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Original Article

EVALUATION OF TOTAL POLYPHENOLS AND ANTIOXIDANT CAPACITY IN MUSHROOM EXTRACTS *PLEUROTUS OSTREATUS* AND *LENTINULA EDODES*

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ABSTRACT

Objective: This research was aimed at assessing the concentration of total polyphenols in ethanolic and methanolic extracts of *Pleurotus ostreatus* and *Lentinula edodes* mushrooms and antioxidant activity.

Methods: Polyphenols were determined by the Folin Ciocalteu method, using lyophilized mushroom samples for the preparation of extracts and antioxidant activity by the TBARS method.

Results: Extracts prepared from mushrooms showed appreciable values of polyphenols, and for the ethanolic extract of *Pleurotus ostreatus* and *Lentinula edodes* values of 102.78 and 81.83 mg of gallic acid/100 g of the sample respectively, comparable to those obtained in some fruits For methanolic extracts, values of 100.45 and 78.92 mg of gallic acid/100 g of sample were obtained. Polyphenol concentration values for the *Pleurotus* were higher in the two types of extracts and lower for the *Lentinula edodes*.

Conclusion: When evaluating the antioxidant activity, high antioxidant activity was found for the two types of mushroom, *Pleurotus ostreatus* and Lentinula edodes, presenting peroxidase inhibition values of 88.04 and 89.49% respectively.

Keywords: Polyphenols, Antioxidants, Extracts, *P. ostreatus, L. edodes*

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INTRODUCTION

Knowing that mushrooms are present in all habitats due to adaptability in almost any substrate and climate. There are on average 200,000 species of which only 7,000 of them are known. World production of cultivated mushrooms exceeds 6.2 million tons, the value of which is close to 30 billion dollars. The growth rate is 11% and this is due to research on its medicinal and nutritional properties. This is the reason for the high demand for edible mushroom derived products [1].

The Orellana mushroom (*Pleurotus ostreatus*) is one of the edible mushrooms with the highest productivity growth during the last ten years due to its nutritional properties and the high percentage of proteins that allow replacing those of animal origin [2]. The mushroom is normally produced in organic matter and is considered as an alternative for the use of agro-industrial waste at low cost [3].

These mushrooms form a large group with very diverse species, differentiated by: color (yellow, white, gray, brown, pink), shapes, flavor and technical requirements [4]. *Lentinula edodes* is one of the most important edible mushrooms in the world from the point of view of production and is one of the most popular cultivated mushrooms [5].

It is known that the extracts of some mushrooms inhibit antioxidant activity by the natural aging process resulting from the action of free radical products of metabolism, which have antioxidant activity and whose study has focused on the kingdom plantae [6].

The research focused on studying the body of *Pleurotus ostreatus* and *Lentinula edodes* in order to exploit the presence of polyphenols, as well as the determination of their antioxidant activity, these compounds could be responsible for the presence of such action in the extracts of these mushrooms.

The objective of this research work was to evaluate the total polyphenols and antioxidant capacity in extracts of *Pleurotus ostreatus* and *Lentinula edodes* mushrooms.

MATERIALS AND METHODS

This work was carried out in the Molecular Biology laboratory of the Research Department, as well as an air-conditioned room, with temperature and humidity control, Bolivar State University. To carry out this research, strains of edible mushrooms were used *Pleurotus ostreatus* (716/12) and *Lentinula edodes* strain L-SSC.

Experimental measurements

In the powdered mushrooms (*Pleurotus ostreatus* and *Lentinula edodes*) physical analyzes were performed as: Humidity, It was performed under the international standard (AOAC925.10); Ashes, using the technique determined by the international standard (AOAC923.03); Elemental analysis of Carbon and Nitrogen, using an elemental analyzer (various macro cube/1922261/120V, USA), this according to the Dumas methodology.

Extracts preparation

To obtain extracts rich in phenolic compounds, extraction was carried out using two types of solvents: methanol and ethanol due to their polarity. For which a block design with factorial arrangement, AxB was applied (table 1). For the process, previously heavy mushrooms (3 g), dehydrated and pulverized by lyophilization and with a humidity of 4-6% were placed in amber glass bottles, then 25 ml of each of the solvents (80%). Each of the dilutions was stirred in a Thermo shaker (YVIMEN TR100-G, USA) for 15 min, then stored for 24 h. At the end of this stage, stirring was repeated to facilitate extraction for 10 min at 25 °C in a cellular ultrasonic chamber with moderate agitation. Finally, the extracts were centrifuged for 12 min at 6000 rpm, at 10 °C, the supernatant was filtered through Whatman # 1 filter paper, the extracts were stored.

Statistical analysis

For this an analysis of variance (ANOVA) was applied to establish the differences between the treatments, also, to know the

differences between the means of the treatments, the 5% Tukey test was applied for averages and factors under study.

Determination of the concentration of total polyphenols

The concentration of total polyphenols in extracts was measured by spectrophotometry, based on a colorimetric oxide-reduction reaction. The oxidizing agent used was the folin-Ciocalteu reagent. The calibration curve was performed using a standard solution of gallic acid (0.1 mg/ml) in volumes from 0 µl to 160 µl at 20 µl intervals. For the concentration of total polyphenols, 250 µl of 1N folin-ciocalteu reagent was added to each of the previously prepared standards and samples and sonicated for 5 min. Subsequently, 250 µl of 7.5% Na_2CO_3 was added and allowed to stand for 1 h. The absorbance at 750 nm was measured.

Table 1: Factors under study

Factor	Code	Levels
Types of mushroom	A	a1 = *Pleurotus ostreatus* a2 = *Lentinula edodes*
Types of solvent	B	b1 = Methanolic Extract b2= Ethanolic Extract

Three grams of mushrooms were used for each treatment with three repetitions.

Determination of antioxidant activity

The antioxidant activity was performed under the methodology described by Rojano *et al.* [7], with modifications, for which different concentrations of the samples (100, 200, 500 and 1000 µg/ml) were prepared, from the prepared dilutions 500 µl were taken and mixed with 500 µl of olive oil (previously oxidized using the TBARS method) in 2 ml eppendorf tubes. In addition, BHT standards (Butyl hydroxytoluene) were prepared at the same concentrations as the sample and similarly mixed with 500 µl of oxidized oil. Samples and standards remained at 28 °C for 8 h in the micro incubator with constant agitation at 400 rpm. After this incubation process, 1 ml of 1% thiobarbituric acid was added to each sample. The prepared samples remained in the micro incubator at 95 °C for 60 min at 400 rpm, then they were cooled and the spectrophotometer was measured at a wavelength of 532 nm. The antioxidant activity was expressed as % oxidation inhibition by the following equation:

$$\text{Ec. 1. } \%\ \textbf{Oxidationinhibition} = \frac{At-Ac}{Ac} * 100$$

Where: At: Sample Absorbance, Ac: Control Absorbance

RESULTS AND DISCUSSION

Physical-chemical characterization of lyophilized and powdered mushrooms table 2 shows the results of the chemical-physical characterization and presents the average values of the samples of the lyophilized and powdered mushrooms.

Table 2: Mean values and standard deviation of the composition of mushrooms

Powdered mushroom	Moisture (%)	Ash (%)	N (%)	C (%)	C/N (%)
Pleurotus	4.00 (0.20)	4.28 (0.25)	5.33 (0.13)	37.56 (2.26)	7.05 (1.28)
Lentinula	3.13 (0.14)	3.47 (0.22)	4.87 (0.22)	38.87 (2.17)	7.98 (2.01)

Values between () represents the standard deviation

The moisture content in the two types of mushroom is low as a lyophilization effect, 4% for the *Pleurotus ostreatus* and 3.13 for the *Lentinula edodes*, therefore, the moisture content in the two types of mushrooms has a variation of 0.87%. Gómez *et al.*, [8], states that the humidity of lyophilized mushrooms should not exceed 5%. Atehortúa, [9], considers that mushrooms grown in vegetable waste contribute an ash content between 3 and 5%, producing an increase in their macro content (sodium, potassium, calcium and phosphorus) and microelements (iron, iodine, copper, zinc). In our study, the *Pleurotus* presented a value of 4.28% while *Lentinula* 3.47%, in fact, both studied mushrooms comply with the established parameters. In reference to the nitrogen content in mushrooms, *Pleurotus* presented the highest percentage with 5.33% followed by Lentinula with 4.87%. In a work developed by, Vega and Franco, [10], considers that an edible mushroom must be greater than 3.5% because this minimum nitrogen content allows the formation of amino acids, proteins and fiber, in our study, *Pleurotus* has the highest percentage with 5.33% followed by the *Lentinula* with 4.87%, values that are within the established bibliographic ranges. Likewise, the same authors consider that the carbon content must be greater than 30%, in this case, the *Lentinula* mushroom had the highest percentage with 38.87%. Cortez *et al.* [11], establishes that freeze-dried and powdered mushrooms must have carbon/nitrogen ratio values greater than 6.5%; As shown in our work, the two types of mushrooms have values higher than those mentioned in the bibliography.

Polyphenol concentration

The results obtained show that the content of polyphenols predominates in the a1b1 treatment corresponding to the mushroom *Pleurotus*+extraction with ethanolic and the lowest value corresponds to treatment 4 (78.92 mg/100g) (table 3).

Table 3: Polyphenol concentration of lyophilized and powdered mushrooms

Code	Description	Total polyphenols (mg of gallic acid/100 g of sample)
a1b1	*Pleurotus M*+Ethanolic extract	102.78
a1b2	*Pleurotus M*+Methanolic extract	100.45
a2b1	*Lentinula M*+Ethanolic extract	81.83
a2b2	*Lentinula M*+Methanolic extract	78.92

According to Radice *et al.* [12], the content of polyphenols in food and should exceed 92 mg/100 g of sample, which shows that the *Pleurotus* mushroom meets this parameter, this is very favorable in the consumer mainly in the treatment of cardiovascular diseases since it has vasodilatory effects.

By means of the analysis of variance that is evidenced in the table 4 that exists in relation to the experimental response total polyphenols due to the effect of factor A: type of mushroom and the effect of factor B: type of solvent. This shows that there is no correlation between these two study variables, that is to say, they

are independent in presenting highly significant differences in polyphenol content.

Table 4: Analysis of variance for the response variable of total polyphenols

Font	Gl	Sum of squares	Medium squares	F-Reason	P-Value
A: Type of mushroom	2	12.64	6.32		0.000
B: Type of solvent	3	3007.77	1002.59		0.000
Interaction AB	6	9.52	1.59	639.7**	0.000
Total	11	3029.93			

**Highly significant difference

The results are verified by Orús [13], which establishes that theoretical F values greater than 500 determine a widely marked difference between the different study factors. Having values that have statistical significance (639.7), the Tukey test was carried out to establish the difference in the means between the treatments.

Table 5: Comparison of means according to tukey at 5% for the treatments of the polyphenol response variable expressed in mg. gallic acid/100 g

Treatments	Means	Ranges
1	102.78	a
2	100.45	b
3	81.83	c
4	79.82	c

After Tukey's analysis, three homogeneous groups were identified, confirming the existence of statistically significant differences between the 4 treatments applied. Treatment 1 (*Pleurotus ostreatus* ethanolic extract) has the highest polyphenol value with 102.78 mg, followed by treatment 2 (*Pleurotus ostreatus* methanolic extract) with 100.45 mg per 100 g, as noted by Radice et to the. [12], the content of polyphenols in foods and zetas should exceed 92 mg per 100 g of sample.

Table 6: Antioxidant activity of the mushroom samples evaluated

Sample	% Peroxidase inhibition
Pleurotus ostreatus	89.41
Lentinula edodes	88.04

A high antioxidant power and inhibition of lipid peroxidation were found in the mushroom *Lentinula edodes* by Liu *et al.* (15), who reported values between 80.32-93.73%. Jin *et al.* (16), found high antioxidant activity in the fruiting bodies of *Pleurotus ostreatus*

Analysis of antioxidant activity

The antioxidant activity of the mushrooms evaluated by the TBARS method, among which the capacity of the mushroom *Pleurotus ostreatus* stands out, since having the highest value 89.41%, inhibits oxidative degradation thanks to its ability to react with free radicals (table 6), these values agree with what Guzmán *et al.* [14], in which it determines that the percentage of peroxidase inhibition of a mushroom must be greater than 85% because in this way the product would act to promote the activity of antioxidant enzymes preventing the mushrooms from deteriorating.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declare none

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