Antioxidant and Anti-Inflammatory Potential of Shiitake Culinary–Medicinal Mushroom, *Lentinus edodes* (Agaricomycetes), Sporophores from Various Culture Conditions

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ABSTRACT: *Lentinus edodes* (= *Lentinula edodes*) is an edible mushroom grown and marketed for centuries due to its nutritional and medicinal properties. *L. edodes* has multiple pharmacological activities as an antioxidant and anti-inflammatory. Few studies were performed taking into account the influence of culture conditions to optimize the biological properties of *L. edodes* on human health. Our work focused on the comparison of antioxidant capacity and anti-inflammatory activity of *L. edodes* fruit bodies cultivated by three mushroom producers in the French Occitanie region using the same strain in various growing conditions (organic and nonorganic). Sequential extraction was performed on freeze-dried fungal materials. All extracts have a quantifiable but moderate antioxidant activity measured via DPPH and ORAC tests. The anti-inflammatory activity of the ethanol and aqueous extracts was evaluated on a model of inflammatory macrophages. The ethanol extracts inhibit NO production in a dose-dependent manner when the cells are pretreated for 4 h with a 24 h stimulation time.

KEY WORDS: *Lentinus edodes*, anti-inflammatory, antioxidant, DPPH, food health benefit, J774.A1 macrophages, medicinal mushrooms, nitric oxide, ORAC, pharmacology

ABBREVIATIONS: APPH, 2,2'-azo-bis(2-methypropionamide); **CA**, chlorogenic acid; **DMSO**, dimethyl sulfoxide; **DPPH**, 1,1-diphenyl-2-picrylhydrazyl; **IFN**, interferon gamma; **LPS**, lipopolysaccharide; **NO**, nitric oxide; **ORAC**, oxygen radical absorbance capacity; **ROS**, reactive oxygen species; **RPMI**, Rosewell Park Memorial Institute; **TE**, trolox equivalent; **TNF-α**, tumor necrosis factor-alpha

I. INTRODUCTION

Shiitake culinary–medicinal mushroom, *Lentinus edodes* (Berk.) Singer (= *Lentinula edodes* [Berk.] Pegler, Marasmiaceae, Agaricomycetes) is among the most edible cultivated mushrooms in the world.[1–3] Its production is intensive in Asia, the United States, and in Europe.[4,5] *L. edodes* can be grown using a wide range of conditions and substrates. *L. edodes* is appreciated for its fragrant taste and nutritional properties, and as a medicinal mushroom.[6–8]

L. edodes is a source of bioactive agents as ergosterol,[9] ergothioneine,[10] phenolic compounds, and polysaccharides,[11,12] responsible for therapeutic activities (anti-inflammatory, antioxidant, antitumor, hypoglycemic).[13–15] Several studies have attributed to this fungus the ability to inhibit or slow down the production of free radicals and to protect lipids against peroxidation due to its content in antioxidant molecules.[16–20]

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Other data reported that *L. edodes* has an inhibitory effect on NO production from LPS/IFNγ activated macrophages, explaining its anti-inflammatory potential.[21]

The aim of our work is to compare the influence of *L. edodes*' growing conditions using the same strain (cultivated by organic and nonorganic French mushroom professionals) on its antioxidant and anti-inflammatory properties.

II. MATERIAL AND METHODS

A. Chemicals

Trolox (98%), NaH_2PO_4, 2-amino-ethyl diphenyl borinate and acetic acid are from Fluka Chemicals. DPPH and AAPH radicals, chlorogenic acid (95%), cyclohexane (99.8%), acetonitrile, and dimethyl sulfoxide (DMSO; 99.9%) were purchased from Sigma-Aldrich. Fluorescein is from Panreac. Ethanol (96%) and methanol (99.9%) were obtained from VWR. Chloroform (99%) and Na_2HPO_4 (99%) are from Honeywell Research Chemicals.

RPMI medium 1640 GlutaMAX®, penicillin–streptomycin, murine recombinant interferon γ, Hanks Balanced Salt Solution (HBSS) and fetal bovine serum were obtained from Gibco. LPS *E. coli* 055:B5 and sodium nitroprusside were purchased from Sigma Aldrich. MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) was purchased from Promega France. PMS (phenazinemethosulfate) was purchased from ICN Biomedical.

B. Mushroom Material

Mycelia-3782 strain of *L. edodes* was cultivated by three producers located in Occitanie (France) on sterilized substrate blocks from his own engineering (producer B) or provided from commercial suppliers (producers A and C). The *L. edodes* sporophores from producer A (Fontiès-d'Aude) grow on a mixture of wood chips and straw (nonorganic conditions; substrate of Eurosubstrat©) with temperatures ranging from 10°C to 20°C and 80% hygrometry. The mushrooms generated by producer B (Saint-André-de-Lancize) are cultivated in the Cevennes National Park on organic sawdust of chestnut, wheat bran, and rye (organic conditions) with temperatures ranging from 18°C to 21°C and 60–70% hygrometry. The mushrooms from producer C (Saint-Bonnet-de-Salendrinque) grow on a mixture of wood chips, oak sawdust, and straw (nonorganic conditions, substrate of "Lentin de la buche" SA©) and temperatures vary from 15°C to 17°C and 100% hygrometry.

C. Sample Preparation

Five kg of *L. edodes* from each producer were cleaned, sliced, gauged, and carefully packaged in plastic bags and snap frozen. Then, they were lyophilized in a RP2V lyophilizer.

D. Extract Production Process

A sequential process based on solvents of increasing polarity is used as previously described.[22] First, 50 g of each lyophilized mushroom sample are crushed with a Thermomix Vorwerk crusher. Then, 5 g of crushed mushroom are placed in 50 ml cyclohexane, sonicated for 90 min at 30°C, and then filtered using a Büchner device. The cyclohexanic filtrate is stored for subsequent evaporation procedure: extract 1. Retentate is then submitted successively to extractions with chloroform (50 mL; extract 2), ethanol (50 mL; extract 3), and water (50 mL; extract 4) under the same conditions.[22] The sequential

extraction is carried out in triplicate. After extractions, solvents were evaporated to dryness. To fully dry aqueous extracts, a lyophilization is performed. All dried extracts were stored in the darkness at 4°C.

Extraction yields (R) were calculated according to the following:

$$R = \frac{\text{Mass of dried extract in g} * 100}{\text{Mass of dried mushroom in g}} \quad (1)$$

The values were expressed as a percentage.

Total yield was calculated as (Sum of masses of dried extracts/Mass of dried mushroom in g) × 100, and was expressed as a percentage.

E. Antioxidant Activity

1. DPPH Test

Antioxidant activity is assessed using the DPPH as previously described.[22] Extracts are solubilized in DMSO (4 mg/mL) and then diluted in absolute ethanol to reach a concentration range of 0.2, 0.5, and 1 mg/mL. Ethanol is used as blank. A standard curve with Trolox is performed (12.5, 25, 50, and 75 µM). *Rosmarinus officinalis* ethanol extract (0.2 mg/mL) and chlorogenic acid (0.01 mg/mL) are used as positive controls. On a 96-well plate, 100 µL of either positive control or mushroom extract is deposited in each well. The assay is run in triplicate at each tested concentration. Then, 75 µL of absolute ethanol and 25 µL of extemporaneously prepared DPPH (0.4 mg/mL) solution are introduced in each well. Plate is incubated for 30 min at room temperature and protected from light. Absorbance reading is performed at 550 nm with a microplate reader (MDS Inc., Toronto, Canada). Results are expressed as mean plus or minus standard deviation of three independent experiments and are expressed as Trolox equivalents (TE µmoles per gram of dry extract). Results are also expressed as inhibition percentage (% inhibition) and calculated as follows:

$$\%\text{inhibition} = \frac{\text{DO blank} - \text{DO extract}}{\text{DO blank}} * 100 \quad (2)$$

2. ORAC Test

ORAC dosage is performed as previously described.[22] *L. edodes* samples are solubilized in DMSO to a concentration of 1 mg/mL. They are then diluted to 25 µg/mL using a phosphate buffer solution (75 mM) at pH 7.4. On a 96-well microplate are deposited Trolox standard curve solutions (20 µL at 6.25, 12.5, 25, 50, and 75 µM) or chlorogenic acid (0.01 mg/mL), or rosemary ethanol extract (12.5 µg/mL) as positive control, or shiitake extracts from all producers at a concentration of 25 µg/mL. Then 100 µL of phosphate buffer and 100 µL of extemporaneously prepared fluorescein solution (0.1 µM in phosphate buffer) are added. Microplate is incubated at 37°C for 10 min under stirring. Reaction is initiated with 50 µL of AAPH extemporaneously prepared. Fluorescence is recorded at an excitation wavelength of 485 nm and an emission wavelength of 535 nm, for 70 min using a microplate reader Tristar LB 941. Final ORAC values are calculated using a regression equation between Trolox concentration and the area under the curve of fluorescein decreasing. Data are expressed as µmoles of Trolox equivalents per gram of dry extract. Results are expressed as mean plus or minus standard deviation (n = 3).

F. Anti-Inflammatory Assay

1. Macrophage Culture and Cell Treatment

The J774.A1 macrophage cell line (ATCC, TIB67) was obtained from LGC Standards. Cells were cultured in RPMI medium 1640 GlutaMAX® supplemented with streptomycin (100 µg/mL) and penicillin (100 Units/mL) and 10% heat inactivated fetal bovine serum (complete RPMI medium), and incubated at 37°C in a humidified incubator containing 5% CO_2. For NO production, the cells (5×10^5 cells/well) were seeded onto a 24-well culture plate in complete RPMI medium and pretreated with different concentrations of ethanol or aqueous extracts (50 to 6.25 µg/mL) for 4 h. After, cells were activated with LPS (100 ng/mL) and interferon γ (10 ng/mL) and incubated at 37°C for another 18 h.

2. Cell Viability by MTS Assay

To test the cytotoxicity, 10^5 cells/well were seeded in a 96-well culture plate in complete RPMI medium and incubated at 37°C with different concentrations of ethanol or aqueous extracts (50 to 6.25 µg/mL) for 20 h. After incubation, 20 µL/well of tetrazolium salt, MTS, mixed with an electron coupling reagent, PMS in HBSS, was added. The plate was incubated for another 4 h and the absorbance at 490 nm was measured in a microplate reader (Molecular Devices) as previously described.[23]

3. Nitrite Determination

Ethanol and aqueous extracts were used for nitrite determination. The presence of nitrite, a stable oxidized product of nitric oxide, was determined in cell culture media as previously described.[24] Briefly, 100 µL of supernatant was combined with 100 µL of Griess reagent in a 96-well plate, incubated 10 min at room temperature. Nitrite concentration was determined by measuring the absorbance at 550 nm and by using a standard curve of $NaNO_2$ (1–100 µM).

G. Statistical Analyses

Values are presented as mean ± standard error of the mean (SEM). Statistical analysis of the data was carried out using Prism® software by two-way ANOVA followed by Bonferroni post-test. P values < 0.05 were considered to be significant.

III. RESULTS

A. Extraction and Extraction Yield

A sequential extraction was used to get the broader spectrum of bioactive molecules from the shiitake materials. Total yield of the fourth extractions was 21.88%, 28.58%, and 24.73% for producers A, B, and C, respectively. For all producers, the highest extraction yields are obtained for aqueous extracts (extract 4 ranging from 13.45% for producer A to 19.52% for producer B) followed by the ethanol extracts (extract 3). No significant differences of extraction yields were found between the three producers for extracts 1, 2 and 3; the aqueous extract yield for organic producer B was significantly higher than that of the other two producers (Fig. 1).

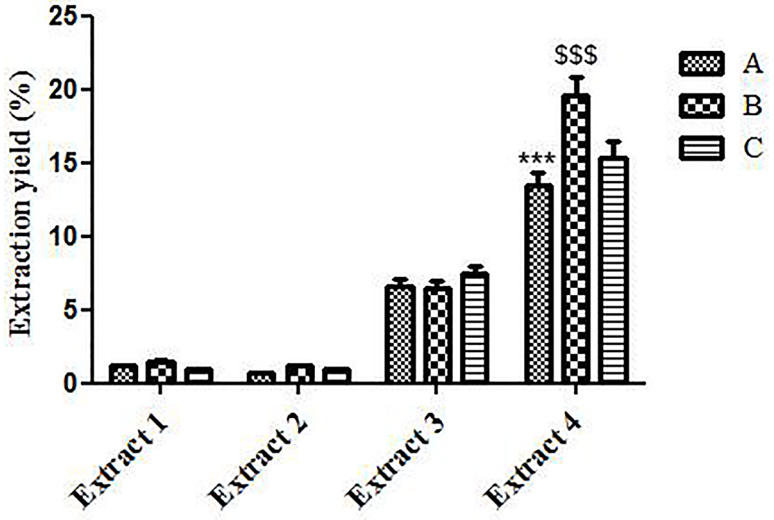

FIG. 1: Extraction yields for producers A, B, and C. Values are presented as mean ± SEM ($n = 3$). Extract 1 = cyclohexane extracts; Extract 2 = chloroform extracts; Extract 3 = ethanol extracts; Extract 4 = aqueous extracts. Extraction yields (R) were calculated according to this formula: (Mass of dried extract in g/mass of dried mushroom in g) × 100, and was expressed as a percentage; ***$p < 0.001$ for producer A vs. producer B; $^{\$\$\$}p < 0.001$ for producer B vs. producer C.

B. Antioxidant Activities

The DPPH and ORAC tests results on our extracts (Table 1) were compared with two positive controls in terms of antioxidant activity: an ethanolic extract of *R. officinalis* that has been considered as a food antioxidant additive (E392) and chlorogenic acid (CA), a caffeic acid derivative.[22] Rosemary extract was used as a reference of natural crude extract and CA as a natural purified standard.

1. DPPH Antioxidant Activity

Results from the DPPH test (Table 1, Fig. 2) are expressed as percentage of inhibition and as Trolox equivalents (TE). Trolox is used as positive control. Shiitake extracts demonstrate an antioxidant effect ranging from 11.54 to 34.73 µmol TE/g of extract. These values are, on average, 22 times lower than rosemary extract antioxidant potential (261.67 µmol TE/g of extract). If all mushroom extracts (1, 2, 3, 4) demonstrate moderate DPPH antioxidant activity, aqueous extracts appear to be the most antioxidant regardless of producer when compared with the organic extracts: 34.73 µmol TE/g for producer A, 25.82 µmol TE/g for producer B, and 30.64 µmol TE/g for producer C. The average value is 8.6 times lower than rosemary extract antioxidant effect and 100 times lower than CA antioxidant effect (3077.4 µmol TE/g of standard) (Fig. 2). In addition, cyclohexane, chloroform, and ethanol extracts from producer A have statistically the highest DPPH antioxidant ability compared with producer C. Cyclohexane and aqueous extracts from producer A have statistically the highest DPPH antioxidant ability compared with producer B (Fig. 2). Cyclohexane extract from producer B and chloroform extract from producer C have the lowest DPPH antioxidant capacities with 12.88 and 11.54 µmol TE/g, respectively. It should be noted that when comparing producers, the highest DPPH antioxidant properties are recorded with nonorganic producer A, whatever the type of extract. However, there is no significant difference between producers A and B regarding their ethanol extracts with values of 26.63 and 22.78 µmol TE/g, respectively. As for organic

TABLE 1: Antioxidant capacity of *Lentinus edodes* extracts from organic and nonorganic French producers

Producers	Extracts	DPPH			ORAC
		EC_{50} (mg/mL)	Inhibition (%) at 1 mg/mL	µmol TE/g EDW	µmol TE/g EDW
A	Extract 1	> 1	18 ± 1.14	25.88 ± 1.71	520 ± 25
	Extract 2	> 1	16.37 ± 0.14	21.7 ± 0.65	442 ± 16
	Extract 3	> 1	17.6 ± 0.84	26.63 ± 1.96	494 ± 6
	Extract 4	> 1	25.23 ± 3.44	34.73 ± 2.82	684 ± 52
B	Extract 1	> 1	10.03 ± 1.02	12.88 ± 0.98	549 ± 15
	Extract 2	> 1	12.41 ± 0.65	16.94 ± 0.67	578 ± 30
	Extract 3	> 1	17.16 ± 0.95	22.78 ± 0.77	528 ± 44
	Extract 4	> 1	18.4 ± 1.09	25.82 ± 0.27	749 ± 38
C	Extract 1	> 1	11 ± 0.21	15.52 ± 0.83	752 ± 22
	Extract 2	> 1	8.7 ± 0.54	11.54 ± 0.89	859 ± 22
	Extract 3	> 1	10.31 ± 0.63	14.01 ± 0.79	747 ± 28
	Extract 4	> 1	24 ± 1.18	30.64 ± 2.03	892 ± 21
Rosmarinus officinalis		0.32 ± 0.01	n.d.	261.67 ± 0.65	2560 ± 216
Chlorogenic acid		n.d.	n.d.	3077.4 ± 129.94	11515 ± 994

Data are mean ± SEM (n = 3). EC_{50}, half-maximal effective concentration; EDW, extract dry weight; TE, Trolox equivalent; n.d., not determined.

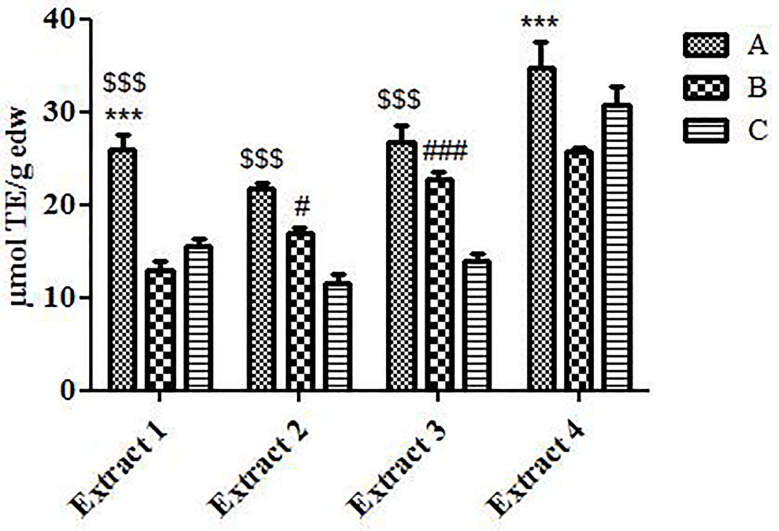

FIG. 2: DPPH values for producers A, B, and C. Values are presented as mean ± SEM (n = 3). Extract 1 = cyclohexane extracts; Extract 2 = chloroform extracts; Extract 3 = ethanol extracts; Extract 4 = aqueous extracts. ***$p < 0.001$ for producer A vs. producer B; $^{\$\$\$}p < 0.001$ for producer A vs. producer C; $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ for producer B vs. producer C.

producer B, DPPH antioxidant potential of extracts increases with extract polarity whereas no correlation can be done between polarity of the extracts and DPPH values for nonorganic producers A and C.

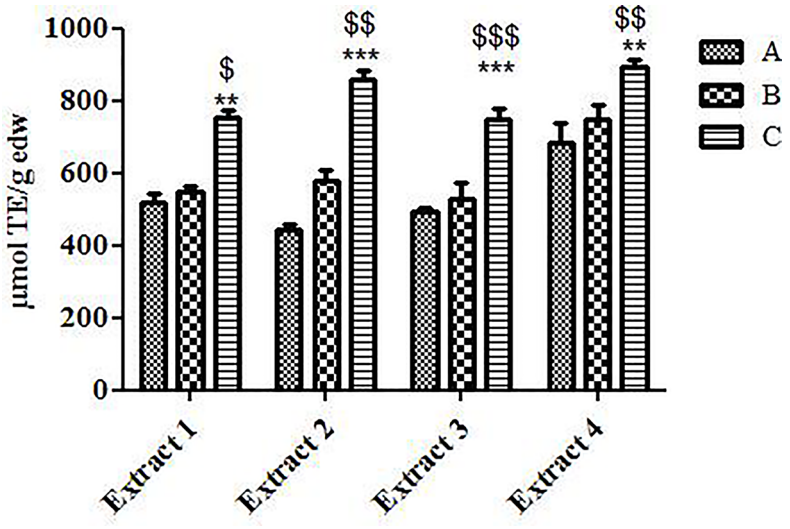

FIG. 3: ORAC values for producers A, B, and C. Values are presented as mean ± SEM ($n = 3$). Extract 1 = cyclohexane extracts; Extract 2 = chloroform extracts; Extract 3 = ethanol extracts; Extract 4 = aqueous extracts. $^{**}p < 0.01$, $^{***}p < 0.001$ for producer A vs. producer C; $^{\$}p < 0.05$, $^{\$\$}p < 0.01$, $^{\$\$\$}p < 0.001$ for producer B vs. producer C.

2. Oxygen Radical Absorbance Capacity (ORAC) Antioxidant Activity

Results are expressed in µmol of Trolox equivalents (TE) per gram of dry matter. Similarly to DPPH, results from ORAC indicate that aqueous extracts have the highest ORAC antioxidant activity for all producers (684, 749, and 892 µmol TE/g for producers A, B, and C, respectively) (Table 1). These values are followed by those of the cyclohexane extract for producer A and chloroform extracts for producers B and C. There is no significant difference between ORAC values of cyclohexane, chloroform, and ethanol extracts from producers A (nonorganic) and B (organic) as shown (Fig. 3). All extracts from nonorganic producer C have significantly higher ORAC values when compared with two other producers. The highest average value of ORAC antioxidant activity for shiitake extracts were three times lower than rosemary activity (2560 µmol TE/g EDW) and 15 times lower than CA (11515 µmol TE/g EDW), respectively.

C. Anti-Inflammatory Activity

Apolar extracts were not investigated for their anti-inflammatory properties due to poor solubility at 10 mg/mL in DMSO.

1. L. edodes Aqueous and Ethanol Extracts Effects on Cell Viability

DMSO was used to solubilize the extracts at 10 mg/mL and then diluted in RPMI to reach a concentration range of 50–6.25 µg/mL. *L. edodes* extract assay on cell viability, performed on macrophage cell line J774.A1 using the MTS/PMS method, demonstrates the absence of cytotoxic effect on cells. Indeed, none of the concentrations tested, ranging from 50 to 6.25 µg/mL during 4 h incubation, impaired cell culture compared with control cells (data not shown). Consequently, these extract concentrations are tested on NO production by activated macrophages *in vitro*.

2. *L. edodes* Aqueous and Ethanol Extracts Effects on Nitric Oxide (NO) Production

NO is an unstable mediator of inflammation. Upon production by cells it is readily converted in nitrites that can be dosed by Griess colorimetric method. Anti-inflammatory effects of ethanol extracts are assessed on stimulated macrophage J774.A1. After cells stimulation by LPS/IFNγ, nitrite production is quantified in the cell culture supernatant. Extracts concentrations range from 50 to 6.25 µg/mL. We record that LPS/IFNγ strongly stimulates NO production by macrophages as expected.

Ethanol extracts from the three producers significantly inhibit NO production in a concentration dependent manner (Fig. 4). At 50 µg/mL, inhibition reaches 29.66%, 31.30%, and 27.56% for producers A, B, and C, respectively, on cells pretreated by extracts for 4 h with stimulation time of 24 h. It should be noted that NO production is also concentration dependently decreased after the 48-h stimulation condition but in this case the effect is globally more important at the highest concentration (41.6%, 42.6%, and 39% for producers A, B and C, respectively). When comparing producers, anti-inflammatory potential appears equivalent, showing no statistical differences. Results presented in Fig. 4 show that cell pretreatment with shiitake aqueous extract induced a low inhibition of nitrite production and this effect was concentration dependent. The highest values after 4 h pretreatment with 24 h of stimulation were 14.23%, 15.60%, and 20.93% for producers A, B, and C, respectively at 50 µg/mL. At about 48 h stimulation, results indicate 17.1%, 15.22%, and 18.71% for producers A, B, and C at higher concentration, respectively. When comparing producers or stimulation time (24 h and 48 h), we found no statistically significant differences (data not shown).

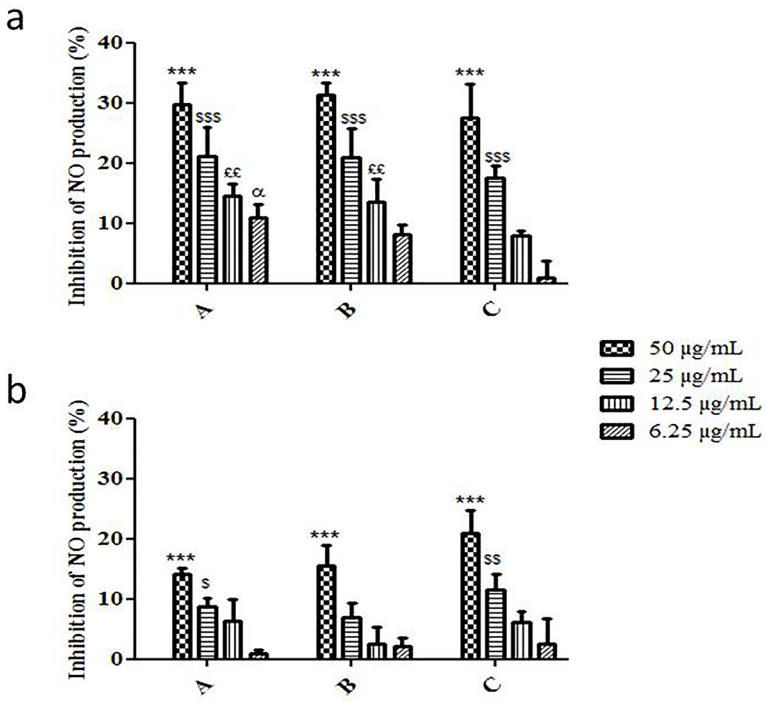

FIG. 4: The bar graphs summarize the NO inhibition for producers A, B, and C after 4 h pretreatment with 24 h of stimulation, (a) by ethanolic extracts or (b) by aqueous extracts. Values are presented as mean ± SEM ($n = 3$). ***$p <$ 0.001, inhibition resulting from 50 µg/mL of extract vs. no inhibition; $^{\$}p < 0.05$, $^{\$\$}p < 0.01$, $^{\$\$\$}p < 0.001$, inhibition resulting from 25 µg/mL of extract vs. no inhibition; $^{\pounds\pounds}p < 0.01$, inhibition resulting from 12.5 µg/mL of extract vs. no inhibition; $^{\alpha}p < 0.05$, inhibition resulting from 6.25 µg/mL of extract vs. no inhibition.

The NO inhibition activities of aqueous and ethanol extracts of *L. edodes* were compared. They were the highest for the ethanol extracts whatever the producer or concentration used; a significant difference was only observed between producers A and B ($p < 0.05$) at the concentration of 50 µg/mL (data not shown).

IV. DISCUSSION

To extract bioactive molecules from various cultivated *L. edodes* strain (Mycelia-3782) conditions, a sequential extraction was performed using cyclohexane, chloroform, ethanol, and water. Aqueous and ethanol extracts generated the highest extraction yields. Differences of total extraction efficiency for each solvent and producer may be explained by the influence of growing conditions, stage of harvest, as well as mushroom size.[25] Methanol, ethanol, water, and hydroethanolic mixes are the most commonly used solvents, for the extraction of bioactive molecules, since they allow to reach high yields.[26] A few studies reported chloroform and cyclohexane to extract secondary metabolites on *L. edodes* mushroom.[26,27] Extraction yields for water extracts from producers A, B, and C (13.45%, 19.52%, and 15.36%, respectively) were similar to data from Cheung et al.[16] (16.2%) but lower than those reported by Da Silva and Jorge (30%).[28] Our results for chloroform extracts ranged from 0.68% to 1.15% and from 6.49% to 7.49% for ethanol extracts. More recently, Ferrari et al.,[29] using a nonsequential extraction, recorded extraction yields of 11%, 13.6%, 14%, 27.2%, 32.7%, and 33.8% with ethanol, methanol, acetone, ethanol–water, methanol–water, and acetone–water, respectively. The differences between our data and the literature may be explained by differences in extraction methods, mushroom strain, and growth conditions when compared with literature.

In our study, we used two complementary methods, i.e., DPPH as the mixed mode assay (hydrogen atom transfer and electron transfer reaction-based assay) and ORAC as a hydrogen atom transfer reaction-based assay to evaluate antioxidant properties of shiitake.[22] DPPH assay results indicate that all shiitake extracts demonstrate a moderate but quantifiable antioxidant activity. With an extract concentration of 1 mg/mL, the inhibition levels of the ethanolic *L. edodes* extracts (17.6%, 17.16%, and 10.31% from producers A, B, and C, respectively; Table 1) were similar to those reported by Da Silva and Jorge.[28] Regarding cyclohexane extracts, our results suggest a lower inhibitory ability for mushroom material from producers A, B, and C (18%, 10%, and 11%, respectively) compared with literature.[27] A large variety of shiitake extracts were tested for their antioxidant potential using DPPH assay; nonetheless, most studies were performed on aqueous, ethanolic, and hydroethanolic extracts.[7,16,20] Regarding antioxidant efficacy against DPPH radicals, hydroethanolic extract demonstrated the highest potential.[29]

Few investigations have been performed on *L. edodes* with the ORAC assay. Our results ranged from 442 to 892 µmol TE/g of extract (Table 1). Previous studies recorded various ORAC values expressed with different units.[30–32] DPPH and ORAC are quantitative assays allowing to compare radical scavenging capacities between extracts as well as producers. Our results, and their comparison to data from the literature, clearly indicate that shiitake biological activity as antioxidant capacity may vary significantly as a function of mushroom strain, growth conditions, extraction process, and experimental conditions of biological activities evaluation. Plant extracts (such as rosemary extract, the E392) or purified natural compounds (such as chlorogenic acid) may be used as food additives. Several authors demonstrated indeed that chlorogenic acid and related compounds can mitigate oxidative and inflammatory stresses and show several health promoting properties.[33] That is why they were used as positive controls in our investigations for oxidant and inflammatory potential of *L. edodes*.

In addition to antioxidant effects, shiitake ethanol extracts demonstrate an inhibitory activity on NO production from LPS/IFNγ activated macrophages J774.A1. This result indicates that shiitake extracts have an anti-inflammatory potential *in vitro*. Ethanol extracts from the three producers, at a concentration of 50 µg/mL, inhibit NO production in concentration dependent manner (41.6%, 42.6%, and 39% inhibition for producers A, B, and C, respectively) on 4-h pretreated cells with a stimulation time of 48 h. Phenolic

compounds and ergothioneine extracted by ethanolic or hydroethanolic solvent might be responsible for this effect as previously suggested.[19,21] These results are in agreement with those reported by authors[34,35] studying the effect of an ethanolic shiitake extract on NO inhibition.

Several mechanisms of action for inhibition of NO production are described in the literature, such as NF-κB, a transcription factor composed of a protein complex, located in cytoplasm and associated with the inhibitor IκB.[36,37] In response to various stimuli such as pro-inflammatory cytokines (IL-1β), LPS, or other forms of stress, IκB inhibitor is phosphorylated by the IKK kinase and then degraded.[36,38] This degradation promotes the activation of NF-κB and its release to join the nucleus where it attaches to inflammatory genes and activates the transcription of specific genes.[39,40] This will lead to synthesis of cytokines and inflammatory mediators including TNF-α and NO.[38] NF-κB transcription factor plays an important role in immune response and then inflammation. Consequently, its deregulated activation contributes to the pathogenic processes of various inflammatory diseases.[40,41] Therefore, inhibition of this pathway prevents or decreases the synthesis of pro-inflammatory cytokines.[37,38,41]

In our work, the inhibition would be associated with a decreased expression of iNOS (induced nitric oxide synthase) responsible for NO production.[42] A cytotoxic impact of the shiitake extracts on cells was ruled out by the cell viability test that produced negative results. Because the inflammation reaction is complex process involving many mediators such as cytokines, in addition to NO, further molecular investigations are required to fully understand the pharmacology of antioxidant and anti-inflammatory properties of *L. edodes* extracts.

V. CONCLUSIONS

Our study focused on the antioxidant and anti-inflammatory potential of *L. edodes* fruit bodies cultivated by nonorganic and organic mushroom professionals using the same strain. The *L. edodes* extracts from the three French mushroom producers obtained by a sequential extraction showed moderate antioxidant activity with greater free radical inhibition in aqueous extracts. Regarding the anti-inflammatory property of the polar extracts tested, all ethanolic extracts strongly inhibit NO production in a concentration-dependent manner thus suggesting *L. edodes*' potential use in the treatment of certain types of noncommunicable diseases. On the other hand, the inhibition observed for aqueous extracts remains low regardless of whether producers are organic or nonorganic. Concerning the shiitake growing conditions used, our study shows some significant differences in the antioxidant and anti-inflammatory activities among the *L. edodes* extracts from the three mushroom producers. Several shiitake strains and various substrates will need to be tested under both organic and nonorganic conditions to improve differences. In addition, further investigations should be carried out to identify the main bioactive components of cultivated *L. edodes* extracts responsible for the antioxidant capacity and anti-inflammatory activity, and especially to study their mechanism of action.

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