International Journal of Medicinal Mushrooms, 20(1):13–29 (2018)

Antiproliferative and Antioxidant Activities of Wild Boletales Mushrooms from France

Sylvie Morel,[1,*] Stéphanie Arnould,[2] Manon Vitou,[1] Frédéric Boudard,[3] Caroline Guzman,[3] Patrick Poucheret,[4] Françoise Fons,[1] & Sylvie Rapior[1]

[1]Laboratoire de Botanique, Phytochimie et Mycologie, Faculté de Pharmacie, CEFE UMR 5175 CNRS Université de Montpellier Université Paul-Valéry Montpellier EPHE, Montpellier, France; [2]Institut de Recherche en Cancérologie de Montpellier, U1194 INSERM/Université de Montpellier/ICM, Montpellier, France; [3]Laboratoire d'Immunologie, Faculté de Pharmacie, UMR 95 Qualisud CIRAD Université de Montpellier, Montpellier, France; [4]Laboratoire de Pharmacologie and Physiopathologie Expérimentale, Faculté de Pharmacie, UMR 95 Qualisud CIRAD Université de Montpellier, Montpellier, France

*Address all correspondence to: Sylvie Morel, Laboratoire de Botanique, Phytochimie et Mycologie, Faculté de Pharmacie, CEFE UMR 5175 CNRS Université de Montpellier Université Paul-Valéry Montpellier EPHE, 15 Avenue Charles Flahault, F-34093 Montpellier Cedex 5, France; sylvie.morel@umontpellier.fr

ABSTRACT: We selected edible and inedible mushrooms growing in the Mediterranean area of France to screen their biological activity: *Caloboletus calopus*, *Rubroboletus lupinus*, *R. pulchrotinctus*, *R. satanas*, *Gyroporus castaneus*, *Suillus luteus*, and *Omphalotus olearius*. Mushrooms were sequentially extracted using cyclohexane, chloroform, ethanol, and water. The antiproliferative activity against the HCT116 colon adenocarcinoma cell line and the antioxidant properties (DPPH radical scavenging assay, Folin-Ciocalteu assay, and oxygen radical absorbance capacity) of the Boletales extracts were evaluated and compared. Among the 28 mushroom extracts evaluated, 11 presented antiproliferative activity against HCT116 cells. These activities were not linked to antioxidant capacity. Among the antioxidant extracts, most were aqueous extracts in the oxygen radical absorbance capacity assay, whereas the highest values on the Folin-Ciocalteu and DPPH assays were noted for chloroform, ethanol, or aqueous extracts, depending on the mushroom species. Further studies are necessary to identify bioactive compounds and to valorize the mushrooms—for edible species, directly as health foods, or, for inedible mushrooms, as ingredients in the pharmaceutical and food industries.

KEY WORDS: activity, antioxidant, antiproliferative, Boletales, *Boletus*, *Caloboletus*, colon adenocarcinoma, *Gyroporus*, HCT116, medicinal mushrooms, *Omphalotus*, ORAC, phenolics, *Rubroboletus*, *Suillus*

ABBREVIATIONS: DMSO, dimethyl sulfoxide; **EC_{50}**, half-maximal effective concentration; **ET**, electron transfer; **F-C**, Folin-Ciocalteu; **GAE**, gallic acid equivalent; **HAT**, hydrogen atom transfer; **IC_{50}**, half-maximal inhibitory concentration; **ORAC**, oxygen radical absorbance capacity; **ROS**, reactive oxygen species; **TE**, Trolox equivalent

I. INTRODUCTION

A total of 110,000 fungal species have been described[1]; among them, macrofungi have been recently studied for bioactive metabolites.[2] Fungal metabolites represent promising therapeutic agents.[1–4] Edible and inedible mushrooms possess interesting and varied compounds, including high molecular weight compounds (e.g., homopolysaccharides, heteropolysaccharides, glycoproteins, glycopeptides, proteins) and low molecular weight compounds (e.g., polyphenols, amines, triacylglycerols, terpenes, steroids, minerals).[5] These compounds are mainly related to anti-Alzheimer, antidiabetes, antimalarial, antimicrobial, antioxidant, antiviral, hypocholesterolemic, and antitumor activities.[1,6–13]

Several medicinal mushroom polysaccharides have immunomodulatory properties and can be useful as immune chemotherapy adjuvants in treating different types of cancer[13–16]: lentinan isolated from *Lentinus edodes*, schizophyllan from *Schizophyllum commune*, and D- and MD-fractions from *Grifola frondosa*.[13–18]

Lectins, as high molecular weight compounds, are glycoproteins or proteins with interesting antitumor, mitogenic, and immunoenhancing activities.[19] Other low molecular weight compounds isolated from mushrooms act as potential antitumor agents through different mechanisms: antioxidant capacity, inhibition of nuclear factor-κB and protein kinases, upregulation of enzymes involved in replication processes, detoxification of mutagenic compounds, direct cytotoxicity against tumor cells, and inhibition of tumor angiogenesis.[5] For example, more than 300 triterpenoids with antiproliferative activity were isolated from mushrooms in the *Ganoderma* genus.[18,20]

Free radicals are atoms or molecules that possess unpaired electrons within their outer orbit. They are generally unstable and very reactive and are produced during normal natural metabolism in aerobic cells, mostly as reactive oxygen species (ROS). Reactive nitrogen species are also present. Physiological concentrations of ROS are involved in cell signaling and regulation and exert beneficial effects. Beyond the physiological level, most free radicals are neutralized by cellular antioxidants such as enzymes and nonenzymatic molecules. Overproduction of ROS or a decrease in antioxidant defense is known as oxidative stress and has been related to many diseases, including several kinds of cancer, diabetes, cirrhosis, cardiovascular diseases, and neurological disorders, as well as to the aging process.[21,22] Antioxidant substances isolated from plants or mushrooms can be useful to reduce oxidative damage in an organism. Moreover, antioxidants can be used to protect food against oxidative deterioration; for example, the European Commission considers rosemary extract to be a food antioxidant.[23] An antioxidative agent can scavenge reactive species (ROS, reactive nitrogen species, etc.) to stop radical chain reactions via the transfer of a hydrogen atom or an electron to reactive species.[24,25] *In vitro* antioxidant capacity assays can be divided into 2 major categories: hydrogen atom transfer (HAT) reaction–based assays and single electron transfer (ET) reaction–based assays.[25,26] Because mushroom extracts are complex mixtures of different substances, antioxidant capacity must be evaluated using complementary methods based on the different reactions involved (ET and HAT). In this study we used 3 assays: oxygen radical absorbance capacity ORAC) as the HAT reaction–based assay, Folin-Ciocalteu (F-C) assay as the ET reaction–based assay, and DPPH as the mixed-mode assay.

Mushrooms are an interesting source of bioactive compounds. Most studies of the order Boletales concern edible mushrooms[22,27–29] such as *Boletus edulis*[30–34] or *B. aereus*.[35] Inedible mushrooms related to Boletales can be interesting sources for bioactive components used in the food and pharmaceutical industries. Chemical composition has been studied or biological screening evaluated for few inedible species, but interesting compounds have been isolated, such as bolesatine from *Rubroboletus satanas*.[36] We therefore evaluated the antioxidant capacity and the antiproliferative activity against HCT116 cells of 6 edible and inedible wild species from Boletales.

II. MATERIALS AND METHODS

A. Mushroom Material

Mushrooms were collected from their natural habitats in the Montpellier area in 2012–2013 and 2014. Three authors (F.F., S.M., and S.R.) taxonomically identified sporocarps based on several publications.[37,38] Information about the wild species collected is provided in Table 1. *Rosmarinus officinalis* was collected in Montpellier in 2014. Fresh mushrooms were cleaned, sliced, frozen, and kept at −20°C until they were freeze-dried. A voucher sample was kept at the Laboratoire de Botanique, Phytochimie et Mycologie, Faculté de Pharmacie, Montpellier. Lyophilized mushrooms were ground before extraction.

TABLE 1: Information About the Wild Mushrooms Analyzed[37–47]

Current Name, by Family	Synonym	Edibility	Habitat	Ecology	Main Compounds Reported
Boletaceae					
Caloboletus calopus (Pers.) Vizzini	*Boletus calopus* Pers.: Fr.	Not considered edible (very bitter taste)	Broadleaf, mixed, and coniferous forests	Mycorrhizal with *Quercus* sp., *Fagus* sp., *Picea* sp., *Abies* sp., or *Pinus* sp.,	δ-lactones (calopin, calopin B, cyclocalopin)[40]
Rubroboletus lupinus (Fr.) Costanzo, Gelardi, Simonini et Vizzini	*B. lupinus* Fr.	Toxic	Warm broadleaf forests	Mycorrhizal with *Quercus* sp. and *Castanea* sp.	None
R. pulchrotinctus (Alessio) Kuan Zhao & Zhu L. Yang	*B. pulchrotinctus* Alessio *Suillellus pulchrotinctus* (Alessio) Blanco-Dios	Toxic	Warm broadleaf forests in calcareous soil	Mycorrhizal with *Quercus* sp.	None
R. satanas (Lenz) Kuan Zhao & Zhu L. Yang	*B. satanas* Lenz	Toxic	Warm broadleaf forests in calcareous soil	Mycorrhizal with *Quercus* sp., *Fagus* sp., *Castanea* sp., *Carpinus* sp., or *Tilia* sp.	Lectin (bolesatine)[36] Amino acids (hydroxynorvaline derivatives)[42]
Suillaceae					
Suillus luteus (L.: Fr.) Roussel	*B. luteus* L.	Edible	Coniferous or mixed forests, artificial plantations	Mycorrhizal with *Pinus* sp.	Ceramides (suillumide derivatives)[41]
Gyroporacaea					
Gyroporus castaneus (Bull.: Fr.) Quélet	*B. castaneus* Bull.	Suspect	Broadleaf or coniferous forests	Mycorrhizal, mostly with *Quercus* sp., but also with *Fagus* sp., *Castanea* sp., or *Betula* sp.	None
Omphalotaceae					
Omphalotus olearius (DC.) Singer	*Clitocybe olearia* (DC.) Maire	Toxic	In broadleaf and mixed forests	Tufted on stumps of deciduous trees, most often *Quercus* sp. or *Fagus* sp.	Sesquiterpenes (illudins)[43,44]

B. Materials

DPPH and AAPH (97%) radicals, gallic acid (97.5%), chlorogenic acid (95%), trichloroacetic acid, sulforhodamine B, Tris buffer, camptothecin, cyclohexane (99.8%), chloroform (99%), and dimethyl sulfoxide (DMSO; 99.9%) were purchased from Sigma-Aldrich (Steinheim, Germany). Anhydrous Na_2CO_3 (99.8%) was purchased from Acros Organics (Geel, Belgium). Trolox (98%) and acetic acid were purchased from Fluka Chemicals (Steinheim, Switzerland). Fluorescein and the F-C reagent were purchased from Panreac (Barcelona, Spain). Ethanol (99.9%) and methanol (99.8%) were purchased from VWR/BDH Prolabo (Radnor, PA).

C. Extract Preparation

Sequential extraction was performed with solvents with increasing polarity (cyclohexane, chloroform, ethanol, water) to extract both nonpolar and polar compounds (10 mL solvent/g dried mushroom). The extraction was conducted under sonication (90 minutes) and temperature was maintained below 30°C. After filtration, the solvents were removed using a vacuum rotary evaporator to dryness (water bath maintained at 30°C) and yielded 4 extracts per mushroom: a cyclohexane extract (CC1), a chloroform extract (CC2), an ethanol extract (CC3), and an aqueous extract (CC4) for *Caloboletus calopus*. These were labeled RL1, RL2, RL3, and RL4, respectively, for *R. lupinus*; RP1, RP2, RP3, and RP4, respectively, for *R. pulchrotinctus*; RS1, RS2, RS3, and RS4, respectively, for *R. satanas*; GC1, GC2, GC3, and GC4, respectively, for *Gyroporus castaneus*; OO1, OO2, OO3, and OO4, respectively, for *Omphalotus olearius*; and SL1, SL2, SL3, and SL4, respectively, for *Suillus luteus*. Powdered extracts were kept at −20°C until testing.

Extraction yields were calculated as (Mass of dried extract in g/Mass of freeze-dried mushroom in g) × 100, and were expressed as a percentage. Total yield was defined as (Sum of masses of dried extracts/Mass of dried mushroom in g) × 100, and was expressed as a percentage. The proportion of each solvent extract was calculated per mushroom as (Mass of dried extract in g/Sum of masses of the 4 dried extracts in g) × 100, and was also expressed as a percentage.

R. officinalis was extracted with ethanol under sonication for 90 minutes at a temperature below 30°C (10 mL ethanol/g dried rosemary leaves). After filtration, evaporation to dryness under reduced pressure yielded a powdered ethanol extract, which was used as a positive control in the DPPH and ORAC assays.

D. Sample Preparation

Crude extracts were extemporaneously prepared in DMSO at 20 mg/mL before the antiproliferative assay in HCT116 colon cancer cells: 1 mg/mL for antioxidant capacity (DPPH, ORAC) and 4 mg/mL for the F-C assay. DMSO solutions were then diluted in culture medium (Dulbecco's modified Eagle's medium for HCT116 cells), in ethanol (for DPPH and ORAC), or in water (for F-C assay) at adequate concentrations.

E. Antiproliferative Activity Against the HCT116 Colon Adenocarcinoma Cell Line

The cytotoxicity assay was performed in HCT116 cells, as described by Skehan et al.,[48] using the sulforhodamine B technique. Briefly, on day 1, 2000 cells/well were seeded in 96-well plates. On day 2, cells were exposed to diluted extracts at a final concentration of 0, 10, 20, 50, or 100 μg/mL. Camptothecin

was used as a quality control (final concentrations: 0.00125, 0.0025, 0.0125, 0.025, 0.125, 0.25, 1.25, or 2.5 μmol/L) in a separate plate. After 24 hours of exposure (37°C in a humidified atmosphere containing 5% CO_2), the medium was removed, wells were washed with phosphate-buffered saline, and drug-free medium was added to each well (150 μL Dulbecco's modified Eagle's medium supplemented with 10 % fetal bovine serum, 100 μg/mL streptomycin, and 100 U/mL penicillin). Cells were grown for another 72 hours and were precipitated with 50 μL ice-cold 50% (w/v) trichloroacetic acid at 4°C for 60 minutes, rinsed 6 times with water, and air-dried. Fixed cells were stained for 30 minutes with 50 μL of 0.4% (w/v) sulforhodamine B solution in 1% (v/v) acetic acid, rinsed 5 times with 1% acetic acid solution, and air-dried. Sulforhodamine B was dissolved with 150 μL of 10 mmol/L Tris buffer (pH 10.5). After stirring for 30 minutes, optical densities at 540 nm were measured using a Tecan Infinite 200 PRO multimode reader (Männedorf, Switzerland). Growth inhibition curves were plotted as a percentage of control cells, and the half-maximal inhibitory concentrations (IC_{50}; the concentration of a sample that is required for 50% inhibition *in vitro*) were determined. The results are presented as the mean ± SD of 3 independent experiments (3 wells/concentration for each experiment).

F. DPPH Radical Scavenging Assay

Radical scavenging activity was evaluated using DPPH free radicals according to the method described by Morel et al.,[49] with some modifications. Tested extracts were diluted in absolute ethanol at different concentrations (0.2, 0.5, or 1 mg/mL) of 4 mg/mL stock solutions in DMSO. Ethanol was used as a blank, and 10, 25, 50, and 75 μmol/L Trolox (hydrophilic α-tocopherol analogue) were used as calibration solutions. A sample of 0.02 mg/mL chlorogenic acid and an ethanol extract of *R. officinalis* (0.1, 0.2, 0.5 mg/mL) were used for quality control. Sample or standard solutions (100 μL) were placed in 96-well plates in triplicate for each concentration tested. Absolute ethanol (75 μL) was added. The reaction was initiated by adding 25 μL freshly prepared DPPH solution (1 mmol/L) to obtain a final volume of 200 μL/well. After 30 minutes in the dark at room temperature, absorbance was determined at 550 nm with a Molecular Devices UV Max microtiter plate reader (MDS Inc., Toronto, Canada). The DPPH scavenging activity of the tested compounds was compared with the Trolox calibration curve. Results are expressed as Trolox equivalents (TEs; micromoles of TEs per gram of dry extract). Results are also expressed as the effective concentration at which 50% of DPPH radicals are scavenged (EC_{50}; milligrams per milliliter).[5,21] The results are the mean ± SD of 3 independent experiments (3 wells/concentration for each experiment).

G. F-C Assay

Total phenolic and other oxygen radical scavengers in crude extracts were quantitated using the F-C method as described by Pawar and Dasgupta,[50] with some modifications. Standard solutions of gallic acid (1.56, 3.125, 6.25, 12.5, 25, 50, 75 μg/mL) were prepared in distilled water. Mushroom and *R. officinalis* extracts were prepared in DMSO (4 mg/mL), then diluted in water to obtain a concentration of 1 mg/mL (0.5 mg/mL for the ethanol extract of *C. calopus*). Standard, mushroom extract, and *R. officinalis* extract were deposited in a 96-well plate in triplicate (50 μL/well). Distilled water (50 μL) and then 50 μL of 10% F-C phenol reagent and 50 μL sodium carbonate solution (1 M) were added to each well. The plate was incubated in the dark at room temperature with continuous stirring for 60 minutes. The absorbance was measured at 650 nm with a Molecular Devices UV Max microtiter plate reader. Results of the F-C assay are expressed as milligrams of gallic acid equivalents (GAEs) per gram of dry extract. The results are the mean ± SD of 3 independent experiments (3 wells/concentration for each experiment).

H. Oxygen Radical Absorbance Capacity

ORAC assays were carried out according to the method of Lavaud et al.,[51] with some modifications. Samples were diluted to 25 µg/mL using a 1 mg/mL stock solution in DMSO. A 75 mmol/L phosphate buffer solution (pH 7.4) was used as a blank, and Trolox concentrations of 6.25, 12.5, 25, 50, and 75 µmol/L were used as the calibration solutions. An 8.8 µmol/L chlorogenic acid sample and an ethanol extract of *R. officinalis* (12.5 µg/mL) were used as quality controls. The sample or standards solutions (20 µL) were deposited in triplicate in an opaque-walled 96-well plate. To the plate were added 100 µL of 75 mmol/L phosphate buffer and 100 µL freshly prepared fluorescein solution (0.1 µmol/L in phosphate buffer). The plate was then preincubated at 37°C for 10 minutes, with stirring. The reaction was started by adding freshly prepared AAPH solution (103.2 mg/mL in phosphate buffer). Fluorescence was then measured and recorded at 485 nm excitation and 535 nm emission for 70 minutes using a TriStar LB 941 Multimode Microplate Reader (Berthold Technologies, Bad Wildbad, Germany). The final ORAC values were calculated using a regression equation between the Trolox concentration and the net area under the fluorescein decay curve, and were expressed as micromoles of TEs per gram of dry matter. The area under the curve was calculated using the trapezoidal rule. The results are the mean ± SD of 3 independent experiments (3 wells/concentration for each experiment).

III. RESULTS AND DISCUSSION

A. Extraction

Solvents with different polarities (cyclohexane, chloroform, ethanol, and water) were used sequentially to extract various compounds—both nonpolar and polar—from the mushrooms. Extraction yields were calculated for each mushroom and are presented in Table 2. The average yield of the extraction procedure was around 30% (w/w), with the advantage of being performed at a low temperature (< 30°C) to avoid thermodegradation and within a reasonable extraction time (6 hours for the 4 extracts from a single mushroom). Proportions of each solvent extract per mushroom are presented in Fig. 1. Extraction profiles were identical among the tested Boletales, with major proportions of aqueous extracts (> 50%) except for *C. calopus* and *R. pulchrotinctus*, which presented low proportions of aqueous extracts (25% of the total extracted material). Ethanol extracts represented around 30% (50% for *C. calopus* and *R. pulchrotinctus*). Extraction yields for *R. lupinus* were within the same range as those obtained previously by

TABLE 2: Extraction Yields of Mushrooms upon Isolation by Different Solvents

Mushroom	Extraction Yield, % (w/w)				
	Cyclohexane	Chloroform	Ethanol	Water	Total
Caloboletus calopus	4.03	2.06	15.53	7.09	28.71
Rubroboletus lupinus	3.46	1.80	12.30	15.98	33.54
R. pulchrotinctus	1.69	3.55	17.98	8.11	31.33
R. satanas	1.31	1.62	6.53	13.19	22.65
Suillus luteus	2.04	1.48	14.85	16.70	35.07
Gyroporus castaneus	1.64	1.05	4.47	16.91	24.07
Ompalotus olearius	3.62	1.13	2.61	20.77	28.12

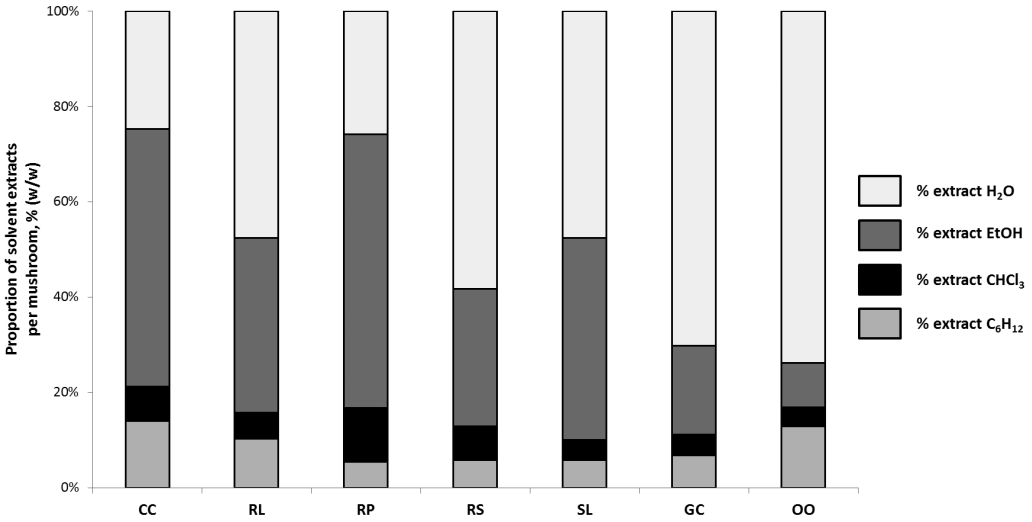

FIG. 1: Representative proportions of solvent extracts per mushroom. CC, *Caloboletus calopus*; GC, *Gyroporus castaneus*; OO, *Omphalotus olearius*; RL, *Rubroboletus lupinus*; RP, *Rubroboletus pulchrotinctus*; RS, *Rubroboletus satanas*; SL, *Suillus luteus.*

Smolskaite et al.[52] using cyclohexane, dichloromethane, and methanol. Furthermore, *O. olearius* possessed a different profile from the other mushrooms, with a small proportion of ethanol extract (9%). *O. olearius* belongs to a distant mushroom genus. The genus *Omphalotus* was historically classified in the order Boletales because Boletales pigments were detected in the species.[53] Singh[54] effectively isolated from *O. illudens* atromentic acid, a pigment also present in Paxillaceae (Boletales). The family Omphalotaceae was later established, in particular because these mushrooms contain specific sesquiterpenes (illudins), contrary to Paxillaceae.[53] Illudin derivatives display anticancer potential.[55,56] We added this species to our study as a quality control in the antiproliferative assay in order to compare its activity with that of edible and inedible Boletales.

B. Antiproliferative Activity Against the HCT116 Colon Adenocarcinoma Cell Line

We tested the potential antiproliferative activity of 28 extracts against the HCT116 colon adenocarcinoma cell line (Table 3). Consistent with criteria from the National Cancer Institute and previous studies,[57,58] 6 extracts presented antiproliferative activities with an $IC_{50} < 20$ µg/mL, and 5 extracts possessed moderate activity (20 µg/mL < IC_{50} < 100 µg/mL), whereas 17 extracts were inactive ($IC_{50} > 100$ µg/mL). The highest antiproliferative activities were obtained for *S. luteus* and *O. olearius*, which are known to have effects against different cell lines.[41,56,59]

O. olearius has been reported as a species with anticancer potential. The molecules responsible for the activities were purified and identified as illudins. These compounds were tested by the National Cancer Institute and had significant antitumor activity but a poor therapeutic index. Irofulven (a semisynthetic derivative) was tested in clinical trials, but its administration resulted in serious side effects.[60,61] New derivatives are currently being synthesized to increase the therapeutic index,[55,56] and new compounds are being isolated.[43,44] In this study, the chloroform, ethanol, and aqueous extracts had antiproliferative activity against the HCT116 cell line (Table 3). Illudin derivatives, as sesquiterpenic compounds, were mainly isolated from chloroform and ethyl acetate extracts,[43,62] which suggests that the aqueous extract may contain new antiproliferative compounds.

TABLE 3: Antiproliferative Activity and Antioxidant Capacity of Mushroom Extracts in Different Solvents

Extracts	IC_{50} (µg/mL) against HCT116 Cells	DPPH		F-C Assay (mg GAE/g EDW)	ORAC (µmol TE/g EDW)
		EC_{50} (mg/mL)	TE (µmol TE/g EDW)		
Caloboletus calopus					
CC1	> 100	> 1	15.19 ± 2.35	6.34 ± 0.43	631.53 ± 30.23
CC2	> 100	0.14 ± 0.01	221.21 ± 18.12	27.18 ± 2.96	745.68 ± 108.76
CC3	> 100	0.43 ± 0.04	110.49± 8.07	90.16 ± 5.89	1083.39 ± 109.88
CC4	> 100	0.62 ± 0.04	102.55 ± 5.54	34.90 ± 3.82	1278.90 ± 64.60
Rubroboletus lupinus					
RL1	84.37 ± 9.43	> 1	22.53 ± 3.27	9.95 ± 1.23	560.07 ± 32.61
RL2	> 100	> 1	30.27 ± 1.31	5.12 ± 0.71	953.63 ± 35.86
RL3	> 100	> 1	59.81 ± 4.39	10.17 ± 1.09	500.49 ± 45.04
RL4	> 100	> 1	68.20 ± 9.04	25.51 ± 3.58	1002.83 ± 148.60
R. pulchrotinctus					
RP1	67.99 ± 8.35	> 1	20.39 ± 2.55	4.22 ± 0.13	382.75 ± 39.57
RP2	> 100	> 1	25.63 ± 3.03	10.90 ± 1.55	579.91 ± 86.35
RP3	> 100	> 1	45.60 ± 4.02	22.27 ± 1.17	641.83 ± 45.64
RP4	> 100	> 1	57.63 ± 8.33	33.08 ± 2.00	1166.79 ± 167.37
R. satanas					
RS1	64.41 ± 8.42	> 1	14.80 ± 2.13	8.22 ± 1.09	523.83 ± 17.35
RS2	> 100	> 1	30.93 ± 4.51	14.31 ± 1.50	927.82 ± 114.26
RS3	> 100	0.91 ± 0.04	72.75 ± 5.98	17.46 ± 1.86	542.65 ± 61.10
RS4	> 100	> 1	59.09 ± 7.35	30.52 ± 4.16	862.37 ± 68.49
Suillus luteus					
SL1	3.45 ± 0.13	0.35 ± 0.02	178.72 ± 12.69	19.03 ± 1.97	495.08 ± 57.17
SL2	3.69 ± 0.13	0.26 ± 0.04	209.84 ± 7.80	25.81 ± 1.99	768.32 ± 50.28
SL3	15.31 ± 5.99	> 1	51.70 ± 6.85	10.30 ± 0.51	510.22 ± 21.86
SL4	> 100	> 1	41.23 ± 5.66	17.38 ± 1.98	1030.61 ± 70.85
Gyroporus castaneus					
GC1	67.22 ± 6.63	> 1	23.22 ± 0.55	3.57 ± 0.31	368.18 ± 35.84
GC2	74.48 ± 12.44	> 1	22.77 ± 3.33	3.83 ± 0.47	864.15 ± 122.43
GC3	> 100	0.72 ± 0.05	87.02 ± 5.36	17.16 ± 1.94	904.98 ± 91.46
GC4	> 100	> 1	49.28 ± 3.07	18.07 ± 3.09	1077.72 ± 106.09
Ompalotus olearius					
OO1	> 100	> 1	23.58 ± 0.51	4.46 ± 0.17	457.08 ± 66.51
OO2	5.75 ± 0.10	> 1	11.76 ± 1.37	3.88 ± 0.17	265.29 ± 17.22
OO3	3.90 ± 0.27	> 1	41.42 ± 2.80	4.14 ± 0.23	613.75 ± 42.30
OO4	8.19 ± 0.35	> 1	23.64 ± 1.59	9.05 ± 1.26	874.88 ± 107.49

TABLE 3: (*continued*)

Rosmarinus officinalis					
Ethanol extract	n.d.	0.32 ± 0.02	259.82 ± 33.06	47.71±6.94	2560.95 ± 374.31
Chlorogenic acid	n.d.	n.d.	3236.57 ± 305.97	n.d.	11515.38 ±1721.66
Camptothecin	0.08 ± 0.01	n.d.	n.d.	n.d.	n.d.

Values are mean ± standard deviation (n = 3). EC_{50}, half-maximal effective concentration; EDW, extract dry weight; F-C, Folin-Ciocalteu; GAE, gallic acid equivalent; IC_{50}, half-maximal inhibitory concentration; n.d., not determined; ORAC, oxygen radical absorbance capacity; TE, Trolox equivalent.

A methanol extract of *S. luteus* was cytotoxic to 2 murine cancer cell lines (L1210 and 3LL; IC_{50} = 18.1 and 40.3 µg/mL, respectively).[59] Another methanol extract had potent activity against the human HCT-15 colon cancer cell line, with an IC_{50} of 17.75 µg/mL. The same extract had moderate activity against the NCI-H460 lung cancer cell line (IC_{50} = 30.33 µg/mL), MCF-7 breast cancer cell line (IC_{50} = 32.25 µg/mL), and AGS gastric cancer cell line (IC_{50} = 30.30 µg/mL). The extract was not cytotoxic to primary porcine PLP2 hepatocytes (IC_{50} > 400 µg/mL). In the same study, ethanol and aqueous extracts of *S. luteus* had no antiproliferative activity (IC_{50} > 400 µg/mL).[63] From an ethanol extract of *S. luteus*, Leon et al.[41] isolated suillumide, a ceramide with potent cytotoxicity against the human SK-MEL-1 melanoma cell line, with an IC_{50} around 10 µmol/L. Further experiments will be performed to identify compounds responsible for the cytotoxic activity of our *S. luteus* extracts. Nevertheless, because the activity is concentrated in nonpolar fractions, suillumide or related compounds could be responsible for the activity against HCT116 cells.

Our results are consistent with those published for *S. luteus* and *O. olearius*.[56,59,63] Unfortunately, no additional mushroom presented important antiproliferative activity, but 5 mushroom extracts (from *R. lupinus*, *R. pulchrotinctus*, *R. satanas*, and *G. castaneus*) did have moderate activity, with IC_{50} between 20 and 100 µg/mL (Table 3). Moreover, the antiproliferative activity of these mushroom extracts has, to our knowledge, never been tested. Activity was concentrated within the nonpolar cyclohexane extracts (and in the chloroform extract, in the case of *G. castaneus*). As *R. lupinus*, *R. pulchrotinctus*, and *R. satanas* belong to the new genus *Rubroboletus* (corresponding to the *Satanas* group of *Boletus* in another classification),[37] one may suppose the bioactive compounds are similar in these 3 species.

Bolesatine, a mitogenic lectin, was previously isolated from *R. satanas*[36] and can account for the toxicity of this mushroom. This glycoprotein inhibits protein synthesis in liver and kidney cells[54,64] and can be considered a nucleoside triphosphate phosphatase.[65] Bolesatine also induces secretion of interleukins 1α and 2 from mononuclear cells.[66] Hydroxynorvaline derivatives were also previously isolated from a methanol extract of *R. satanas*.[42] No antiproliferative activity was explored for these compounds. The antiproliferative activity of the cyclohexane extract RS1 therefore cannot be attributed to bolesatine, which is a polar compound, but rather to unidentified nonpolar compounds such as triterpenoids, steroids, ceramides, or lactones. Thus further studies must be conducted to identify the compounds involved in these activities for *R. satanas* and for *R. pulchrotinctus*, *R. lupinus*, and *G. castaneus*.

C. Antioxidant Properties

Studies of antioxidant properties of some Boletales are described in the literature.[21,28,30,33] The DPPH test is commonly used to assess antioxidant capacity,[21,27,28,30,33,52,67–69] with wide interlaboratory variability in methodology (DPPH concentration, extract concentrations, duration of reaction, etc.), which makes comparison difficult.[70] Furthermore, results are expressed in different ways: mainly TEs and EC_{50}, but also as an

antioxidant activity index[71] or antioxidant activity unit.[72] We therefore chose in this study to present results with the 2 most commonly used expressions (TEs and EC_{50}) so as to make easier the comparison with data from the literature. ORAC, as a HAT-based assay, is less used in the literature in the case of mushrooms, but it complements the antioxidant description of mushrooms. Furthermore, the F-C assay is presented for each extract, and comments are given about their significance. The F-C assay, which commonly measures total phenolic content, is based on an ET reaction and measures the reducing capacity of a sample.[25] This method is generally used to quantify the phenolic content in plant extracts, as most phenolics react with the F-C reagent. But the term *total phenolic content* is partly incorrect because the F-C reagent measures the total reducing capacity of a sample, not solely the reducing capacity of phenolic compounds. It also reacts with some nitrogen-containing compounds, thiols, vitamins, nucleotide bases, and carbohydrates.[73]

Our results were compared with data obtained from an ethanol extract of *R. officinalis* that was accepted as a food antioxidant (E 392) in 2010.[23] As presented in Table 3, among 28 mushroom extracts that were evaluated with the DPPH assay, 7 had an EC_{50} < 1 mg/mL—namely, chloroform, ethanol, and aqueous extracts of *C. calopus*; ethanol extracts of *R. satanas* and *G. castaneus*; and cyclohexane and chloroform extracts of *S. luteus*. The ORAC assay revealed 6 extracts with high values (> 1000 µmol TE/g dry extract): ethanol and aqueous extracts of *C. calopus* and the aqueous extracts of *R. lupinus*, *R. pulchrotinctus*, *G. castaneus*, and *S. luteus*. The highest F-C assay value was obtained for the ethanol extract of *C. calopus*. Except for *R. lupinus*,[52] no record of ORAC or F-C assay for these mushrooms is, to our knowledge, currently available in the literature.

Results from these 3 assays show that *C. calopus* is the most interesting mushroom, with values in accordance with those from the literature.[40,74] Indeed, Macakova et al.[74] evaluated a *C. calopus* ethanol extract using the DPPH assay and found an EC_{50} of 0.32 mg/mL. Moreover, the bioactive fractionation of a methanol extract of this mushroom led to the isolation of calopin, calopin B, and cyclocalopin A, which have antioxidant properties and EC_{50} values of 2.2, 5.4, and 2.0 µg/mL, respectively, in the DPPH assay.[40] Our results show that DPPH values were not proportionally linked with the F-C assay (Figs. 2–8), as the highest DPPH value was obtained with the chloroform extract (EC_{50} = 0.14 mg/mL), whereas the F-C assay value was higher for the ethanol extract (90.16 mg GAE/g extract dry weight). Furthermore,

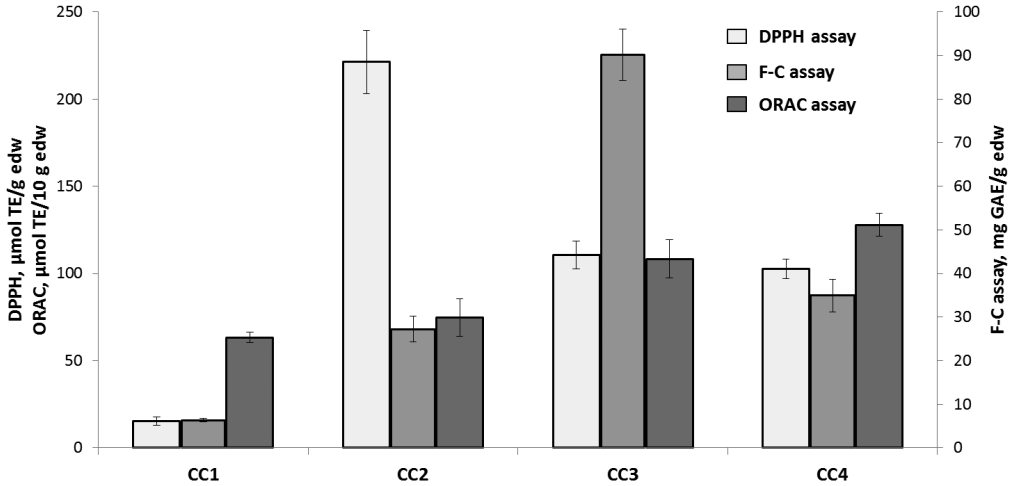

FIG. 2: Antioxidant profile for *Caloboletus calopus*. Data are presented as the mean ± SD (n = 3). CC1, cyclohexane extract of *C. calopus*; CC2, chloroform extract of *C. calopus*; CC3, ethanol extract of *C. calopus*; CC4, aqueous extract of *C. calopus*; F-C, Folin-Ciocalteu; GAE, gallic acid equivalent; ORAC, oxygen radical absorbance assay; TE, Trolox equivalent.

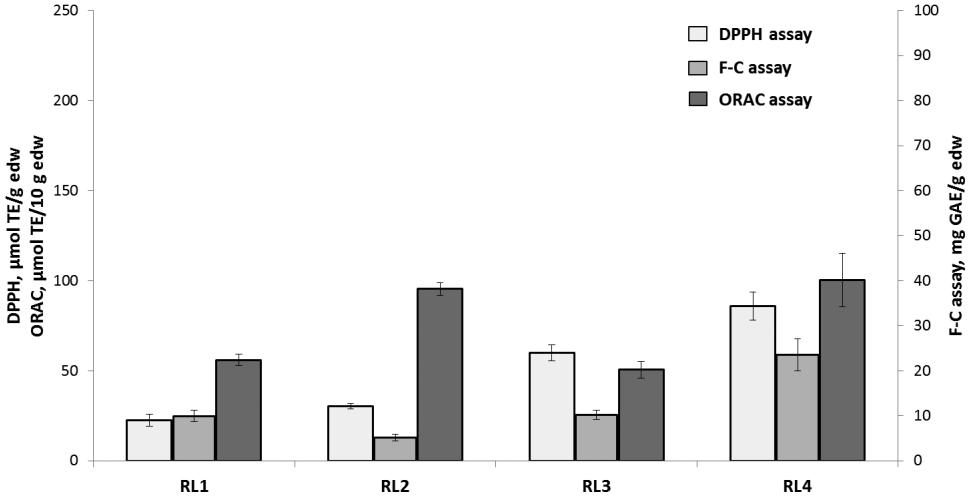

FIG. 3: Antioxidant profile for *Rubroboletus lupinus*. Data are presented as the mean ± SD (n = 3). F-C, Folin-Ciocalteu; GAE, gallic acid equivalent; ORAC, oxygen radical absorbance assay; RL1, cyclohexane extract of *R. lupinus*; RL2, chloroform extract of *R. lupinus*; RL3, ethanol extract of *R. lupinus*; RL4, aqueous extract of *R. lupinus*; TE, Trolox equivalent.

with the ORAC assay, the ethanol and aqueous extracts had higher values than the chloroform extract (Fig. 2). We can therefore suppose that the antioxidant potential of this mushroom is attributable to a mix of phenolic and other compounds. Lactones certainly contribute to the antioxidant capacity observed. Further studies will be conducted to confirm these results with other antioxidant assays, to identify the active compounds, and, finally, to obtain an extract that is enriched with antioxidants. *C. calopus* is not toxic, but it is not deemed edible because it has a very bitter taste.

S. luteus also displayed interesting antioxidant properties in the 3 assays, in particular the chloroform extract in the DPPH assay and the aqueous extract in the ORAC assay. A methanol extract of *S. luteus*

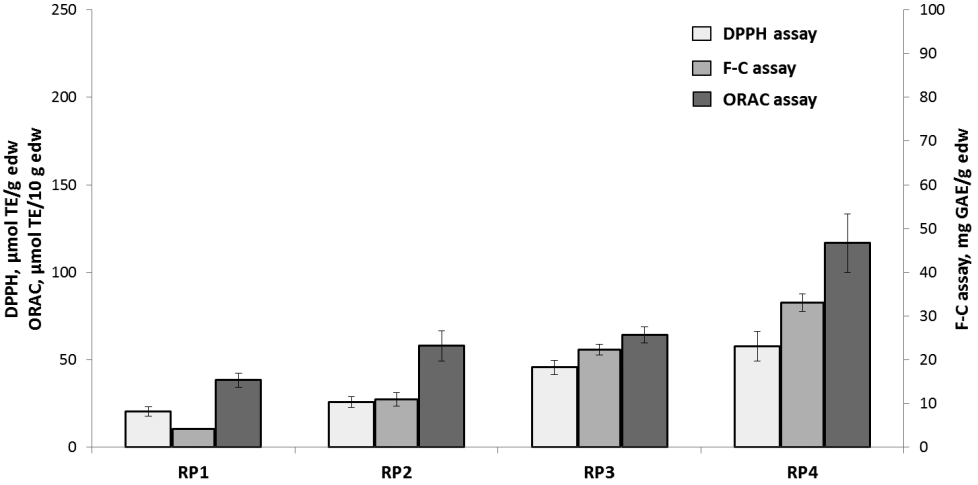

FIG. 4: Antioxidant profile for *Rubroboletus pulchrotinctus*. Data are presented as the mean ± SD (n = 3). F-C, Folin-Ciocalteu; GAE, gallic acid equivalent; ORAC, oxygen radical absorbance assay; RP1, cyclohexane extract of *R. pulchrotinctus*; RP2, chloroform extract of *R. pulchrotinctus*; RP3, ethanol extract of *R. pulchrotinctus*; RP4, aqueous extract of *R. pulchrotinctus*; TE, Trolox equivalent.

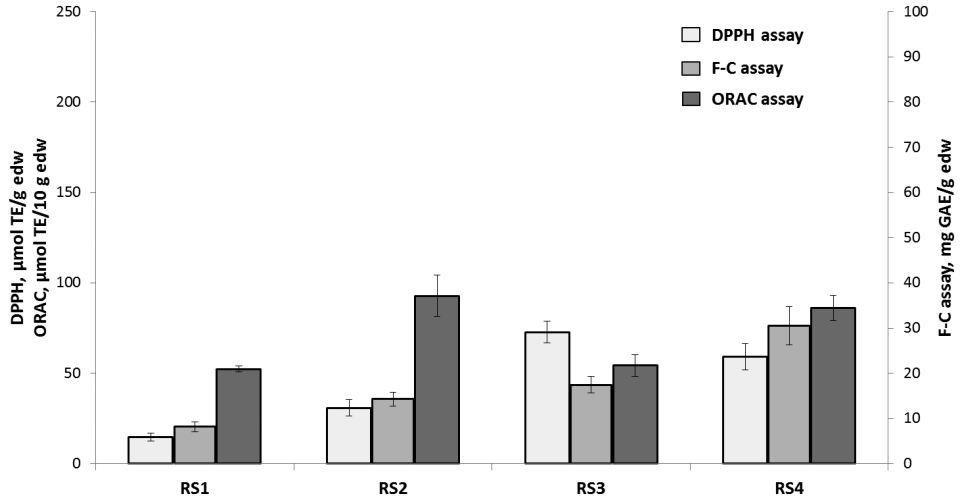

FIG. 5: Antioxidant profile for *Rubroboletus satanas*. Data are presented as the mean ± SD (n = 3). F-C, Folin-Ciocalteu; GAE, gallic acid equivalent; ORAC, oxygen radical absorbance assay; RS1, cyclohexane extract of *R. satanas*; RS2, chloroform extract of *R. satanas*; RS3, ethanol extract of *R. satanas*; RS4, aqueous extract of *R. satanas*; TE, Trolox equivalent.

was previously tested using DPPH and displayed a moderate antioxidant property, with an EC_{50} of 1.92 mg/mL,[75] whereas an ethanol extract of this mushroom had an EC_{50} of 0.66 mg/mL.[74] We found a higher antioxidant capacity for cyclohexane and chloroform extracts (EC_{50} = 0.35 and 0.26 mg/mL, respectively, on the DPPH assay), which suggests that antioxidant capacity is not linked with phenolic content (Fig. 6), but rather with other compounds. As ceramide compounds have been isolated from *S. luteus*,[41] it would be interesting to evaluate the antioxidant potential of suillimide and related compounds.

An extract of *R. satanas* using acetone and water (80:20) had a moderate antioxidant capacity, with an EC_{50} of 0.43 mg/mL.[76] An ethanolic extract of *R. satanas* was not active upon DPPH testing, with an EC_{50} > 1 mg/mL.[74] In our study, only the ethanol extract had a notable antioxidant property upon DPPH

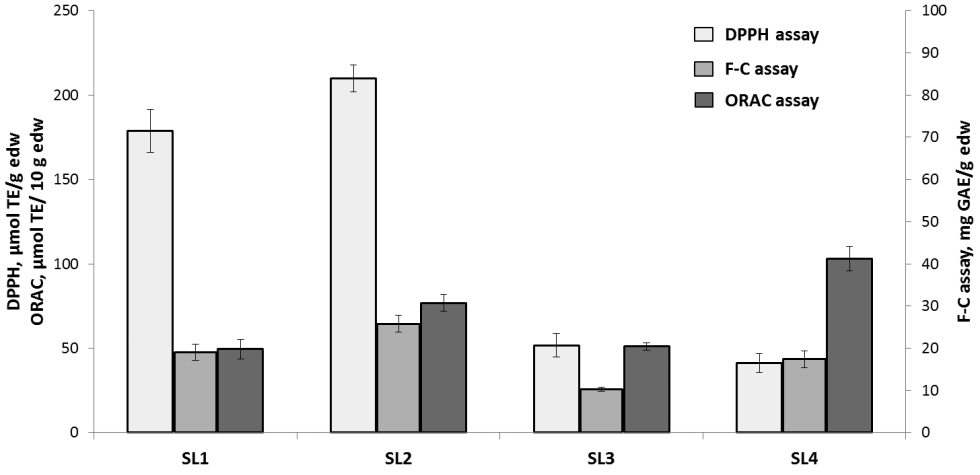

FIG. 6: Antioxidant profile for *Suillus luteus*. Data are presented as the mean ± SD (n = 3). F-C, Folin-Ciocalteu; GAE, gallic acid equivalent; ORAC, oxygen radical absorbance assay; SL1, cyclohexane extract of *S. luteus*; SL2, chloroform extract of *S. luteus*; SL3, ethanol extract of *S. luteus*; SL4, aqueous extract of *S. luteus*; TE, Trolox equivalent.

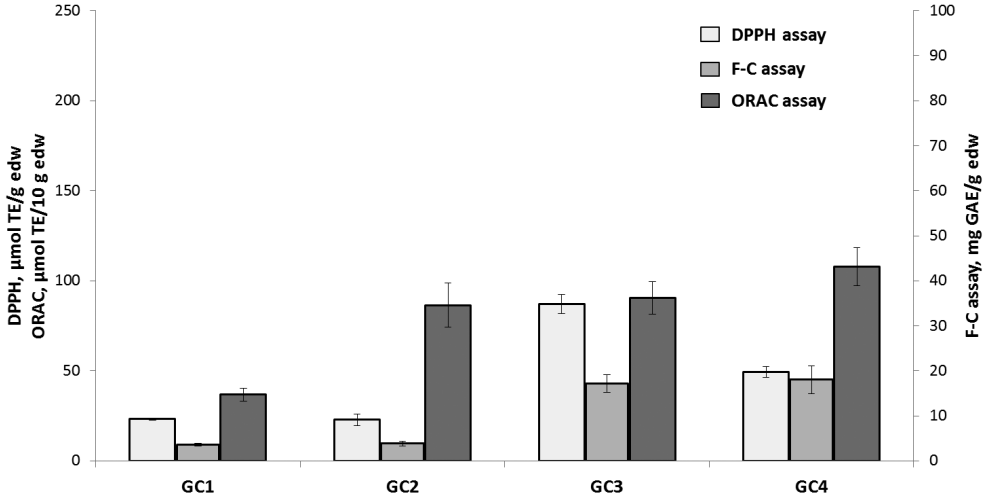

FIG. 7: Antioxidant profile for *Gyroporus castaneus*. Data are presented as the mean ± SD (n = 3). F-C, Folin-Ciocalteu; GAE, gallic acid equivalent; GC1, cyclohexane extract of *G. castaneus*; GC2, chloroform extract of *G. castaneus*; GC3, ethanol extract of *G. castaneus*; GC4, aqueous extract of *G. castaneus*; ORAC, oxygen radical absorbance assay; TE, Trolox equivalent.

testing (EC_{50} = 0.91 mg/mL), whereas the chloroform and aqueous extracts had higher values in the ORAC assay (927.82 and 862.37 µmol TE/g extract dry weight, respectively) (Fig. 5).

The antioxidant capacity of sporophores of *O. olearius* has, to our knowledge, never been investigated. Asatiani et al.[77] evaluated a hot water and an ethanolic extract from submerged *O. olearius* mycelia with the DPPH assay, with EC_{50} of 6 and 0.9 mg/mL, respectively. Kalyoncu et al.[78] reported the antioxidant capacity of chloroform, ethanol, and aqueous mycelial extracts from *O. olearius*. At 1 mg/mL, the percentages of inhibition of DPPH were 47.11%, 60.25%, and 22.97%, respectively. Our results confirm the low antioxidant capacity of *O. olearius* (Fig. 8).

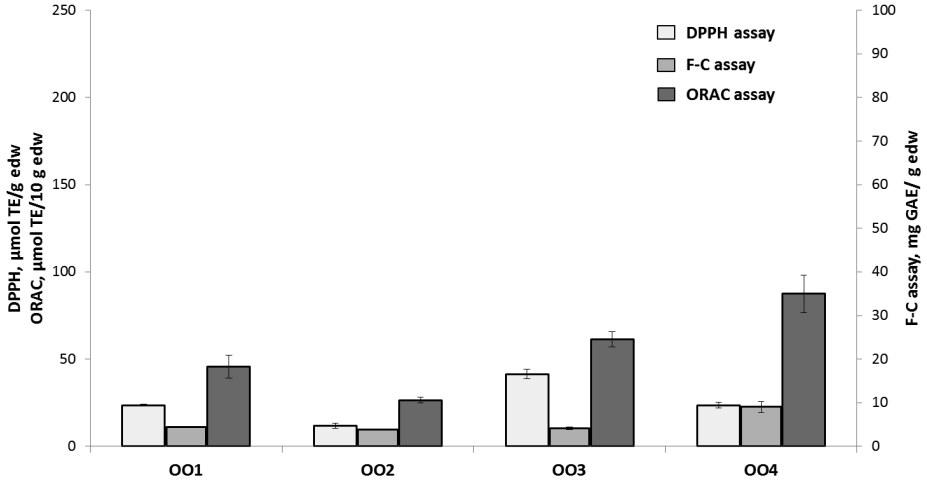

FIG. 8: Antioxidant profile for *Omphalotus olearius*. Data are presented as the mean ± SD (n = 3). F-C, Folin-Ciocalteu; GAE, gallic acid equivalent; OO1, cyclohexane extract of *O. olearius*; OO2, chloroform extract of *O. olearius*; OO3, ethanol extract of *O. olearius*; OO4, aqueous extract of *O. olearius*; ORAC, oxygen radical absorbance assay; TE, Trolox equivalent.

Smolskaite et al.[52] recently performed DPPH, ORAC, and F-C assays with 3 extracts of *R. lupinus* that were obtained using a Soxhlet apparatus with cyclohexane, dichloromethane, and methanol (5-hour extraction for each solvent). Our results for the F-C assay are in perfect accord with those previously obtained. Some differences were observed upon DPPH and ORAC testing, but these can be explained by the differences in extract preparation or in the procedures used to measure antioxidant capacity (Fig. 3).

R. pulchrotinctus and *G. castaneus* have, to our knowledge, never been evaluated for their antioxidant potential. The aqueous extract of *R. pulchrotinctus* had higher scores than the other extracts on the 3 assays (Fig. 4). The ethanol extract of *G. castaneus* displayed the best values on the DPPH assay, and the aqueous extract also had a significant antioxidant capacity as determined with the F-C and ORAC assays (Fig. 7).

Finally, in the DPPH assay, the chloroform extract from *C. calopus* and the cyclohexane and chloroform extracts from *S. luteus* had values within the same range as the ethanol extract from rosemary (values around 200 µmol TE/g extract dry weight). On the ORAC assay, the highest values were generally obtained for aqueous extracts (~1000 µmol TE/g extract dry weight), albeit significantly lower than rosemary extracts (2560.95 µmol TE/g extract dry weight). In the F-C assay, the ethanol extract of *C. calopus* displayed the highest values (90.16 mg GAE/g extract dry weight). Our results show no link between the DPPH and F-C assays, which suggests that various classes of compounds are involved in the antioxidant capacity of these mushroom species.

IV. CONCLUSIONS

This investigation focused on the antiproliferative and antioxidant properties of 6 wild mushrooms from the order Boletales. Sequential extractions under sonication led to yields around 30%. *C. calopus* and *R. pulchrotinctus* had similar extraction profiles, with important proportions of ethanol extracts. For the other mushrooms, aqueous extracts gave the highest extraction yields. The obtained extracts were evaluated for antioxidant capacity and antiproliferative activity. The antiproliferative activity was not linked with the antioxidant potential of these mushrooms. In this study, *C. calopus* seems to be the most promising mushroom for antioxidant applications (therapeutics or foods). Three extracts had antioxidant capacity—namely, the chloroform extract in the DPPH assay, the ethanolic extract in the F-C assay, and the aqueous extract in the ORAC assay. The mushroom contains a mix of both nonpolar and polar antioxidant compounds. *S. luteus*, in particular the chloroform extract, seems to be a good source of antioxidant agents and had a high value in the 3 assays. *R. lupinus*, *R. pulchrotinctus*, and *R. satanas* have recently been classified in the genus *Rubroboletus*.[47] In this study, cyclohexane extracts of these 3 mushrooms had moderate activity against the HCT116 cancer cell line, suggesting the 3 extracts contain bioactive compounds that could be related. While antioxidant capacity was concentrated in aqueous extracts for *R. lupinus* and *R. pulchrotinctus* in the 3 assays, it was disperse in the chloroform, ethanol, and aqueous extracts of *R. satanas*. Cyclohexane and chloroform extracts of *G. castaneus* displayed moderate antiproliferative activities, whereas the ethanol and aqueous extracts presented the highest values upon antioxidant testing. This mushroom has, to our knowledge, never been tested and studied before, so it will be interesting to identify its bioactive compounds. As a whole, these results indicate that Boletales species may be sources of bioactive compounds with promising applications in medicine and nutrition supplementation.

ACKNOWLEDGMENTS

The authors are grateful to Jacqueline Casadebaig and Luc Maury for collecting some of the mushroom samples.

REFERENCES

1. Wasser SP. Medicinal mushroom science: history, current status, future trends, and unsolved problems. Int J Med Mushrooms. 2010;12(1):1–16.
2. De Silva DD, Rapior S, Sudarman E, Stadler M, Xu J, Alias SA, Hyde KD. Bioactive metabolites from macrofungi: ethnopharmacology, biological activities and chemistry. Fungal Divers. 2013;62(1):1–40.
3. Poucheret P, Fons F, Rapior S. Biological and pharmacological activity of higher fungi: 20-year retrospective analysis. Cryptogam Mycol. 2006;27(4):311–33.
4. Fortin H, Tomasi S, Delcros JG, Bansard JY, Boustie J. In vivo antitumor activity of clitocine, an exocyclic nucleoside isolated from Lepista inversa. Chem Med Chem. 2006;1:189–96.
5. Ferreira ICFR, Vaz JA, Vasconcelos MH, Martins A. Compounds from wild mushrooms with antitumor potential. Anticancer Agents Med Chem. 2010;10(5):424–36.
6. Gargano ML, van Griensven LJLD, Isikhuemhen OS, Lindequist U, Venturella G, Wasser SP, Zervakis GI. Medicinal mushrooms: valuable biological resources of high exploitation potential. Plant Biosyst. 2017;151(3):548–65.
7. De Silva DD, Rapior S, Hyde KD, Bahkali AH. Medicinal mushrooms in prevention and control of diabetes mellitus. Fungal Divers. 2012;56(1):1–29.
8. De Silva DD, Rapior S, Fons F, Bahkali AH, Hyde KD. Medicinal mushrooms in supportive cancer therapies: an approach to anti-cancer and putative mechanisms of action. Fungal Divers. 2012;55(1):1–35.
9. Popovic V, Zivkovic J, Davidovic S, Stevanovic M, Stojkovic D. Mycotherapy of cancer: an update on cytotoxic and antitumor activities of mushrooms, bioactive principles and molecular mechanisms of their action. Curr Top Med Chem. 2013;13(21):2791–806.
10. Froufe HJC, Abreu RMV, Ferreira ICFR. Virtual screening of low molecular weight mushrooms compounds as potential Mdm2 inhibitors. J Enzym Inhib Med Chem. 2013;28(3):569–75.
11. Durgo K, Koncar M, Komes D, Belscak-Cvitanovic A, Franekic J, Jakopovich I, Jakopovich N, Jakopovich B. Cytotoxicity of blended versus single medicinal mushrooms extracts on human cancer cell lines: contribution of polyphenol and polysaccharide content. Int J Med Mushrooms. 2013;15(5):435–48.
12. Synytsya A, Novak M. Structural diversity of fungal glucans. Carbohydr Polym. 2013;92(1):792–809.
13. Wasser SP. Medicinal mushrooms in human clinical studies. Part I. Anticancer, oncoimmunological, and immunomodulatory activities: a review. Int J Med Mushrooms. 2017;19(4):279–317.
14. Aleem E. β-glucans and their applications in cancer therapy: focus on human studies. Anticancer Agents Med Chem. 2013;13:709–19.
15. Ruthes AC, Smiderle FR, Iacomini M. D-glucans from edible mushrooms: a review on the extraction, purification and chemical characterization approaches. Carbohydr Polym. 2015;117:753–61.
16. Zhu F, Du B, Bian Z, Xu B. β-glucans from edible and medicinal mushrooms: characteristics, physicochemical and biological activities. J Food Compost Anal. 2015;41:165–73.
17. Meng X, Liang H, Luo L. Antitumor polysaccharides from mushrooms: a review on the structural characteristics, antitumor mechanisms and immunomodulating activities. Carbohydr Res. 2016;424:30–41.
18. Ivanova TS, Krupodorova TA, Barshteyn VY, Artamonova AB, Shlyakhovenko VA. Anticancer substances of mushroom origin. Exp Oncol. 2014;36(2):58–66.
19. Singh RS, Kaur HP, Kanwar JR. Mushroom lectins as promising anticancer substances. Curr Protein Pept Sci. 2016;17:797–807.
20. Luo Q, Yang XH, Yang ZL, Tu ZC, Cheng YX. Miscellaneous meroterpenoids from Ganoderma applanatum. Tetrahedron. 2016;72:4564–74.
21. Ferreira ICFR, Barros L, Abreu RMV. Antioxidants in wild mushrooms. Curr Med Chem. 2009;16(12):1543–60.
22. Kozarski M, Klaus A, Jakovljevic D, Todorovic N, Vunduk J, Petrović P, Niksic M, Vrvic MM, van Griensven L. Antioxidants of edible mushrooms. Molecules. 2015;20(10):19489–525.
23. European Commission. Commission Directive 2010/67/EU of 20 October 2010 amending Directive 2008/84/EC laying down specific purity criteria on food additives other than colours and sweeteners. Off J Eur Union. 2010;L277:17–19.
24. Apak R, Özyürek M, Güçlü K, Çapanoğlu E. Antioxidant activity/capacity measurement. 2. Hydrogen atom transfer (HAT)-based, mixed-mode (electron transfer (ET)/HAT), and lipid peroxidation assays. J Agric Food Chem. 2016;64(5):1028–45.
25. Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. J Agric Food Chem. 2005;53(6):1841–56.
26. Foti MC. Use and abuse of the DPPH(•) radical. J Agric Food Chem. 2015;63:8765–76.
27. Keleş A, Koca I, Gençcelep H. Antioxidant properties of wild edible mushrooms. J Food Process Technol 2011;2:130. doi:10.4172/2157-7110.1000130.
28. Puttaraju NG, Venjateshaiah SU, Dharmesh SM, Urs SMN, Somasundaram R. Antioxidant activity of indigenous edible mushrooms. J Agric Food Chem. 2006;54:9764–72.

29. Sadi G, Kaya A, Yalcin HA, Emsen B, Kocabaş A, Kartal DI, Altay A. Wild edible mushrooms from Turkey as possible anticancer agents on HepG2 cells together with their antioxidant and antimicrobial properties. Int J Med Mushrooms. 2016;18(1):83–95.
30. Jaworska G, Pogoń K, Skrzypczak A, Bernaś E. Composition and antioxidant properties of wild mushrooms Boletus edulis and Xerocomus badius prepared for consumption. J Food Sci Technol. 2015;52(12):7944–53.
31. Lemieszek MK, Ribeiro M, Alves HG, Marques G, Nunes FM, Rzeski W. Boletus edulis ribonucleic acid – a potent apoptosis inducer in human colon adenocarcinoma cells. Food Funct. 2016;7:3163–75.
32. Lemieszek MK, Nunes FHFM, Sawa-Wejksza K, Rzeski W. A king bolete, Boletus edulis (Agaricomycetes), RNA fraction stimulates proliferation and cytotoxicity of natural killer cells against myelogenous leukemia cells. Int J Med Mushrooms. 2017;19(4):347–53.
33. Novakovic A, Karaman M, Kaisarevic S, Radusin T, Llic N. Antioxidant and antiproliferative potential of fruiting bodies of the wild-growing king bolete mushroom, Boletus edulis (Agaricomycetes), from Western Serbia. Int J Med Mushrooms. 2017;19(1):27–34.
34. Wang D, Sun SQ, Wu WZ, Yang SL, Tan JM. Characterization of a water-soluble polysaccharide from Boletus edulis and its antitumor and immunomodulatory activities on renal cancer in mice. Carbohydr Polym. 2014;105:127–34.
35. Stojković DS, Reis FS, Ćirić A, Barros L, Glamočlija J, Ferreira ICFR, Soković M. Boletus aereus growing wild in Serbia: chemical profile, in vitro biological activities, inactivation and growth control of food-poisoning bacteria in meat. J Food Sci Technol. 2015;52(11):7385–92.
36. Kretz O, Creppy EE, Boulanger Y, Dirheimer G. Purification and some properties of bolesatine, a protein inhibiting in vitro protein synthesis, from the mushroom Boletus satanas Lenz (Boletaceae). Arch Toxicol Suppl. 1989;13:422–7.
37. Courtecuisse R, Duhem B. Champignons de France et d'Europe. Paris (France): Delachaux et Niestlé; 2013.
38. Eyssartier G, Roux P. Le guide des champignons: France et Europe. Paris (France): Belin; 2013.
39. Binder M, Hibbett DS. Molecular systematics and biological diversification of Boletales. Mycologia. 2006;98(6):971–81.
40. Kim JW, Yoo ID, Kim WG. Free radical-scavenging δ-lactones from Boletus calopus. Planta Med. 2006;72(15): 1431–2.
41. Leon F, Brouard I, Torres F, Quintana J, Rivera A, Estevez F, Bermejo J. A new ceramide from Suillus luteus and its cytotoxic activity against human melanoma cells. Chem Biodivers. 2008;5(1):120–5.
42. Matzinger P, Catalfomo P, Eugster CH. Isolation of (2S,4S)-(+)-gammahydroxynorvaline and (2S,4R)-(-)-gammahydroxynorvaline from Boletus satanas Lenz [article in German]. Helv Chim Acta. 1972;55(5):1478–90.
43. McMorris TC, Lira R, Gantzel PK, Kelner MJ, Dawe R. Sesquiterpenes from the basidiomycete Omphalotus illudens. J Nat Prod. 2000;63:1557–9.
44. McMorris TC, Kashinatham A, Lira R, Rundgren H, Gantzel PK, Kelner MJ, Dawe R. Sesquiterpenes from Omphalotus illudens. Phytochemistry. 2002;61:395–8.
45. Moreau PA, Courtecuisse R, Bellanger JM. Les noms qui changent. (2) Agaricales, Boletales et Tricholomatales [article in French]. Doc Mycol. 2015;36:85–101.
46. Nuhn ME, Binder M, Taylor AFS, Halling RE, Hibbett DS. Phylogenetic overview of the Boletineae. Fungal Biol. 2013;117(7–8):479–511.
47. Zhao K, Wu G, Yang Z. A new genus, Rubroboletus, to accommodate Boletus sinicus and its allies. Phytotaxa. 2014;188(2):61–77.
48. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR. New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst. 1990;82(13):1107–12.
49. Morel S, Landreau A, Nguyen VH, Derbré S, Grellier P, Le Pape P, Pagniez F, Litaudon M, Richomme P. Preparative isolation, fast centrifugal partition chromatography purification and biological activity of cajaflavanone from Derris ferruginea stems. Phytochem Anal. 2012;23:152–8.
50. Pawar SS, Dasgupta D. Quantification of phenolic content from stem-bark and root of Hugonia mystax Linn. using RP-HPLC. J King Saud Univ Sci. 2016 Sept 12 [Epub ahead of print]
51. Lavaud A, Richomme P, Gatto J, Aumond MA, Poullain C, Litaudon M, Andriantsitohaina R, Guilet D. A tocotrienol series with an oxidative terminal prenyl unit from Garcinia amplexicaulis. Phytochemistry. 2015;109:103–10.
52. Smolskaite L, Talou T, Venskutonis PR. Comprehensive evaluation of the antioxidant potential of coastal dune mushroom species from the Southwest of France. Int J Med Mushrooms. 2016;18(11):1023–35.
53. Kirchmair M, Poder R, Huber CG, Miller OK. Chemotaxonomical and morphological observations in the genus Omphalotus (Omphalotaceae). Persoonia. 2002;17(4):583–600.
54. Singh P. Atromentic acid from Clitocybe illudens. Phytochemistry. 1971;10:3259–62.
55. McMorris TC, Chimmani R, Alisala K, Staake MD, Banda G, Kelner MJ. Structure-activity studies of urea, carbamate, and sulfonamide derivatives of acylfulven. J Med Chem. 2010;53(3);1109–16.
56. Staake MD, Kashinatham A, McMorris TC, Estes LA, Kelner MJ. Hydroxyurea derivatives of irofulven with improved antitumor efficacy. Bioorg Med Chem Lett. 2016;26(7):1836–8.

57. Baharum Z, Akim AM, Taufiq-Yap YH, Hamid RA, Kasran R. In vitro antioxidant and antiproliferative activities of methanolic plant part extracts of Theobroma cacao. Molecules. 2014;19:18317–31.
58. Manosroi A, Akazawa H, Akihisa T, Jantrawut P, Kitdamrongtham W, Manosroi W, Manosroi J. In vitro anti-proliferative activity on colon cancer cell line (HT-29) of Thai medicinal plants selected from Thai/Lanna medicinal plant recipe database "MANOSROI III". J Ethnopharmacol. 2015;161:11–7.
59. Tomasi S, Lohezic-Le Devehat F, Sauleau P, Bezivin C, Boustie J. Cytotoxic activity of methanol extracts from Basidiomycete mushrooms on murine cancer cell lines. Pharmazie. 2004;59(4):290–3.
60. Berg WJ, Schwartz L, Yu R, Mazumdar M, Motzer RJ. Phase II trial of irofulven (6-hydroxymethylacylfulvene) for patients with advanced renal cell carcinoma. Invest New Drugs. 2001;19(4):317–20.
61. Thomas JP, Arzoomanian R, Alberti D, Feierabend C, Binger K, Tutsch KD, Steele T, Marnocha R, Smith C, Smith S. MacDonald J, Wilding G, Bailey H. Phase I clinical and pharmacokinetic trial of irofulven. Cancer Chemother Pharmacol. 2001;48(6):467–72.
62. Anchel M, Hervey A, Robbins WJ. Production of illudin M and of a fourth crystalline compound by Clitocybe illudens. Proc Natl Acad Sci U S A. 1952;38(11):927–8.
63. Dos Santos T, Tavares C, Sousa D, Vaz JA, Calhelha RC, Martins A, Almeida GM, Ferreira ICFR, Vasconcelos MH. Suillus luteus methanolic extract inhibits cell growth and proliferation of a colon cancer cell line. Food Res Int. 2013;53(1):476–81.
64. Kretz O, Barbieri L, Creppy EE, Dirheimer G. Inhibition of protein synthesis in liver and kidney of mice by bolesatine: mechanistic approaches to the mode of action at the molecular level. Toxicology. 1992;13:297–304.
65. Ennamany R, Lavergne JP, Reboud JP, Dirheimer G, Creppy EE. Mode of action of bolesatine, a cytotoxic glycoprotein from Boletus satanas Lenz. Mechanistic approaches. Toxicology. 1995;100:51–5.
66. Licastro F, Morini MC, Kretz O, Dirheimer G, Creppy EE, Stirpe F. Mitogenic activity and immunological properties of bolesatine, a lectin isolated from the mushroom Boletus satanas Lenz. Int J Biochem. 1993;25(5):789–92.
67. Arora S, Goyal S, Balani J, Tandon S. Enhanced antiproliferative effects of aqueous extracts of some medicinal mushrooms on colon cancer cells. Int J Med Mushrooms. 2013;15(3):301–14.
68. Ebrahimzadeh MA, Nabavi SM, Nabavi SF, Eslami S. Antioxidant and free radical scavenging activities of culinary-medicinal mushrooms, golden chanterelle Cantharellus cibarius and angel's wings Pleurotus porrigens. Int J Med Mushrooms. 2010;12(3):265–72.
69. Kovacs B, Zomborszki ZP, Orban-Gyapai O, Csupor-Loffler B, Liktor-Busa E, Lazar A, Papp V, Urban E, Hohmann J, Vanyolos A. Investigation of antimicrobial, antioxidant, and xanthine oxidase–inhibitory activities of Phellinus (Agaricomycetes) mushroom species native to central Europe. Int J Med Mushrooms. 2017;19(5):387–94.
70. Xie J, Schaich KM. Re-evaluation of the 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) assay for antioxidant activity. J Agric Food Chem. 2014;62(19):4251–60.
71. Scherer R, Teixeira Godoy H. Antioxidant activity index (AAI) by the 2,2-diphenyl-1-picrylhydrazyl method. Food Chem. 2009;112(3):654–8.
72. Deng J, Cheng W, Yang G. A novel antioxidant activity index (AAU) for natural products using the DPPH assay. Food Chem. 2011;125:1430–5.
73. Everette JD, Bryant QM, Green AM, Abbey YA, Wangila GW. Thorough study of reactivity of various compound classes toward the Folin-Ciocalteu Reagent. J Agric Food Chem. 2010;58(14):8139–44.
74. Macakova K, Opletal L, Polasek M, Samkovac V, Jahodar L. Free-radical scavenging activity of some European Boletales. Nat Prod Commun. 2009;4(2):261–4.
75. Reis FS, Heleno SA, Barros L, Sousa MJ, Martins A, Santos-Buelga C, Ferreira ICFR. Toward the antioxidant and chemical characterization of mycorrhizal mushrooms from northeast Portugal. J Food Sci. 2011;76(6):824–30.
76. Heleno SA, Barros L, Sousa MJ, Martins A, Santos-Buelga C, Ferreira ICFR. Targeted metabolites analysis in wild Boletus species. LWT Food Sci Technol. 2011;44(6):1343–8.
77. Asatiani MD, Elisashvili VI, Wasser SP, Reznick AZ, Nevo E. Free-radical scavenging activity of submerged mycelium extracts from higher Basidiomycetes mushrooms. Biosci Biotechnol Biochem. 2007;71(12):3090–2.
78. Kalyoncu F, Oskay M, Kayalar H. Antioxidant activity of the mycelium of 21 wild mushroom species. Mycology. 2010;1(3):195–9.