

Article

Insights into the Chemistry and Functional Properties of Edible Mushrooms Cropped in the Northeastern Highlands of Puebla, Mexico

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Featured Application: Based on our results, the studied edible resources may be included in the local eat-well plate through diets to ameliorate some disorders of the metabolic syndrome.

Abstract: Herein, we present an integrative investigation of the nutritional and nutraceutical potential of *Lactarius indigo*, *Clitocybe nuda*, *Clitocybe subclavipes*, *Russula delica*, *Russula brevipes*, *Clitocybe squamulosa*, and *Amanita jacksonii*, which are edible mushrooms consumed in the northeastern highlands of Puebla, Mexico. The content of protein oscillated from 4.8 to 10.9 g 100 g⁻¹ fresh weight (FW) whereas that of fiber ranged from 8.8 to 19.7 g 100 g⁻¹ FW. The edible species presented low amounts of fat (1.5–3.4 g 100 g⁻¹ FW) and reducing sugars (0.8–2.9 g 100 g⁻¹ FW), whereas the content of vitamin C oscillated from 6.5 to 84.8 mg 100 g⁻¹ dry weight (DW). In addition, four vitamins of B complex (thiamine, riboflavin, vitamin B₆, and folate) were determined in different concentrations. A high abundance of potassium (92.3–294.3 mg 100 g⁻¹ DW), calcium (139.1–446.9 mg 100 g⁻¹ DW), and magnesium (81.3–339.1 mg 100 g⁻¹ DW) was determined in most of the edible mushrooms, as well as detectable levels of *p*-hydroxybenzoic acid (2.2–48.7 mg 100 g⁻¹ DW), protocatechuic acid (0.5–50.8 mg 100 g⁻¹ DW), oleic acid (14.2–98.3 mg 100 g⁻¹ DW), linoleic acid (748–1549.6 mg 100 g⁻¹ DW), and linolenic acid (from 9.1 to 83.6 mg 100 g⁻¹ DW). The total phenol content and antioxidant capacity significantly ($p < 0.05$) varied among the studied species, and their capacity to inhibit enzymes involved in glucose, lipid, and polyamine metabolism. Nevertheless, the hydroalcoholic extracts from *A. jacksonii* and *L. indigo* efficiently inhibited alpha-glucosidase and ornithine decarboxylase (IC₅₀ < 50 µg mL⁻¹), respectively. The evaluation of the same extracts on microorganisms associated with the gastrointestinal tract showed negligible toxicity on probiotics (MIC > 500 µg mL⁻¹) and moderate toxicity against pathogenic bacteria (MIC < 400 µg mL⁻¹). Based on the studied parameters, principal component analysis and orthogonal partial least squares discriminant analysis clustered these edible mushrooms into two main groups with similar biological or chemical properties.

Keywords: edible mushrooms; northeastern highlands of Puebla; nutrients; nutraceuticals; phenols content; antioxidant capacity; antimicrobial activity; enzyme inhibitors



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1. Introduction

Wild edible mushrooms represent an inherent part of Mexico's biological and cultural diversity [1]. Knowledge of the edible and medicinal uses of these species comes from ethnic legacy inherited from one generation to another. Despite these foods being overlooked for nutrients and nutraceuticals, they can provide multiple benefits to native consumers [1]. Edible fungi store noteworthy levels of fiber and protein, with low levels of reducing sugars and fat. These features make edible mushrooms a good ingredient for balanced diets against obesity and related comorbidities [1]. In addition, the local market of wild mushrooms earns significant income for marginal communities worldwide [1]. The formal consumption of these sources has always been controversial because of their concealed toxicity to humans. However, it has been calculated that 2200 mushrooms are routinely consumed raw or pre-treated in 99 different countries worldwide before being eaten [1]. Nevertheless, highly marginalized places from third-world countries would stock unnoticed edible species. Approximately 2006 species are considered safe if consumed raw, and 183 species require a specific pre-treatment to be innocuous [1]. The regulation of the wild edible mushroom market has been delayed in most countries for decades. That is why elucidation of their biological activity and basic chemistry would help consumers to know about their potential benefits and promote their revalorization and conservation.

The nutraceutical activity of edible mushrooms on human health is well known [2]. These organisms contain polyphenols with potent antioxidant, hypoglycemic, antitumor, and immune-stimulating properties demonstrated in biochemical and murine models [2]. They accumulate non-assimilable glucans and terpenoids with proven antiviral, hypcholesterolemic, antibacterial, and immune-modulating activities [2]. It has been proposed that 371 species of wild mushrooms are consumed in Mexico [3,4]. This number emerges from records obtained in the provinces of Puebla, Oaxaca, and Mexico state. Nevertheless, almost half of the Mexican territory contains unexplored populations surrounded by cloud forests, oak forests, high evergreen forests, and evergreen lowland forests, which may increase the number of edible fungi. The consumption of macromycetes is a millenary practice in several municipalities of the northern and northeastern highlands of Puebla (NHP), Mexico. This geographical zone harbors a great diversity of edible species eaten for centuries because of Totonac, Nahuatl, Otomi, and Tepehua ancestries. Many of these species are still being sold during the rainy period in local markets, also known as "tianguis" (Figure 1).

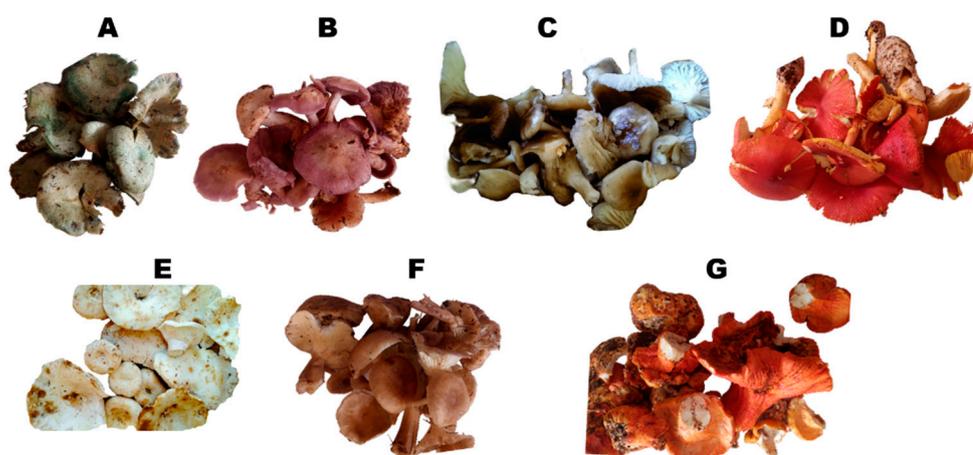


Figure 1. Some edible mushrooms sold in local markets of the northeastern highlands of Puebla, Mexico. (A) *Lactarius indigo*. (B) *Clitocybe nuda*. (C) *Clitocybe subclavipes*. (D) *Russula delica*. (E) *Russula brevipes*. (F) *Clitocybe squamulosa*. (G) *Amanita jacksonii*.

To the best of our knowledge, there are no available data on the basic nutritional content and nutraceutical activity of edible mushrooms eaten in the NHP so far. Nevertheless,

previous studies have revealed that *Russula* spp. and *Amanita* spp. contain substantial levels of oxalic acid, quinic acid, malic acid, citric acid, fumaric acid, alpha-tocopherol, and fatty acids [5]. In the same context, the presence of phenolic acids such as protocatechuic, *p*-hydroxybenzoic, *p*-coumaric, and cinnamic acids was endorsed in ethanolic extracts, which additionally showed evident antioxidant, antibacterial, and antifungal activities [5]. Due to the latter points, this investigation aimed to generate data on the nutritional and nutraceutical potential of seven edible mushrooms commercialized and eaten in the NHP as a continuation of the chemical and biological characterization of traditional foods from the highlands of Puebla, Mexico.

2. Materials and Methods

2.1. Chemicals

Solvents for chromatography were from J.T. Baker[®]. Rezasurin, procyanidin B2, abietic acid, alpha-glucosidase (AG), alpha-amylase (AA), 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase; catalytic domain kit), standard mixture of elements (zinc, calcium, magnesium, sodium, iron, and potassium), *p*-hydroxybenzoic acid, *p*-coumaric acid, protocatechuic acid, *trans*-cinnamic acid, oleic acid, linoleic acid, linolenic acid, 2,2'-azo-bis (2-amidinopropane) dihydrochloride (AAPH), resazurin, and fluorescein were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Pancreatic lipase (PL) was from Affymetrix/USB and human ornithine decarboxylase (ODC) was obtained from MyBiosource (MBS967514).

2.2. Plant Material

The edible mushrooms included in this investigation were purchased in the “tianguis” (local markets) of Atempan and Zaragoza, Puebla, located at 19°50'17" N 97°27'23" O (2049 masl) and 19°46'15" N 97°33'18" O (2300 masl), respectively. Fruiting bodies from seven different fungal morphologies (five kilograms each) were transported to the laboratory in paper bags and superficially washed with sterile distilled water and dried with sterile wipes for subsequent analysis and/or lyophilization. For this purpose, a FreeZone 2.5 L – 84C Benchtop Freeze Dryer—Labconco (Kansas, MO, USA) was used. Mushroom identification was obtained by the mycologist Fermin Tavares at the medicinal mushroom collection of the Northern Highlands Association–Mexico through basic dichotomous keys [6–8]. The molecular identity of the macromycetes was endorsed by sequencing the internal transcribed spacer (ITS) of the 18S ribosomal gene as described by Coyotl-Pérez et al. [9]. Amplicons were sequenced employing the commercial services of Macrogen Inc. (Seoul, Republic of Korea) and the LANBAMA from IPICYT-México. The resulting sequences were validated by BLASTn software version 2.2.24 and deposited in the nucleotide database of the National Center for Biotechnology Information (NCBI). The local name and scientific name supported by voucher code, as well as the NCBI accessions for each fungal isolate, are described in Table 1.

Table 1. Some edible mushrooms eaten in the northeastern highlands of Puebla, Mexico.

Local Name	Scientific Name	Voucher	¹ ITS Accession
Tzenzon	<i>Infundibulicybe squamulosa</i>	NM-0981	OR656586
Tecomate	<i>Amanita jacksonii</i>	NM-0982	OR663657
Ilimorado	<i>Lepista nuda</i>	NM-0983	OR631737
Borrego	<i>Russula delica</i>	NM-0984	OR659898
Xochinacatl	<i>Russula brevipes</i>	NM-0985	OR659899
Quesquec	<i>Lactarius indigo</i>	NM-0986	OR631739
Talistac	<i>Clitocybe subclavipes</i>	NM-0986	OR635626

¹ Partial sequence of the Internal Transcribed Spacer of the small-subunit ribosomal RNA gene (rRNA) deposited at the NCBI nucleotide data base.

2.3. Basic Nutrients Content

The amounts of protein, fat, fiber, and reducing sugars were determined by the methods 920.23, 920.39, 962.09, and 945.66, respectively [10]. These parameters were estimated on the basis of fresh material. The content of vitamin C, folate, thiamine, total vitamin B₆, and riboflavin were estimated by the methods 967.21/90, 944.12, 942.23, 961.15, and 970.65, respectively [10]. These parameters were calculated using lyophilized plant material [10]. The concentration of selected mineral elements (zinc, calcium, magnesium, sodium, iron, and potassium) was obtained by atomic absorption spectroscopy using an A3F flame atomic absorption spectrometer and the conditions previously reported by Pacheco-Hernández et al. [11]. All assays were performed in quintuplicate two times ($n = 10$).

2.4. Molecules with Nutraceutical Activity and Antioxidant Potential

These procedures were basically performed in accordance with a previous study performed by Pacheco-Hernández et al. [11] with slight modifications. The contents of *p*-hydroxybenzoic acid, *p*-coumaric acid, protocatechuic acid, and *trans*-cinnamic acid were estimated from hydroalcoholic extracts. These extracts were prepared from 100 g of lyophilized material homogenized in High Speed CONSFly Version FSH-2A (St. Louis, MO, USA) using 200 mL 80% EtOH. The extracts were maintained at 4 °C for 48 h in the dark and filtered with Whatman No. 1 to be reduced to dryness using a rotary evaporator and immediate lyophilization. The extract was resuspended in pure methanol for HPLC analysis using the conditions reported by Vaz et al. [12]. Calibration curves (1–200 $\mu\text{g mL}^{-1}$; $R^2 = 0.99$) were designed with authentic standards of *p*-hydroxybenzoic acid, *p*-coumaric acid, protocatechuic acid, and *trans*-cinnamic acid using procyanidin B2 as internal standard to normalize chromatograms (Figure S1). The presence of the three major fatty acids (linoleic acid, linolenic acid, and oleic acid) was determined from lyophilized material (1 g) extracted with petroleum ether (20 mL) and subjected to ultrasonication (VEVOR Ultrasonic Bath) at 40 kHz ultrasonic frequency and with 70 W transducers for 30 min. The analysis was carried out by GC-MS according to the same authors [11,13]. For quantification, calibration curves (1–100 $\mu\text{g mL}^{-1}$; $R^2 = 0.98$) were designed with authentic standards of linoleic acid, linolenic acid, and oleic acid using abietic acid as the internal standard to normalize chromatograms (Figure S2). Total phenolic content and antioxidant capacity were estimated in accordance with Awika et al. [14], using the modified conditions of Pacheco-Hernández et al. [11]. The results were expressed as gallic acid equivalents (GAE mg g^{-1} DW) and Trolox equivalent antioxidant capacity (TEAC $\mu\text{M g}^{-1}$ DW), respectively. The results were expressed in $\text{mg } 100 \text{ g}^{-1}$ DW, and all assays were replicated 15 times ($n = 15$).

2.5. Inhibitory Activity on Key Enzymes with Therapeutic Potential

The enzymatic assays were performed with the five enzymes described in Section 2.1. The reactions were performed with the same lyophilized hydroalcoholic extracts (80% EtOH) described in Section 2.4. The dose-response curves (10–300 $\mu\text{g mL}^{-1}$), substrates, and units used in each enzymatic assay were the same as those reported in standardized tests reported from previous studies [11]. The IC_{50} was determined by linear regression, considering the maximum specific activity of AG (0.034 mM min^{-1}), AA (0.063 mM min^{-1}), HMG-CoA reductase (0.021 mM min^{-1}), PL (0.085 mM min^{-1}), and ODC (0.046 mM min^{-1}). The tests were carried out 25 times ($n = 25$) for each enzyme.

2.6. In Vitro Antimicrobial Activity

The broth microdilution method enriched with resazurin was conducted to determine the minimum inhibitory concentration (MIC) [9]. For this purpose, dose-response curves (10–700 $\mu\text{g mL}^{-1}$) using each lyophilized hydroalcoholic extract were considered. The assayed species were *Helicobacter pylori* ATCC53504, *Escherichia coli* ATCC25922, *Enterococcus faecalis* ATCC 29212, *Saccharomyces cerevisiae* INVSc1, *Lactobacillus reuteri* ATCC55730, *Lactobacillus acidophilus* ATCC4356, and *Bifidobacterium bifidum* ATCC 29521. Each curve point was assayed 25 times ($n = 25$).

2.7. Statistical Analysis

Analysis of variance coupled with Tukey's test was performed to determine statistically significant differences in the amounts of bioactive molecules using GraphPad Prism 8.0 software. For enzymatic tests, IC_{50} was stated by linear regression using the same software and the maximum specific activity described in Section 2.5. To understand biological properties convergences among the studied edible mushrooms, principal component analysis (PCA) using four components and orthogonal partial least squares discriminant analysis (OPLS-DA) using one predictive component and four orthogonal components were performed. These statistical analyses were carried out with SIMCA-Sartorius version 18.

3. Results and Discussion

3.1. Nutritional Content

The proximate analysis performed on the seven edible mushrooms revealed that all studied samples contained nutrients potentially usable to cover the basic dietary reference intakes of Mexican older adults [15]. According to our results, *A. jacksonii* was the best source of protein compared with the other species (Figure 2a). The levels of protein oscillated from 4.8 to 10.9 g 100 g⁻¹ FW, which were comparable to those found in other edible mushrooms such as *Polyporus tenuiculus*, *P. dictyopus*, *Laetiporus sulphureus*, *Tremella fuciformis*, *Auricularia auricula-judae*, *Tremella fuciformis*, and *Lentinula edodes* (shiitake), which are considered alternative sources of protein [16,17]. The amounts of fiber ranged from 8.8 to 19.7 g 100 g⁻¹ FW, which was substantially higher than those found in *Grifola frondosa*, *Cantharellus cibarius*, *Boletus edulis*, and *Agaricus bisporus* (Figure 2b) [17]. Crude fiber was particularly abundant in *C. subclavipes*, *C. squamulose*, and *R. delica* (Figure 2b). As a general finding, all studied mushrooms had low levels of crude fat (1.5–3.4 g 100 g⁻¹ FW) (Figure 2c). These results were coincident with the fat levels reported in other edible sources such as *Tuber melanosporum*, *Hericium erinaceus*, *Grifola frondosa*, and *Cantharellus cibarius* [17]. Similarly, the levels of reducing sugars were low (1–3 g 100 g⁻¹ FW) as reported for most edible mushrooms consumed worldwide [16,17]. Interestingly, *C. subclavipes*, *R. delica*, and *R. brevipes* presented no more than 3 g 100 g⁻¹ FW reducing sugars (Figure 2d). These results strongly suggest that edible mushrooms can be safely administered to diabetics and persons with diseases associated with metabolic syndrome. Previous experimentation using in vitro and in vivo models suggested that fresh edible mushrooms and their extracts have therapeutic properties in human health as they possess many properties such as anti-obesity, cardioprotective, and anti-diabetic effects [18].

The fatty acid profile revealed that *C. squamulosa* contained approximately 1.5 g 100 g⁻¹ DW linoleic acid, whereas *C. subclavipes* and *L. indigo* accumulated around 1.5 g 100 g⁻¹ DW linoleic acid (Figure 3a). The levels of this unsaturated fatty acid in *C. nuda*, *R. delica*, *R. brevipes*, and *A. jacksonii* did not surpass 1.5 g 100 g⁻¹ DW. Linoleic acid is one of the most abundant fatty acids in several edible mushrooms [19]. The amounts of this nutraceutical in the seven edible mushrooms consumed in the NHP was higher than those determined in *L. eddoes* and *Laetiporus sulphureus* as well as comparable to those determined in *P. ostreatus*, *A. bisporus*, and *A. campestris* harvested in Ethiopia [19]. Linolenic acid has shown cardiovascular-protective, anti-cancer, neuro-protective, anti-osteoporotic, anti-inflammatory, and antioxidant effects [20]. In addition, this fatty acid is involved in the biosynthesis of longer-chain omega-3 fatty acids, eicosapentaenoic acid, and docosahexaenoic acid, which shows neuroprotective activity [20]. The levels of linolenic acid were below 100 mg 100 g⁻¹ DW in all of the edible mushrooms studied (Figure 3b). *R. brevipes*, *C. squamulose*, and *Clitocybe nuda* contained more levels of this unsaturated fatty acid than the other mushrooms analyzed. These levels were higher than those reported in *P. ostreatus*, *L. eddoes*, *A. bisporus*, *A. campestris*, *Termitomyces microcarpus*, and *Tabernaemontana letestui* harvested in Ethiopia [20]. The levels of oleic acid were similar to those observed for linolenic acid (Figure 3c). Nevertheless, *L. indigo*, *C. nuda*, and *R. delica* contained more levels than *C. subclavipes*, *R. brevipes*, *C. squamulose*, and *A. jacksonii*. These concentrations

were higher than those found in *L. eddoes* and *A. bispours* but lower than those determined in *T. microcarpus*, *T. letestui*, and *P. ostreatus* [19].

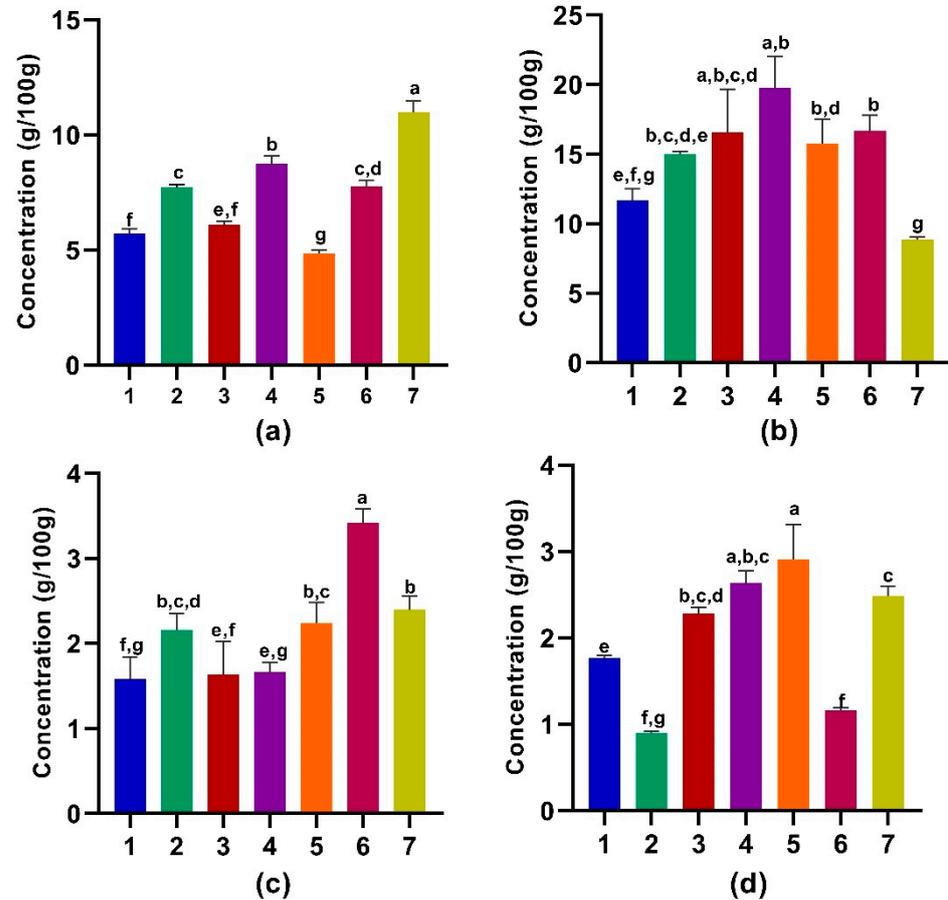


Figure 2. Contents of protein (a), fiber (b), fat (c), and reducing sugars (d) in seven edible mushrooms consumed in the northeastern highlands of Puebla Mexico. 1, *Lactarius indigo*. 2, *Clitocybe nuda*. 3, *Clitocybe subclavipes*. 4, *Russula delica*. 5, *Russula brevipes*. 6, *Clitocybe squamulosa*. 7, *Amanita jacksonii*. Different letters indicate means ($n = 10$) with statistically significant differences by ANOVA-Tukey ($p < 0.05$). Raw data can be consulted in Table S1. The concentrations are presented in fresh weight.

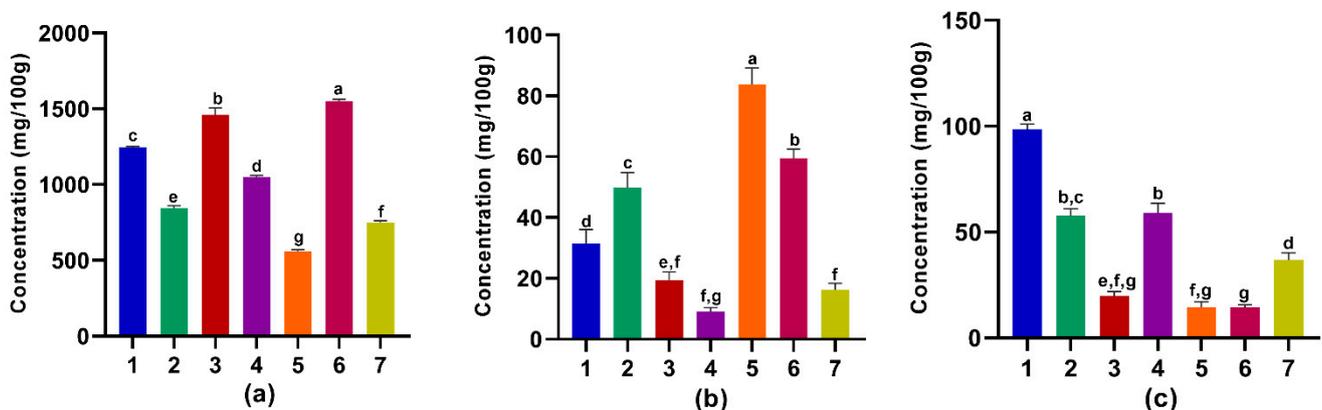


Figure 3. Contents of linoleic acid (a), linolenic acid (b), and oleic acid (c) in seven edible mushrooms consumed in the northeastern highlands of Puebla Mexico. 1, *Lactarius indigo*. 2, *Clitocybe nuda*. 3, *Clitocybe subclavipes*. 4, *Russula delica*. 5, *Russula brevipes*. 6, *Clitocybe squamulosa*. 7, *Amanita jacksonii*. Different letters indicate means ($n = 10$) with statistically significant differences by ANOVA-Tukey ($p < 0.05$). Raw data are presented in Table S5. The concentrations are presented in dry weight.

3.2. Selected Vitamin Content

Our results revealed that the seven edible mushrooms contained moderate amounts of ascorbic acid (vitamin C) (Figure 4a). Nevertheless, *R. brevipes* showed the highest levels ($>80 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$), whereas the lowest levels were found in *L. indigo* and *C. nuda* ($<10 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$). These results suggest that the studied mushrooms are an overlooked source of vitamin C and possess higher levels than those reported in common edible sources such as *Agaricus bisporus*, *Lentinus edodes*, and *Pleurotus ostreatus* [21]. The content of thiamine (vitamin B₁) was abundant in *C. subclavipes* ($>200 \text{ } \mu\text{g } 100 \text{ g}^{-1} \text{ DW}$), *L. indigo* ($>100 \text{ } \mu\text{g } 100 \text{ g}^{-1} \text{ DW}$), and *C. nuda* ($>100 \text{ } \mu\text{g } 100 \text{ g}^{-1} \text{ DW}$) (Figure 4b). The other species contained less than $90 \text{ } \mu\text{g } 100 \text{ g}^{-1} \text{ DW}$ thiamine. Interestingly, these levels surpassed those described for *A. bisporus*, *L. edodes*, and *P. ostreatus* [21]. The levels of riboflavin were remarkably higher in *L. indigo* ($>300 \text{ } \mu\text{g } 100 \text{ g}^{-1} \text{ DW}$) and *C. nuda* ($>400 \text{ } \mu\text{g } 100 \text{ g}^{-1} \text{ DW}$) in comparison with those observed in the other five species, which oscillated from 30 to $100 \text{ } \mu\text{g } 100 \text{ g}^{-1} \text{ DW}$ (Figure 4c). These levels were comparable to those already reported for *A. bisporus*, *L. edodes*, and *P. ostreatus*, which are considered common edible mushrooms consumed worldwide [21]. The content of bioactive forms of vitamin B₆ in edible mushrooms is still a big issue to be addressed, since little information is available in the current scientific literature. However, the levels of this vitamin in *Flammulina velutipes*, *Grifola frondosa*, *A. bisporus*, and *P. ostreatus* oscillate from 20 to $100 \text{ } \mu\text{g } 100 \text{ g}^{-1} \text{ DW}$ [22]. Our results suggest that *C. nuda* contains almost fourfold more levels of vitamin B₆ ($>400 \text{ } \mu\text{g } 100 \text{ g}^{-1} \text{ DW}$) than the latter selected mushrooms frequently eaten in North America (Figure 4d). *L. indigo* and *C. subclavipes* contained $\sim 200 \text{ } \mu\text{g } 100 \text{ g}^{-1} \text{ DW}$ vitamin B₆, whereas the other four species showed levels below $100 \text{ } \mu\text{g } 100 \text{ g}^{-1} \text{ DW}$ vitamin B₆ (Figure 4d). The folate content was higher in *R. brevipes* ($\sim 100 \text{ } \mu\text{g } 100 \text{ g}^{-1} \text{ DW}$) than in the other mushrooms studied (Figure 4e). *L. indigo* and *C. nuda* showed levels over $50 \text{ } \mu\text{g } 100 \text{ g}^{-1} \text{ DW}$ folate, whereas the other four species presented below $50 \text{ } \mu\text{g } 100 \text{ g}^{-1} \text{ DW}$ folate. The content of this vitamin was higher than that reported for *F. velutipes*, *G. frondosa*, and *A. bisporus*, but lower than that described for *L. edodes*, and *P. ostreatus* [21,22].

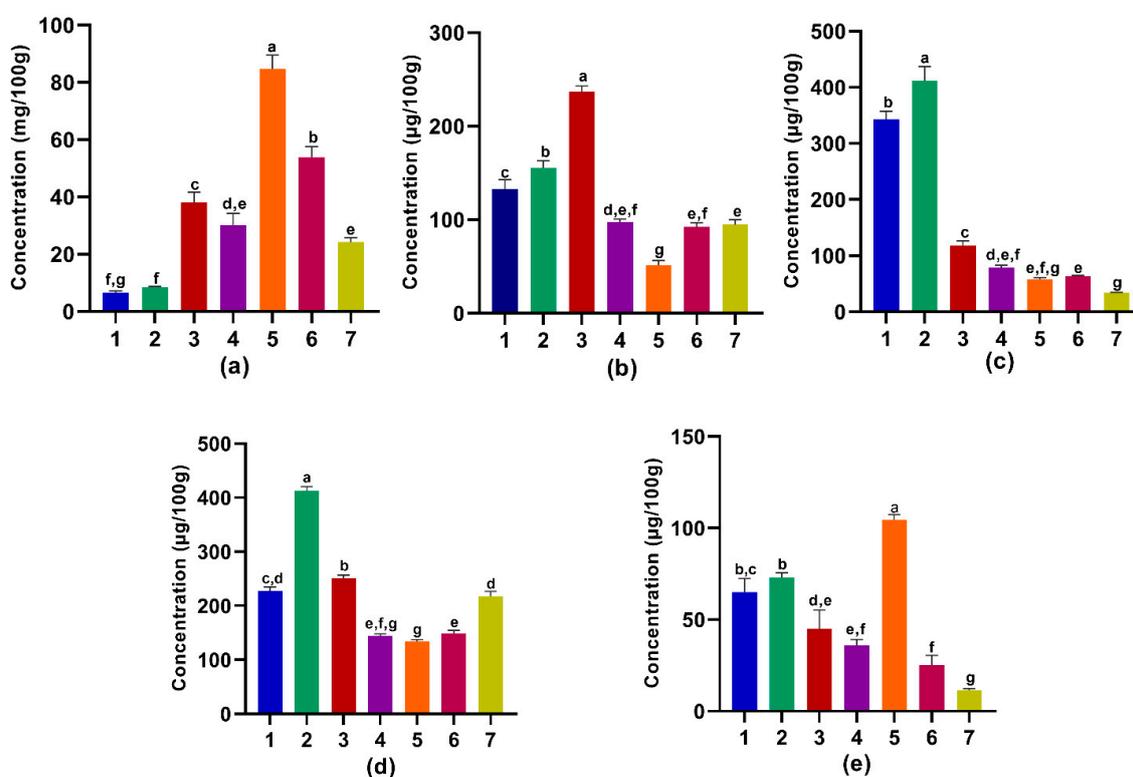


Figure 4. Contents of vitamin C (a), thiamine (b), riboflavin (c), vitamin B₆ (d), and folic acid (e) in seven edible mushrooms consumed in the northeastern highlands of Puebla Mexico. 1, *Lactarius*.

indigo. 2, *Clitocybe nuda*. 3, *Clitocybe subclavipes*. 4, *Russula delica*. 5, *Russula brevipes*. 6, *Clitocybe squamulosa*. 7, *Amanita jacksonii*. Different letters indicate means ($n = 10$) with statistically significant differences by ANOVA-Tukey ($p < 0.05$). Raw data are presented in Table S2. The concentrations are presented in dry weight.

3.3. Mineral Content

According to our results, the potassium content was higher in *C. squamulose*, *L. indigo*, and *R. brevipes* ($200 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$) than in the other four analyzed mushrooms (Figure 5a). *C. nuda*, *C. subclavipes*, *R. delica*, and *A. jacksonii* presented low levels of this element ($<200 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$) in comparison with those determined in *L. edodes*, *Pleurotus florida*, and *P. djamor*, which are frequently consumed in India [23]. Our results support that edible mushrooms can be a rich source of potassium, as stated in previous investigations [23–25]. Calcium was another abundant element in the seven studied samples (Figure 5b). However, *L. indigo* stored more endogenous levels of this mineral than the other mushrooms ($>400 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$). *C. nuda*, *C. subclavipes*, *C. squamulose*, and *A. jacksonii* stored levels higher than $300 \text{ mg } 100 \text{ g}^{-1}$, whereas *R. delica* and *R. brevipes* accumulated approximately 200 and $100 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$ calcium, respectively. These levels were higher than those reported in *L. edodes*, *P. florida*, and *P. djamor* [23]. The iron levels were high in all analyzed mushrooms (Figure 5c). *Clitocybe subclavipes* accumulated more amounts of this micronutrient ($>70 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$) than the other mushrooms. *L. indigo* stored approximately $50 \text{ mg } 100 \text{ g}^{-1}$ iron, whereas *C. nuda*, *R. delica*, *R. brevipes*, *C. squamulosa*, and *A. jacksonii* accumulated less than $40 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$. These results strongly suggest that these traditional foods may contribute to the recommended daily iron intake ($20\text{--}30 \text{ mg/day}$) [23]. This finding could be useful in formulating new diets based on these foods to prevent or treat anemia, which shows a high incidence in children from rural areas of Mexico [24]. Nevertheless, the consumption of sources rich in iron should be carefully standardized to avoid possible toxic effects for consumers and extensive studies should be performed to demonstrate their effect to prevent or alternatively treat anemia. The amount of sodium ranged from 5 to $40 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$, and this range was lower than that observed for *Boletus badius* and *B. edulis*, which are popularly consumed in Poland [25]. Interestingly, *A. jacksonii* was the mushroom with the lowest levels of this mineral (Figure 5d). The zinc levels ranged from 70 to $160 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$ (Figure 5e). *C. nuda* and *A. jacksonii* were rich sources of this mineral, and their levels were comparable to those found in *B. badius* and *B. edulis* [25]. The magnesium concentration was noticeably high in *L. indigo* ($300 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$). The levels of this element were similar in *C. nuda*, *C. subclavipes*, and *R. delica* (approximately $200 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$), whereas those found in *R. brevipes*, *C. squamulosa*, and *A. jacksonii* were below $150 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$ (Figure 5f). These levels were threefold higher than those determined in *B. badius* and *B. edulis* [25].

3.4. Phenols with Nutraceutical Activity

The seven mushrooms analyzed contained four phenolic acids with known antioxidant activity. *p*-Hydroxybenzoic acid (4-hydroxybenzoic acid) was significantly abundant in *A. jacksonii* (approximately $50 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$), whereas its levels in *R. brevipes* slightly surpassed $20 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$ (Figure 6a). The other five mushrooms studied contained less than $10 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$ *p*-hydroxybenzoic acid. These amounts were tenfold higher than those reported in *Agaricus arvensis*, *A. bisporus*, and *A. romagnesii* consumed in Portugal [26]. Beyond its known antioxidant activity, this compound has shown potent antimicrobial, anti-algal, anti-mutagenic, anti-estrogenic, hypoglycemic, anti-inflammatory, anti-platelet aggregating, nematocidal, and antiviral activities [27]. On the other hand, *p*-coumaric acid (4-coumaric acid) was more abundant in *R. brevipes* and *C. squamulosa* ($15 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$) than in the other mushrooms studied (Figure 6b), which presented low ($<3 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$) or undetectable levels (*R. delica*) of this phenolic compound. Nevertheless, the levels of *p*-coumaric acid found in these edible mushrooms consumed in the NHP were higher

than those reported in *Agaricus arvensis*, *A. silvicola*, and *A. bisporus* [28]. *p*-Coumaric acid showed low toxicity in mice ($LD_{50} = 2850 \text{ mg kg}^{-1}$ body weight) and is a precursor of other phenolic compounds [28]. *p*-Coumaric acid is a well-known nutraceutical with anti-cancer, antimicrobial, antiviral, anti-inflammatory, antiplatelet aggregation, anxiolytic, antipyretic, analgesic, and anti-arthritis activities [28]. It ameliorates diabetes, obesity, and hyperlipaemia under in vivo and in vivo conditions [28]. Protocatechuic acid was abundant in *L. indigo* (approximately $50 \text{ mg } 100 \text{ g}^{-1}$ DW), whereas low levels ($<10 \text{ mg } 100 \text{ g}^{-1}$ DW) were detected in *C. nuda* and *C. subclavipes* (Figure 6c). Traces or negligible levels of this phenolic acid were determined in *R. delica*, *R. brevipes*, *C. squamulosa*, and *A. jacksonii*. Previous studies have reported detectable levels of protocatechuic acid in *L. nuda* consumed in Portugal [26]; however, the levels of this compound were threefold higher in the same species harvested in the NHP. Protocatechuic acid has shown in vitro antiproliferative activity and in vivo chemopreventive properties against different types of cancer [29]. In addition, this natural product has shown antifungal, antibacterial, antispasmodic, anti-inflammatory, hepatoprotective, cardioprotective, and antiatherosclerotic properties [29]. Cinnamic acid was abundant in *R. delica* (approximately $15 \text{ mg } 100 \text{ g}^{-1}$ DW) and levels below $10 \text{ mg } 100 \text{ g}^{-1}$ DW were detected in *L. indigo*, *C. nuda*, *C. subclavipes*, *C. squamulosa*, and *A. jacksonii* (Figure 6d). These levels were higher than those reported in *A. arvensis*, *A. silvicola*, and *A. bisporus* [26]. Undetectable levels of cinnamic acid were observed in *R. brevipes*. Cinnamic acid and its derivatives have shown antibacterial, antimalarial, and anticancer activity under in vivo conditions as well as exhibited inhibitory potential for acetylcholinesterase and butyrylcholinesterase as neuroprotective agents [30].

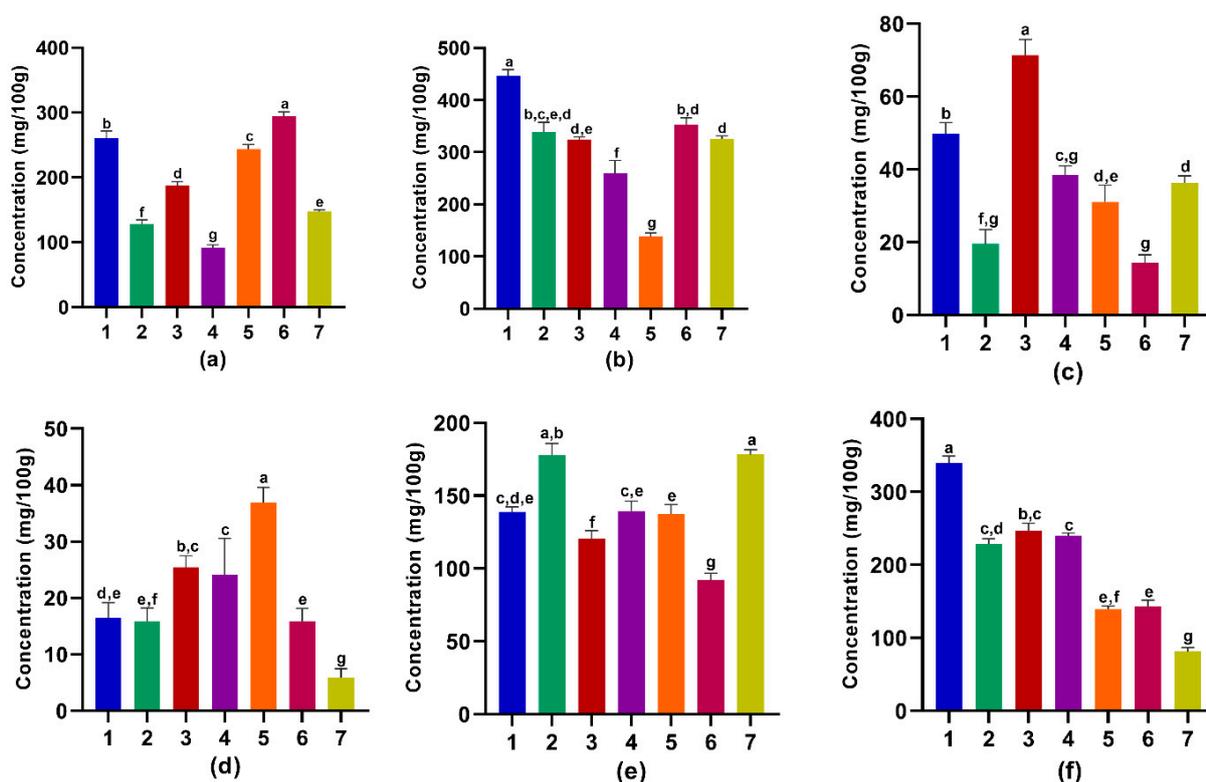


Figure 5. Contents of potassium (a), calcium (b), iron (c), sodium (d), zinc (e), and magnesium (f) in seven edible mushrooms consumed in the northeastern highlands of Puebla, Mexico. 1, *Lactarius indigo*. 2, *Clitocybe nuda*. 3, *Clitocybe subclavipes*. 4, *Russula delica*. 5, *Russula brevipes*. 6, *C. squamulosa*. 7, *Amanita jacksonii*. Different letters indicate means ($n = 10$) with statistically significant differences by ANOVA-Tukey ($p < 0.05$). Raw data are presented in Table S3. The concentrations are presented in dry weight.

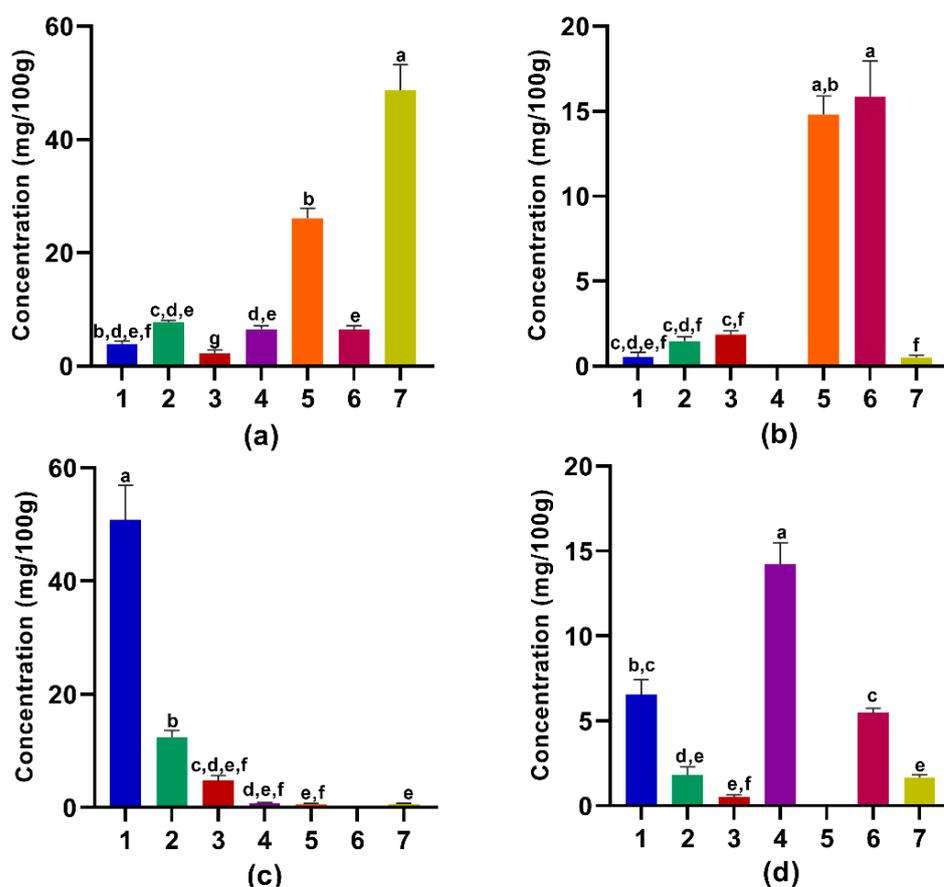


Figure 6. Contents of *p*-hydroxybenzoic acid (a), *p*-coumaric acid (b), protocatechuic acid (c), and cinnamic acid (d) in seven edible mushrooms consumed in the northeastern highlands of Puebla Mexico. 1, *Lactarius indigo*. 2, *Clitocybe nuda*. 3, *Clitocybe subclavipes*. 4, *Russula delica*. 5, *Russula brevipes*. 6, *Clitocybe squamulosa*. 7, *Amanita jacksonii*. Different letters indicate means ($n = 10$) with statistically significant differences by ANOVA-Tukey ($p < 0.05$) and spaces without bars indicate non-detectable compounds under assayed conditions. Raw data are presented in Table S4. The concentrations are presented in dry weight.

3.5. Antioxidant Potential

The seven mushrooms showed remarkable antioxidant potential (Figure 7). *A. jacksonii* was the source with the highest phenolic content ($>100 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$), whereas *R. delica* and *R. brevipes* showed similar levels ($<100 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$) (Figure 7a). Such activity was comparable to that found in native “quelites” consumed in this geographical region [11]. Interestingly, there was no correlation with the antioxidant capacity since *L. indigo* and *R. delica* showed the highest antioxidant capacity without statistically significant differences between them. According to our results, the edible mushroom with the lowest phenol content was *C. subclavipes*, but its antioxidant capacity surpassed $200 \mu\text{M/g TEAC}$. This trend strongly suggests that other compounds with different structures to phenolics may influence divergences in the total antioxidant capacity. As is known, edible mushrooms contain at least 1000 metabolites of different chemical natures and many of them could exert antioxidant activity [5]. Considering that the present investigation was limited to determining specific phenols and fatty acids with proven nutraceutical properties, the synergistic antioxidant activity of other compounds accumulated in the edible mushroom cannot be discarded. Remarkably, the total phenol content of the seven edible mushrooms consumed in the NHP was higher than those consumed in other regions of Mexico [31]. According to our results, these fungi presented three to fourfold higher levels of phenols than *A. bisporus*, *Macrolepiota procera*, and *Boletus edulis* commercialized and consumed in

Chihuahua, Mexico [31]. Similarly, the antioxidant potential of the seven edible mushrooms consumed in the NHP was evidently higher than that reported for *Russula integra*, *R. nigricans*, *R. vesca*, and *Sarcodon imbricatus* consumed in Tlaxcala, Mexico [32].

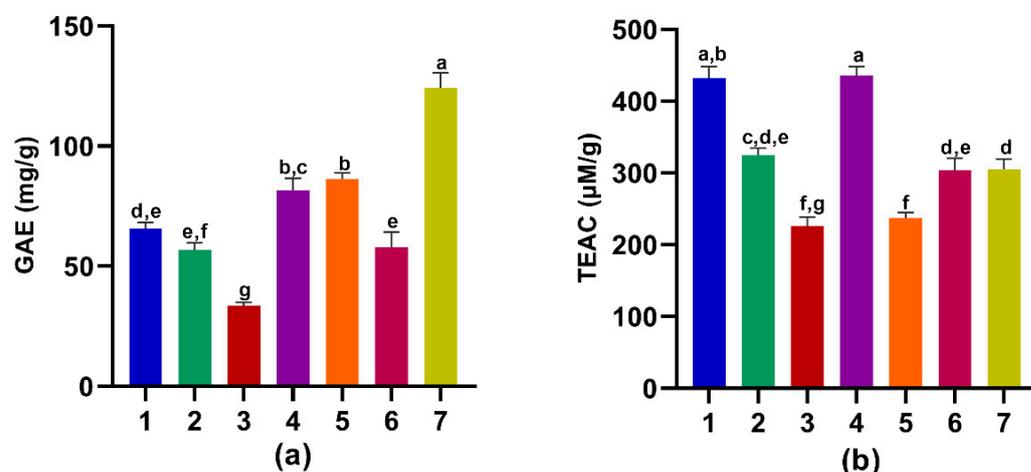


Figure 7. Antioxidant potential of seven edible mushrooms consumed in the northeastern highlands of Puebla, Mexico. 1, *Lactarius indigo*. 2, *Clitocybe nuda*. 3, *Clitocybe subclavipes*. 4, *Russula delica*. 5, *Russula brevipes*. 6, *Clitocybe squamulosa*. 7, *Amanita jacksonii*. The total phenol content expressed in gallic acid equivalents (GAE; (a)) and the antioxidant capacity expressed in trolox equivalents (TEAC µM/g; (b)) are shown. Different letters indicate means ($n = 15$) with statistically significant differences by ANOVA-Tukey ($p < 0.05$). Raw data are presented in Table S6. The concentrations are presented in dry weight.

3.6. Inhibitory Activity on Key Enzymes

To confirm the nutraceutical potential of the studied mushrooms, their hydroalcoholic extracts were evaluated on their capacity to inhibit LP, AG, AA, HMG-CoA reductase, and ODC (Figure 8). The hydroalcoholic extract of *A. jacksonii* had lower IC_{50} ($<50 \mu\text{g mL}^{-1}$) on PL than that of the other mushrooms assayed (Figure 8a). Currently, few inhibitors of PL have been obtained from edible mushrooms. However, a stable inhibitor was successfully isolated from the fruiting bodies of *Phellinus linteus* [33]. AG and AA activities were mainly affected by the hydroalcoholic extract of *L. indigo* and *C. nuda* ($<200 \mu\text{g mL}^{-1}$ and $<150 \mu\text{g mL}^{-1}$, respectively). In contrast, the extracts of the other mushrooms showed moderate inhibitory activity against these enzymes (Figure 8b,c). A previous study revealed that the organic extracts of *Amanita hemibapha*, *A. hemibapha*, *A. pseudoprinceps*, and *A. subhemibapha* (consumed in Thailand) produced 20 to 30% inhibition on AG [34]. The conversion of the IC_{50} obtained for the seven edible mushrooms consumed in the NHP revealed that they can inhibit 50 to 70% AG activity. Then, a deep exploration of the chemistry of *L. indigo* and *C. nuda* should be further performed. In the same context, previous investigation sustained that the alcoholic extract of *P. ostreatus* inhibits AA at a concentration of $383 \mu\text{g mL}^{-1}$, being less effective than the hydroalcoholic extracts of *L. indigo* and *C. nuda* [35]. The same investigation revealed that glycoproteins and catechins are possible candidates involved in the biological activity [35]. Then, our results on the inhibitory activity of the seven mushrooms consumed in the NHP on AA and AG may indicate that these sources can be tagged as a natural alternative to prevent hyperglycemia. Likewise, HMG-CoA reductase was strongly inhibited by the extracts of *L. indigo* ($<100 \mu\text{g mL}^{-1}$) and *C. subclavipes* ($<150 \mu\text{g mL}^{-1}$) (Figure 8d). Nevertheless, the extracts of *C. nuda*, *R. delica*, and *R. brevipes* produced moderated inhibition on the enzyme ($>200 \mu\text{g mL}^{-1}$). Previous studies have demonstrated that low levels of statins may be accumulated in the fruiting bodies of some edible mushrooms, such as *P. ostreatus* [36]. It has been observed that the hypocholesterolemic effect of edible mushrooms is produced by variable biochemical processes

such as impairing dietary cholesterol absorption or inhibiting the endogenous cholesterol metabolism by the biological activity of chitin and beta-glucans [36]. In any case, the rates of cholesterol absorption decrease by fecal excretion of bile [36]. In addition, the synergic activity of phenols and other bioactive metabolites, such as eritadenine (an adenosine analog alkaloid), can contribute to reducing cholesterol levels [36]. To our knowledge, studies have demonstrated the effect of edible mushroom extracts on HMG-CoA reductase. As is known, ODC is a relatively new and promising anti-proliferative target [37]. The hydroalcoholic extracts of *C. nuda*, *C. subclavipes*, and *A. jacksonii* produced moderate inhibitory activity in ODC ($180\text{--}250\ \mu\text{g mL}^{-1}$), whereas that of *L. indigo* ($<50\ \mu\text{g mL}^{-1}$) exerted the most potent inhibition of this enzyme (Figure 8e). Little is known about the inhibitory activity of individual molecules or organic extracts from edible mushrooms in ODC. Nevertheless, our results suggest that dissolved molecules in the hydroalcoholic extracts of *L. indigo* have similar inhibitory potency to that observed for pelargonidin-3-O-rutinoside accumulated in *R. pompana* berries [37].

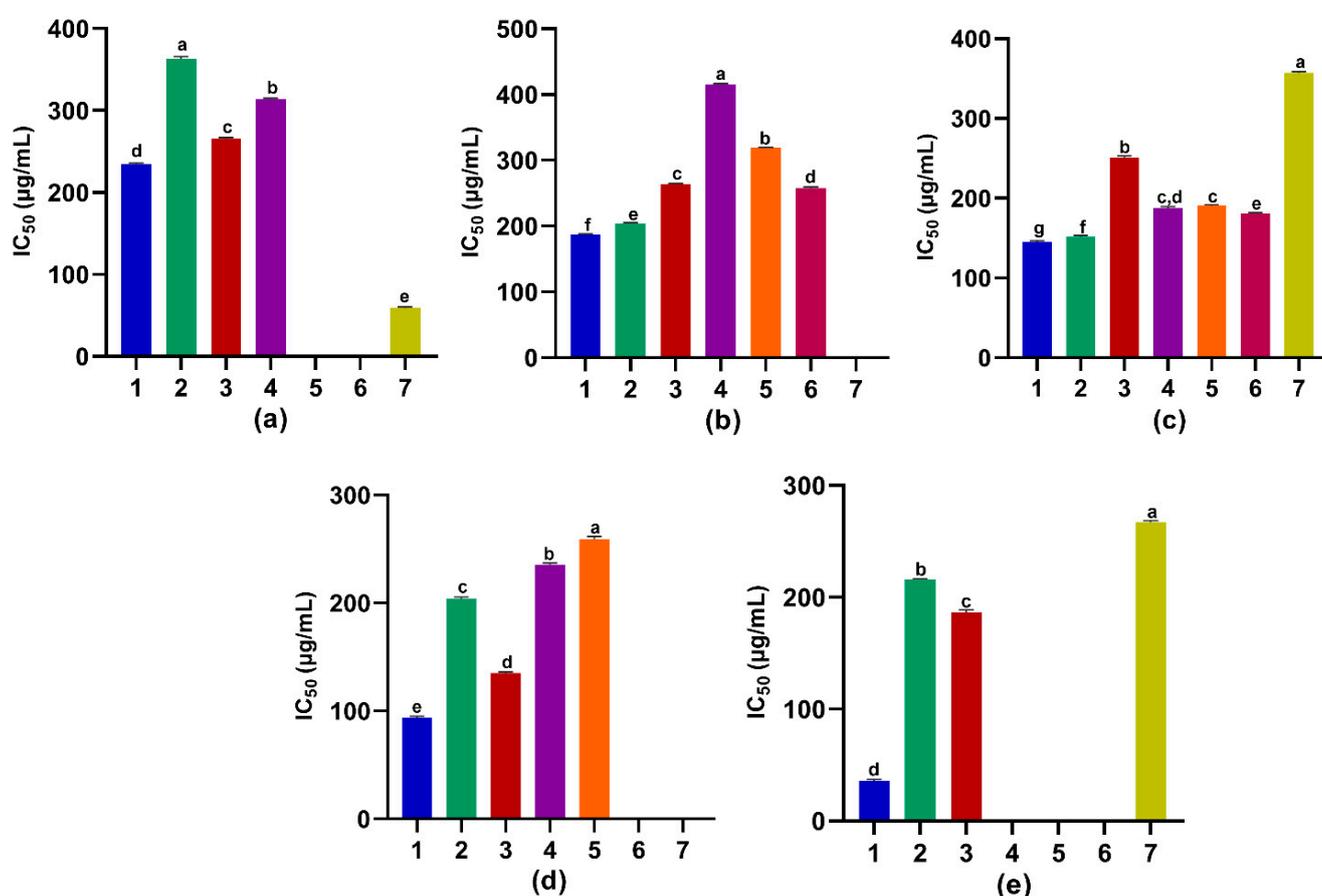


Figure 8. Inhibitory activity (IC_{50}) of the ethanolic extracts from seven edible mushrooms consumed in the northeastern highlands of Puebla, Mexico on lipase (a), AG (b), AA (c), HMG-CoA reductase (d), and ODC (e). 1, *Lactarius indigo*. 2, *Clitocybe nuda*. 3, *Clitocybe subclavipes*. 4, *Russula delica*. 5, *Russula brevipes*. 6, *Clitocybe squamulosa*. 7, *Amanita jacksonii*. Different letters indicate means ($n = 25$) with statistically significant differences by ANOVA-Tukey ($p < 0.05$) and spaces without bars indicate undetectable activity under assayed conditions. Raw data can be consulted in Table S7.

3.7. Antimicrobial Activity

Overall, the results of antimicrobial activity revealed differential potential against pathogenic and beneficial microorganisms associated with the gastrointestinal tract (Table 2). Interestingly, the hydroalcoholic extract of *L. indigo* and *C. nuda* showed strong anti-*H. pylori* activity (MIC < 160 $\mu\text{g mL}^{-1}$), and these results should be considered as a valuable background to perform further exploration for elucidating putative bioactive compounds and their mechanisms of antimicrobial action. Contrarily, the extract from the six edible mushrooms showed weak or negligible activity on the assayed strain of *E. coli* (>300 $\mu\text{g mL}^{-1}$). Similar results were observed for *S. cerevisiae*, *L. reuteri*, *L. acidophilus*, and *B. bifidum* where the hydroalcoholic extracts had no significant toxic effects. This evidence strongly suggests that consuming these sources could probably be safe for some probiotics associated with the human gut lumen. On the other hand, the extracts of *L. indigo*, *C. nuda*, *C. subclavipes*, *R. delica*, and *A. jacksonii* produced a noticeable inhibitory effect on *E. faecalis* whereas those of *L. indigo*, *C. nuda*, and *A. jacksonii* were highly effective against *S. typhi*. Previous works focused on evaluating ethanolic or hydroalcoholic extracts from edible mushrooms suggest the possible use of these foods as a source of natural inhibitors of pathogenic bacteria as well as agents to promote the proliferation of beneficial probiotics [38,39].

Table 2. Minimum inhibitory concentration ($\mu\text{g mL}^{-1}$) of the hydroalcoholic extracts from seven edible mushrooms consumed in the northeastern highlands of Puebla, Mexico.

	LI ¹	CN ¹	CS ¹	RD ¹	RB ¹	CQ ¹	AJ ¹
<i>Helicobacter pylori</i> (ATCC53504)	156.8	135.9	>500	>500	>500	>500	156.8
<i>Escherichia coli</i> ATCC25922	>500	>500	>500	>500	356.8	458.7	>500
<i>Enterococcus faecalis</i> (ATCC 29212)	130.4	158.9	197.8	238.2	>500	>500	135.4
<i>Salmonella typhi</i> (ATCC 6539)	98.7	217.9	>500	318.7	>500	>500	106.5
<i>Saccharomyces cerevisiae</i> (INVS _c 1)	>500	>500	>500	>500	>500	>500	>500
<i>Lactobacillus reuteri</i> (ATCC55730)	>500	>500	>500	>500	>500	>500	>500
<i>Lactobacillus acidophilus</i> (ATCC4356)	>500	>500	>500	>500	>500	>500	>500
<i>Bifidobacterium bifidum</i> (ATCC 29521)	>500	>500	>500	>500	>500	>500	>500

¹ Acronym of *Lactarius indigo* (LI), *Clitocybe nuda* (CN), *Clitocybe subclavipes* (CS), *Russula delica* (RD), *Russula brevipes* (RB), *Clitocybe squamulosa* (CQ), and *Amanita jacksonii* (AJ).

3.8. Clustering of Edible Mushrooms Based on Their Biological Properties

The results of PCA analysis revealed two main groups of edible mushrooms which were clustered on the bases of metabolite content, total phenol content, antioxidant capacity, and ability to inhibit enzymes visualized as therapeutic targets (Figure 9A). One group (group 1) contained *R. brevipes*, *C. squamulosa*, and *A. jacksonii*, whereas the other (group 2) was comprised of *R. delica*, *C. subclavipes*, *C. nuda*, and *L. indigo*. This model explained 81.4% of total variability, while OPLS-DA confirmed the presence of the two main groups produced by PCA (Figure 9B). The OPLS-DA model was validated with 999 permutations and values of $R^2 = 1$ and $Q^2 = 0.997$ (Figure 9C). These results suggested that the differential consumption of the different groups might produce similar biological effects. Group 1 had outstanding amounts of *p*-hydroxybenzoic acid, *p*-coumaric acid, vitamin C, and substantial levels of phenols (Figure 9D). Group 2 had remarkable anti-lipase activity as well as substantial levels of Mg, thiamine, riboflavin, and oleic acid (Figure 9D). Interestingly, the variable important plot in each fungal species reveal that lipase activity fat, Mg, and *p*-coumaric, *p*-hydroxybenzoic acid, thiamine, and vitamin C contents influenced the results of OPLS-DA model and should be considered as the most relevant properties of the edible mushrooms studied (Figures S3–S9).

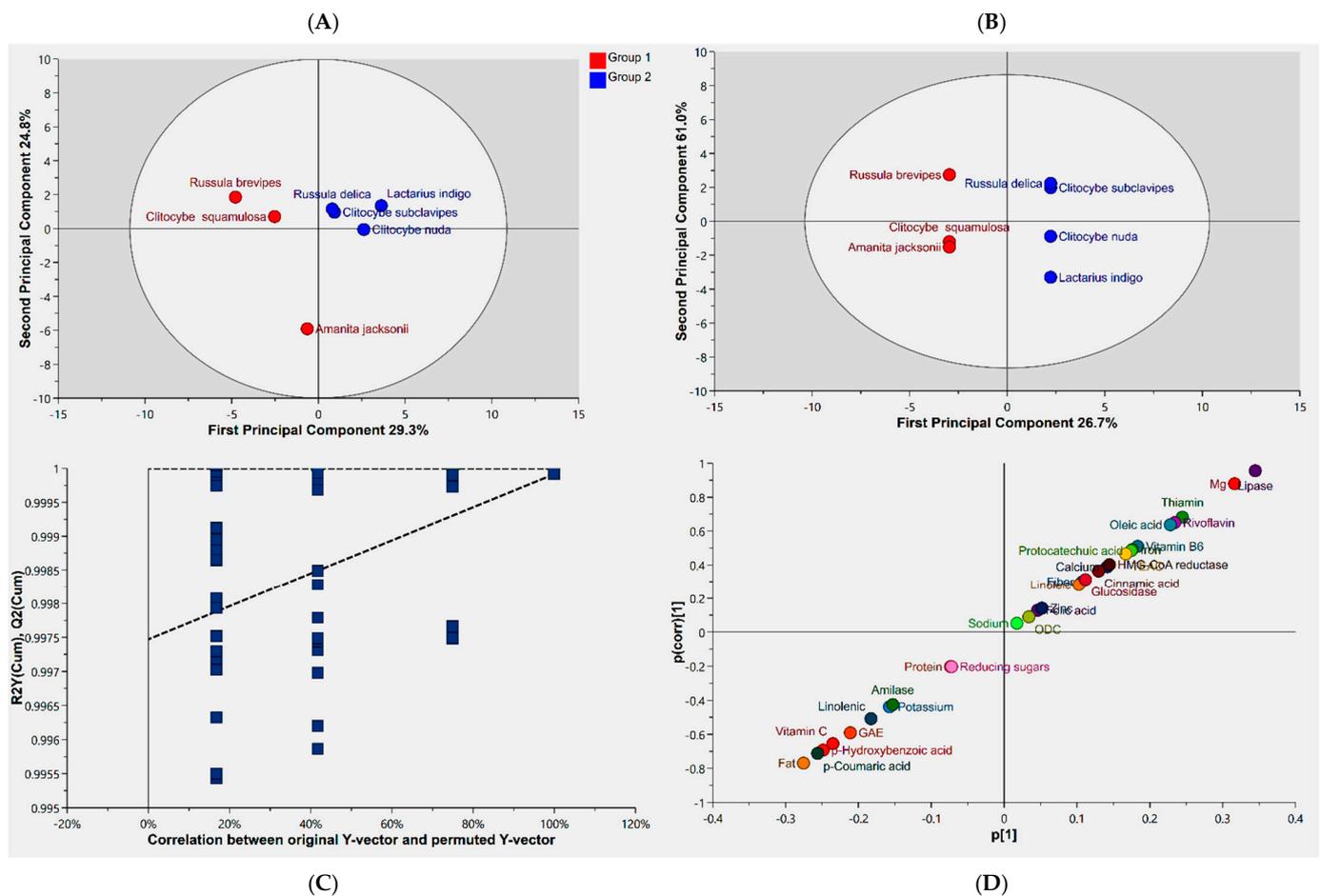


Figure 9. Discriminant statistical analysis for the edible mushrooms *Lactarius indigo*, *Clitocybe nuda*, *Clitocybe subclavipes*, *Russula delica*, *Russula brevipes*, *Clitocybe squamulosa*, and *Amanita jacksonii*. (A) principal component analysis. (B) orthogonal partial least squares discriminant analysis. (C) validation of orthogonal partial least squares discriminant analysis ($R^2 = 1$ and $Q^2 = 0.997$). (D) S-plot of orthogonal partial least squares discriminant analysis. (C) validation of orthogonal partial least squares discriminant analysis showing the dispersion of studied variables.

4. Conclusions

The seven edible mushrooms addressed in this investigation showed substantial levels of protein, fiber, selected vitamins, and minerals as well as low amounts of reducing sugars and fat. These native foods contained fatty acids and phenols with proven nutraceutical activity. The phenol content, antioxidant capacity, and inhibitory activity of hydroalcoholic extracts obtained from the studied mushrooms on target enzymes linked to lipid, glucose, and cell proliferation demonstrate their potentiality as natural agents to prevent or control diseases associated with the metabolic syndrome, diverse types of cancer, as well as to maintain the equilibrium of gut microbiota. The results of the PCA and OPLS-DA models suggested that two groups of edible mushrooms might exert similar biological effects. Group 1 (*R. brevipes*, *C. squamulosa*, and *A. jacksonii*) had outstanding amounts of *p*-hydroxybenzoic acid, *p*-coumaric acid, vitamin C, and substantial levels of phenols. Group 2 (*R. delica*, *C. subclavipes*, *C. nuda*, and *L. indigo*) had remarkable anti-lipase activity as well as substantial levels of Mg, thiamine, riboflavin, and oleic acid. This research could be useful for more profound studies focused on the evaluation of bioactive molecules from these edible sources and their mechanisms of action using *in vitro* and *in vivo* models.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/app14062520/s1>, Table S1: Macronutrient profile in seven mushrooms consumed in the northeastern highlands of Puebla-Mexico; Table S2. Vitamin content in seven mushrooms consumed in the northeastern highlands of Puebla-Mexico; Table S3. Mineral content in mushrooms consumed in the northeastern highlands of Puebla-Mexico. Table S4. Nutraceutical content in seven mushrooms consumed in the northeastern highlands of Puebla-Mexico; Table S5. Fatty acids content in seven mushrooms consumed in the northeastern highlands of Puebla-Mexico. Table S6. Total phenolics and antioxidant capacity of seven mushrooms consumed in the northeastern highlands of Puebla-Mexico. Table S7. IC₅₀ for the ethanolic extracts from edible organs of seven mushrooms consumed in the northeastern highlands of Puebla-Mexico. Figure S1. HPLC profiling of the hydroalcoholic extract from *Lactarius indigo*. 1, *p*-coumaric acid. 2, protocatechuic acid. 3, *p*-hydroxybenzoic acid. 4, cinnamic acid. IS, procyanidin B2 as internal standard. Figure S2. GC-MS profiling of the petroleum ether extract from *Lactarius indigo*. 1, TMS-linoleic acid. 2, TMS-oleic acid. 3, TMS-linolenic acid. IS, abietic acid as internal standard. Figure S3. Variable importance plot (VIP) plot for discriminate analysis for the parameters of *L. indigo*. Figure S4. Variable importance plot (VIP) plot for discriminate analysis for the parameters of *C. nuda*. Figure S5. Variable importance plot (VIP) plot for discriminate analysis for the parameters of *C. subclavipes*. Figure S6. Variable importance plot (VIP) plot for discriminate analysis for the parameters of *R. delica*. Figure S7. Variable importance plot (VIP) plot for discriminate analysis for the parameters of *R. brevipes*. Figure S8. Variable importance plot (VIP) plot for discriminate analysis for the parameters of *C. squamulosa*. Figure S9. Variable importance plot (VIP) plot for discriminate analysis for the parameters of *A. jacksonii*.

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