



Study of antiradical and antihyperglycaemic activities of mushrooms *Pleurotus pulmonarius* and *Pleurotus floridanus* currently consumed in Cameroon

Etoundi Omba Cunégonde Blanche¹; Mbang Mbarga Audrey Judith¹, Kayo Tayou Cynthia Valère¹, Gouado Inocent¹

¹ Laboratory of Biochemistry, University of Douala-Cameroon

Received: June 2018

Revised: August 2018

Accepted: September 2018

Available online: September 2018

Abstract

Diabetes is a chronic syndrome due to a high level blood of carbohydrates. This pathology is increasingly becoming widespread worldwide with more than 100 million people affected every year. It is essential to find new active natural compounds because of the high cost and side effects of current antihyperglycemic drugs. Hence our interest in functional foods like edible mushrooms. Indeed, according to the literature, edible mushrooms are more accessible than chemical drugs and have antioxidant properties that regulate blood sugar levels. This study aimed to evaluate the antiradical and antihyperglycaemic activities of aqueous, ethanolic, hydroethanolic and hexane extracts of the mushroom *Pleurotus pulmonarius* and *Pleurotus floridanus*. Thus, mushroom samples were macerated and dried for obtaining crude extracts. Afterward, these extracts were used for performing DPPH-based anti-radical activity and determining polyphenol content. The best antioxidant extracts were selected for evaluation of antihyperglycaemic activity at different doses (400 mg/kg BW and 800mg/kg BW). Aqueous and apolar extracts from *P. floridanus* exhibited both a high antiradical activity ($IC_{50} = 1.28$ and 0.021 respectively) and a phenolic compound content (10.196 ± 3.663 mg EC/g and 22.358 ± 8.832 mg EC/g respectively) compare to Vit C used as control. These findings were different from those obtained with *P. pulmonarius* where the best antiradical and antihyperglycaemic results were obtained using alcoholic and hydroalcoholic extracts. Results based on antihyperglycaemic activity pointed out that the both extracts elicited a higher inhibitory effect in the increase of postprandial blood glucose at dose of 800 mg/kg BW. These results reveal that our extracts have interesting anti-radical and antihyperglycaemic activities and outline their possible use as medicines.

ISSN 1029–2225c2016 Sciences, Technologies et Développement

Key words: Benin; dendrométric characteristic; phytodistricts; African Star Apple; diametric structure.

1. Introduction

Numerous are the metabolic diseases that continuously cause a very high number of deaths worldwide each year. According to some studies, good foods intake (a balanced diet, mainly made up of fruits and vegetables) and regular physical activities seem to be helpful to reduce the rate of mortality and morbidity (Furtado de Souza et al., 2012). It was demonstrated that fruits and vegetables are abundant in antioxidants compounds. Findings proved the efficiency of the latter by exhibiting their properties such as antiradical, antibacterial, antifungal, anti-aging, inflammatory, antihyperglycaemic and many others (Bazzano et al., 2003).

A high blood glucose level enhances the growth of free radicals that could no more being neutralized by the antioxidants naturally produced by the body. This imbalance will thus cause an oxidative stress in the cell with a decreased in enzymatic antioxidant defenses, leading to diabetes (Mohora et al., 2007). An antioxidant supplementation will be therefore very essential in the inhibition of the harmful action of free radicals. Also, this will improve the damage related to the insulin secretion, and so, balanced the glycaemic index (Dalle-Donne et al., 2006).

Diabetes is a cardiovascular risk associated with a chronic level in the

blood glucose (Girard, 2008). In 2013, the International Diabetes Federation (IDF) estimated that 382 million of people (precisely 8.3 % adults) have diabetes worldwide with 46 % undiagnosed. Also, in Africa nearly 20 million people have diabetes, a prevalence of 4.9 %. These estimations are expected to double by 2035 to reach 41.5 million: an increase of 109 % (IDF, 2013). Concerning the Cameroon, diabetes is increasing over time: 93 thousand people aged 60-79 years have diabetes with 229 thousand undiagnosed (IDF, 2013) and in 2015, the data showed that among Cameroonians diagnosed 10 % of the population suffers from diabetes (CAMERPOST, 2015).

The term diabetes covers two different diseases insulin-dependent diabetes (type1) and non-insulin-dependent diabetes (type 2) (Boitard, 2002). The latter increases with age, urbanization, sedentarisation and obesity (Grimaldi, 2000).

Cameroon, which is a miniature Africa, is full of a very diverse flora of vegetables including mushrooms. Their consumption particularly that of the family of oyster mushrooms appears here as an alternative solution, taking into account their accessibility, low productivity cost, high yield, which can be cultivated at any time of the year ... (Ninkwango, 2007). The mushrooms represent a very specific realm called fungi (Courtecuisse, 2011). In culinary context, they can be

*Corresponding author address: ++237 695418979 E-mail address: blancheetoundi@hotmail.fr

considered as vegetables in their own right. According to literature, studies on mushrooms, such as the *pleurotus* genus (found in a myciculture in our country), showed that they are very good antioxidants and thus, prevent from some metabolic diseases such as diabetes (Dubost and al., 2007). Since few scientific data are available in our country on the prevention of diabetes using oyster mushrooms, we have focused on this study through antiradical and antihyperglycaemic tests to contribute to the management of this disease.

2. Materials and methods

2.1.Preparation of the vegetal material

The mushrooms precisely *Pleurotus pulmonarius* and *Pleurotus floridanus* harvested in a myciculture at PK 21 in February 2016 (a suburb of the city of Douala) were dried in the sun for three days and then crushed. The powder obtained have been macerated in different solvent (distilled water, ethanol, water and ethanol (1/1) and hexan) following the proportion 1/8 (100 grams of powder of mushrooms in 800 mL of solvent). Afterwards, were dried in an oven to obtain the aqueous, hydroethanolic, ethanolic and hexanic extracts respectively.

2.2.Evaluation of antiradical activity and the content of phenolic compound:

2.2.1. Evaluation of antiradical activity by the DPPH method

The antiradical activity of the extracts was determined according to the DPPH method. This method is based on the decrease of absorbance as describe by Katalinie and al. in 2003 and is measured at 517 nm. Thus, a DPPH solution was prepared in absolute methanol to obtain a concentration of 0.11 mg/ml. 1 mL of this solution was added to 50 µl of the extracts prepared at different concentrations: 0.025; 0.05; 0.1; 0.3; 0.5; 1; 3; 5; 7 and 10 mg/ml. Ascorbic acid was prepared the same concentrations the extracts was used to plot the calibration curve.

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

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

A1: absorbance of control (DPPH solution without extract)

A2: absorbance in the presence of extract.

2.2.2. Determination of phenolic compound content

The determination of phenolic compound content is based on the reduction of a phosphomolybdic - tungstin chromogen by an antioxidant and the resulting coloration is read at an absorbance of 750 nm. The reagent was prepared by taking 5 ml of Folin from the stock solution of 2 N concentration and introduced into a 50 ml volumetric flask. The volume was then supplemented with distilled water till 50 ml in order to obtain a 0.2 N solution. Catechin was used as a standard. The best antiradical and high phenolic compound extracts were retained for further antihyperglycemic tests (Singleton and Rossi, 1965).

2.3. Evaluation of antihyperglycaemic activity of mushroom extracts overload in normal rats

Thirty normal *Wistar*-type rats aged two and a half months whose weight ranged from 170 to 200 kg were divided into six groups of 5 rats. These rats were then fasted 12 hours before the start of the experiment. They were divided as follows in table 1.

After a slight puncture of the distal tip of the tail with a lancing device, blood samples were collected to determine the glycaemic rate. Blood glucose was taken on fasting state (basal glucose). Afterwards, the extracts were administered by gavage 30 minutes prior to the administration of glucose. Subsequently, the glycaemia was determined successively at 30, 60, 90 and 120 min. During these measurements, the animals were kept on fasting state.

3. Results and discussions

3.1. Evaluation of the antiradical activity of oyster mushroom extracts

3.1.1. DPPH antiradical activity

Evaluation of antioxidant activity by DPPH antiradical method allowed us to plot the curves representing the variation of the concentration of the various extracts as a function of the percentage of inhibition. The plot of these curves makes it possible to highlight the IC₅₀ of each extract which is the concentration for which the antiradi

Table 1. Distribution of the animals for the antihyperglycaemic test

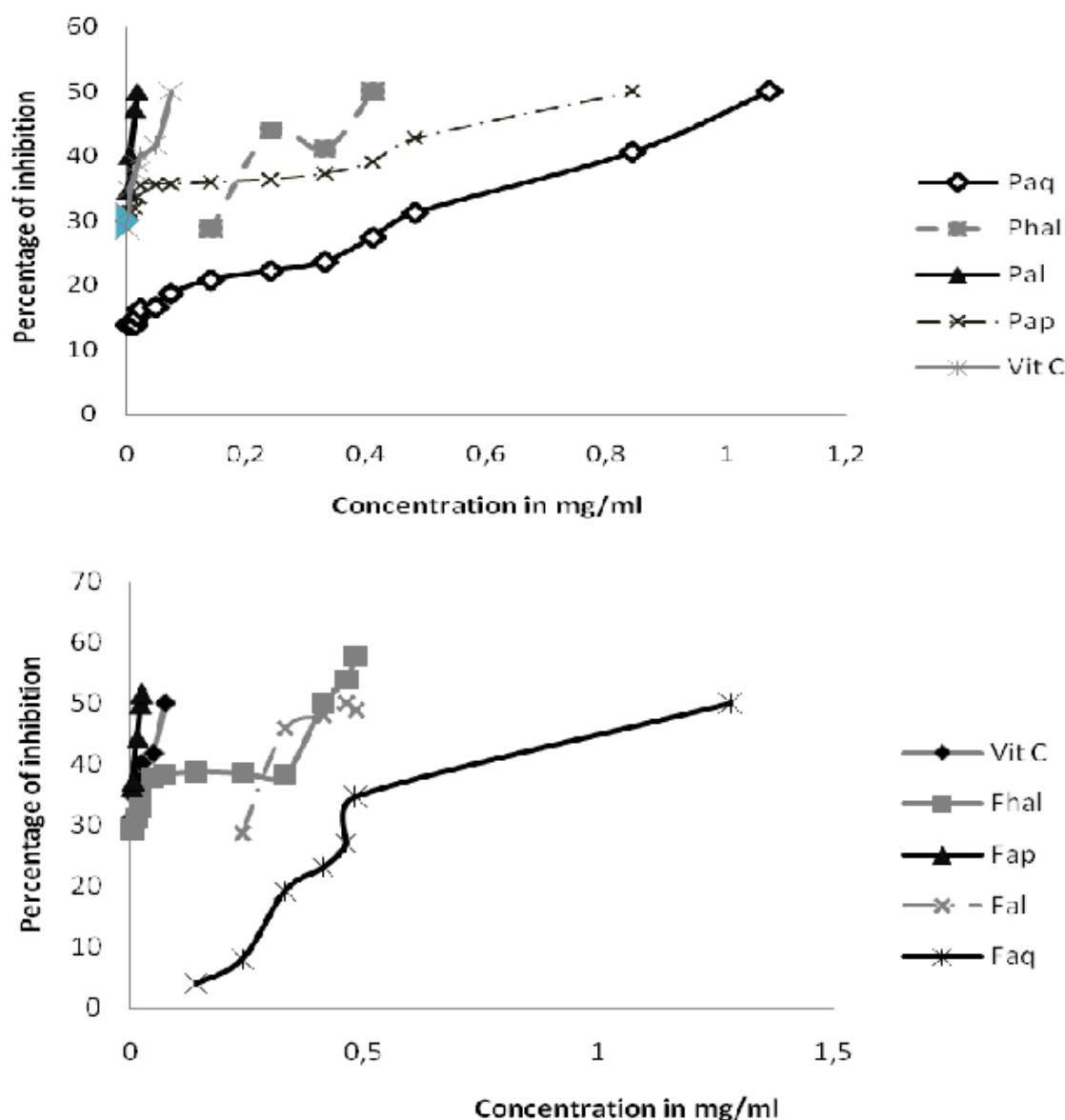
GROUPS	DESCRIPTION
1-negative control	Normal rats treated with distilled water
2-positive control	Normal rats treated with distilled water + glucose
3- test 1	Normal rats treated with The aqueous extract of <i>Pleurotus floridanus</i> + glucose
4- test 2	Normal rats treated with the apolar extract of <i>Pleurotus floridanus</i> + glucose
5- test 3	Normal rats treated with the alcohol extract of <i>Pleurotus pulmonarius</i> + glucose
6- test 4	Normal rats treated With the hydroalcoholic extract of <i>Pleurotus pulmonarius</i> + glucose

cal agent present in the extract inhibits 50 % of the DPPH solution (Figure1).

Figure 1 allowed us to highlight the table showing the different IC₅₀ extracts and standard. Hence the following table 2.

According to table 2, the hydroalcoholic, aqueous, alcoholic and apolar extracts of *Pleurotus pulmonarius* have an inhibitory concentration of 0.41 mg/ml, 1.07 mg/ml, 0.018 mg/ml and 0.842 mg/ml respectively. Similarly, the hydroalcoholic, aqueous, alcoholic and apolar extracts of *Pleurotus florida* have an IC₅₀ of 0.430 mg/ml, 1.28 mg/ml, 0.46 mg/ml and 0.021 mg/ml, respectively.

Compared to ascorbic acid used as standard, which shows an IC₅₀ = 0.073 mg/ml, the alcohol extracts of *Pleurotus pulmonarius* and apolar of *Pleurotus florida* have a more appreciable inhibitory activity than the standard. The strong capacity of these two extracts to inhibit DPPH and to fix the oxidizing metal ions could be due to the presence of high reactive hydroxyl group in the alcohol extract of *Pleurotus pulmonarius*. As for the non polar extract of *Pleurotus florida*, the inhibitory potency may be due to the high micronutrient content of *Pleurotus florida*, as Favier (2003) suggests that micronutrients are also essential to the activity enzyme



Phal, Paq, Pal and Pap are the hydroalcoholic, aqueous, alcoholic and apolar extracts of *Pleurotus pulmonarius* and Fhal, Faq, Fal and Fap respectively are the hydroalcoholic, aqueous, alcoholic and apolar extracts of *Pleurotus florida*.

Figure 1. Trapping variation of DPPH-free radical as a function of the antioxidant concentration of the various extracts and Vit C.

antioxidant. Table 2 also shows that the aqueous extracts of *Pleurotus pulmonarius* and *Pleurotus floridanus* have the weakest inhibitory activities.

3.1.2. Phenolic compound content

Analysis of extracts of *Pleurotus pulmonarius* and *Pleurotus floridanus* by the Folin method presents a significant difference between the species of oyster mushrooms and between the extracts of the same species. Indeed, in terms of significance between species, there was a significant difference in phenolic compound between the apolar extract of *Pleurotus floridanus* and the apolar extract of *Pleurotus pulmonarius* (22.358 ± 8.832 mg EC/g* and 7.341 ± 4.763 mg EC/g respectively). Similarly, with regard to the significant difference within the extracts of the same species, there was a significant difference in polyphenol content between the alcoholic extract, and the aqueous and hydroalcoholic extracts (22.358 ± 8.832 mg EC/g \neq 10.196 ± 3.663 mg EC/g and 11.994 ± 7.456 mg EC/g respectively). Also, a significant difference in phenolic content was observed between the alcohol and apolar extracts of *Pleurotus pulmonarius* (17.282 ± 4.759 mg EC/g and 7.341 ± 4.763 mg EC/g respectively). The richest extracts in phenolic compounds exhibited in Table 3 were therefore the apolar extract of *Pleurotus floridanus* and the alcohol extract of *Pleurotus pulmonarius*. The lowest extracts in phenolic compounds were the aqueous extract of *Pleurotus floridanus* with a content in polyphenol of 10.196 ± 3.663 mg EC /g and the apolar extract of *Pleurotus pulmonarius* with a rate of 7.341 ± 4.763 mg EC /g. From Table 3, we can say that our two species of mushroom are rich in polyphenols. These results corroborate those found by Marsi and al. (2015).

The mushrooms studied showed antiradical activity, likewise, the phenolic compounds they contained can act as an antioxidant by breaking the radical chains of more stable products in liver microsomal membranes, with the ability to protect low-density lipoproteins and heavy macrophages (Wei et al., 2008). Therefore, since antiradicals and phenol rich compounds have the ability to neutralize the damaging effects of free radicals while stabilizing blood glucose, the best antiradical extracts and phenol rich compounds have been retained for antihyperglycaemic test.

- Choice of extracts for the rest of the work

The antiradical dosage and the phenol compound determined by the Folin method allowed us to retain certain extracts compared to others for the rest of the antihyperglycemic test. The choice of the extracts was made on the basis of the most inhibitory extracts and the richest extracts in polyphenols. It is therefore the apolar extract of *Pleurotus floridanus* and the alcohol extract of *Pleurotus pulmonarius* which were the best extracts in both tests. Then, we also retained the hydroalcoholic extract of *Pleurotus pulmonarius* which also responded favorably to these two tests. Finally, we retained the aqueous extract of *Pleurotus floridanus* which exhibited not only a low inhibitory activity but also a low content of phenolic compounds. Indeed, our choice on this last extract is due to the fact that since we did not carry out a phytochemical screening, we wanted to know if despite the fact that this extract has a low DPPH-antiradical activity, it could not contain a compound which will allow him to be active for the antihyperglycemic test.

3.2. Evaluation of antihyperglycaemic activity in normal rats

Administration of a dose of 400 mg/kg of body weight (BW) to the rats of various tests followed by the administration of glucose by gavage after 30 minutes allowed us to obtain the following figure 2.

Figure 2 presents six curves of which the first curve with the most low blood glucose level over time represents the negative control batch. Indeed, the latter has a very low blood sugar level compared to the others. This is due to the fact that the other batches of rats have ingested the extract and / or the glucose; meanwhile, the rats of the negative control only ingested distilled water and even, were still young. Thus, the negative control curve is initially normal for fasting subjects for more than 12 hours; But after, the curve decreases, this could be explained by the fact that the rats having been fasting for longer have entered hypoglycemia. The second curve represents the batch of the positive control to which distilled water and glucose have been administered. This curve is initially increasing because of the hyperglycaemic effect of glucose and then, over time, we observed that the curve decreases. This decrease could be explained by the fact that over time the organisms of the rats metabolized glucose and used it as energy necessary for the different metabolic voices. On the

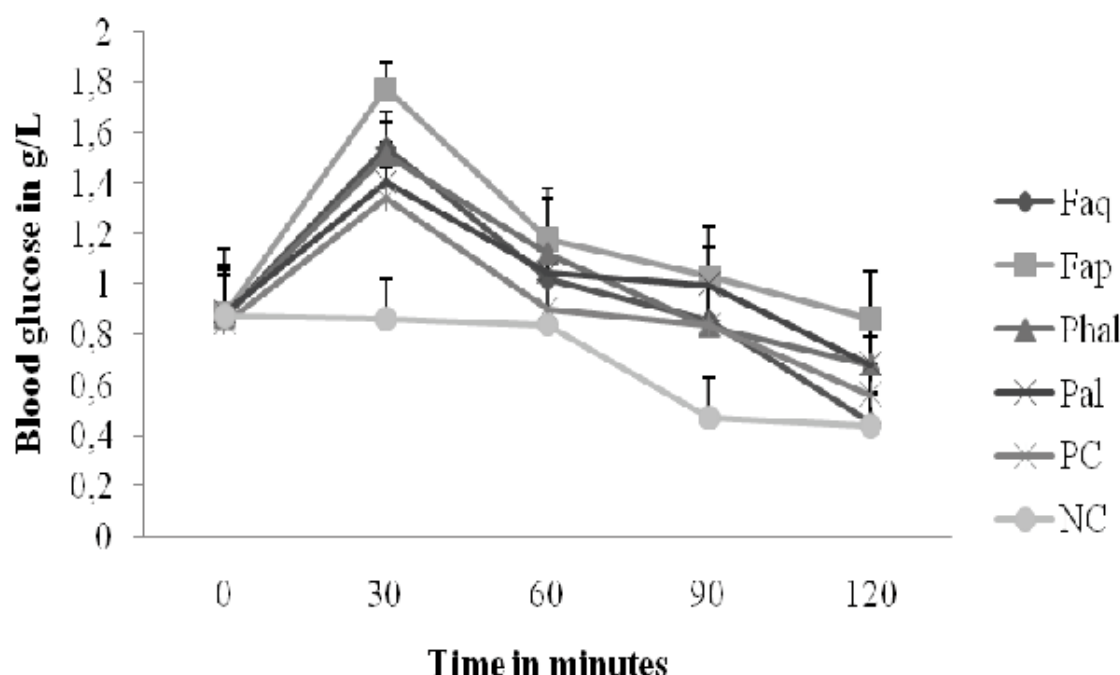
Table 2. Variation of IC₅₀ according to the different extracts and the standard

Extracts and standard	IC ₅₀
Phal	0.41 mg/ml
Pal	0.018 mg/ml
Paq	1.07 mg/ml
Pap	0.842 mg/ml
Fhal	0.43 mg/ml
Fal	0.46 mg/ml
Faq	1.28 mg/ml
Fap	0.021 mg/ml
Vit. C	0.073 mg/ml

Table 3. Variation of the phenol content as a function of the type of extract

Types of oyster mushrooms	Extracts	Content of polyphenols (mg EC /g)
<i>P.flo.</i>	Fal	15.801±2.698
	Fap	22.358±8.832
	Faq	10.196±3.663
	Fhal	11.994±7.456
<i>P.pul.</i>	Pal	17.282±4.759
	Pap	7.341±4.763
	Paq	17.176±6.961
	Phal	15.061±3.17

*mg EC / g =catechin equivalent per gram of mushroom



Phal and Pal are the hydroalcoholic, alcoholic extracts of *Pleurotus pulmonarius* and Faq, and Fap respectively are the aqueous, and apolar extracts of *Pleurotus floridanus*; PC = positive control; NC = Negative Control

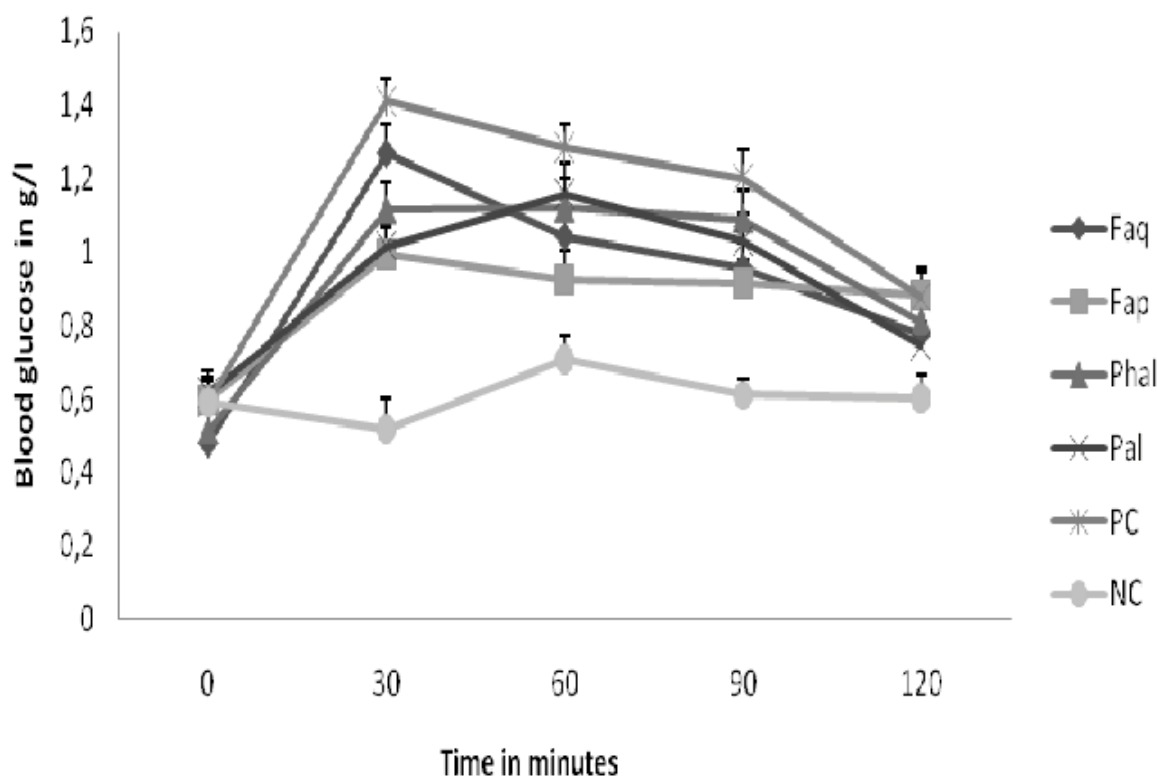
Figure 2. Evolution of blood glucose as a function of time after administration of a dose of 400 mg/kg of BW of the extracts followed by a gavage of glucose at a dose of 2000 mg/kg of BW.

Indeed, after administration of 800 mg/kg of BW on normal rats followed by glucose gavage after 30 minutes, the change in blood glucose as a function of time was different from that obtained with a dose of 400 mg/Kg BW.

With regard to figure 3, six curves are observed as previously in figure 2, with the negative control being the first curve. Thus, the negative control curve initially shows a slight decrease in blood glucose that could be due to fasting. Then this curve increases and then decreases; the growth of the curve could be explained by the phenomenon of gluconeogenesis and the decrease by the fact that the glucose having been produced by other non-glucose compounds (gluconeogenesis) has already been exhausted. The four curves

following the negative control represent the tests. Indeed, the fact that the extracts appear with low glycemia compared to the positive control could be due to the fact that the dose of extract administered to the rats was high and therefore at this dose of 800 mg/kg of BW the extracts of oyster mushrooms act.

The apolar extract of *Pleurotus floridanus* appears to be the extract having a better antihyperglycaemic activity and the aqueous extract of *Pleurotus floridanus* as the least antihyperglycemic extract compared to the positive control. In all cases, the aqueous extract of *P. floridanus* was the least inhibitory extract with a low content of phenolic compound. fig. 3 shows that, despite its low antiradical activity, the aqueous extract of *Pleurotus floridanus*-



Phal and Pal are the hydroalcoholic and alcoholic extracts of *Pleurotus pulmonarius*; Faq, and Fap respectively are the aqueous and apolar extracts of *Pleurotus floridanus*; PC = positive control; NC = Negative Control

Figure 3. Evolution of blood glucose as a function of time after administration of a dose of 800 mg/kg of BW of the extracts followed by a gavage of glucose at a dose of 2000 mg/kg of BW.

may nevertheless have antihyperglycaemic activity. This result could suggest that, there is one or many compound in this extract that would have the ability to inhibit the increase in blood glucose after a meal.

These results are similar to those of Wahab et al. (2014) and could be explained by the fact that four proteins linked to antidiabetic agents, namely proteins of the profilin type, proteins of glyceraldehyde-3-phosphate dehydrogenase type, proteins of the trehalose phosphorylase (TP) type, and catalase proteins. Therefore, mushroom have a high potential to lower the level of glucose in the blood, which reduces insulin resistance and vascular complications (Wahab et al., 2014).

4. Conclusion

At the end of our study, which evaluated the antiradical and antihyperglycaemic activity of two species of mushroom commonly consumed in Cameroon, *Pleurotus pulmonarius* and *Pleurotus floridanus*, it appears that:

- *Pleurotus pulmonarius* introduced for these best extracts an IC₅₀ of 0.018 and 0.41 respectively for ethanolic and hydroethanolic extracts against *Pleurotus floridanus* which presented an IC₅₀ of 0.021 and 0.43, respectively for these best extracts hexanic and hydroethanolic. These two species of mushrooms are also particularly rich in phenolic compounds, there is an average of 17.282 ± 4.759 mg EC/g for the most phenolic *Pleurotus pulmonarius*

extract and an average of 22.358 ± 8.832 mg EC/g for the most phenolic *Pleurotus floridanus* extract.

- The above mentioned extracts of *Pleurotus pulmonarius* and *Pleurotus floridanus* revealed after the antihyperglycemic test that, at a dose of 800 mg/kg of BW, our two species of mushrooms inhibit the increase in postprandial blood glucose and hence, the most antihyperglycemic extracts are the apolar extract of *Pleurotus floridanus* and the alcoholic extract of *Pleurotus pulmonarius*. In view of the above, we can say that *Pleurotus pulmonarius* and *Pleurotus floridanus* consumed in Cameroon exhibit DPPH antiradical and antihyperglycaemic activity. This suggests their use as a functional food.

Acknowledgments

The authors would like to thank the Laboratory of Biochemistry of the Faculty of Sciences of the University of Douala where most of this work has been done.

References

- Bazzano, Serdula, Liu (2003). Dietary intake of fruits and vegetables and risk of cardiovascular disease. *Curr Atheroscler Rep* November; 5 (6): 492-9.
- Boitard (2002). The origin of type 1 diabetes: an autoimmune disease? *Diabetes Metab Diabetes Care*, 18: 807 - 816.
- Camerpost (2015). Cameroon tribune by Yvette MBASSI-BIKELE;

- 37% of the population are affected by Arterial hypertension in Cameroon. [http:// www. Camerpost.com/cameroun-statistics-37-de-la-population-sont-atteint-de-lhypertension-arterielle-17042015/](http://www.Camerpost.com/cameroun-statistics-37-de-la-population-sont-atteint-de-lhypertension-arterielle-17042015/).
- Courtecuisse, Duhem (2011). Guide to mushrooms of France and Europe. 1752 species described and illustrated. Paris: Delachaux and Niestlé, 544p.
- Dalle-Donne, Rossi, Colombo, Giustarini, Milzani (2006). Biomarkers of oxidative damage in human disease. *Clin Chem* ; 52 : 601-623.
- Dubost, Or, Beelman (2007). Quantification of polyphenols and ergothioneine in total antioxidant capacity. *Food Chemistry*.
- Favier (2003). Conceptual and experimental interest in the understanding of disease mechanisms and therapeutic potential. *The Chemical Industry*, 108 - 115.
- Furtado de Souza, Dalzochio, de Oliveira, Gross, Leitao (2012). Glucose tolerance status is a better predictor of diabetes and cardiovascular outcomes than metabolic syndrome : a prospective cohort study. *Diabetol Metab Syndr*; 4: 4-25
- Girard (2008). The incretins: from the concept to their use in the treatment of type 2 diabetes. Part A : Incretins : concept and physiological functions. *Diabetes Metabolism*; 34 : 550-9.
- Grimaldi (2000). Physiopathology of type 2 diabetes. Pierre et Marie Curie University: Diabetology. Internship Question No. 331, p.22 / 142.
- IDF (2013). Diabetes Atlas, Sixth edition, www.idf.org/diabetesatlas (prevalence of diabetes in Africa).
- Katalinić, Milos, Modun, Musi, and Boban (2004). Antioxidant effectiveness of selected wines In comparison with (+) - catechin. *Food Chemis.* 86, 593-600.
- Mohora, Greabu , Muscurel, Du, Totan (2007). The sources and the targets of oxidative stress in the etiology of diabetic complications. *Romanian J. Biophys*, 17 (2); 63 - 84.
- Ninkwango (2007). The cultivation of oyster mushrooms. Cameroon. 2nd edition, 47 pages
- Singleton and Rossi (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Technology and Viticulture.* 16, 144-153.
- Wahab, Nurul, Abdullah, Noorfidah, Aminudin, Norhaniza (2014). Characterization of proteins related to the potential antidiabetic of *Pleurotus pulmonarius* (Fr.) (Gray oyster mushrooms)