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# ETHNONUTRITIONAL INVESTIGATION, ANTIOXIDANT ACTIVITIES AND SECONDARY METABOLITE CONTENT OF A RANGE OF MUSHROOM TEAS BASED ON *PLEUROTUS PULMONARIUS, PLEUROTUS OSTREATUS* AND *PLEUROTUS FLORIDANUS*

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ABSTRACT: Oyster mushrooms are considered functional foods due to their structure. Our work focused on assessing the antioxidant power and determining the secondary metabolite content of mushroom teas formulated from the bestknown, cultivated and eaten species. We carried out an ethnonutritional survey, and the species selected were used for the rest. We implemented a mixing plan after which 13 formulations were selected to prepare the various herbal teas. Pleurotus pulmonarius, Pleurotus ostreatus and Pleurotus floridanus were the most cultivated, known, and consumed species. DPPH test revealed that T5, T6 and T2 exhibited the best CI\_{50}: 17.39  $\pm$  0.35<sup>b</sup>, 17.65  $\pm$  1.20<sup>b</sup>, and 18.93  $\pm$  0.31<sup>bc</sup> respectively compared to the standard which had a  $CI_{50}$  of:  $3.09 \pm 1.56^{a}$ . In the FRAP test, T3, T9 and T11 displayed the best IC50 values:  $7.72 \pm 2.51^{b}$ ,  $10.16 \pm$  $0.14^{c}$ ,  $11.00 \pm 0.17^{cd}$ , respectively, while the standard had an IC<sub>50</sub> of 1.80  $\pm$  $1.06^{a}$ . In the hydrogen peroxide test, T1, T3 and T4 had the best CI<sub>50</sub> values:  $23.44 \pm 2.94^{b}$ ,  $24.98 \pm 4.79^{b}$  and  $26.37 \pm 0.30^{b}$ . Whereas the standard was  $5.82 \pm$ 1.27<sup>a</sup>. The determination of secondary metabolite levels showed that: T2 (41.48  $\pm$  9.47<sup>g</sup>), T4 (26.45  $\pm$  0.77<sup>f</sup>) and T1 (19.64  $\pm$  0.92<sup>e</sup>) had higher total polyphenol content, T9 (258.24  $\pm$  11.37<sup>c</sup>), T2 (253, 39  $\pm$  16,37<sup>bc</sup>) and T4 (252, 48  $\pm$  42,76<sup>bc</sup>) exhibited higher condensed tannin levels, while T7 (173.07  $\pm$  0.58<sup>i</sup>), T13  $(154.07 \pm 3.51^{\text{h}})$ , T3  $(152.07 \pm 5.03^{\text{h}})$  had higher total flavonoid content.

**INTRODUCTION:** The world is increasingly experiencing the resurgence of metabolic diseases <sup>1</sup>. One approach to alleviate this is the use of functional foods. Edible mushrooms of the oyster mushroom genus are considered as such, as several studies have elucidated their antioxidant, anti-free radical and antihyperglycemic properties <sup>2</sup>. On the other hand, herbal tea is defined as a drink obtained by maceration, decoction, infusion, and percolation of a material in hot or cold water.



It has proven to be an effective means for quick, easy, and efficient extraction of bioactive compounds <sup>4</sup>. Our objective was to evaluate the antioxidant activities and secondary metabolite content of a range of herbal teas based on *Pleurotus pulmonarius, Pleurotus ostreatus* and *Pleurotus floridanus.* 

# **MATERIAL AND METHODS:**

**Plant Material:** The oyster mushroom species (*Pleurotus pulmonarius*, *Pleurotus ostreatus* and *Pleurotus floridanus*) used in our study were collected from a mushroom farm located at the Faculty of Science, University of Douala.

# Ethno-nutritional Survey:

Survey site: We selected various locations within the city of Douala, organized by district. Indeed,

Douala is situated in the country of Cameroon within the coastal region of the Wouri department  $(04^{\circ}03'N, 009^{\circ}41'E)$ . Located in the southern part of the west of the country, it has an altitude of 13 m above sea level and covers an area of 210 km<sup>2</sup>. The city experiences a tropical monsoon climate (according to the Koppen climate classification), characterized by alternating hot and cold periods with abundant rainfall, particularly in July and August. Built largely on the alluvial basin of the Wouri River, Douala's main characteristic is its

rainfall, i.e. 4000 mm/year. Its average temperature is 26.7°C. Speaking of its relief, Douala is characterized by ecosystems such as: dense humid forests, mangroves, swamps. Its geographical and cultural diversity suggests that Douala could be likened to as Cameroon in miniature. Additionally, it has a cosmopolitan and highly dynamic population with an annual growth rate of 5%, spread over an area of 20,000 hectares. Hence the interest in this city.



FIG. 1: LOCATION OF THE CITY OF DOUALA. (Source: http://www.afrique.com/cameroun/image/carte-regions-cameroun-G.gif)

Procedure and Analysis of the Forms: We conducted an initial survey of the population, visiting localities daily to gather information about oyster mushrooms, focusing on the best-known species, consumed, cultivated and forms of consumption. During these visits, we conducted interviews in French, English and local languages with both producers and community members. The material used for this purpose was a semiquestionnaire consisting of structured the respondent' profiles and open-ended/closed-ended questions. Additionally, participants were provided with a catalogue containing images of commonly and cultivated mushrooms encountered in Cameroon to aid in their recognition, targeting consenting persons aged at least 21 years. The second survey was conducted among producers aiming to gather priority information about the most cultivated and sold species.

Formulation and Preparation of a Range of Herbal Teas: We carried out cubic mixing design with three factors corresponding to three oyster mushroom species and six responses. Corresponding to Antioxidant and free Radical Scavenging Activities: DPPH. FRAP and Peroxide). Additionally, the harvested mushrooms had been dried (in a dryer at 30°C) and coarsely ground using a food processor. We weighed 3 grams of each formulation (generated by the introduced into software) that we teabags purchased from the central market (Douala-Cameroon). Each teabag of each formulation was introduced into 100 ml of water previously brought to a boil for 25 min (percolation).

## **Antioxidant Activities of Mushroom Teas:**

**Trapping of the free Radical DPPH**°: The antifree radical activity of mushroom teas reflects their ability to scavenge free radicals in the body. The activity was evaluated using the 2,2-diphenyl-1picrylhydrazyl spectrophotometric method. The principle is based on the reduction of the DPPH radical from purple (DPPH) to yellow (DPPH-H). This reduction capacity is determined by a decrease in absorbance induced by anti-free radical substances. A calibration range obtained from a stock solution of extract at 1mg/ml in methanol was used to evaluate the anti-free radical activity of the concentrates. The reaction medium consists of 0.75 mL of mushroom teas and 1.5 mL of 0.04 mg/mL DPPH methanolic solution. A control containing no concentrate (sample blank) is prepared with methanol. The tubes were incubated in the dark for 30 min at room temperature and the optical densities measured at 517 nm were used to calculate the percentage of DPPH radical inhibition proportional to the anti-free radical strength of the sample <sup>5</sup>.

The FRAP Method. The FRAP (Ferric Reducing Antioxidant Power) method is based on the ability of Mushroom teas to reduce the ferric ion (Fe3+) present in the potassium ferricyanide complex [K3Fe(CN)6] to the ferrous ion (Fe2+) by an antioxidant. The reaction is indicated by the change from the yellow color of ferric iron (Fe3+) to the blue-green color of ferrous iron (Fe2+). The intensity of this color change, corresponding to the antioxidant power of each concentrate, was evaluated by the spectrophotometric method at 700 nm. To 2 mL of each sample (100  $\mu$ g/mL), we added 2 mL of phosphate buffer (0.2 M, pH 6.6) and 2 mL of aqueous solution (1%) of potassium hexacyanoferrate [K3Fe(CN)6]. The mixture was incubated at 50°C for 20 min followed by the addition of 2ml of trichloroacetic acid (10%). The mixture was centrifuged at 3000 rpm for 10 minutes to collect the top layer of the solution. 2 ml of the supernatant was then mixed with the same volume of distilled water and 0.4 ml of a freshly prepared aqueous solution of FeCl3 (0.1%) was added. After 10 minutes of reaction, the absorbance was read at 700 nm. The reducing power was deduced from a calibration curve (y = 0.028x -0.024; R2 = 0.995) established with ascorbic acid (0 - 100mg/l). The reducing power is expressed in umol Ascorbic Acid Equivalent per gram of dry extract (µmol EAA/g). Ascorbic acid was used as a reference <sup>5</sup>.

The free radical scavenging activity of mushroom teas on hydrogen peroxide radicals was determined using a modified method (Matthew, 2006; Kuntal Das *et al.*, 2017). A 40 mM hydrogen peroxide solution was used to screen the antioxidant activity of mushroom teas. The peroxide method. The free radical scavenging activity was determined using a modified method <sup>5</sup>. A 40 mM hydrogen peroxide

solution was used to screen the antioxidant activity of mushroom teas. The hydrogen peroxide solution (40 mM) was prepared in phosphate buffer with a pH of 7.4 and stored until use. 0.6 mL of hydrogen peroxide solution was added at different concentrations of mushroom teas and to the standard. The absorbance was measured at 230 nm. A phosphate buffer (pH 7.4) without hydrogen peroxide was used as white. The percentage of free radical scavenging activity of mushroom teas and standard compounds with respect to hydrogen peroxide radicals was calculated using the formula below:

The % of hydrogen peroxide scavenging activity =  $[(AC - AS)/AC] \times 100 \text{ AC}$  is the absorbance of the phosphate buffer (white) and AS is the absorbance in the presence of mushroom teas or standards.

# Secondary Metabolite Levels:

Total Polyphenols: Total phenolic content was estimated by colorimetry using the Folin-Ciocalteu procedure and gallic acid as reference molecules. Briefly, from the mushroom teas, a 1 mg/ml aqueous solution was prepared for this analysis, while the reaction mixtures were prepared by sampling 0.5 mL of sample, 2.5 mL of the 10% Folin-Ciocalteu reagent dissolved in water, and 2.5 mL of the 7.5% NaHCO3 aqueous solution. The mixture was then incubated for 45 minutes. Absorbance obtained using was a spectrophotometer at a wavelength of 765 nm. The same method was repeated for gallic acid and then the required calibration curve was constructed. Results were expressed as gallic acid equivalent per mg of sample (GAEq/mg)  $^{\circ}$ .

**Flavonoids:** The flavonoids were measured using a calibration curve for quercetin used as a reference molecule. Indeed, a stock solution with a concentration of 100 mg/ml was prepared and then diluted to concentrations of 2.5; 5; 10; 20; 40mg/ml. Subsequently, we mixed 1 ml of each solution with 0.3 ml of 10% AlCl3 solution, 1 ml of distilled water, 1 ml of 2 M NaOH and 0.3 ml of NaNO2. These prepared samples were incubated for 30 minutes at room temperature. The resulting colorimetric absorbance values were estimated using a UV-visible spectrophotometer at a wavelength of 510 nm. After obtaining the quercetin calibration curve, the flavonoid contents

of mushroom teas were expressed in mg quercetin equivalent per gram (mg QUE/g)  $^{5}$ .

**Condensed Tannins:** The condensed tannins were determined here by the acidic vanillin method used by Mignanwandé and et *al* (2020). This method is based on the ability of vanillin to react with condensed tannin units in the presence of acid to produce a color complex. Vanillin's reactivity with tannins involves only the first unit of the polymer. Stock solutions of mushroom teas were prepared at a concentration of 1mg/ml in methanol.

Next, we took 400µl of each stock solution and added 3ml of 4% vanillin to it. We added 1500µl of hydrochloric acid (HCl) to this mixture. The blank was prepared by replacing the reagent with the methanol-acid mixture. The tubes are kept at 30°C for twenty minutes in a darkroom.

The absorbance was read at 500nm. Condensed tannin contents were determined using a linear calibration curve (y = 0.002; R2 = 0.994) plotted using specific concentrations of gallic acid (0 - 60mg/l, R2 = 0.994) or catechin as the reference substance. The results were calculated according to the equation: C = (c\*D/Ci) \*100; and expressed in

**Socio-demographic Characteristics:** 

milligrams gallic acid equivalent per 100 milligrams of mushroom teas  $(mgEAG/100mg)^5$ .

C = Condensed tannin concentration in mg EAG/100 mg dry matter

C = Sample Concentration Read

D = Dilution Factor

Ci = Concentration of the initial solution

**Statistical Analysis:** The analysis of the cards consisted of using the citation frequencies related to each question, as a function of the sample size; data were fed into an Excel spreadsheet and then subjected to a one-way ordered analysis of variance (ANOVA) and Duncan's post-hoc test were used to make comparisons between herbal teas. For the same column, numbers with different letters are statistically significant at the 5% level. Data are presented as mean  $\pm$  standard deviation (SD).

## **RESULTS:**

**Ethno-nutritional Survey:** Our survey aimed to assess levels of knowledge, consumption, culture and forms of consumption. We interviewed a total of 398 people.



FIG. 2: SOCIODEMOGRAPHIC CHARACTERISTICS (GENDER, AGE, LEVEL OF EDUCATION AND REGION OF AFFILIATION)

It shows that the proportion of women (68.6%) was higher than that of men. The most represented age

group was 31-40 years (42.0%); we surveyed mainly Western nationals (28.4%) and the most

frequent level of education was secondary school with 45.7%.

**Eating Oyster Mushrooms:** Fig. 3 below shows the level of consumption of oyster mushrooms according to the sociodemographic parameters of the respondents. It shows that the most well-known and most consumed species were: *Pleurotus sajor caju* (16.6%), *Pleurotus floridanus* (32.7%), *Pleurotus pulmonarius* (47.7%) and *Pleurotus*  ostreatus (61.1%). Based on Pearson's and Fisher's exact chi-square independence tests, we compared the different percentages. It was found that: the consumption of *Pleurotus pulmonarius* was significantly dependent (p value 0.0001) on the level of education. On the other hand, that of *Pleurotus floridanus* was significantly dependent on the age group (p value 0.02).



FIG. 3: LEVEL OF CONSUMPTION OF OYSTER MUSHROOM SPECIES AS A FUNCTION OF SOCIODEMOGRAPHIC PARAMETERS OF POPULATIONS

**Table 1** below presents the results of the producer survey. It shows that *Pleurotus ostreatus* and *Pleurotus pulmonarius* were the most cultivated species among all growers, i.e. 100%. However, *Pleurotus floridanus* and *Pleurotus sajor caju* were produced at 80% and 50% respectively.

TABLE	1:	CULTURE	LEVEL	OF	OYSTER
MUSHR	DOM	S			

Crop species	Cultivation level (%)
Pleurotus ostreatus	100
Pleurotus pulmonarius	100
Pleurotus floridanus	80
Pleurotus sajor caju	50

**Form of Consumption: Fig. 4** below shows the different forms of consumption. It shows that: herbal tea was the weakest form of herbal tea, at 0.8%.





below shows the results obtained from the mixing plan. We used the Statgraphics centurion software version XVI. We obtained 13 formulations. Indeed, these 13 formulations represented 13 herbal teas.

|--|

	Factors		
N°	Pleurotus pulmonarius	Pleurotus ostreatus	Pleurotus floridanus
1	0.33	0.33	0.33
2	0.4	0.3	0.3
3	0.37	0.32	0.32
4	0.37	0.33	0.3
5	0.3	0.33	0.37
6	0.3	0.37	0.33
7	0.33	0.37	0.3
8	0.32	0,32	0.37
9	0.3	0.3	0.4
10	0.3	0.4	0.3
11	032	0.37	0.37
12	0.32	0.3	0.33
13	0.33	0.3	0.37

## Antioxidant Testing:

Antioxidant Power of mushroom Teas using the DPPH Method (2,2-diphenyl-1-picrylhydrazyl): DPPH (2, 2-diphenyl 1-picrylhydrazyl) is a free radical. The DPPH test measures the antioxidant power of a substance through its ability to scavenge DPPH free radicals. Fig. 5 and Table 3 below show the percentage of inhibition (% I) and the inhibitory concentration 50 (CI 50), respectively, of the different herbal teas and the standard (Ascorbic Acid).



FIG. 5: INHIBITORY POWER OF MUSHROOM TEAS AND ASCORBIC ACID

 TABLE 3: INHIBITORY CONCENTRATIONS 50 (CI50) IN MG/L OF MUSHROOM TEAS AND ASCORBIC ACID

 FOR DPPH TEST

Mushroom teas	IC <sub>50</sub> mg / L
T1	$19.77 \pm 0.42^{ m bc}$
T2	$18.93 \pm 0.31^{ m bc}$
Τ3	$24.69 \pm 0.20^{ m ef}$
T4	$22.15 \pm 1,20^{cde}$
T5	$17.39 \pm 0.35^{\rm b}$
T6	$17.65 \pm 1,20^{\rm b}$
Τ7	$19.44 \pm 1,00^{ m bc}$
Τ8	$20.06 \pm 1,20^{ m bc}$
Т9	$23.70 \pm 1,22^{def}$
T10	$25.44 \pm 2,02^{ m f}$
T11	$20.56 \pm 0,60^{ m bc}$
T12	$30.12 \pm 5{,}02^{ m g}$
T13	$21.31 \pm 1,56^{cd}$
Aas	$3.09 \pm 1.56^{a}$

Data are presented as a mean  $\pm$  standard deviation (SD). The one-factor Ordered Analysis of Variance (ANOVA) and Duncan's post-hoc test were used to make comparisons between herbal teas. For the same column, numbers with different letters are statistically significant at the 5% level.

It can be seen from this figure that the % I of our mushroom teas on DPPH varied between 0 and 62% for concentrations ranging from 0 to 1000 ppm. However, among our herbal teas, the ones

with the best % I were included: T5 (61.7% per 1000 ppm), T2 (55.09% per 1000 ppm) and T6 (55.03% per 1000 ppm). The data obtained by determining the 50 inhibitory concentrations

allowed us to learn more. This is because the smaller the  $IC_{50}$  of a compound, the higher the capacity of a compound.

It can be seen from the table above that T5, T6 and T2 had the best  $CI_{50}$ :  $17.39 \pm 0.35^{b}$ ,  $17.65 \pm 1.20^{b}$ , and  $18.93 \pm 0.31^{b}$ , respectively. Based on Anova's 01-factor test, we found that these differences between the best herbal teas mentioned above were not statistically significant.

In addition, compared to the data in the standard (Ascorbic Acid), we realize that it appears with a % I of 78% per 1000 ppm and a  $CI_{50}$  of  $3.09 \pm 1.56^{a}$ . A comparison was made between our best mushroom teas and ascorbic acid, we obtained statistically significant differences at p-value < 5%.

Antioxidant Power of Mushroom teas by the FRAP Method: The FRAP method is used to

determine the ability of our herbal teas to reduce the ferric ion (Fe3+) to the ferrous ion (Fe2+). **Fig. 6** and **Table 4** below show the percentages of inhibition and IC<sub>50</sub> of the different herbal teas and the standard, respectively.



FIG. 6: REDUCING POWER OF MUSHROOM TEAS AND ASCORBIC ACID

TABLE 4: INHIBITORY 50 MG/L CONCENTRATIONS OF MUSHROOM TEAS AND ASCORBIC ACID FOR THE TEST OF FRAP

Mushroom teas	IC <sub>50</sub> mg / L
T1	$15.22 \pm 0.03^{ m hi}$
Τ2	$13.53 \pm 0,69$ fg
Т3	$7.72 \pm 2,51^{ m b}$
Τ4	$13.15 \pm 0.40^{ m efg}$
Т5	$13.64 \pm 0.06^{ m fg}$
T6	$16.39 \pm 0.06^{ m i}$
Τ7	$11.96 \pm 0.30^{de}$
Τ8	$12.75 \pm 0.77^{ m ef}$
Т9	$10.16 \pm 0.14^{\circ}$
T10	$15.33 \pm 0.03^{ m hi}$
T11	$11.00 \pm 0.17^{\rm cd}$
T12	$14.48 \pm 0.76^{ m gh}$
T13	$15.63 \pm 0.03^{ m hi}$
Aas	$1.80\pm1.06^{\mathrm{a}}$

Data are presented as a mean  $\pm$  standard deviation (SD). The one-factor Ordered Analysis of Variance (ANOVA) and Duncan's post-hoc test were used to make comparisons between herbal teas. For the same column, numbers with different letters are statistically significant at the 5% level.

It can be seen from this figure that the percentages of Reduction (% R) of our mushroom teas for the FRAP test varied between 0 and 91.33% for concentrations ranging from 0 to 1000 ppm. However, among our herbal teas, the ones with the best % R were: T3 (91.01% Rper 1000 ppm), T9 (90.88% R per 1000 ppm) and T2 (89.18% R per 1000 ppm) compared to the % R of Ascorbic Acid which was 91.33. The data obtained by determining the inhibitory concentrations 50 allowed us to better appreciate the effect of our mushroom teas on the reduction of the ferric ion (Fe3+) present in the potassium ferricyanide complex [K3Fe (CN)6] to the ferrous ion (Fe2+). It can be seen from the table above that T3, T9 and T11 mushroom teas had IC50 namely:  $7.72 \pm 2.51^{\text{b}}$ ,  $10.16 \pm 0.14^{\text{c}}$  and  $11.00 \pm 0.17^{\text{cd}}$ , compared to that of the standard either:  $1.80 \pm 1.06^{\text{a}}$ .

Based on the Anova 01-factor test, we found that the differences between T3 and T9 teas on the one hand and T3 and T11 were statistically significant p-value < 5%. In addition, the standard CI 50 of  $1.80 \pm 1.06^{a}$  was statistically different and significant compared to other herbal teas with a pvalue < 5%. Antioxidant Power of Mushroom teas by the Peroxide Method: Hydrogen peroxide is an oxidizing agent, capable of causing several damages within the body. We evaluated the ability of our mushroom teas compared to that of the standard to neutralize hydrogen peroxide.

Fig. 7 and Table 5 below show the inhibition percentages and CI<sub>50</sub>. It can be seen from this figure that the percentages of inhibition (% I) of our mushroom teas for the peroxide test varied between 0 and 74.28% for concentrations ranging from 0 to 1000 ppm. However, among our mushroom teas, the ones with the best % I were in particular: T1 (37.05% per 1000 ppm), T3 (35.98% per 1000 ppm), T4 (32.19% per 1000 ppm) and T11 (32.19% per 1000 ppm) compared to % I of Ascorbic Acid which was 74.28%. The data obtained by determining the 50 inhibitory concentrations allowed us to have an overview of the effect of our mushroom teas on peroxide inhibition.



FIG. 7: INHIBITORY POWER OF MUDHROOM TEAS AND ASCORBIC ACID ON HYDROGEN PEROXIDE

<b>TABLE 5: INHIBITORY CONCENTRATIONS 50 IN</b>	
MG/L OF MUSHROOM TEAS AND ASCORBIC ACID	
FOR PEROXIDE TEST	

Mushroom teas	IC <sub>50</sub> mg / L
T1	$23.44 \pm 2,94^{b}$
T2	$32.59 \pm 11,46^{\rm bc}$
Т3	$24.98 \pm 4,79^{ m b}$
T4	$26.37 \pm 0.30^{ m b}$
T5	$29.32 \pm 1,06^{ m bc}$
Τ6	$30.87 \pm 0.71^{\rm bc}$
Τ7	$41.46 \pm 10,57^{ m d}$
Τ8	$30.23 \pm 4,69^{\rm bc}$
Т9	$27.37 \pm 1,89^{b}$
T10	$27.88 \pm 2.13^{b}$
T11	$26.37 \pm 0.30^{b}$
T12	$28.74 \pm 2,52^{ m bc}$
T13	$37.98 \pm 2,94^{cd}$
Aas	$5.82 \pm 1.27^{a}$

Data are presented as a mean  $\pm$  standard deviation (SD). The one-factor Ordered Analysis of Variance (ANOVA) and Duncan's post-hoc test were used to make comparisons between herbal teas. For the same column, numbers with different letters are statistically significant at the 5% level.

It can be seen from the table above that the mushroom teas with the best CI50 were: T1 (23.44  $\pm 2.94^{\text{b}}$ ), T3 (24.98  $\pm 4.79^{\text{b}}$ ), T4 (26.37  $\pm 0.30^{\text{b}}$ ) and T11 (26.37  $\pm 0.30^{\text{b}}$ ). We compared the IC<sub>50</sub> of the best mushroom teas, we noticed that they are not statistically different. In addition, the IC50 value of Ascorbic Acid was 5.82  $\pm 1.27^{\text{a}}$ , which was statistically different and significant at p-value < 5%.

**Secondary Metabolite Levels:** The table below summarizes the data from the determination of the total polyphenols, total flavonoids, and condensed tannins of mushroom teas.

Herbal teas	Polyphenols (mg Eq AG/100 mg)	Tannins (mg Eq Cat/100 mg)	Flavonoids (mg Eq Quer/100 mg)
T1	$19,64 \pm 0,92^{\rm e}$	$251,58 \pm 15,54^{\rm bc}$	$136,73 \pm 1,15^{de}$
T2	$41,\!48 \pm 9,\!47^{ m g}$	$253,39 \pm 16,37^{\rm bc}$	$134,07 \pm 5,69^{cde}$
T3	$12,83 \pm 0,74^{bcd}$	$247,64 \pm 5,45^{\rm bc}$	$152,07 \pm 8,02^{ m h}$
T4	$26,\!45\pm0,\!77^{ m f}$	$252,48 \pm 42,76^{\rm bc}$	$141,40 \pm 4,36^{\rm ef}$
T5	$6,16 \pm 0,96^{\mathrm{a}}$	$225,82 \pm 8,77^{ m ab}$	$139,73 \pm 1,53^{\rm ef}$
T6	$3,54 \pm 0,12^{ m a}$	$230,97 \pm 3,78^{ m abc}$	$128,40 \pm 2,65^{ m abc}$
Τ7	$16,23 \pm 2,34^{de}$	$244,00 \pm 1,57^{\rm bc}$	$173,07 \pm 0,58^{\rm i}$
T8	$7,72 \pm 0,21^{ m ab}$	$234,00 \pm 11,02^{\rm bc}$	$152,07 \pm 5,03^{ m h}$
Т9	$8,86\pm2,02^{\rm abc}$	$258,24 \pm 11,37^{\circ}$	$125,\!40\pm4,\!36^{\mathrm{ab}}$
T10	$7,72 \pm 0,43^{ m ab}$	$243,09 \pm 9,23^{\rm bc}$	$122,73 \pm 2,31^{a}$
T11	$7,72 \pm 0,37^{ m ab}$	$203,39 \pm 15,14^{\rm a}$	$131,07 \pm 2,31^{bcd}$
T12	$13,33 \pm 1,28^{\rm cd}$	$242,79 \pm 14,72^{\rm bc}$	$146,73 \pm 4,51^{ m gh}$
T13	$5.24 \pm 0.89^{a}$	$244.61 \pm 11.51^{\rm bc}$	$154.07 \pm 3.51^{\rm h}$

Data are presented as a mean  $\pm$  standard deviation (SD). The one-factor Ordered Analysis of Variance (ANOVA) and Duncan's post-hoc test were used to make comparisons between herbal teas. For the same column, numbers with different letters are statistically significant at the 5% level.

In terms of total polyphenol content, the best mushroom teas were: T2 ( $41.48 \pm 9.47^{g}$ ), T4 (26.45  $\pm 0.77^{f}$ ) and T1 (19.64  $\pm 0.92^{e}$ ). We note that these data are statistically significant at p-value < 5%.

Speaking of condensed tannins, the best mushroom teas included: T9 ( $258.24 \pm 11.37^{\circ}$ ), T2 ( $253.39 \pm 16.37b^{\circ}$ ) and T4 ( $252.48 \pm 42.76b^{\circ}$ ). However, these values were not statistically significant and still statistically different from each other.

Total flavonoid levels varied from one mushroom teas to another; however, the best herbal teas were T7  $(173.07 \pm 0.58^{i})$ , T13  $(154.07 \pm 3.51^{h})$ , T3 and T8  $(152.07 \pm 5.03^{h})$ . T13, T3 and T8 teas were not statistically different and significant; in contrast, the flavonoid content of T7 tea was statistically significant at p-value < 5% and statistically different from other herbal teas.

**DISCUSSION:** The results of the ethno-nutritional survey showed that the rate of women surveyed was higher than that of men. Indeed, confirming the place of women in society. Additionally, we observed that the most represented age group was 31-40 years old (42%), and 45.7% of the population had a high school education. These findings are consistent with those of several authors  ${}^{6,7}$ .

Furthermore, the survey results revealed that Pleurotus ostreatus and Pleurotus pulmonarius were the most widely cultivated, known and consumed species due to their morphology. the least represented form Moreover, of consumption was herbal tea (decoction or infusion). Previous work by Ninkwangodemonstrated that oyster mushrooms contain the 8 amino acids essential for the proper functioning of the body, unsaturated fatty acids, mineral salts, and vitamins, ergosterol, which helps to reduce cholesterol levels in the blood, cellulosic fibers that help alleviate heart disease, control overweight and blood sugar levels, and chitinous fibers that facilitate mechanical digestion<sup>8, 9</sup>. The results showed that total polyphenols, flavonoids, and condensed tannins were all present in our mushroom teas, at different concentrations <sup>10</sup>. Total polyphenols and flavonoids were statistically significant at P-value < 5%. Additionally, we obtained a non-negligible CI<sub>50</sub>.

The work of Mbang had shown that the aqueous extracts of mixtures of three oyster mushroom species had remarkable antioxidant capacities than any other extract given their water content, thus facilitating the extraction of polar compounds such as total polyphenols. These results justify our choice of mushroom teas. In the same vein, Etoundi's work had shown that the individual extraction of oyster mushroom species had limitations, it had obtained phenolic compound contents of the order of  $22 \pm 8.83$  mgEC/g and  $17.28 \pm 4.75$  mgEC/g; these values were lower than the values obtained by Mbang who had obtained a value of 40 mgEC/g. All these values were lower than those obtained by us. Indeed, the T2 mushroom tea had a value of:  $41 \pm 9.47$  mg Eq AG/100 mg. In addition, we noted that several authors had shown that the substrate on which the mushrooms were grown could significantly influence the content of secondary metabolites and, in turn, the antioxidant capacity  $^{2,3}$ .

**CONCLUSION:** Oyster mushrooms, as we mentioned earlier, are functional foods due to their composition. Indeed, they are endowed with different secondary metabolites. namely: polyphenols, flavonoids and tannins. Additionally, they also contain vitamins (A and B), proteins, minerals, fibre and trace fats. The results of the ethnonutritional survey revealed that Pleurotus ostreatus and Pleurotus pulmonarius were the most well-known and consumed species. Oyster mushroom teas exhibited antioxidant capacities and non-negligible levels of secondary metabolites. In short, herbal teas based on 03 species of oyster mushrooms can have a positive effect on long-term health.

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## **CONFLICTS OF INTEREST:** Nil

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