

EVALUATION OF THE ACTIVITY OF ALPHA AMYLASE AND ANTIOXIDANT POTENTIAL FORMULATIONS OF THREE VARIETIES OF OYSTER MUSHROOMS: *PULMONARIUS*, *FLORIDANUS* AND *SAJOR-CAJU*

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RESUME

Le présent travail a pour but d'apporter une contribution à l'amélioration du traitement contre le diabète. Pour ce faire, trois variétés de champignons comestibles du genre pleurote ont été mélangées (ou formulées) afin d'étudier le potentiel antioxydant des extraits bruts issus de ces champignons formulés par les tests DPPH, FRAP et Folin et d'en déterminer ensuite l'influence de ces extraits sur l'activité de l'alpha amylase. En termes de résultats, on a obtenu 4 formulations et 16 extraits. Pour ce qui est du test antioxydant, le test par le DPPH a permis d'obtenir un meilleur IC₅₀ de 4,77 mg/ml contre 0,61 mg/ml chez le standard. Aussi, pour ce qui est du test de FRAP on a obtenu des extraits qui présentent une activité supérieure au standard avec des valeurs de 2853,33mgEC/g et de 2805,94mgEC/g contre 2781,85 mgEC/g du standard. Et, pour ce qui est du test de Folin, bien que le standard a présenté un taux de 57,03±0,55mgEC/g, les extraits renferment néanmoins une bonne teneur en composés phénoliques avec un taux de 37,89±1,48mgEC/g pour l'extrait le plus riche en composés phénoliques. Enfin, pour ce qui est du test de l'alpha amylase, les extraits précisément aqueux, éthanolique et hydroéthanolique de chaque formulation ont présentés une meilleure activité inhibitrice que le standard. Ces résultats suggèrent que les extraits issus des champignons formulés présentent une activité antioxydante considérable et une bonne capacité à inhiber l'activité de l'alpha amylase.

Mots clés : Diabète, champignons pleurotes, antioxydants et alpha amylase

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ABSTRACT

The aim of this work is to contribution to improving treatment for diabetes. To do this, three varieties of edible mushrooms of the genus oyster mushrooms were mixed (or formulated) to study the antioydan potential of raw extracts from these mushrooms formulated by the DPPH, FRAP and Folin tests and then to determine the influence of these extracts on alpha amylase activity. In terms of results, 4 formulations and 16 extracts were obtained. For the antioxidant test, the DPPH test resulted in a better IC_{50} of 4.77 mg/ml compared to 0.61 mg/ml in the standard. Also, for the FRAP test, extracts were obtained that showed above-standard activity with values of 2853.33mgEC/g and 2805.94mgEC/g versus 2781.85 mgEC/g of standard. And, for the Folin test, although the standard had a rate of 57.03 ± 0.55 mgEC/g, the extracts nevertheless contain a good phenolic compound content with a rate of 37.89 ± 1.48 mgEC/g for the most phenolic compound extract. Finally, for the alpha amylase test, the precisely aqueous, ethanolic and hydroethanolic extracts of each formulation showed better inhibitory activity than the standard. These results suggest that extracts from formulated mushrooms exhibit considerable antioxidant activity and a good ability to inhibit alpha amylase activity.

Keywords: Diabetes, mushrooms, antioxidants and alpha amylase

INTRODUCTION

Diabetes is a chronic disease that occurs when the body is unable to produce enough insulin or use insulin effectively (WHO, 2015). In the case of diabetes, hyperglycemia increases the increased growth of free radicals thus creating a state of oxidative stress in the body: this condition is usually the cause of complications associated with diabetes or even death of the patient. A poor balance of diabetes is responsible for the development of cardiovascular complications by alteration of blood vessels, and represents: 3 to 6 times more risk of developing heart disease, 25% of kidney damage requiring dialysis, the leading cause of blindness, and 50% of lower limb amputations (Kaleem *et al.*, 2006).

According to the IDF, the number of adults living with diabetes worldwide in 2000 was estimated at 151 million. By 2009, it had risen to 8.8%, or 285 million. Today, according to calculations, 9.3% of adults aged 20 to 79, or 463 million people, live with diabetes (IDF, 2019). In Africa, WHO estimates that diabetes has killed more than 300,000 Africans in 2017 and is projected to reach 41 million people with diabetes by 2045 on the continent with a 156% increase in cases in less than 30 years, and Africa will then be the region of the world with the highest increase in the disease (WHO, 2018). In the face of this pandemic, Cameroon is not left behind, according to WHO statistics in 2016, Cameroon recorded 2,340 deaths due to diabetes in adults aged 30 to 69 and 2060 deaths in adults over 70 years, with a percentage of 9.2% of adults suffering from diabetes (WHO, 2016).

Antioxidant supplementation would be essential. Although there are anti-diabetic pharmaceuticals to regulate blood sugar and avoid a state of oxidative stress, these have limitations such as the presence of side effects, high cost: not accessible to all... hence our interest in finding functional foods like edible mushrooms. The work

carried out by Etoundi *et al.* in 2017 on edible mushrooms specifically the genus of oyster mushrooms (which is the subject of myciculture in our country) has shown that oyster mushrooms have antiradical and hypoglycemic properties that can overcome certain metabolic diseases such as diabetes which is growing in size (Dubost *et al.*, 2007). However, the limit of this work was to find that the effect of these mushroom properties was only observed at high concentrations. In order to make our contribution on diabetes therapy, it has been proposed to increase the effectiveness of oyster mushrooms by formulations of three different varieties of oyster mushrooms namely *pleurotus pulmonarius*, *pleurotus floridanus* and *pleurotus sajor caju*. In this work, it will be a question of studying the antioxidant activity of extracts of these edible mushroom formulations and also studying their influence on the activity of alpha amylase which is an enzyme of the digestion of starch (glucose polymer whose degradation in the body increases the level of glucose in the blood).

MATERIALS AND METHODS

I-Preparation of experimental plant material:

I-1-Harvest and mushroom formulation:

Fresh mushrooms were harvested in Douala in a myciculture in Yassa and then dried and crushed finally to get mushroom powder. The different powders obtained from each mushroom were subsequently distributed in the following proportions in order to obtain the mushroom formulations: 1/1/1; 2/0.5/0.5; 0.5/2/0.5 and 0.5/0.5/2.

I-2-preparation of raw mushroom extracts:

The aqueous, hydroethanolic, ethanolic and hexanic extracts were prepared at the Biochemistry Laboratory at the University of Douala. Indeed, the different formulation powders were macerated each in proportions 1/8 respectively with the following solvents: distilled water, water/ethanol mixture 1:1, ethanol and hexane and then dried in the oven for 4 days at 45°C temperature.

I-3- Evaluation of antioxidant activity of formulation extracts:

I-3-1- Evaluation of antiradical Activity DPPH (2,2-diphenyl-1-picrylhydrazyl):

The DPPH test was carried out here by the method described by **Katalinić *et al* in 2003** with some modifications. An 1ml intake of a DPPH solution (0.11 mg/ml) prepared in absolute ethanol was added to 50 µl of extract at concentrations of 1; 3; 5; 7; 10; 15; 20 and 25mg/ml. Ascorbic acid prepared in the same concentrations as the extracts served as a standard. The absorbances were measured at 517 nm after incubation of the solutions for 30 minutes in the dark. Control was the DPPH solution without extract.

The trapping percentage of the DPPH radical was calculated according to the following equation:

$$\% \text{of trapping} = [A1 - A2] / A1 \times 100$$

A1: absorption of control.

A2: absorption in the presence of extract.

I-3-2- Determination of polyphenolic concentration by Folin method:

This method (**Singleton and Rossi, 1965**) is based on the reduction of a phosphomolybdic chromogen - tungstic by an antioxidant. The polyphenolic concentration of the extract was measured using the Folin-ciocalteu 2N reagent diluted 10 times before use to achieve a concentration of 0.2N. After adding 30 µl of solution of each extract prepared at the concentration of 100mg/ml in test tubes, 1 ml of Folin solution diluted 10 times was introduced, the absorption was measured at 750 nm after 30 minutes of reaction using a spectrophotometer. The catechin was used to trace the calibration curve.

I-3-3- Iron-reducing activity (FRAP):

The reductive power of the extracts was determined using the **Oyaisu method (1986)** and modified by **Bakchiche and Gherib in 2014**. In

each test tube containing 0.1 ml of sample solution (extract or BHT prepared at different concentrations: 1; 5; 10; 15 and 20mg/mL), 2ml of distilled water and 2 ml of potassium hexacyanoferrate [K₃Fe (CN)₆] (10g/l) were added. The whole thing was incubated in the bath marie at 50°C for 30 minutes. A volume of 2 ml of trichloroacetic acid (100 g/l) was then added and the mixture was centrifuged to 3000 rpm for 10 minutes.

After centrifugation, 2ml of the supernatant is mixed with 2ml of distilled water and 0.4 ml of iron chloride [FeCl₃] (1g/l). The reading was measured at 700 nm. Negative control was prepared under the same conditions as the test tubes with the only difference that the sample solution was replaced by methanol. BHT (Butyl hydroxytoluene) was used for positive control.

I-4 Evaluation of the effect of mushroom formulations on the enzyme associated with glucose metabolism: pancreatic alpha amylase

The inhibitory activity of the extract's amylase was carried out using the standard procedure with minor modification of **Thalapaneni *et al.* in 2008**. In suitable tubes, 100 µl of extract and acarbose extract prepared at concentrations of 1; 5; 10; 15 and 20 mg/ml are added in the reaction medium; the reaction medium being a mixture of 20µl of enzyme (30 µg/ml) and 1380 µl of tris-HCl pH 6.8 buffer. The set was then pre-cooked at 30° C. for 20min. Then the reaction was initiated by adding 100 µl of starch (1%) solution. After 20 minutes of incubation, the enzymatic reaction was stopped by adding 2ml of acidified iodine, the absorption was read at 500nm.

The inhibitory activity of amylase was measured using the formula:

$$\% \text{ inhibition of amylase} = [1 - (A/B)] \times 100$$

Where A - absorption of the test well (extract or acarbose)

B - absorption of enzymatic control

STATISTICAL ANALYSIS:

The data were entered into an Excel spreadsheet (Microsoft Office 2007) and then subjected to an orderly analysis of one-factor variances (ANOVA) and the results were expressed, in the form of an average-standard deviation. The normality of the distribution of values was assessed with the Shapiro-Wilk test. The differences between the sample averages were compared and discriminated using the Tukey test

when the values had a normal distribution or the Games-Howell test if not. All the scans were done with the SPSS 20.0 software for Windows, and the significance threshold was set at $P < 0.05$.

RESULTS

I- Obtaining extracts of formulations:

I-1 Preparation of different mushroom formulations:

The different mushroom powders obtained after drying and grinding the three different species of oyster mushrooms resulted in Table I:

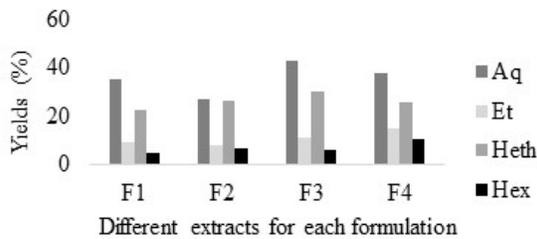
Table I: Table of obtaining different formulations

Type of formulations	Proportions	Combination	Types of extracts of each formulation	Codification extracts
F1	1 : 1 : 1	A : B : C	Aqueous	F1aq
			Ethanolic	F1et
			Hydroethanolic	F1heth
			Hexanic	F1hex
F2	2 : 0.5 : 0.5	A : B : C	Aqueous	F2aq
			Ethanolic	F2et
			Hydroethanolic	F2heth
			Hexanic	F2hex
F3	0.5 : 2 : 0.5	A : B : C	Aqueous	F3aq
			Ethanolic	F3et
			Hydroethanolic	F3heth
			Hexanic	F3hex
F4	0.5 : 0.5 : 2	A : B : C	Aqueous	F4aq
			Ethanolic	F4et
			Hydroethanolic	F4heth
			Hexanic	F4hex

Table I results in four formulations of mushrooms, each of which has been macerated in four different solvents: distilled water, water/ethanol mixture: 1/1, ethanol and hexane making four extracts for each formulation. Hence, in the end, we obtained sixteen extracts of formulations.

I-2 Extraction yield:

Figure 1 below presents four formulations, each of which contains four excerpts. This figure shows that water extracts of any formulation have the best yields, then there are the hydroalcoholic extracts, then alcoholic extracts and finally hexanic extracts that have the lowest yields.



F1 ,F2, F3, and F4 = different formulations; Aq, Et, Heth and Hex are respectively the various extracts Aqueux, Ethanolic, Hydroethanolic and Hexanic

Figure1: Extraction performance of different formulation extracts.

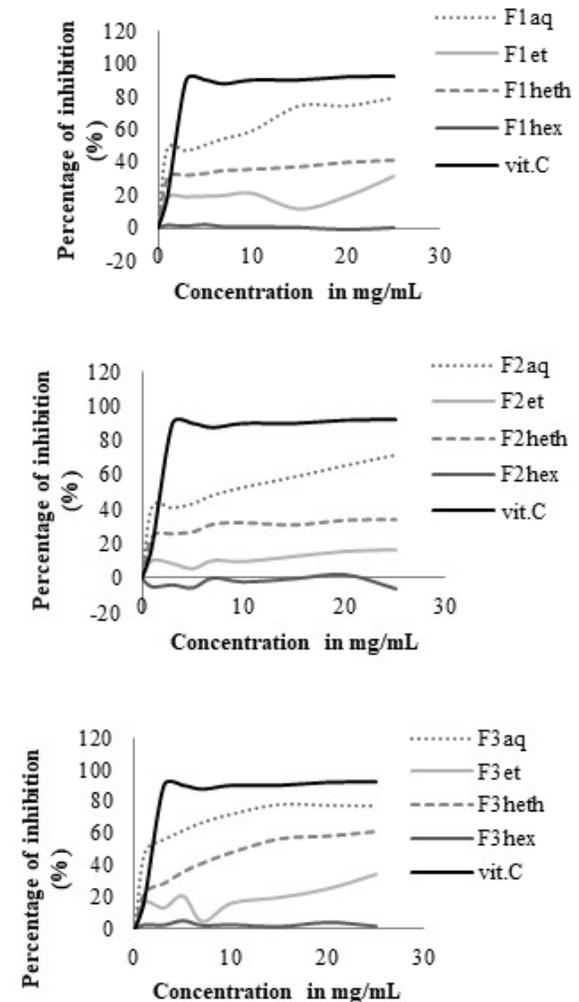
II- Evaluation of antioxidant activity of formulation extracts:

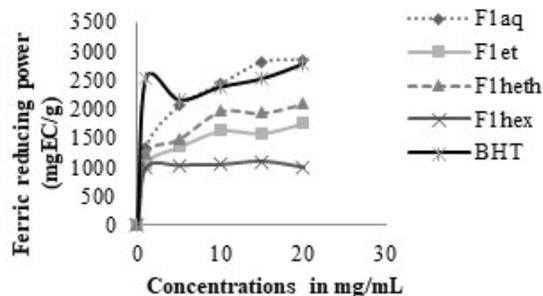
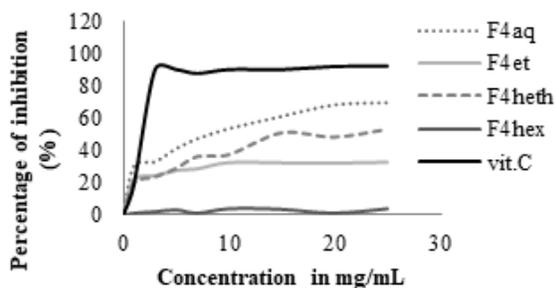
II-1 Evaluation of antiradical activity DPPH (2,2-diphenyl-1-picrylhydrazyl):

The various figures below in Figure 2 show the percentages of inhibitions of the extracts of each formulation according to concentration. Like extraction yield, it is apparent from these four figures that, whatever formulation is used, the water extract compared to the standard that is vitamin C appears to be the extract with the most inhibitory activity, then the hydroethanolic extract, then the ethanolic extract and finally the hexanic extract which has lower activity. However, at $P < 0.05$, whether for formulation 1, formulation 2 or formulation 4, there is a significant difference between the different aqueous extracts and the standard only formulation 3 does not present a significant difference between its aqueous extract and the standard.

Also, from Figure 2 comes Table II which presents the inhibitory concentrations 50 (IC_{50}): a

concentration for which the antioxidants present in the extracts inhibit 50% of free radicals. It is determined graphically according to the logarithmic regression equation of inhibition percentages based on logarithm of increasing concentrations of each of the samples tested. Note that the antioxidant capacity of a compound is all the higher as its IC_{50} is small. Thus compared to the standard that has an IC_{50} of 0.61 mg/mL, the best extract from this DPPH test is the aqueous extract of formulation 3 (F3aq) with an IC_{50} of 4.77 mg/mL followed by the aqueous extract of formulation 1 (F1aq) with an IC_{50} of 7.70 mg/mL. It is therefore noted from the different figures 2 and the tableau of IC_{50} that the best formulations are formulations 1 and 3 (F1 and F3).





F1, F2, F3, and F4 are respectively the different formulations 1, 2, 3 and 4; Aq, And, Heth and Hex are respectively the various extracts aqueous, ethanolic, hydroethanolic and hexanic, each formulation contains these 4 extracts

Figure 2: Percentage inhibition of extracts from each formulation based on concentration and compared to standard (vitamin C)

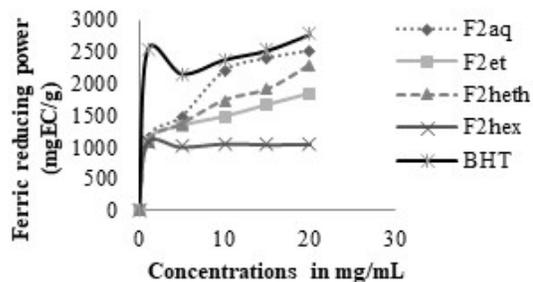
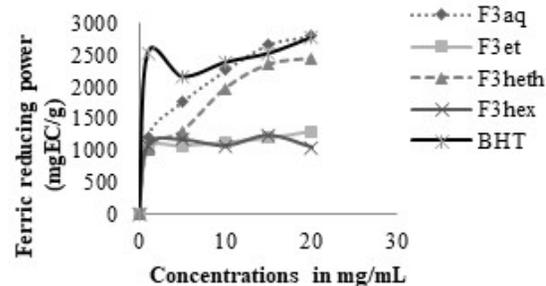


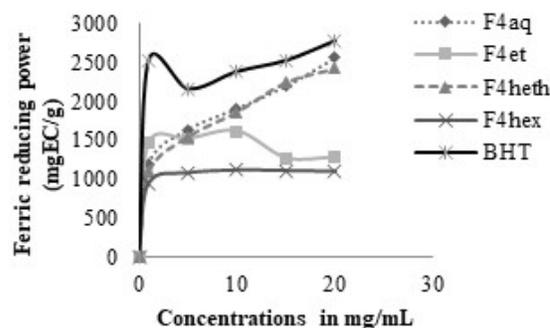
Table II: f IC₅₀ (in mg/mL) of different formulation extracts

Formulations Extracts	F1	F2	F3	F4
Aq.	7.70	11.34	4.77	11.74
Heth.	29.13	39.06	14.81	18.99
Et.	66.69	91.38	45.48	40.73
Hex.	0	1749.5	2166.18	545.27
Vit. C	0.61			



II- 2 Iron-reducing activity (FRAP):

The different figures 3 below show the reductive power of iron according to concentration. Like the inhibitory activity of DPPH, it is apparent from these four figures that, whatever formulation is used, the water extract compared to the standard that is BHT appears to be the extract with the highest reducing power, then the hydroethanolic extract then the ethanolic extract and finally the hexanic extract that has the lowest activity. It is also observed in Figure 3 that the best formulations are formulations 1 and 3 where extracts are observed that have a reducing power greater than the standard, notably the F1aq and F3aq extracts that presented respectively values 2853.33mgEC/g and 2805.94mgEC/g against 2781.85mgEC/g BHT, however, for P<0.05, there is no significant difference between these aqueous extracts and BHT.



F1, F2, F3, and F4 are respectively the different formulations 1, 2, 3 and 4; Aq, And, Heth and Hex are respectively the various extracts aqueous, ethanolic, hydroethanolic and hexanic, each formulation contains these 4 extracts

Figure3: Iron-reducing power of extracts from each formulation based on concentration and compared to standard

II-3 Phenolic compound content by Folin method:

Table 3 below shows the different levels of phenolic compounds in formulation extracts. This table shows that at $P < 0.05$, although there is a significant difference between the vitamin C used here as standard with the different formulation extracts, the extracts nevertheless have a considerable content of phenolic compounds, particularly the ethanol extract of formulation 1 and the aqueous extract of formulation 2. Hence formulations 1 and 2 are the best formulations because contain the extracts with the best phenolic compound content. In terms of formulation 1, there is a polyphenol level of 37.89 ± 1.48 mgEC/g (catechine equivalent milligram per gram of mushroom) for ethanolic extract and a rate of 36.66 ± 0.46 mgEC/g for the aqueous extract of formulation 2. Hexanic extracts compared to other extracts appear to be the extracts with the lowest phenolic compound content.

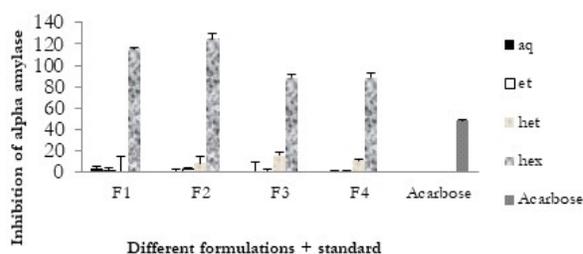
Table III: Phenolic compound content of extracts and standard in mgEC/g

Formulations Extracts	F1	F2	F3	F4
Aq.	23.84 ± 10.35	36.66 ± 1.48	32.61 ± 1.23	28.42 ± 0.56
Heth.	33.69 ± 2.27	26.21 ± 2.30	25.16 ± 1.10	17.85 ± 1.42
Et.	37.89 ± 0.47	36.20 ± 1.68	30.24 ± 4.42	32.88 ± 2.38
Hex.	6.65 ± 0.94	1.57 ± 3.05	12.55 ± 2.47	8.59 ± 0.61
Vit. C	57.03 ± 0.55			

III- Evaluation of the effect of mushroom formulations on alpha amylase activity:

The effect of mushroom formulations on alpha amylase activity was shown in Figure 4 below. Indeed, Figure 4 presents the behavior of alpha amylase in the presence of formulation extracts and acarbose used here as standard. Thus, in Figure 4 there is a good inhibition of alpha amylase by extracts from formulations that exhibit a higher activity than the standard at the

exception of hexanic extracts. It therefore resorts from the statistical analysis of this figure that at $P < 0.05$ there is no significant difference between the standard and the formulations of mushroom with respect to the average inhibitions. However, there is a significant difference between the standard and the formulation extracts at $P < 0.05$. Thus, in terms of formulation 1, there is a significant difference between the standard and ethanolic, hydroethanolic and hexanic extracts. In contrast, formulations 2, 3 and 4 are rather a significant difference between the standard and the aqueous and hexanic extracts. Formulations 3 and 4 are the best formulations.



F1,F2,F3, and F4 = different formulations; Aq, Et, Heth and Hex are respectively the various extracts Aqueux, Ethanolic, Hydroethanolic and Hexanic; Acarbose = standard

Figure 4: Inhibition of alpha amylase activity by formulation extracts and acarbose

DISCUSSION

Mushrooms have great pharmacological potential and there is a lot of research in this area, although it is still insufficient. The most well-known historical and accidental discovery is that of antibiotics by Sir Alexander Fleming from strains of Penicillium, a microscopic fungus. The medical fields in which mushrooms are used are numerous: antibiotic therapy, oncology, parasitology, cardiology, dermatology, endocrinology, diabetology, etc. (Courtecuisse, 2011).

Extraction yields varied depending on the type of extract. Whether for formulation 1, 2, 3 or 4, the water extracts showed the best extraction yields than all the other extracts. Indeed, during extraction, solvents diffuse into solid plant matter

and solubilize compounds with a similar polarity (**Ncube *et al.*, 2008**); the selection of the extraction solvent will thus depend on the nature of the desired phytochemical, its solubility in this solvent, its polarity and its size. Thus, the higher yields observed in water extracts could be explained by the fact that the mushrooms being made up of nearly 90% water, most of these bioactive compounds would be hydrophilic, from which the water extracts due to their polarity fixed more active compounds; which gives them a higher yield than other extracts. These results were also obtained by **Hip *et al.* in 2009** who worked on the extraction of oyster mushrooms with two types of extracts: water extract and hydroethanolic extract and showed that extraction yield with distilled water was higher than extraction yield with water/ethanol mixture. In addition, previous studies have shown that alcohol (methanol or ethanol) and their mixture at different ratios are the most commonly used solvents for high recovery of phenolic compounds (**Sahreem *et al.*, 2010**) and obtaining better antioxidant activity (**Barros *et al.*, 2010**).

Antioxidants can reduce, inhibit, or delay the deleterious effect of free radicals on the body's macro-molecules and organs by trapping free radicals. Natural products from mushrooms or plants in particular with this property have been shown to play a protective beneficial role against oxidative stress caused in many chronic and degenerative diseases (**Kaur and Arora, 2009**). According to **Ceriello in 2003**, some anti-diabetic plants affect the functioning of pancreatic cells and the secretion of insulin through the suppression of oxidative stress following diabetes. This would be one of the potential mechanisms of action of mushrooms or antidiabetic medicinal plants.

The results obtained from the antioxidant activity of the mushroom formulations studied show that

the water extracts have a remarkable antioxidant effect than all other extracts, this was observed with the test of DPPH and FRAP: this would indicate the presence in the extracts analyzed of antioxidant molecules that could intervene by these two types of mechanism of action. On the other hand, the antiradical capacity of hexanic extracts is very low. These differences in antioxidant potential between extracts may be due to variation in the composition of extracts into anti-oxidant molecules (**Gulcin, 2006**), in other words, these differences in activity could be attributed to the various compounds extracted by solvents of different polarity. Indeed, as observed on extraction yield, the mushrooms being very rich in water would have for most of these bioactive compounds, polar compounds; thus the aqueous extracts because of their polarity fixed more active compounds and therefore more phenolic compounds from which their inhibitory power is higher than that of other extracts.

In addition **Kintzios and colleagues in 2010** showed that water extracts are believed to contain polar compounds such as flavonoids and glycosides that may be responsible for their antioxidant activity. These results suggest that mushroom extracts contain free radical trapping agents acting as primary antioxidants. The action of these antioxidants is believed to be due to their ability to donate hydrogen atoms or electrons derived mainly from the A-cycle hydroxyl of flavonoids (**Le *et al.*, 2007**).

In terms of phenolic compound content, the dosage of total polyphenols was done by the Folin-Ciocalteu method. This method is considered the best method of determining the rate of total polyphenols of plant extracts (**Djeridane *et al.*, 2010**). For this test, water extracts compete with ethanol extracts. In terms of formulations 2 and 3, water extracts contain the best levels of phenolic compounds, and for formulations 1 and

4 it is ethanol extracts that contain the best levels of phenolic compounds.

The oxidative processes are multiple and the nature of antioxidant activity can be multifaceted and attributed to different mechanisms such as free radical entrapry, chelation of transition metal ions, prevention of the initiation of a chain of reactions producing reactive oxygen species and peroxide decomposition (**Ozen, 2009**). Thus, the combination of several complementary antioxidant tests is useful in assessing the antioxidant potential of extracts (**Ksouri et al., 2009**).

These results from the DPPH and Folin test, based on previous work by **Etoundi et al** in 2017 that worked on isolated mushrooms of *Pleurotus pulmonarius* and *Pleurotus floridanus*, are observed by difference in results for the DPPH test; however, in terms of the Folin test, there is an importance of the formulation. Indeed, it was apparent from this work that the extract richest in phenolic compounds had a rate of $22.35 \pm 8.83 \text{ mgEC/g}$ followed by a next richer extract with a rate of $17.28 \pm 4.75 \text{ mgEC/g}$. On the other hand here with the formulations it is clearly observed that there are extracts which approach a rate of 40 mgEC/g with several extracts above 30 mgEC/g .

In light of ethnopharmacological data and experimental work, medicinal plants can carry out their anti-diabetic activities by inhibiting digestive enzymes (amylase and glucosidase) thus decreasing postprandial hyperglycemia (**kaleem et al., 2006**).

Indeed, the results obtained on the test of amylase, reveal that among the extracts of mushroom studied for the evaluation of the antidiabetic effect, some show their effectiveness in inhibiting the digestive enzyme hydrolyzing

dietary sugars (starch) in glucose. These are precisely the aqueous, ethanol and hydroethanol extracts of formulations 1, 2, 3 and 4 that showed better activity than the standard. Only hexanic extracts showed low activity. The inhibition of alpha amylase by extracts could be explained by **Fischer and Steinen** in 1964 by the fact that alpha-amylase being a calcium metallo-enzyme, inhibition or absence of calcium by a compound present in the extract contributes to the inactivation of this enzyme. Thus, according to **Faiveley** in 2010, the non-degradation of starch by the enzyme is rather due to the absence of the formation of the enzyme/substrate complex due to the phenolic compounds of the extracts that prevented the formation of the hydrogen bond between the amino acids of the enzyme fixation site and the polar groups (OH) of the carbon chain of the starch.

CONCLUSION

At the end of this work, which involved assessing the antioxidant activity of extracts from formulations from three species of oyster mushrooms, it appears that:

- Extracts from mushroom formulations, especially water extracts from F1, F2, F3 and F4 formulations, showed the best antioxidant activities followed by hydroethanolic extracts compared to the standard. Any time the F3 formulation appears to be the best formulation of all formulations.
- Extracts from mushroom formulations specifically aqueous, ethanol and hydroethanol extracts from F1, F2, F3 and F4 formulations have a higher potential than the standard to inhibit alpha amylase activity.

Considering these findings, it can be said that formulated mushrooms could be very good functional foods in diabetes therapy.

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