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Antiradical Activity and Ferric Reducing Antioxidant Power of *Pleurotus pulmonarius*, *Pleurotus floridanus* and *Pleurotus sajor-caju* Formulations Extracts *in Vitro*

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Abstract

Edible mushrooms, known for many years and considered as functional foods, contain a wide variety of biomolecules with nutritional and medicinal properties. *Pleurotus* species, especially *Pleurotus pulmonarius*, *Pleurotus floridanus* and *Pleurotus sajor-caju* are the most commonly consumed in Cameroon. The present work aims to study the "*in vitro*" antiradical activity and ferric reducing antioxidant power of extracts of formulations of these three species. Mushrooms were harvested in two mycicultures from Cameroon, then dried and crushed finely to obtain powders. In well-defined proportions, ten different formulations were made and their aqueous, ethanol and hydroethanol extracts prepared. These were used to determine total phenolic content by using Folin-Ciocalteu reagent method, the antioxidant activity determined by 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging activity and ferric-reducing antioxidant power (FRAP) assays, with vitamin C (ascorbic acid) as standard. The results obtained from the ten formulations, including thirty extracts, showed that all the extracts had quite significant ferric reducing power at C = 1 mg/ml, (7.35 - 26.02 mg EAA/g). They also had good percentages of DPPH radical inhibition at C = 25 mg/ml (60.40% - 77.72%) and a significant polyphenol content at C = 100 mg/ml (13.73 - 21.15 mg CE/g). The aqueous extracts had the best activities compared to the standard. In addition, a strong positive linear correlation exists between polyphenol content and the scavenging property (R^2 = 0.9063; p = 0.0003) also with the ferric reducing test (R^2 = 0.9253; p = 0.0001); suggesting the responsibility of these compounds for these activities. This study suggests that these mushrooms may be used as a natural source of potential antioxidants for food supplements and their consumption is strongly recommended

for their beneficial effects on health through, the protection of the human body against the oxidative damage involved in the occurrence of many chronic diseases.

Keywords

Scavenging Activity, Ferric Reducing Antioxidant Power, Edible Mushrooms

1. Introduction

Mushrooms, species known for many years (5000-4000B.C.), play and continue to play an important role in many aspects of human activity. Edible mushrooms, for example, are widely used in human food because of their excellent nutritional and medicinal value [1].

The true nutritive value of mushrooms has rapidly become known and recognized not only by mushroom researchers and farmers but also by the general consumers [2]. In addition to their good flavour, mushrooms possess favourable chemical components with high amounts of functional proteins, low total fat level, and the high proportion of polyunsaturated fatty acids (PUFA), making them well suited for low-calorie diets. Edible mushrooms provide a nutritionally significant content of vitamins (B1, B2, B12, C, D, and E) [3] [4]. Moreover, mushrooms have a low glycaemic index, and high mannitol, making them particularly beneficial for diabetics. Mushrooms have very low sodium (Na) concentration, which is beneficial for hypertensive patients and a high content of potassium (K) and phosphorus (P), which is an important orthomolecular aspect [2]. In Asia, mushrooms are used as important source of home remedies against various diseases elicited by oxidative stress [5].

There is no easy distinction between edible and medicinal mushrooms because many of the common edible species have therapeutic properties [6] [7]. Besides antioxidant properties, mushrooms have received considerable attention for their biological activities, such as antitumor, antiviral, anticomplementary, anticoagulant, antidiabetic, hypolipidemic, hepatoprotective, immunostimulant and immunological activities, which made them suited for uses in food, cosmetics, biomedicine, agriculture, environmental protection and wastewater management [8] [9] [10]. For many reasons, mushrooms are also considered as a good source of natural antioxidants and seem useful as a natural source of potential antioxidant additives [11].

To date, numerous edible wild mushroom species, growing in various ecological conditions are known. Those of the highest economic value are usually produced under artificial conditions, *i.e.*, on a well-defined substrate and under full climatization. These are mostly *Agaricus bisporus* (button mushroom), *Lentinula edodes*, *Pleurotus spp.*, and *Flammulina velutipes* [12].

The genus *Pleurotus* is the most commonly studied nowadays and it accounts

for about 25% of world mushroom production. It gathers about 40 species that are edible and about ten of which are grown for consumption. In Cameroon, the most cultivated are *P. ostreatus*, *P. pulmonarius*, *P. sajor-caju*, *P. floridanus*, *P. citrinopileatus*, and *P. salmoneo-stramineus*. Three species, *P. pulmonarius*, *P. floridanus*, *P. sajor-caju*, have been choose as sample for this study because they are commonly consumed due to their availability, their low cost of production, their high product yield and their easy cultivation procedures [13]. Also, individually, they have shown significant antiradical, antihyperglycémic and antidiabetic activity [14] [15]. In view of their functional properties, the formulation of these fungi in proportions defined according to a formula could optimize their various properties. Based on the hypothesis that the formulations of these three fungal species could optimize their antioxidant properties, we aim tostudy "*in vitro*", the antiradical activity and ferric reducing antioxidant power of extracts of formulations of *Pleurotus pulmonarius*, *Pleurotus floridanus* and *Pleurotus sajor-caju*.

2. Material and Methods

2.1. Plant Material

Three species of oyster mushroom, *P. pulmonarius*, *P. floridanus* and *P. sajor-caju* were harvested during early mature fruiting stage in two mycicultures from Cameroon, at Pk 21, a city's suburb of Douala and at Obala during the dry season. After collection, the mushrooms were individually cleaned and air-dried until dryness. Then each species of dried mushroom sample was ground to obtain fine powder.

2.2. Extraction

To obtain formulations, the powders of the three species of mushrooms were mixed for 150 g of total powder in three proportions defined in Table 1 below.

Table 1. Defined proportions for each formulation.

Proportion for 150 g of total powder (w:w:w)	Formulations (F)	
50:50:50	F1	*Pp.Pf.Ps*
100:25:25	F2	*Pp.Pf.Ps*
	F3	*Pf.Ps.Pp*
	F4	*Ps.Pp.Pf*
75:50:25	F5	*Pp.Pf.Ps*
	F6	*Pp.Ps.Pf*
	F7	*Ps.Pp.Pf*
	F8	*Ps.Pf.Pp*
	F9	*Pf.Ps.Pp*
	F10	*Pf.Pp.Ps*

Pp. Pleurotus pulmonarius; *Pf. Pleurotus floridanus*; *Ps. Pleurotus sajor-caju*.

The aqueous, ethanolic and hydroethanolic extracts of the various formulations were prepared at the Biochemistry Laboratory of the University of Douala. Formulations of mushrooms powders (100 g) were macerated in 600 ml of solvent (proportion 1w:6v), respectively distilled water, ethanol and water/ethanol (1v:1v) during 48 h. After, the salters were filtered using a filter paper, then the filtrate was dried in a drying oven (brand binder) at 45˚C until dryness to obtain the crude extract. The dry raw extracts were stored at 4˚C in glass bottles until analysis.

2.3. Antioxidant Activity

2.3.1. Scavenging Activity of DPPH (2,2-Diphényl-1-Picrylhydrazyl) Radical

DPPH radical scavenging activity was determined according to Ivette [16], with some modifications. The reaction mixture consisted of 100 µL of 25 mg/mL of each formulation extract and 1 mL of 0.3 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical solution in ethanol. Each test was repeated 3 times. After incubation for 30 min in the dark, at room temperature, absorbance was determined by a spectrophotometer at 517 nm. The results were presented as percentage DPPH radical inhibition (%*I*), calculated according to the formula:

$$\%I = \left[\left(A_{\text{control}} - A_{\text{sample}}\right) / A_{\text{control}}\right] \times 100$$

A_{control} = Absorbance of negative control at the moment of solution preparation.

A_{sample} = Absorbance of sample after 30 mins.

2.3.2. Determination of Total Phenolic Content

The total phenolic compound content in dry mushroom extracts was determined by Folin-Ciocalteu procedure [17], using catechin as standard. 30 µL of sample at 100 mg/ml was added to 1 ml of 1N Folin-Ciocalteu's Reagent diluted 10 times and mixed before being incubated for 1 hour at room temperature. Each test was repeated 3 times. Absorbance was measured at 765 nm. Total phenolic compound content has been expressed as mg of catechin equivalent (CE) per g of dry mushroom extract (mg CE/g).

2.3.3. Ferric Reducing Antioxidant Power (FRAP)

FRAP assay was measured according to the procedure described by Benzie and Strain [18]. The FRAP reagent contained 2.5 mL of a 10 mM TPTZ solution in 40 mM HCl, 2.5 mL of 20 mM $FCEl_3 \cdot 6H_2O$, and 25 mL of 300 mM acetate buffer (pH 3.6). It was freshly prepared and warmed at 37˚C. Then, to 2 mL of FRAP reagent, 75 µL of each extract at 1 mg/mL was added. The reaction mixture was incubated at room temperature for 30 min and the absorbance was measured at 593 nm. The results expressed as mg of Ascorbic acid equivalent (AAE) per g of dry mushroom extract (mg AAE/g).

2.4. Statistical Analysis

All assays were carried out in triplicates and results expressed as mean ± stan-

dard deviation (SD). The data was introduced in an EXCEL spreadsheet (Microsoft Office 2013) and then analyzed with the STATGRAPHICS Centurion XV version 17.1.12 software. Statistical analysis was carried out with one-way analysis of variance (ANOVA) followed by Fisher LSD post hoc test. Values of $p < 0.05$ were considered significant.

3. Results

The ten formulations established following three different proportions resulted in thirty extracts including ten aqueous, ten ethanolic and ten hydro-ethanolic. These extracts were used for the evaluation of scavenging activity of DPPH, the total polyphenol content and the ferric reducing antioxidant power.

3.1. Scavenging Activity of DPPH (2,2-Diphényl-1-Picrylhydrazyl) Radical

The evaluation of the scavenging activity of extracts at a concentration of 25 mg/ml by the DPPH assay was expressed as percentage inhibition as presented in the graph below (Figure 1). It appears that, for each formulation, there is a significant difference ($p < 0.05$) between its different extracts and the extract with the highest percentage of inhibition is the aqueous extract (Ea) followed by the hydro-ethanol extract (Eh) and the ethanol extract (Ee) with the lowest percentage inhibition. Similarly, there is a significant difference ($p < 0.05$) between the percentages of inhibition of Ea, Ee, and Eh of the different formulations. Ea of all formulations have the largest percentages of inhibition. However, at this concentration, there was a significantly high difference ($p < 0.05$) between the percentages of inhibition of these extracts. It is also noted that Ea of F1, F3, F8, and F9 had the highest inhibition percentages (77.72% ± 0.97%; 75.16% ± 0.52%; 73.79% ± 0.79% and 72.31% ± 0.30%, respectively). But compared to Vit C used as standard for the same concentration, only the Ea of F1, F3, and F8 had higher percentages of inhibition ($p < 0.05$) than Vit C (71.74% ± 1.14%).

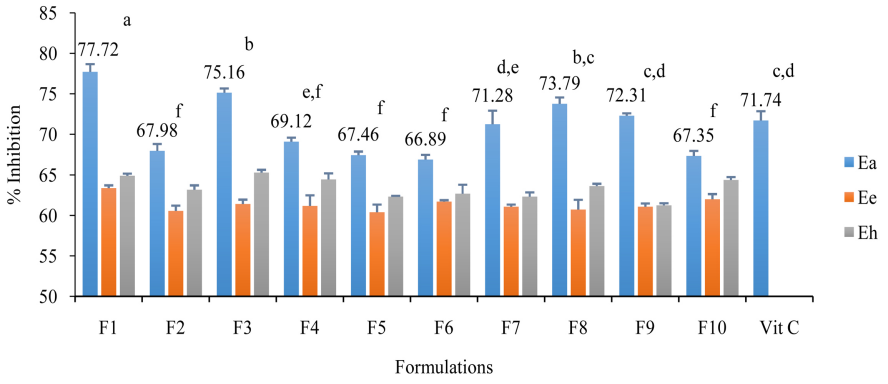

Figure 1. DPPH radical scavenging activity of different extracts according to the formulations and comparatively with vitamin C (ascorbic acid used as standard). Ea: aqueous extract; Ee: ethanol extract; Eh: hydro-ethanol extract; F1-10: Formulations; Vit C: vitamin C; $p < 0.05$.

3.2. Total Phenolic Content

The total polyphenol content of the extracts of the different formulations were expressed in mg of catechin equivalent per g of extracts (mg CE/g) at a single concentration (100 mg/ml). These different levels have resulted in the graph below (Figure 2). It shows that there is a significant difference ($p < 0.05$) between the polyphenol content of the extracts of the same formulation. Moreover, for all these formulations, Ea exhibited higher polyphenol levels than those of Eh, which are themselves higher than those of Ee. In addition, there is a significant difference ($p < 0.05$) between the levels of polyphenols of Ea, Ee and Eh of the different formulations. The Ea of all formulations with the highest content, the comparison between them showed that, the Ea of F1, F3, F8 and F9 had the highest levels of polyphenols with 21.15 ± 0.58 mg CE/g; 19.23 ± 0.18 mg CE/g; 18.65 ± 0.35 mg CE/g and 17.95 ± 0.59 mg CE/g, respectively.

3.3. Ferric Reducing Antioxidant Power

The reducing capacities of formulations extracts were measured according to the FRAP assay and expressed as mg AAE/g, are shown in Figure 3. It shows that

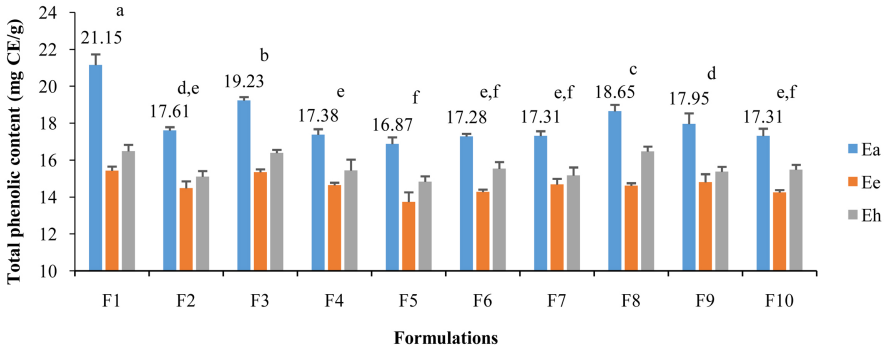

Figure 2. Total phenolic content of different extracts according to the formulations. Ea: aqueous extract; Ee: ethanol extract; Eh: hydro-ethanol extract; F1-10: Formulations; $p < 0.05$.

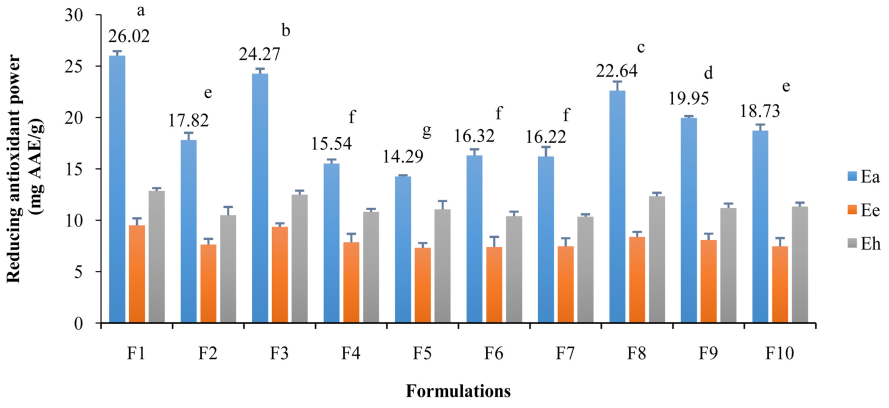

Figure 3. Ferric reducing antioxidant power (FRAP) of different extracts according to the formulations. Ea: aqueous extract; Ee: ethanol extract; Eh: hydro-ethanol extract; F1-10: Formulations; $p < 0.05$.

there is a significant difference ($p < 0.05$) between the reducing antioxidant powers of the extracts of the same formulation. Additionally, for all these formulations, Ea exhibited higher antioxidant power than those of Eh, which are higher than those of Ee. Furthermore, there is a significant difference ($p < 0.05$) between the reducing capacities of Ea, Ee and Eh of the different formulations. The Ea of all formulations with the highest content, the comparison between them showed that, the Ea of F1, F3, F8 and F9 had the highest reducing powers with 26.02 ± 0.45 mg AAE/g; 24.27 ± 0.49 mg AAE/g; 22.64 ± 0.88 mg AAE/g and 19.95 ± 0.20 mg AAE/g.

4. Discussion

The evaluation of the antioxidant activity of the extracts of the different formulations prepared, by DPPH assay, Total phenolic content and Ferric reducing antioxidant power showed that the aqueous extracts of all the formulations had the best antioxidant activities followed by the hydroethanolic and ethanolic extracts. These results could be explained by the fact that the yield efficiency of bioactive compounds is higher for water than for ethanol because of their polarity; Water being more polar than ethanol will extract more compounds than the latter. In addition, the hydroethanol mixture had a higher yield than that of ethanol. In fact, Hip *et al*., [19] had shown that in edible fungi, oyster, in particular, water yielded better extraction of antioxidant compounds than 20% (V/V) hydroethanol and 60% (V/V) Hydroethanol and thus a better Yield than 50% (V/V) hydroethanol used in our study. Furthermore, they demonstrated that 60% (V/V) hydroethanol had a better yield than 100% ethanol. More specifically, Prabu *et al*. [20] showed that, in *P. floridanus* species, water had a better extraction yield than ethanol.

In addition, there is a linear relationship between the three assays. This was justified by the Pearson test which showed a very high and highly significant positive correlation ($p < 0.001$) between the DPPH radical scavenging assay and the polyphenol contents of the aqueous extracts with a coefficient of Correlation $R^2 = 0.9063$ for a probability $p = 0.0003$. Moreover, the correlation was also significant between DPPH and FRAP assays ($R^2 = 0.8840$, $p = 0.0007$) and between Total phenolic content and Ferric reducing antioxidant power assays ($R^2 = 0.9253$, $p = 0.0001$). This correlation was also significant ($p < 0.05$) for the hydroéthanolic extracts with $R^2 = 0.6554$ and $p = 0.0397$ between DPPH assay and the polyphenol contents, $R^2 = 0.8745$ and $p = 0.0009$ between FRAP assay and Total phenolic content and $R^2 = 0.6389$ and $p = 0.0464$ between DPPH and FRAP assays. However, it was even less high and not significant ($p > 0.05$) for ethanolic extracts with $R^2 = 0.5640$ and $p = 0.0895$ between DPPH assay and the polyphenol contents, and $R^2 = 0.5469$ and $p = 0.1018$ between DPPH and FRAP assays, but significant between FRAP assay and Total phenolic content with $R^2 = 0.8855$ and $p = 0.0007$.

In light of the foregoing, the genus *Pleurotus* would have more water-soluble

compounds than in ethanol as demonstrated by Lee *et al*. [21] (Lee *et al*., 2006). As part of this study, water is, therefore, the best solvent for extraction of total polyphenols, a result justified by Pinelo *et al*. [22] and Ghosh *et al*. [23] which claimed that the best yields of total phenols are obtained using polar solvents such as water, methanol and ethanol.

Moreover, in general, antioxidant power is strongly dependent on the concentration of phenolic compounds [24]. This fact corroborates the study of Trabelski *et al*. [25] that showed a significant and positive correlation between phenolic compound levels and antiradical activity.

5. Conclusion

In conclusion, the results indicate that all the formulations' extracts had antioxidants properties. Furthermore, water was the best extraction solvent for phenolic compounds present in the studied mushrooms and, as a consequence, water extracts of all formulations had better antioxidant properties. However, among the ten formulations prepared, the water extract of the formulation one had the highest concentration of phenolic compounds, the highest reducing power, and the best radical-scavenging properties. That may be due to the presence of the three species of mushroom in the same proportion in the formulation. Though other antioxidants were probably present in the mushroom extracts, phenolic compound in the extracts was responsible for their effective antioxidant properties. Antioxidant activity of the edible mushrooms has significant importance because this activity greatly contributes to their nutraceutical properties, thus enhancing their nutritional value.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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