Journal of Agricultural Chemistry and Environment, 2016, 5, 73-84
Published Online May 2016 in SciRes. http://www.scirp.org/journal/jacen
http://dx.doi.org/10.4236/jacen.2016.52008

Edible Ectomycorrhizal Mushrooms *Russula* spp. of Côte d'Ivoire: Total Phenolic Content, HPLC-Profiles of Phenolic Compounds and Organic Acids, Antioxidant Activities

Kouamé Appolinaire Kouassi, Eugène Jean Parfait Kouadio, Kouakou Martin Djè, Ahipo Edmond Dué, Lucien Patrice Kouamé

Laboratoire de Biocatalyse et des Bioprocédés, UFR des Sciences et Technologie des Aliments, Université Nangui Abrogoua (Ex Université d'Abobo-Adjamé), Abidjan, Côte d'Ivoire
Email: nkouadiop@yahoo.fr

Received 10 February 2016; accepted 6 May 2016; published 9 May 2016

Abstract

The aim of this study was to investigate the phenolic compounds content, HPLC-profiles of phenolic compounds and organic acids, and also antioxidant activities via the ability to scavenge DPPH radical of three wild edible mushrooms belonging to *Russula* genus and being collected in center of Côte d'Ivoire. Total phenolic compounds, flavonoids and tannins contents of methanolic extracts were assessed by colorimetric assays. So, the obtained values of these chemical parameters ranged from 394.05 to 513.50 mg/100 g DW, 94.50 to 139.95 mg/100 g DW and from 124.20 to 165 ± 0.54 mg/100 g DW, respectively. Otherwise, HPLC-profiles of the methanolic extracts revealed that quercetin, salicylic acid and tannin ol were the main phenolic compounds in *R. delica* whereas *R. lepida* contained gallic acid, catechin and protocatechuic acid as main phenolic compounds. Besides, it showed that the phenolic compounds such as salicylic acid, tannin ol and catechin were observed in *R. mustelina*. As for HPLC-profiles of organic acid, the fumaric and malic acid were recorded as the main organic acids in the three species of wild edible mushrooms. However, citric acid content was found to be highest in *R. lepida*. The methanolic extracts of the three mushrooms exhibited high DPPH radical scavenging activities ranging from 74.92% to 58.92%. These wild edible mushrooms could be considered a potential supply source of adequate natural antioxidant for local population.

Keywords

Ectomycorrhizal Mushrooms, Côte d'Ivoire, Phenolic Compounds, Organic Acids, DPPH Scavenging Activities

How to cite this paper: Kouassi, K.A., *et al*. (2016) Edible Ectomycorrhizal Mushrooms *Russula* spp. of Côte d'Ivoire: Total Phenolic Content, HPLC-Profiles of Phenolic Compounds and Organic Acids, Antioxidant Activities. *Journal of Agricultural Chemistry and Environment*, **5**, 73-84. http://dx.doi.org/10.4236/jacen.2016.52008

1. Introduction

According to Chang & Miles [1], mushroom is defined as being a macro-fungus with a distinctive fruiting body, which can be hypogeous or epigeous, large enough to be seen with the naked eye and to be picked by hand. From the taxonomic point of view, mainly basidiomycetes but also some species of ascomycetes are mushroom forming fungi [2]. Among the mushrooms, certain species form symbiotic associations with their host plants. These constitute the so-called ectomycorrhizal mushrooms. Moreover, other species so-called saprobic mushrooms colonize the coarse woody debris.

Since ancient times, mushrooms have been consumed by people from all over the world, for their nutritional and culinary values. Although nowadays, most of edible mushrooms are able to be cultivated and marketed in large commercial surfaces [3]-[6], collecting wild mushrooms for food and commercial purposes remain always a very widespread activity in developing countries and in some developed [7]-[10]. In Africa and particularly in Côte d'Ivoire, wild mushrooms are highly treasured and have always been important in the feed of rural people. Thus, as in Côte d'Ivoire, mushroom cultivation is few known, it is found on rural or urban markets, fresh or dried mushrooms that are collected in the wild.

The consumption of wild edible mushrooms is increasing due to a good proteins content and trace minerals. Mushrooms are valuable healthy foods, low in calories, fats, and essentials fatty acids, and high in vegetable proteins, vitamins and minerals [11] [12]. This high consumption of edible mushrooms demands a better knowledge of their biological potential. Thus currently, mushrooms are a focus of interest of many researchers as a source of bioactive substances such as organic acids and antioxidants compounds comprising phenolic acids, flavonoids and carotenoids [6] [13]-[16]. Phenolic compounds and organic acids are known to influence the organoleptic properties of food matrices, and have also been used for their quality control [17]. Additionally, it is well-established that antioxidant activity is mainly related to their phenolic content [18] [19]. Consequently, several reports focusing on total phenols and antioxidant activities of wild and commercial mushrooms have been published [6] [20]-[24].

Considering these findings, the aim of this study is to investigate three edible ectomycorrhizal mushrooms belonging to *Russula* genus and being collected in the wild in the center of Côte d'Ivoire for their total phenolic content and HPLC-profiles of phenolic compounds and organic acids. In addition, their antioxidant capacities are evaluated using their ability to scavenge DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical. This is the first report about determination of phenolic compounds, organic acids and estimation of antioxidant capacities of ectomycorrhizal mushrooms from Côte d'Ivoire.

2. Materials and Methods

2.1. Reagents and Chemicals

Citric, oxalic, ascorbic, succinic, malic, fumaric, Salicylic and tannic acids, Folin-Ciocalteu were purchased from Sigma-Aldrich (Steinheim, Germany). Caffeic, benzoic, Gallic, o-phosphoric and cinnamic acids, acetonitrile, catechin, quercetin, tannin ol and resveratrol were obtained from Merck (Darmstadt, Germany). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), aluminum chloride and *p*-hydroxybenzoic acids were provided by Sigma Chemical Co (St, Loius, MO, USA). Methanol was purchased from Prolabo.

2.2. Sample Collection

The used mushrooms species in this work were picked from woodlands present in the center of Côte d'Ivoire. Taxonomic identification was achieved by Dr Souleymane Yorou Nourou (Abomey Calavy University of Benin/ Munich University of Germany), as *Russula mustelina*, *R. delica* and *R. lepida*. After picking, the mushrooms were immediately transferred to the laboratory and cleaned.

2.3. Extraction of Phenolic Compounds

The mushrooms were dried at 25˚C for ten days, until constant weight, according Ribeiro *et al.* [17] method slightly modified. Then, each mushroom sample was ground into a fine-dried powder (mill IKA, Germany/ Deutschland). A sample (10 g) of each fine-dried mushroom powder was extracted by stirring with 50 ml of methanol 80% (v/v) at 25˚C for 24 hours and filtered through Whatmann 4 paper. The residue was then extracted with two additional 50 ml portions of methanol. The combined methanolic extracts were evaporated at

35˚C (rotary evaporator HEILDOLPH Laborata 4003 Control, Schwabach, Germany) until 25 ml, and stored at −20˚C prior to further use.

2.4. Extraction of Organic Acids

The organic acids of each dried sample of mushroom were extracted according Hasib *et al.* [25] method by grinding (Waring Blendor, Polychimie, Abidjan, Côte d'Ivoire) in distilled water (1:10, w/v) and clarified by centrifuging at 4000 rpm for 30 minutes. The supernatant was first filtered through Whatmann 4 paper, then through 0.45 µm filter (Millipore; Sartorius AG, Goëttingen, Germany) and stored at −20˚C prior further use.

2.5. Determination of Total Phenolic Compounds Content

Total phenolic compounds content was estimated according The Folin-Ciocalteu method [26]. A volume of 1 mL of methanolic extract of each sample was added to 1 mL of Folin-Ciocalteu's solution in a test tube. After 3 minutes, 1 ml of 20% sodium carbonate solution was added to the mixture and adjusted to 10 ml with distilled water. The mixture was allowed to stand at room temperature in a dark environment for 30 min. Absorbance was measured against the blank reagent at 725 nm. Gallic acid was used for the calibration curve with a concentration range of 50 - 1000 µg/ml. Results were expressed as mg gallic acid equivalent (GAE)/100 g DW (Dry Weight). All experiments were performed in triplicate.

2.6. Determination of Flavonoids

Total flavonoids content was determined according method used by Meda *et al.* [27], but slightly modified. A volume of 0.5 ml of methanolic extract of each mushroom sample was diluted in 0.5 ml of distilled water. Then, 0.5 ml of aluminum chloride 10% (P/V) and the same volume of sodium acetate 1 M were added. Finally, 2 ml of distilled water was added and absorption reading at 415 nm was carried out after 30 min against a blank sample consisting of a 4 ml methanolic extract without aluminum chloride. Quercetin was used for the calibration curve with a concentration range of 0 - 100 µg/ml. Results were expressed as mg of quercetin equivalent (QE)/100 g DW. All experiments were performed in triplicate.

2.7. Determination of Tannins

Tannins content was determined using the method described by Bainbridge *et al.* [28]. A volume of 1 ml of each methanolic extract was collected and mixed with 5 ml of reaction solution [vanillin 0.1 mg/ml in sulphuric acid 70% (V/V)]. The mixture was allowed to stand at room temperature in a dark environment for 20 min. The absorbance was measured at 500 nm against a blank (without extract). Tannic acid was used for the calibration curve with a concentration range of 0 - 100 µg/ml. The results were expressed as mg of tannic acid equivalents (TAE)/100 g DW. All experiments were performed in triplicate.

2.8. HPLC Analysis of Phenolic Compounds

The phenolic extracts previously prepared (50 ml) were diluted in 100 ml of distilled water and 20 µl of each sample was analyzed using an analytical HPLC unit (HPLC (Shimadzu Corporation, Japan) equipped with a binary pump (LC-6A) coupled to a UV-VIS detector (SPD-6A). Phenolic compounds were separated on a column ICSep ICE ORH-801 (length 25 cm) at a temperature set at 30˚C. The mobile phase consisted of 50 mM $NaH_4H_2PO_4$ to pH 2.6 (eluent A), a solution of acetonitrile/$NaH_4H_2PO_4$ (80:20, v/v) (eluent B) and 200 mM acid *o*-phosphoric pH 1.5 (eluent C). The operating time was 70 min with a flow rate of 1 ml/min. Phenolic compounds in methanolic extract of mushroom samples were identified through comparison of their retention times and UV-visible spectra with those obtained by injection of the standard solution under the same conditions. Peak area was used for quantitation purposes, using external calibration with standards.

2.9. HPLC Analysis of Organic Acids

The separation of the organic acids was carried out by using a system consisting of an analytical HPLC unit (Shimadzu Corporation, Japan) in conjunction with a column heating device set at 35˚C with the aid of an oven Meta Therm TM (Interchrom, France), with an ions exclusion column ICSep ICE ORH-801 (40 cm × 5 µm, In-

terchom, France). The system was also coupled to a pump (Shimadzu LC-6A Liquid Chromatograph), a UV detector (Shimadzu SPD-6A UV Spectrophotometric Detector) and an integrator (Shimadzu Chromatopac CR 6A). Elution was carried out isocratically with sulphuric acid 0.04 N, at a solvent flow rate of de 0.6 ml/min and detection was performed at 210 nm. Organic acids in mushroom extracts were identified by comparing the retention times and spectral data obtained from standards under the same conditions. Quantitation was performed by comparing the peak areas with those of the respective external standards.

2.10. Estimation of Antioxidant Activity by DPPH Radical Scavenging

The DPPH scavenging activity was determined using the method described by Shimada *et al.* [29]. Each sample of methanolic extract (2.5 ml) was mixed with 1 ml of a 3 mM DPPH methanol solution. After 30 min incubation at room temperature in the dark, the absorbance of the mixture was determined at 517 nm against a blank containing methanol without DPPH radical. A lower absorbance indicates a higher scavenging activity. Absorbance was converted to the DPPH radical-scavenging rate according to the equation:

$$\text{DPPH radical scavenging rate}(\%) = \left[\left(A_{\text{control}} - A_{\text{sample}}\right)/A_{\text{control}}\right] \times 100.$$

2.11. Statistical Analysis

All chemical analyses and assays were performed in triplicate, unless otherwise indicated. Results were expressed as mean values ± standard deviation (SD). Analysis of variance (ANOVA) followed by Duncan's test was performed to test for differences between means by employing Kyplot (version 2.0 beta 15, ©1997-2001, Koichi Yoshioka) statistical software. Significance of differences was defined at the 5% level ($p < 0.05$).

3. Results and Discussion

3.1. Total Phenoliccompounds, Flavonoids and Tannins Contents

The total phenolic compounds, flavonoids and tannins contents in the three species of wild edible mushrooms were presented in **Table 1**. The total phenolic compounds content ranged from 394.05 ± 1.02 to 513.50 ± 1.06 mg (GAE)/100 g DW. The higest total phenolic compound content was obtained with *R. lepida* while *R. mustelina* contained the lowest total phenolic compound content. Otherwise, the analysis of variance indicated that the species main effect had significant effect ($p \leq 0.05$) on total phenolic compounds content. It appeared also statistically differences ($p < 0.05$) between the total phenolic compounds content of the three species of wild edible mushrooms. Concerning the flavonoid content, the results showed that it varied from 94.50 ± 0.12 to 139.95 ± 0.18 mg/100 g (QE)/100 g DW. *R. lepida* had the lowest value of total flavonoids content whereas the highest value was observed in *R. delica*. Besides, the analysis of variance showed that the species main effect had meaningful effect ($p < 0.05$) on flavonoids content. Thus, the flavonoids content of wild edible mushrooms varied significantly ($p < 0.05$) from one species to another. As for tannin content, it ranged from 124.20 ± 0.25 to 165 ± 0.54 (TAE)/100 g DW. The lowest value of tannin content was found in *R. delica* while the highest value of tannin content was obtained with *R. delica*. Furthermore, the analysis of variance indicated that the species main effect had significant effect ($p \leq 0.05$) on tannin content. Thus, there were significantly differences ($p < 0.05$) between the tannin content in wild edible mushrooms from one species to another. Overall, the results of this work revealed that total phenolic compounds, flavonoids and tannins contents of wild edible mushrooms

Table 1. Total phenolic compounds, flavonoids and tannins contents of three *Russula* species mushroom from Côte d'Ivoire.

Compounds (mg/100 g)	Mushroom samples		
	Russula delica	***Russula lepida***	***Russula mustelina***
Total phenolics	496.35 ± 1.71^{b}	513.50 ± 1.06^{c}	394.05 ± 1.02^{a}
Total flavonoids	139.95 ± 0.18^{c}	94.50 ± 0.12^{a}	122.40 ± 0.2^{b}
Total tannins	165 ± 0.54^{c}	124.20 ± 0.25^{a}	130.05 ± 0.16^{b}

Each value is an average of three replicate. Values are mean ± standard deviation. Means not sharing a similar letter in a line are significantly different $p \leq 0.05$ as assessed by the test of Duncan.

differed significantly ($p < 0.05$) from one species to another. Findings of several reports indicated the total phenolic contents of others wild mushrooms fell within the range of our results [15] [16] [30]-[33]. Nevertheless, total phenolic compounds content of methanolic extracts of *Russula* species from Côte d'Ivoire were found to be lower than that recorded in ethanolic extract of *Russula delica* collected in Turkey (47.01 ± 0.29 $\mu g \cdot mg^{-1}$ of pyrocatechol equivalent) [34]. Considering the fundamental role of phenolic compounds found in food in human diet including antioxidant properties and the role in stabilizing lipid peroxidation [35] [36], high of phenolic compounds contents found in these wild mushrooms could constitute interesting data for population nutrition. Flavonoids probably belong to the most interesting groups of natural phenolic compounds since it is well-established that they can act as an antioxidant by breaking the radical chains and more stable products in the membranes of liver microsomes [37], and also play an important role to the instinctive protection against oxidative stress and side effects of their contribution with vitamins [38]. Our results of flavonoids content in methanolic extracts from the three species were higher than those observed in methanolic extracts of other wild mushrooms studied elsewhere [31]. However, flavonoids content of methanolic extract of some edible wild mushrooms fell within the range of our values [33]. Tannins constitute one class of natural phenolic compounds which contribute in part to the antioxidant properties of plants [23] [36] [39]. The assessed mushroom methanolic extracts displayed high of tannins content compared to some data of literature. Indeed, Tripathy *et al.* [40] and Rajoriya *et al.* [41] reported tannin contents of 0.47 ± 0.02 mg/gm and 0.24 ± 0.04 mg/gm in *Lentinus polychrous* and *Ganoderma* sp. from India, respectively.

3.2. HPLC-Profiles of Phenolic Compounds

The analysis of methanolic extracts from the three mushroom species by HPLC is presented in **Figure 1**. It allowed the identification of ten phenolic compounds such as gallic acid, catechin, benzoic acid, stilbene, quercetin, salicylic acid, tannin ol, cinnamic acid, caffeic acid and ρ-hydroxybenzoic acid for *R. delica* (**Figure 1(a)**). On the other hand, six phenolic compounds such as gallic acid, catechin, benzoic acid, cinnamic acid, caffeic acid, and protocatechuic acid were detected for *R. lepida* (**Figure 1(b)**). Otherwise, seven phenolic compounds that (catechin, Resveratrol, salicylic acid, tannin ol, cinnamic acid, caffeic acid and protocatechuic acid) were observed in *R. mustelina* (**Figure 1(c)**). In sum, all mushroom samples contained phenolic acids (gallic acid, benzoic acid, salicylic acid, protocatechuic acid, ρ-hydroxybenzoic acid), hydroxycinnamic acids (caffeic acid, cinnamic acid), flavonoids (catechin, quercetin), stilbene (resveratrol) and condensed tannin (tannin ol). Other phenolic compounds were detected in all analyzed species, although it was impossible to identify them. All obtained phenolic compounds were reported for the first time in ectomycorrhizal mushrooms from Côte d'Ivoire. In Russula species from Portugal, protocatechuic acid p-hydroxybenzoic acid, were also detected, but not gallic acid, caffeic acid, catechin and p-coumaric [42]. In *R. delica* from Greece, besides p-hydroxybenzoic acid, caffeic acid, catechin and p-coumaric, quercetin and resveratrol were detected, but, not gallic acid and protocatechuic acid [43]. Generally most of the phenolic compounds identified in our mushroom samples were detected in numerous species of wild mushrooms investigated by several authors in many countries [11] [13] [17] [24] [33] [44]. Regarding level of individual phenolic compound (**Table 2**), the results indicated that catechin had relatively high amount in the three species of studied mushroom. Indeed, the catechin contents were 19.52 ± 0.57 mg/kg DW, 91.74 ± 1.40 mg/kg DW and 35.80 ± 080 mg/kg DW for *R delica*, *R. lepida*, *R. mustelia*, respectively. Kim *et al.* [45] had also detected a significant amount of catechin in wild mushroom *Agaricusblazei* from Korea. Gallic acid which was already detected in indigenous wild mushroom *Lactarius deliciosus* [43], was preponderant in *R. delica* and *R. lepida* with respective contents of 176.28 ± 2.64 mg/kg DW and 125.90 ± 2.10 mg/kg DW. Quercetin which was detected in trace amount in *Fistulina hepatica* mushroom [17], was one of the most abundant phenolic compound in *R. delica* (124.10 ± 2.25 mg/100 g DW), but this flavonoid didn't detect in both other mushroom species. As for salicylic acid, its content was high in *R. mustelina* (132.72 ± 2.2 mg/100 g DW) and *R. delica* (85.82 ± 2.40 mg/kg DW), whereas it didn't detect in *R. lepida*. Salicylic acid was also identified as one of the major phenolic compounds in the extracts of the mushroom *Naematoloma sublateritium* [46]. Protocatechuic acid content (42.18 ± 1.84 mg/kg DW) in *R. lepida* was found to be higher than that recorded in *R. mustelina* with a rate of 6.50 ± 0.02 mg/kg DW. It didn't detect in *R. delica*. Barros *et al.* [42] reported that protocatechuic acid was the main phenolic compound in *Lepistnuda* and *Ramaria botrytis* from Portugal [47]. Tannin ol which is constituted by the condensed tannins that are polymers formed by the condensation of flavans was present with moderate contents in *R. delica* (38.04 ± 1.02 mg/kg DW) and *R. mustelina* (49.32 ± 1.22 mg/kg DW). However, it didn't detect in *R. lepida*. Benzoic acid and ρ-hydroxybenzoic acid contents were

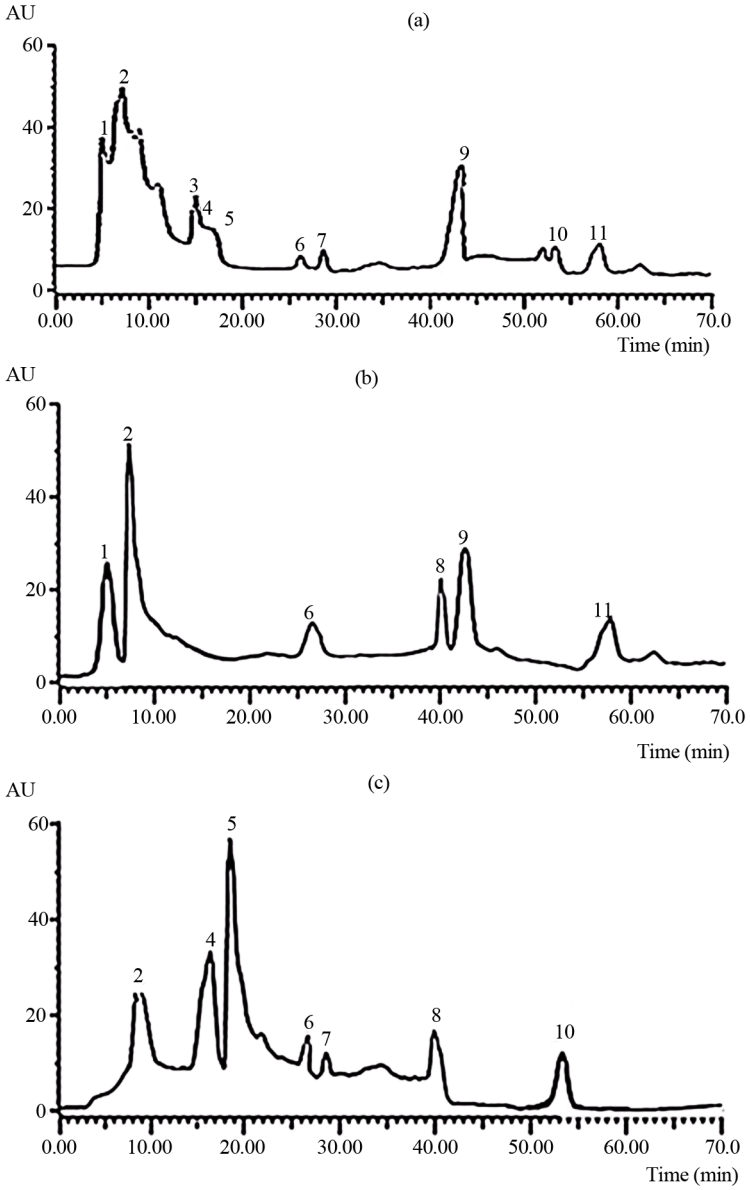

Figure 1. HPLC-profiles of phenolic compounds of methanolic extracts of three *Russula* species mushroom from Côte d'Ivoire (a): *R. delica*, (b) *R. lepida* and (c) *R. mustelina*). Detection at 280 nm: (1) gallic acid; (2) catechin; (3) quercetin; (4) Salicylic acid; (5) tannin ol; (6) cinnamic acid; (7) caffeic acid; (8) protocatechuic acid; (9) *ρ*-hydroxybenzoic acid; (10) resveratrol (11) benzoic acid.

also found to be low in *R. delica* with respective values of 9.97 ± 0.02 and 9.82 ± 0.31 mg/kg DW and moderate in *R. lepida* with respective of 17.84 ± 0.15 and 24.10 ± 1.20 mg/kg DW. Benzoic acid was already detected with high levels in wild mushrooms *Agaricus blazei*, *Sparassis crispa* and *Phellinus linteus* from Korea [45], well as *ρ*-hydroxybenzoic acid in *Amanita rubescens* and *Russula cyanoxantha* from Portugal [46]. Moreover, caffeic acid was available in *R. delica* (6.81 ± 0.51 mg/kg DW) and in *R. mustelina* (18.32 ± 1.02 mg/kg DW) also with very moderate levels and didn't detected in *R. lepida*. Caffeic acid was identified as preponderant phenolic compound in Chanterelle (*Cantharellus cibarius*) mushroom dried at 30˚C for 96 h [11]. Resveratrol already detected in *Sparassis crispa*, and *Inonotus obliquus* from Korea [45], possessed also a moderate content in *R. mustelina* (22.52 ± 0.03 mg/kg DW). However, although detected in *R. delica*, its content was very low. In contrast, it didn't findin *R. lepida*. With regard to cinnamic acid, although it was present in the three mushrooms

Table 2. Individual phenolic compounds contents (mg/kg) DW in three Russula species mushroom from Côte d'Ivoire.

		Mushroom samples		
Phenolic compounds (mg/kg)	**Retention time (min)**	***R. delica***	***R. lepida***	***R. mustelina***
Gallic acid	(5)	176.28 ± 2.64^{b}	125.90 ± 2.10^{a}	nd
Catechin	(8)	19.52 ± 0.57^{a}	91.74 ± 1.40^{c}	35.80 ± 080^{b}
Quercetin	(15)	124.10 ± 2.25	nd	nd
Salicylic acid	(15.80)	85.82 ± 2.40^{a}	nd	132.72 ± 2.2^{b}
Tannin ol	(17.50)	38.04 ± 1.02^{a}	nd	49.32 ± 1.22^{b}
Cinnamic acid	(26.50)	0.27 ± 0.00^{a}	0.55 ± 0.01^{b}	0.74 ± 0.12^{c}
Caffeic acid	(28.50)	6.81 ± 0.51^{a}	nd	18.32 ± 1.02^{b}
Protocatechuic acid	(40)	nd	42.18 ± 1.84^{b}	6.50 ± 0.02^{a}
ρ-Hydroxybenzoic acid	(43)	9.82 ± 0.31^{a}	24.10 ± 1.20^{b}	nd
Resveratrol	(53)	0.82 ± 0.01^{a}	nd	22.52 ± 0.03^{b}
Benzoic acid	(58)	9.97 ± 0.02^{a}	17.84 ± 0.15^{b}	nd

Each value is an average of three replicate. Values are mean ± standard deviation. Means not sharing a similar letter in a line are significantly different $p \leq 0.05$ as assessed by the test of Duncan.

examined, its content was very low.

3.3. HPLC-Profiles of Organic Acids

The organic acids profiles showed that all three species of ectomycorrhizal mushrooms contained oxalic, citric, malic, succinic and fumaric acids (**Figure 2**). **Table 3** showed that the found main organic acids in the three studied species were fumaric acid and malic acid. So, the fumaric acid content was 4241.40 ± 30.03 mg/kg DW, 4183.80 ± 20.25 mg/kg DW and 3477.3 ± 25.35 mg/kg DW for *R. delica*, *R. lepida* and *R. mustelina*, respectively. Besides, *R. delica*, *R. lepida* and *R. mustelina* had respective malic acid content of 3207.6 ± 10.5, 833.2 ± 7.05 and 4230.40 ± 20.40 mg/kg DW, respectively). Fumaric acid is an important organic acid because of its antioxidant, antimicrobial and acidifying properties [48] [49]. Malic acid is a dicarboxylic acid produced in all living organisms, occurring naturally in all fruits and many vegetables. It contributes to the pleasantly sour taste of fruits, and it is used as a food additive. This organic acid was found also in wild mushroom *Macrolepiota procera* from Turkey in high content of 19.40 ± 0.62 g/kg dry DW [50]. Citric acid content was found to be highest in *Russula lepida* (3159.7 ± 8.73 mg/kg), but medium and didn't vary statistically ($p > 0.05$) in both others species. This organic acid is known to have an important role in the prevention of mushroom browning and to extend its shelf life; this is because of its antibacterial and antioxidant properties [48] [49]. Overall, the investigated mushrooms displayed low contents in ascorbic acid, which is known to possess high antioxidant properties (818.20 ± 0.45, 429.40 ± 0.35 and 582.4 ± 0.30 mg/kg for *R. delica*, *R. lepida* and *R. mustelina*, respectively). Oxalic and succinic acids were the minor organic acids in the three examined mushrooms. As for phenolic compounds, all organic acids are also reported for the first time in ectomycorrhizal mushrooms from Côte d'Ivoire. However, it is worth pointing out that there are some minor or major peaks that have not been identified in each of the analyzed mushroom species.

3.4. DPPH Scavenging Activity

The potential antioxidant of the different methanolic extracts of mushroom was evaluated by their DPPH scavenging effect. Indeed, this represented a rapid method to characterize the antioxidant capacity of extracts against oxidation caused by free radicals. According to this method, the color of the mixture reaction changed from mauve to light yellow, depending on the antioxidant capacity of the extracts. Results expressed as the ratio percentage of sample absorbance decrease and the absorbance of DPPH solution in the absence of extract at 517 nm, indicated that methanolic extract from *R. delica* exhibited the highest DPPH scavenging activity with a rate of 74.92% ± 0.94%, followed by *R. lepida* with a rate of 71.18% ± 1.90% (**Figure 3**). Regarding, *R. mustelina*

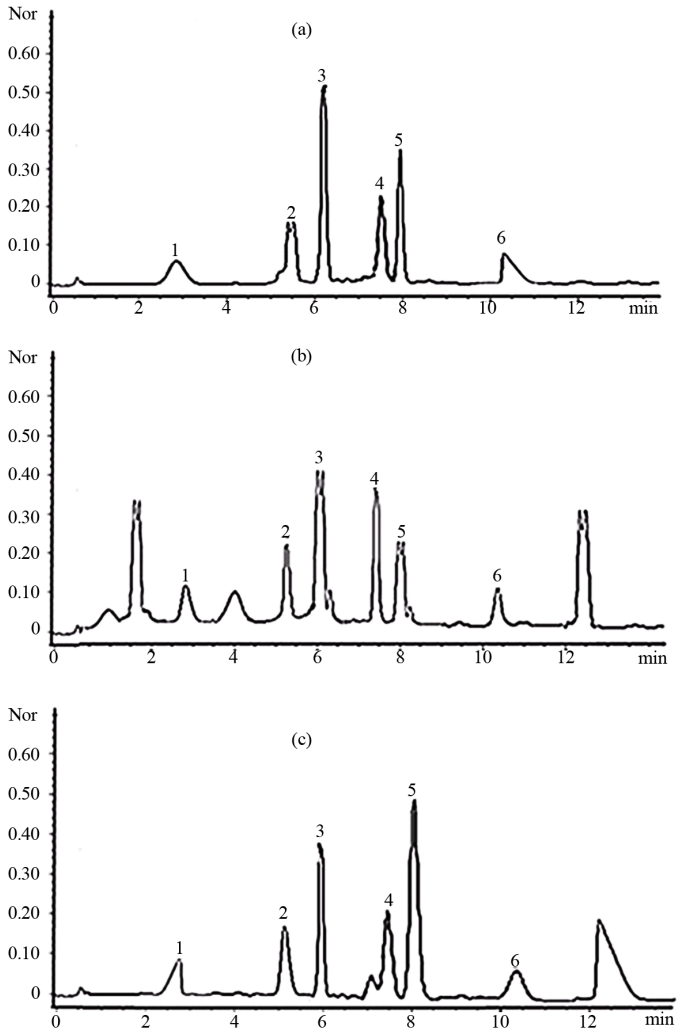

Figure 2. HPLC organic acids profile of extracts of three *Russula* species mushroom from Côte d'Ivoire ((a) *R. delica*, (b) *R. lepida* and (c) *R. mustelina*). Detectionat 210 nm: (1) oxalic acid; (2) ascorbic acid; (3) fumaric acid; (4) citric acid; (5) malic acid; (6) succinic acid.

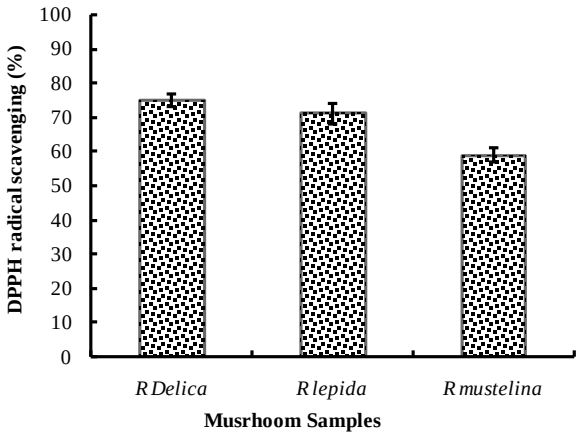

Figure 3. DPPH radical scavenging activities of methanolic extracts of three Russula species mushrooms from Côte d'Ivoire.

Table 3. Individual organic acidcontents (mg/kg DW) in three *Russula* species mushroom from Côte d'Ivoire.

Organic acid (mg/kg)	Mushroom samples			
	Retention time (min)	*R. delica*	*R. lepida*	*R. mustelina*
Oxalic acid	(2.80)	59.40 ± 0.04^{a}	71.50 ± 0.03^{a}	129.6 ± 0.13^{b}
Ascorbic acid	(5.25)	818.20 ± 0.45^{c}	429.40 ± 0.35^{a}	582.4 ± 0.30^{b}
Fumaric acid	(6.00)	4241.40 ± 30.03^{b}	4183.80 ± 20.25^{b}	3477.3 ± 25.35^{a}
Citric acid	(7.50)	2004.1 ± 12.52^{a}	3159.7 ± 8.73^{b}	2148 ± 32.50^{a}
Malic acid	(8.00)	3207.6 ± 10.5^{b}	833.2 ± 7.05^{a}	4230.40 ± 20.40^{c}
Succinic acid	(10.40)	86.50 ± 0.07^{b}	43.50 ± 0.04^{a}	34.3 ± 0.06^{a}

Each value is an average of three replicate. Values are mean ± standard deviation. Means not sharing a similar letter in a line are significantly different $p \leq 0.05$ as assessed by the test of Duncan.

methanolic extract, scavenging activity was 58.92% ± 1.10 % (**Figure 3**). These results showed that methanolic extracts of the studied mushroom species had a significant effect on scavenging free radical. Methanolic extracts of several wild edible mushroom species were also successfully explored for their DPPH scavenging activity [21] [24] [30] [32] [43] [51] [52]. But, more importantly, these results were close to those reported by Keles *et al.* [32] about *Russula nigricans* (78.16%) and *Russula vinosa* (72.21%) from Turkey. However, results of these authors indicated a moderate value of 37.10% for *R. delica*.

4. Conclusion

On completion of this study, the first interesting evidence is the higher rate of total phenolic, flavonoids and tannins in the methanolic extracts of the samples of mushroom from Côte d'Ivoire, since these compounds are included in the antioxidant compounds from mushrooms. Afterward, HPLC profiles of phenolic compounds show the presence of phenolic compounds well known to be involved in the antioxidant properties of plants and mushrooms such as phenolic acids, flavonoids and tannins. Likewise, the HPLC profiles of organic acids in the extracts reveal the presence of fumaric and malic acid in the species of Russula from Côte d'Ivoire, and it is well established that these possess a positive role in the organoleptic properties as well as in antioxidant properties of food. Finally, the methanolic extracts from these three ectomycorrhizal mushrooms picked in the wild of Côte d'Ivoire center display significant antioxidant properties as demonstrated by their strong scavengers of DPPH free radical. Ultimately, these mushroom species can constitute the potential of easily accessible sources of natural antioxidants and other bioactive compounds for local population nutrition.

Acknowledgements

We are grateful to Dr Souleymane Yorou Nourou (Abomey Calavy University of Benin/University of Munich, Tropical Mycology, Department Biology I, Organismic Biology, Germany) for advices and his contribution on taxonomic identification of the mushroom samples.

References

[1] Chang, S.T. and Miles, P.G. (1989) Edible Mushrooms and Their Cultivation. CRC Press, Inc., Florida, 345 p.

[2] Thatoi, H. and Singdevsachan, S.K. (2014) Diversity, Nutritional Composition and Medicinal Potential of Indian Mushrooms: A Review. *African Journal of Biotechnology*, **13**, 523-545. http://dx.doi.org/10.5897/AJB2013.13446

[3] Chang, S.T. and Miles, P.G. (1991) Recent Trends in World Production of Cultivated Edible Mushrooms. *Mushroom Journal*, **504**, 15-18.

[4] Martinez-Carrera, D., Aguilar, A., Martinez, W., Bonilla, M., Morales, P. and Sobal, M. (2000) Commercial Production and Marketing of Edible Mushrooms Cultivated on Coffee Pulp in Mexico; Chapter 45. pp. 471-488. In: Sera, T., Soccol, C.R., Pandey, A. and Roussos, S., Eds., *Coffee Biotechnology and Quality*, Kluwer Academic Publishers, Dordrecht, 625.

[5] Mattila, P., Könkö, K., Eurola, M., Pihlava, J.-M., Astola, J., Vahteristo, L., Hietaniemi, V., Kumpulainen, J., Valtonen, M. and Piironen, V. (2001) Contents of Vitamins, Mineral Elements, and Some Phenolic Compounds in Cultivated

Mushrooms. *Journal of Agricultural and Food Chemistry*, **49**, 2343-2348. http://dx.doi.org/10.1021/jf001525d

[6] Al-Momany, A.M. and Salih, G. (2011) Chemical Compositions and Nutritional Value of Three Edible Mushrooms Widely Consumed in Cyprus. *Jordan Journal of Agricultural Sciences*, **7**, 540-548.

[7] Pilz, D. and Molina, R. (2002) Commercial Harvests of Edible Mushrooms from the Forests of the Pacific Northwest United States: Issues, Management, and Monitoring for Sustainability. *Forest Ecology and Management*, **155**, 3-16. http://dx.doi.org/10.1016/S0378-1127(01)00543-6

[8] Wang, Y. and Hall, I.R. (2004) Edible Ectomycorrhizal Mushrooms: Challenges and Achievements. *Canadian Journal of Botany*, **82**, 1063-1073. http://dx.doi.org/10.1139/b04-051

[9] Egli, S., Peter, M., Buser, C., Stahel, W. and Ayer, F. (2006) Mushroom Picking Does Not Impair Future Harvest-Results of a Long-Term Study in Switzerland. *Biological Conservation*, **129**, 271-276. http://dx.doi.org/10.1016/j.biocon.2005.10.042

[10] Adinya, I.B., Ijoma, J.U., Enil, I., Ewona, G., Anyorah, C.N. and Ogar, N.E. (2012) Analysis of Edible Mushroom Marketing in Three Villages in Central Cross River State, Nigeria. *Global Journal of Agricultural Sciences*, **11**, 73-80.

[11] Valentão, P., Andrade, P.M., Rangel, J., Ribeiro, B., Silva, B.M., Baptista, P. and Seabra, R.M. (2005) Effect of the Conservation Procedure on the Contents of Phenolic Compounds and Organic Acids in Chanterelle (*Cantharellus cibarius*) Mushroom. *Journal of Agricultural and Food Chemistry*, **53**, 4925-4931. http://dx.doi.org/10.1021/jf0580263

[12] Colak, A., Faiz, O. and Sesli, E. (2009) Nutritional Composition of Some Wild Edible Mushrooms. *Turkish Journal of Biochemistry*, **34**, 25-31.

[13] Shirmila, J.G. and Radhamany, P. (2012) Identification and Determination of Antioxidant Constituents of Bioluminescent Mushroom. *Asian Pacific Journal of Tropical Biomedicine*, **2**, S386-S391. http://dx.doi.org/10.1016/S2221-1691(12)60194-4

[14] Sułkowska-Ziaja, K., Muszynska, B., Motyl, P., Pasko, P. and Ekiert, H. (2012) Phenolic Compounds and Antioxidant Activity in Some Species of Polyporoid Mushrooms from Poland. *International Journal of Medicinal Mushrooms*, **14**, 385-393. http://dx.doi.org/10.1615/IntJMedMushr.v14.i4.60

[15] Yap, Y.H., Tan, N., Fung, S., Aziz, A.A., Tan, C. and Ng, S. (2013) Nutrient Composition, Antioxidant Properties, and Anti-Proliferative Activity of *Lignosus rhinocerus* Cooke Sclerotium. *Journal of the Science of Food and Agriculture*, **93**, 2945-2952. http://dx.doi.org/10.1002/jsfa.6121

[16] Johnsy, G. and Kaviyarasan, V. (2014) Evaluation of Antioxidant Activities and Determination of Bioactive Compounds in Two Wild Edible Termitomycetes (*T. microcarpus* and *T. heimii*). *World Journal Dairy & Food Sciences*, **9**, 10-19.

[17] Ribeiro, B., Valentão, P., Baptista, P., Seabra, R.M. and Andrade, P.B. (2007) Phenolic Compounds, Organic Acids Profiles and Antioxidative Properties of Beefsteak Fungus (*Fistulina hepatica*). *Food and Chemical Toxicology*, **45**, 1805-1813. http://dx.doi.org/10.1016/j.fct.2007.03.015

[18] Lee, Y.-L., Jian, S.-Y., Lian, P.-Y. and Mau, J.-L. (2008) Antioxidant Properties of Various Extracts from *Hypsizigus marmoreus*. *Food Chemistry*, **104**, 1-9. http://dx.doi.org/10.1016/j.foodchem.2006.10.063

[19] Soares, A.A., De Souza, C.G.M., Daniel, F.M., Ferrari, G.P., Da Costa, S.M.G. and Peralta, R.M. (2009) Antioxidant Activity and Total Phenolic Content of *Agaricus brasiliensis* (*Agaricus blazei* Murril) in Two Stages of Maturity. *Food Chemistry*, **112**, 775-781. http://dx.doi.org/10.1016/j.foodchem.2008.05.117

[20] Choi, Y., Lee, S.M., Chun, J., Lee, H.B. and Lee, J. (2006) Influence of Heat Treatment on the Antioxidant Activities and Polyphenolic Compounds of Shiitake (*Lentinus edodes*) Mushroom. *Food Chemistry*, **99**, 381-387. http://dx.doi.org/10.1016/j.foodchem.2005.08.004

[21] Ferreira, I.C.F.R., Baptista, P., Vilas-Boas, M. and Barros, L. (2007) Free-Radical Scavenging Capacity and Reducing Power of Wild Edible Mushrooms from Northeast Portugal: Individual Cap and Stipe Activity. *Food Chemistry*, **100**, 1511-1516. http://dx.doi.org/10.1016/j.foodchem.2005.11.043

[22] Alvarez-Parrilla, E., De La Rosa, L.A., Martinez, N.R. and Gonzalez-Aguilar, G.A. (2007) Total Phenols and Antioxident Activity of Commercial and Wild Mushrooms from Chiuahua, Mexico. *Ciencia y Tecnología Alimentaria*, **5**, 329-334. http://dx.doi.org/10.1080/11358120709487708

[23] Hung, P.V. and Nhi, N.N.Y. (2012) Nutritional Composition and Antioxidant Capacity of Several Edible Mushrooms Grown in the Southern Vietnam. *International Food Research Journal*, **19**, 611-615.

[24] Obodai, O., Ferreira, I.C.F.R., Fernandes, A., Barros, L., Mensah, D.L.N., Dzomeku, M., Urben, A.F., Prempeh, J. and Takli, R.K. (2014) Evaluation of the Chemical and Antioxidant Properties of Wild and Cultivated Mushrooms of Ghana. *Molecules*, **19**, 19532-19548. http://dx.doi.org/10.3390/molecules191219532

[25] Hasib, A., Jaouad, A., Mahrouz, M. and Khouili, M. (2002) HPLC Determination of Organic Acids in Moroccan Apricot. *Ciencia y Tecnología Alimentaria*, **3**, 207-211. http://dx.doi.org/10.1080/11358120209487729

[26] Singleton, V.L., Orthofer, R. and Lamuela-Raventos, R.M. (1999) Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. *Methods in Enzymology*, **299**, 152-178. http://dx.doi.org/10.1016/S0076-6879(99)99017-1

[27] Meda, A., Lamien, C.E., Romito, M.J. and Nacoulma, O.G. (2005) Determination of the Total Phenolic, Flavonoid and Proline Contents in Burkina Fasan Honey, as Well as Their Radical Scavenging Activity. *Food Chemistry*, **91**, 571-577. http://dx.doi.org/10.1016/j.foodchem.2004.10.006

[28] Bainbridge, Z., Tomlins, K., Willings, K. and Westby, A. (1996) Methods for Assessing Quality Characteristics of Non-Grain Starch Staple. Part 4 Advanced Methods. National Resources Institute, University of Greenwich, Chatham, **1**, 43- 79.

[29] Shimada, K., Fujikawa, K., Yahara, K. and Nakamura, T. (1992) Antioxidative Properties of Xanthan on the Autoxidation of Soybean Oil in Cyclodextrin Emulsion. *Journal of Agricultural and Food Chemistry*, **40**, 945-948. http://dx.doi.org/10.1021/jf00018a005

[30] Elmastas, M., Isildak, O., Turkekul, I. and Temur, N. (2007) Determination of Antioxidant Activity and Compounds in Wild Edible Mushrooms. *Journal of Food Composition and Analysis*, **20**, 337-345. http://dx.doi.org/10.1016/j.jfca.2006.07.003

[31] Gursoy, N., Sarikurkcu, C., Cengiz, M. and Solak, M.H. (2009) Antioxidant Activities, Metal Contents, Total Phenolics and Flavonoids of Seven Morchella Species. *Food and Chemical Toxicology*, **47**, 2381-2388. http://dx.doi.org/10.1016/j.fct.2009.06.032

[32] Keles, A., Koca, L. and Genccelep, H. (2011) Antioxidant Properties of Wild Edible Mushrooms. *Food Processing Technology*, **2**, 1-6.

[33] Palacios, I., Lozano, M., Moro, C., Arrigo, M., Rostagno, M.A., Martinez, J.A., Garcia-Lafuente, A., Guillamon, E. and Villares, A. (2011) Antioxidant Properties of Phenolic Compounds Occurring in Edible Mushrooms. *Food Chemistry*, **128**, 674-678. http://dx.doi.org/10.1016/j.foodchem.2011.03.085

[34] Türkoğlu, A., Duru, M.E., Mercan, N., Kivrak, I. and Gezer, K. (2007) Antioxidant and Antimicrobial Activities of *Laetiporus sulphureus* (Bull.) Murill. *Food Chemistry*, **10**, 267-273. http://dx.doi.org/10.1016/j.foodchem.2006.01.025

[35] Yen, G.C., Duh, P.D. and Tsai, C.L. (1993) Relationship between Antioxidant Activity and Maturity of Peanut Hulls. *Journal of Agricultural and Food Chemistry*, **4**, 67-70. http://dx.doi.org/10.1021/jf00025a015

[36] Gülçin, I., Oktay, M., Kireçci, E. and Küfrevioglu, O.I. (2003) Screening of Antioxidant and Antimicrobial Activities of Anise (*Pimpella anisum* L.) Seed Extracts. *Food Chemistry*, **83**, 371-382. http://dx.doi.org/10.1016/S0308-8146(03)00098-0

[37] Van Acker, S.A., Van Balen, G.P., Van Den Berg, D.J., Bast, A. and Van Der Vijgh, W.J. (1998) Influence of Iron Chelation on the Antioxidant Activity of Flavonoids. *Biochemical Pharmacology*, **56**, 935-943. http://dx.doi.org/10.1016/S0006-2952(98)00102-6

[38] Arbaayah, H.H. and UmiKalsom, Y. (2013) Antioxidant Properties in the Oyster Mushrooms (*Pleurotus* spp.) and Split Gill Mushroom (*Schizophyllum commune*) Ethanolic Extracts. *Mycosphere*, **4**, 661-673. http://dx.doi.org/10.5943/mycosphere/4/4/2

[39] Hagerman, A.E., Riedl, K.M., Jones, G.A., Sovik, K.N., Ritchard, N.T., Hartzfeld, P.W. and Riechel, T.K. (1998) High Molecular Weight Plant Polyphenolics (Tannins) as Biological Antioxidants. *Journal of Agricultural and Food Chemistry*, **46**, 1887-1892. http://dx.doi.org/10.1021/jf970975b

[40] Tripathy, S.S., Rajoriya, A. and Gupta, N. (2014) Wild Mushrooms of Odisha: Prospective Candidates of Antioxidant Sources. *Advance in Plants & Agriculture Research*, **1**, 00021.

[41] Rajoriya, A., Tripathy, S.S. and Gupta, N. (2015) *In Vitro* Antioxidant Activity of Selected *Ganoderma* Species Foundin Odisha, India. *Tropical Plant Research*, **2**, 72-77.

[42] Barros, L., Dueñas M., Ferreira, I.C.F.R., Baptista, P. and Santos-Buelga, C. (2009) Phenolic Acids Determination by Hplcdad-Esi/Ms in Sixteen Different Portuguese Wild Mushrooms Species. *Food and Chemical Toxicology*, **47**, 1076-1079. http://dx.doi.org/10.1016/j.fct.2009.01.039

[43] Kalogeropoulos, N., Yanni, A.E., Koutrotsios, G. and Aloupi, M. (2013) Bioactive Microconstituents and Antioxidant Properties of Wild Edible Mushrooms from the Island of Lesvos, Greece. *Food and Chemical Toxicology*, **55**, 378-385. http://dx.doi.org/10.1016/j.fct.2013.01.010

[44] Puttaraju, N.G., Venkateshaiah, S.U., Dharmesh, S.M., Urs, S.M. and Somasundaram, R. (2006) Antioxidant Activity of Indigenous Edible Mushrooms. *Journal of Agricultural and Food Chemistry*, **54**, 9764-9772. http://dx.doi.org/10.1021/jf0615707

[45] Kim, M.-Y., Seguin, P., Ahn, J.-K., Kim, J.-J., Chun, S.-C., Kim, E.-H., Seo, S.-H., Kang, E.-Y., Kim, S.-L., Park, Y.-J., Ro, H.-M. and Chung, I.-M. (2008) Phenolic Compound Concentration and Antioxidant Activities of Edible and Medicinal Mushrooms from Korea. *Journal of Agricultural and Food Chemistry*, **56**, 7265-7270.

http://dx.doi.org/10.1021/jf8008553

[46] Li, H., Nam, W.-S., Moon, B. and Lee, C. (2014) Antioxidant Activity and Phenolic Content of Brick Caps Mycelium (*Naematoloma sublateritium*) Extracts. *Food Science and Biotechnology*, **23**, 1425-1431. http://dx.doi.org/10.1007/s10068-014-0195-0

[47] Ribeiro, B., Rangel, J., Valentão, P., Baptista, P., Seabra, R.M. and Andrade, P.B. (2006) Contents of Carboxylic Acids and Two Phenolics and Antioxidant Activity of Dried Portuguese Wild Edible Mushrooms. *Journal of Agricultural and Food Chemistry*, **54**, 8530-8537. http://dx.doi.org/10.1021/jf061890q

[48] Ribeiro, B., Andrade, P.B., Baptista, P., Barros, L., Ferreira, I.C.F.R., Seabra R.M. and Valentão, P. (2008) *Leucopaxillus giganteus* Mycelium: Effect of Nitrogen Source on Organic Acids and Alkaloids. *Journal of Agricultural and Food Chemistry*, **56**, 4769-4774. http://dx.doi.org/10.1021/jf8001526

[49] Barros, L., Pereira, C. and Ferreira, I.C.F.R. (2013) Optimized Analysis of Organic Acids in Edible Mushrooms from Portugal by Ultra-Fast Liquid Chromatography and Photodiode Array Detection. *Food Analytical Methods*, **6**, 309-316. http://dx.doi.org/10.1007/s12161-012-9443-1

[50] Ayaz, F.A., Torun, H., Özel, A., Col, M., Duran, C., Sesli, E. and Colak, A. (2011) Nutritional Value of Some Wild Edible Mushrooms from Black Sea Region (Turkey). *Turkish Journal of Biochemistry*, **36**, 213-221.

[51] Barros, L., Telma, C., Paula, B., Estevinho, L.M. and Ferreira, I.C.F.R. (2008) Wild and Commercial Mushrooms as Source of Nutrients and Nutraceuticals. *Food and Chemical Toxicology*, **46**, 2742-2747. http://dx.doi.org/10.1016/j.fct.2008.04.030

[52] Oboh, G. and Shodehinde, S.A. (2009) Distribution of Nutrients, Polyphenols and Antioxidant Activities in the Pilei and Stipes of Some Commonly Consumed Edible Mushrooms in Nigeria. *Bulletin of the Chemical Society of Ethiopia*, **23**, 391-398. http://dx.doi.org/10.4314/bcse.v23i3.47663