidant activity of wild and cultivated *Ganoderma lucidum*; they found that wild *G. lucidum* has higher terpenoid content than cultivated *G. lucidum*, with a concentration of 23.72 ± 2.17 mg/UAE g dw. This indicates that there are adequate environmental conditions for better production of secondary metabolites in wild areas. Mešić *et al.* (2020) analysed Croatian wild mushroom samples and found concentrations of 16 ± 1 to 61 ± 6 mg/UAE g dw in species such as *Laetiporus sulphureus* and *Polyporus multipedata*. The highest concentration of these natural products in fungi are sesquiterpenes, diterpenoids and triterpenoids in basidiomycetes (Wang *et al.* 2021).

Antioxidant activity

Table 3 presents the IC50 values (mg/mL) obtained from the radical-scavenging activity assays. *H. aurantiaca* presented higher antioxidants effects than *H. macrosporus* in both extraction methods. With the ultrasonic technique, *H. aurantiaca* showed the highest IC_{50} value, 0.053 ± 0.002 mg/mL and 0.047 ± 0.003 mg/mL, in DPPH and ABTS, respectively, at 0.1 mg/mL. The antioxidant properties observed are consistent with the phenolic and triterpenoid levels quantified in this study and with those commonly reported in other wild mushroom species. *H. aurantiaca* presented higher total phenolic content and stronger antioxidant activity, particularly in ultrasonic extracts, which exhibited the lowest IC_{50} values.

Heleno *et al.* (2010) found IC_{50} values (mg/mL) in *H. aurantiaca* of 1.20 ± 0.22 mg/mL. In *H. lactifluorum*, Espejel-Sánchez *et al.* (2021) reported antioxidant capacity values of 5.78 ± 0.03 µmol ET/g on a dry basis in the ABTS assays. Moreover, Woldegiorgis *et al.* (2014) repoted IC_{50} values ranging from 1.4 to 11.4 mg/mL in species like *A. campestris* and *L. sulphureus*, whereas Pereira *et al.* (2012) found values between 0.86 ± 0.02 and 20.02 ± 1.27 mg/mL in wild edible mushrooms such as *Suillus variegatus* and *Hydnum chrysodon*.

According to the obtained results, the antioxidant components which are present in mushrooms that were analysed with both the maceration and ultrasound extraction methods can entrap free radicals DPPH and ABTS and inhibit oxidative changes that are responsible for different types of diseases.

CONCLUSIONS

Wild edible mushrooms, *Hygrophoropsis aurantiaca* and *Hypomyces macrosporus*, collected in La Malinche National Park, were found to contain macronutrients (proteins, sugars, fibre, and lipids) and mycochemical components (polyphenolic compounds, flavonoids, and triterpenes). These findings provide evidence regarding their nutritional and mycochemical composition and highlight their importance as a supplementary food source for rural populations, where access to diverse protein and functional foods is often limited. Their content of macronutrients is

relevant for human nutrition, while the presence of secondary metabolites indicates possible functional properties. However, additional research is required before establishing specific health benefits or recommending them as part of dietary strategies.

ACKNOWLEDGEMENTS

The author Diego Ibarra-Cantún acknowledges the financial support received from the Secretaría de Ciencia, Humanidades, Tecnología e Innovación (SECIHTI) for his postdoctoral fellowship.

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