

# Phenolic compounds, organic acid and antioxidant activity of *Lactarius subsericatus*, *Cantharellus platyphyllus* and *Amanita rubescens*, three edible ectomycorrhizal mushrooms from center of Côte d'Ivoire

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Considering the interest for mushrooms and research of antioxidants in natural food resources, the aim of this study was to investigate the content of phenolic compounds, profiles of phenolic compounds and organic acids and antioxidant activities via the ability to scavenge DPPH radical of three ectomycorrhizal mushrooms identified as *Lactarius subsericatus*, *Cantharellus platyphyllus* and *Amanita rubescens* and collected in central part of Côte d'Ivoire. Contents of total phenolic, flavonoids and tannins of mushrooms methanolic extracts were evaluated by colorimetric assays to ranges of 430.20-610.95 mg (GAE)/100 g DW, 119.55-159.75 mg (QE)/100 g DW and 139.80-232.80 mg (TAE)/100 g DW, respectively. HPLC-profiles of methanolic extracts indicated as main individual phenolic compounds, gallic acid, catechin and quercetin for *L. subsericatus*; gallic acid, *p*-coumaric acid and quercetin for *C. platyphyllus*; gallic acid and benzoic acid for *A. rubescens*. HPLC profiles of organic acids revealed that fumaric and citric acids were the main organic acids in all species. Malic acid was also preponderant in *L. subsericatus*. The methanolic extracts of the three mushrooms exhibited the high DPPH radical scavenging activities ranging from 77.42 to 67.17 %. These data indicated that these mushrooms could constitute a potential good source of natural antioxidant for local population.

**Keywords:** antioxidant activity, ectomycorrhizal mushrooms, organic acids, phenolic compounds

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## INTRODUCTION

Wild mushrooms have a worldwide distribution and are used in human consumption in many countries. In fact, since ancient times mushrooms have been consumed by humans as a part of the normal diet. They have a highly desirable taste and aroma, being also consumed for their texture: they add flavor and texture to a meal [1, 2]. In Côte d'Ivoire, particularly, in the center part, ectomycorrhizal mushrooms such as *Lactarius subsericatus*, *Cantharellus platyphyllus* and *Amanita rubescens* picked in the wild are involved regularly in the local people feed. These wild mushroom species are widely found in woodlands of this part of Côte d'Ivoire where they form symbiotic associations with their host plants. Nowadays, it was acknowledged that wild edible mushrooms contain many bioactive substances such as phenolic acids, flavonoids organic acids, vitamins and vitamin precursors [1, 3, 4]. For these reasons, many researchers have explored wild mushrooms for their phenolic compounds in many countries in Europe [2, 4, 5, 6], Asia [7, 8], Africa [9, 10, 11], America [12]. In most cases, these reports have also focused on the evaluation of antioxidant activity since it is well-established that this activity is mainly related to their phenolic content [13, 14]. Moreover, many wild mushrooms was analyzed for their organic acids contents since these compounds are known to influence the organoleptic properties of food, and have also been used for their quality control [15]. Considering the well-known crucial role of these bioactive compounds in the relationship between food and health, search of their availability in natural food resources has an undeniable interest to many researchers. But, to our knowledge, there are no published data on phenolic compounds and organic acids in wild edible mushrooms from center part of Côte d'Ivoire. Hence, the objective of this study was to investigate on phenolic compounds, organic acids and antioxidant activity in three edible ectomycorrhizal mushrooms from central region of Côte d'Ivoire identified as *Lactarius subsericatus*, *Cantharellus platyphyllus* and *Amanita rubescens*. Thus, we described, apparently for the first time the analysis of phenolic compounds content and the identification and quantification of individual phenolic compounds and organic acids in these species of edible ectomycorrhizal mushrooms from Côte d'Ivoire. Additionally, we evaluated their antioxidant activity via their ability to scavenge DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical.

## MATERIALS AND METHODS

### 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. Louis, MO, USA). Methanol was purchased from Prolabo.

### Sample collection

The species of mushrooms used in this work were picked from woodlands of central Côte d'Ivoire. Taxonomic identification was achieved by Dr Souleymane Yorou Nourou (Abomé Calavy University of Benin/ Munich University of Germany), as *Lactarius subsericatus*, *Cantharellus platyphyllus* and *Amanita rubescens*. After picking, the samples of mushroom were immediately transferred to the laboratory and cleaned.

## Extraction of phenolic compounds

The mushrooms were dried at 25 °C for ten days, until constant weight, according Ribeiro *et al.* [15] 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 Whatman n° 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, prior to phenolic compound contents determination and HPLC analysis.

## Extraction of organic acids

The organic acids of dried mushroom samples were extracted 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 Whatman n° 4 paper, then through 0.45 µm filter (Millipore; Sartorius AG, Goettingen, Germany) prior to HPLC analysis.

## Determination of total phenolic compounds content

Contents of total phenolic compounds were estimated according Folin-Ciocalteu method [16]. 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)/100g DW (Dry Weight). All experiments were performed in triplicate.

## Determination of flavonoids

Total flavonoids content was determined according method used by Meda *et al.* [17], 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 1M 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)/100g DW. All experiments were performed in triplicate.

## Determination of tannins

Tannins content was determined using the method described by Bainbridge *et al.* [18]. A volume of 1 ml of each methanolic extract was collected and mixed with 5 ml of reaction solution [vanillin 0.1mg/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.

## HPLC analysis of phenolic compounds

The phenolic extracts previously prepared (50 ml) were diluted in 100 ml of distilled water and 20  $\mu$ l of each sample were 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  $\text{NaH}_4\text{H}_2\text{PO}_4$  to pH 2.6 (eluent A), a solution of acetonitrile/ $\text{NaH}_4\text{H}_2\text{PO}_4$  (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.

## 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, Japon) 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 x 5  $\mu$ m, Interchom, 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.

## Estimation of antioxidant activity by DPPH radical scavenging

The DPPH scavenging activity was determined using the method described by Shimada *et al.* [19]. 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:

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

## Statistical analysis

All chemical analyses and assays were performed in triplicate, unless otherwise indicated. Results were expressed as mean values  $\pm$  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 ( $p < 0.05$ ).

## RESULTS AND DISCUSSION

### Contents of phenolic compounds, flavonoids and tannins

The total phenolic contents for the investigated wild edible mushrooms estimated by the Folin-Ciocalteu methods using the methanolic extracts are

indicated in Table 1. The values were  $430.20 \pm 1.68$ ,  $610.95 \pm 2.73$  and  $543.45 \pm 2.23$  mg (GAE) / 100 g DW for *L. subsericatus*, *C. platyphyllus* and *A. rubescens*, respectively. In order to obtain more information on the nature of phenolic compounds, the contents of total flavonoids and tannins were also estimated. Total flavonoids contents were found to be  $232.8 \pm 2.52$  (*L. subsericatus*),  $159.75 \pm 1.65$  (*C. platyphyllus*),  $119.55 \pm 1.97$  (*A. rubescens*) mg (QE)/100 DW (Table 1). However, it is worth noting that flavonoids determination is highly selective for flavonoid structure since the isoflavone derivatives give no color with aluminum chloride [20]. Regarding tannin contents, results indicated content values of  $232.8 \pm 2.52$ ,  $144.00 \pm 1.98$  and  $139.8 \pm 1.32$  mg (TAE)/100g DW *L. subsericatus*, *C. platyphyllus* and *A. rubescens*, respectively (Table 1). Overall, for total phenolic, flavonoids and tannins contents, there were significant ( $p < 0.05$ ) differences between the three species of ectomycorrhizal mushrooms from central Côte d'Ivoire. From these results, it was noted that the three analyzed wild edible mushrooms had relatively high contents of total phenolic compounds; this could be attributed in part to the nature of the extraction solvent used. Indeed, several reports have indicated that methanol is considered to be one of the best solvents for extraction of total phenolic compounds in mushrooms [21, 22, 23].

**Table 1:** Total phenolic, flavonoids and tannins of three ectomycorrhizal mushrooms from Côte d'Ivoire (*L. subsericatus*, *C. platyphyllus* and *A. rubescens*)

Compounds (mg/100g)	Mushroom samples		
	<i>Lactarius subsericatus</i>	<i>Cantharellus platyphyllus</i>	<i>Amanita rubescens</i>
Total phenolics	$430.20 \pm 1.68^a$	$610.95 \pm 2.73^c$	$543.45 \pm 2.23^b$
Total flavonoids	$152.55 \pm 1.17^b$	$159.75 \pm 1.65^c$	$119.55 \pm 1.97^a$
Total tannins	$232.80 \pm 2.52^c$	$144.00 \pm 1.98^b$	$139.80 \pm 1.32^a$

Each value is an average of three replicate.

Values are mean  $\pm$  standard deviation.

Means not sharing a similar letter in a line are significantly different  $p < 0.05$  as assessed by the test of Duncan.

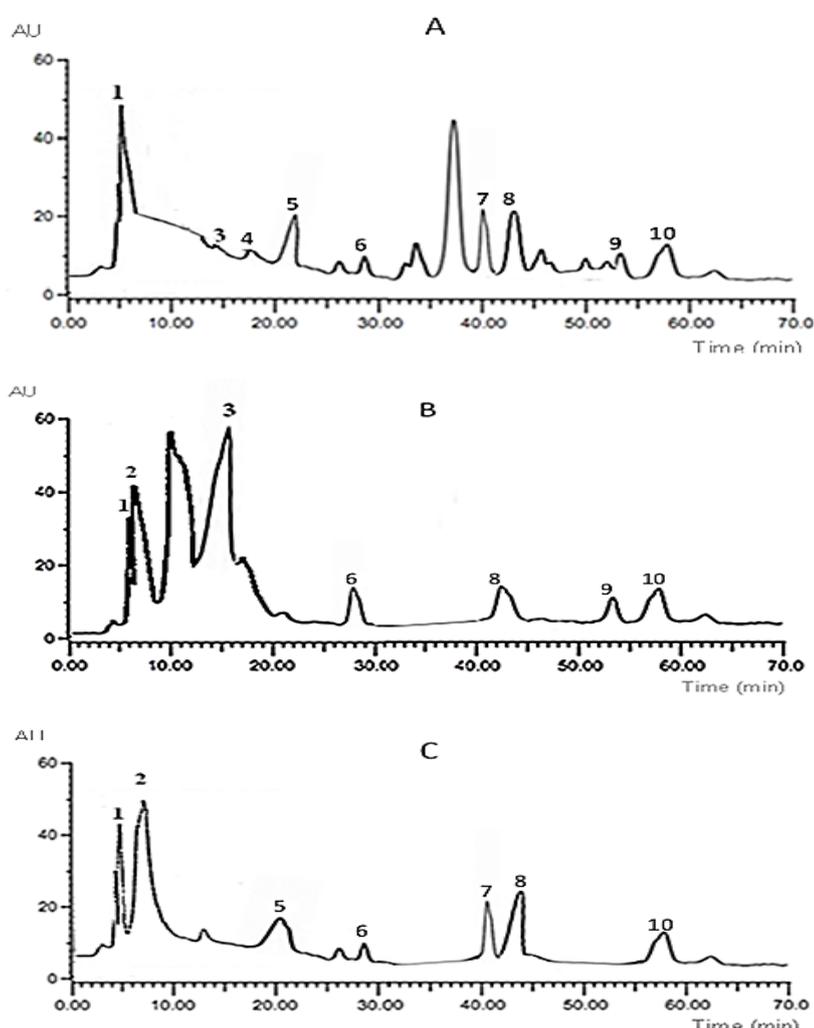
Moreover, total phenolic compounds contents of these three ectomycorrhizal mushrooms were in the range of those reported for others wild mushroom species in others countries [24, 25, 26, 36]. In addition, these high contents of phenolic compounds found in these wild mushrooms could constitute interesting data for population nutrition since it is well-known that these bioactive compounds found in human diet act as the antioxidant compounds and play a role in stabilizing lipid peroxidation [27, 28]. Regarding flavonoids, they probably belong to the most interesting groups of natural phenolic compounds. Indeed, flavonoids are recognized to act as an antioxidant by breaking the radical chains and more stable products in the membranes of liver microsomes [29], and also to play an important role to the instinctive protection against oxidative stress [30]. Contents of flavonoids in methanolic extracts of the three analyzed mushrooms were comparatively higher than those reported for others wild mushrooms studied elsewhere [25, 31]. As for tannins, they constitute another class of natural phenolic compounds which contribute in part to the antioxidant properties of plants [28, 32, 33]. The investigated mushroom methanolic extracts displayed high contents of tannins comparatively to some data of literature [31, 34]. However, the findings of [35] indicated the absence of tannins among phytochemicals in edible wild mushrooms from selected areas in Kenya.

### HPLC-profiles of phenolic compounds

The analysis by HPLC of the methanolic extract of three ectomycorrhizal mushrooms (Fig. 1) revealed the existence of three phenolic acids (gallic acid,  $p$ -

hydroxybenzoic acid and benzoic acid), one hydroxycinnamic acids (caffeic acid), two flavonoids (quercetin, catechin,) and one stilbene (resveratrol) for *L. subsericatus* (Fig. 1A); four phenolic acids (gallic acid, protocatechuic acid,  $\rho$ -hydroxybenzoic acid and benzoic acid), one flavonoid (quercetin), two hydroxycinnamic acids (caffeic acid,  $\rho$ -coumaric acid), one condensed tannin (tannin ol) and one stilbene (resveratrol) for *C. platyphyllus* (Fig. 1b); four phenolic acids (gallic acid, protocatechuic acid,  $\rho$ -hydroxybenzoic acid and benzoic acid), one flavonoid (catechin), two hydroxycinnamic acids (caffeic acid,  $\rho$ -coumaric acid) for *A. Rubescens* (Fig. 1C). Others phenolic compounds were detected in all of the analyzed species, although it was not possible to identify them. All these phenolic compounds are reported for the first time in ectomycorrhizal mushrooms from Côte d'Ivoire.

On the whole, most of the phenolic compounds identified in these samples of mushroom were detected in numerous species of wild mushrooms explored by several authors in many countries [2, 6, 10, 15, 24 36]



**Figure 1:** HPLC-profiles of phenolic compounds in three ectomycorrhizal mushrooms from Côte d'Ivoire (**A:** *C. platyphyllus*, **B:** *L. subsericatus*, **C:** *A. rubescens*)

Detection at 280 nm: **1**(Gallic acid), **2**(Catechin), **3**(Quercetin), **4**(Tannin ol), **5**( $\rho$ -Coumaric acid), **6** (Caffeic acid). **7**(protocatechuic acid), **8** ( $\rho$ -Hydroxybenzoic acid), **9** (Resveratrol), **10** (Benzoic acid)

With regard to the amounts of individual phenolic compounds (Table 2), the main remark was that gallic acid displayed relatively high contents in the three

samples:  $100.96 \pm 3.3$ ,  $149.20 \pm 2.73$ ,  $83.35 \pm 3.20$  mg/kg DW for *L. subsericatus*, *C. platyphyllus* and *A. rubescens*, respectively. Puttaraju *et al.* [36] had also detected significant amounts of gallic acid in indigenous species of mushroom (*Termitomyces heimii* and *T. mummiformis*) from India.

**Table 2:** Phenolic compounds contents (mg/kg DW) in some ectomycorrhizal mushrooms from Côte d'Ivoire: (*L. subsericatus*, *C. platyphyllus* and *A. rubescens*)

Phenolic compounds (mg/kg)	Retention time (min)	Mushroom samples		
		<i>L. subsericatus</i>	<i>C. platyphyllus</i>	<i>A. rubescens</i>
Gallic acid	(5)	$100.96 \pm 3.31^a$	$149.20 \pm 2.73^b$	$83.35 \pm 3.20^c$
Catechin	(8)	$94.53 \pm 1.16^b$	nd	$35.38 \pm 1.05^a$
Quercetin	(15)	$61.47 \pm 2.01^a$	$62.42 \pm 1.21^b$	nd
Tanin ol	(17.50)	nd	$23.74 \pm 1.03$	nd
$\rho$ -Coumaric acid	(20.50)	nd	$69.59 \pm 1.50^b$	$37.55 \pm 1.21^a$
Caffeic acid	(28.50)	$53 \pm 0.20^a$	$15.71 \pm 0.24^b$	$41.87 \pm 2.47^c$
Protocatechuic acid	(40)	nd	$42.47 \pm 1.12^b$	$22.44 \pm 1.01^a$
$\rho$ -Hydroxybenzoic acid	(43)	$5.59 \pm 0.11^a$	$47.61 \pm 1.02^b$	$36.67 \pm 1.10^c$
Resveratrol	(53)	$9.83 \pm 1.08^a$	$35.00 \pm 2.05^b$	nd
Benzoic acid	(58)	$16.14 \pm 1.01^a$	$38.52 \pm 2.05^b$	$73.63 \pm 2.22^c$

Each value is an average of three replicate.

Values are mean  $\pm$  standard deviation.

Means not sharing a similar letter in a line are significantly different  $p < 0.05$  as assessed by the test of Duncan.

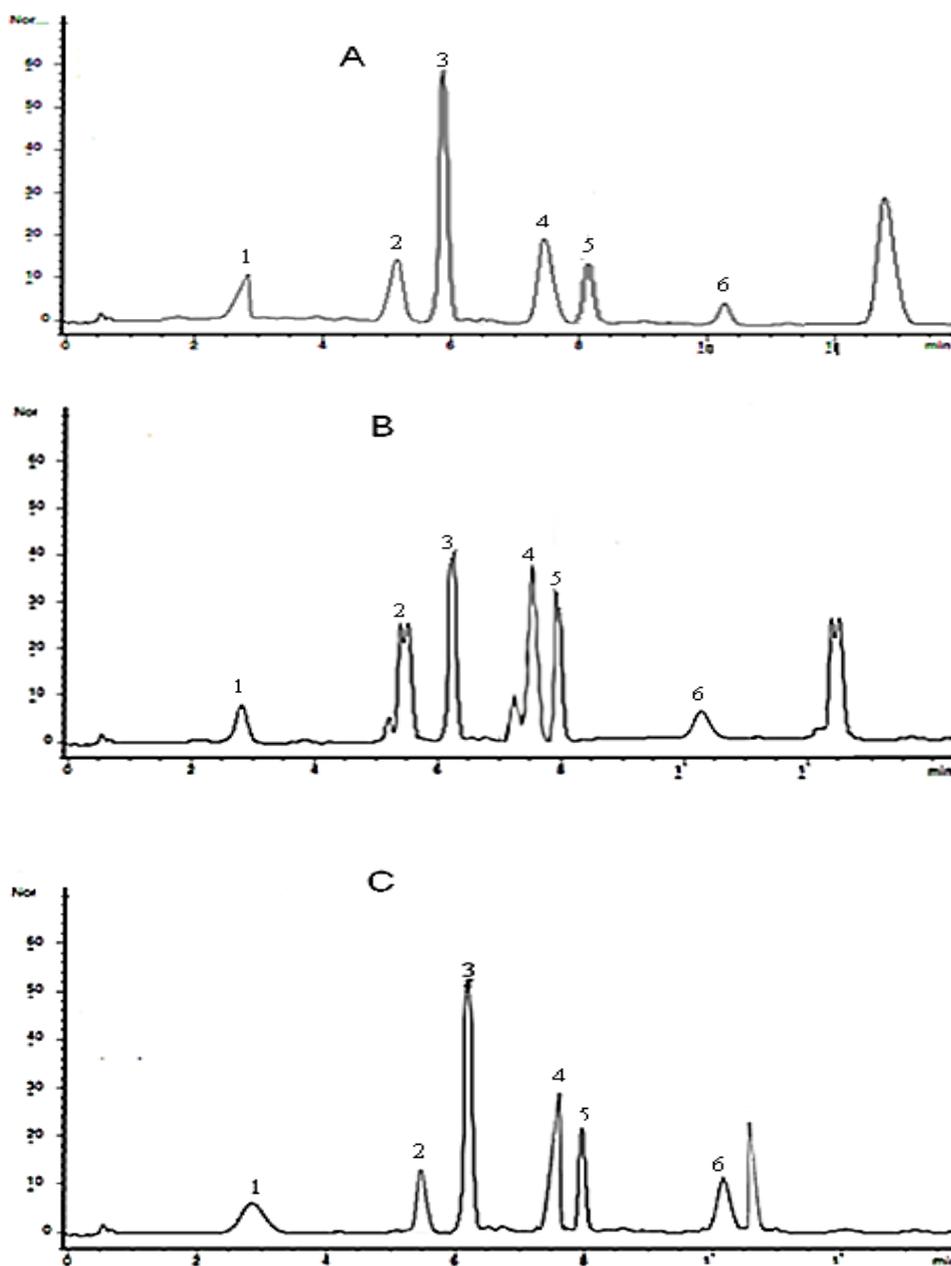
nd: Not Detected

Benzoic acid,  $\rho$ -hydroxybenzoic acid and caffeic acid were found in the three mushrooms with significant levels ranging  $5.59 \pm 0.11$  to  $73.63 \pm 2.22$  mg/kg DW.  $\rho$ -hydroxybenzoic acid was also detected in two species of polyporoid mushrooms (*Fomitopsis pinicola* and *Gloeophyllum sepiarium*) from Poland [37] and Portuguese wild species such as *Agaricus arvensis* and *A. silvicola* [24] and *Fistulina hepatica* [38]. As for caffeic acid, it was successfully explored in *F. hepatica* [14]. Protocatechuic acid was identified in *C. platyphyllus* and *A. rubescens* with content of  $42.47 \pm 1.12$  and  $22.44 \pm 1.01$  mg/kg DW, respectively. This phenolic acid previously reported with relative high content in wild species of mushroom as *Boletus badius* [39], *Lepista nuda* [24], and *F. hepatica* [38]. Catechin which was already detected in wild mushroom *Agaricus blazei* from Korea [40], was preponderant in *L. subsericatus* ( $94.53 \pm 1.16$  mg/kg DW), moderately present in *A. rubescens* ( $35.38 \pm 1.05$  mg/kg DW) and not found in *C. platyphyllus*. Quercetin which was detected in trace amount in mushroom *F. hepatica* [15], was one of the most abundant phenolic compounds identified in *L. subsericatus* ( $61.47 \pm 2.01$  mg/kg DW) and *C. platyphyllus* ( $62.42 \pm 1.21$  mg/kg DW). *C. platyphyllus* and *A. rubescens* presented contents of  $\rho$ -Coumaric acid estimated to  $69.59 \pm 1.50$  and  $37.55 \pm 1.21$  mg/kg DW, respectively. However, it was not detected in *L. subsericatus*. This hydroxycinnamic acid derivative was abundantly detected in several edible wild mushrooms [2, 6, 15, 24]. Tannin ol which is constituted by the condensed tannins that are polymers formed by the condensation of flavans was only present in *C. platyphyllus* with amount of  $23.74 \pm 1.03$  mg/kg DW. Regarding stilbene resveratrol, it was successfully analyzed in *L. subsericatus* ( $9.83 \pm 1.08$  mg/kg DW) and in *C. platyphyllus* ( $35.00 \pm 2.05$  mg/kg DW). Resveratrol was previously detected in *Sparassis crispa* and *Inonotus obliquus* from Korea [40].

### HPLC-profiles of organic acids

Fig. 2 shows the organic acids profiles of three analyzed species of ectomycorrhizal mushrooms from Côte d'Ivoire. As seen, all the three species

contained oxalic, citric, malic, succinic, ascorbic and fumaric acids. Table 3 shows that the main organic acids found in the three studied species were fumaric (3353.30±16.07, 4890±12.76 and 3526.7±30.03 mg/kg DW, for *L. subsericatus*, *C. platyphyllus* and *A. rubescens*, respectively) and citric acids (3323.30±11.15, 12060±08.7 and 2823.30±19.50 mg/kg DW for *L. subsericatus*, *C. platyphyllus* and *A. rubescens*, respectively).



**Figure 2:** HPLC chromatograms of identified organic acids in some ectomycorrhizal mushrooms from Côte d'Ivoire

(**A:** *C. platyphyllus*, **B:** *L. subsericatus*, **C:** *A. rubescens*)

1: oxalic acid; 2: ascorbic acid; 3: fumaric acid; 4: citric acid; 5: malic acid; 6: succinic acid

Fumaric acid is an important organic acid because of its antioxidant, antimicrobial and acidifying properties [41, 42]. Citric acid is known to be very

important in the prevention of mushroom browning and to extend its shelf life; this is because of its antibacterial and antioxidant properties [41].

**Table 3:** Contents (mg/kg DW) of organic acid composition in some ectomycorrhizal mushrooms from Côte d'Ivoire: (*L. subsericatus*, *C. platyphyllus* and *A. rubescens*)

Organic acids (mg/kg DW)	Retention Time (min)	Mushroom samples		
		<i>L. subsericatus</i>	<i>C. platyphyllus</i>	<i>A. rubescens</i>
Oxalic acid	(2.80)	10.20±0.05 <sup>b</sup>	22.30±0.12 <sup>c</sup>	06.10±0.03 <sup>a</sup>
Ascorbic acid	(5.25)	90.40±0.19 <sup>c</sup>	75.10±01.54 <sup>b</sup>	50.60±0.08 <sup>a</sup>
Fumaric acid	(6.00)	3353.30±16.07 <sup>a</sup>	4890±12.76 <sup>c</sup>	3526.7±30.03 <sup>b</sup>
Citric acid	(7.50)	3323.30±11.15 <sup>c</sup>	12060±08.7 <sup>a</sup>	2823.30±19.50 <sup>b</sup>
Malic acid	(8.00)	2156.70±17.09 <sup>c</sup>	390±06.24 <sup>a</sup>	976.70±04.04 <sup>b</sup>
Succinic acid	(10.40)	13.80±0.11 <sup>b</sup>	07.70±0.04 <sup>a</sup>	20.40±0.07 <sup>c</sup>

Each value is an average of three replicate.

Values are mean ± standard deviation.

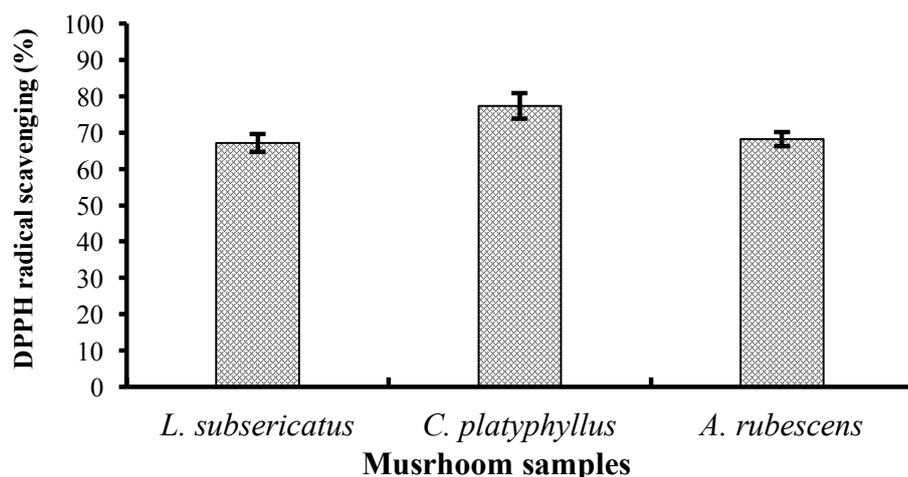
Means not sharing a similar letter in a line are significantly different  $p < 0.05$  as assessed by the test of Duncan

But, also malic acid was detected in important amount in *L. subsericatus* (2156.70±17.09 mg/kg DW) and in *A. rubescens* (976.70±04.04 mg/kg DW). This organic acid which is well-known to contribute to the pleasantly sour taste of fruits and to be used as a food additive, was also described in wild mushroom *Macrolepiota procera* from Turkey in high content of 19.40± 0.62 g/kg dry DW [43]. Ascorbic acid which is known to possess high antioxidant properties was very moderately present in the three mushrooms (90.40±0.19, 75.10±01.54, 50.60±0.08 mg/kg DW for *L. subsericatus*, *C. platyphyllus* and *A. rubescens*, respectively). Oxalic and succinic acids were the minor organic acids in the three investigated mushrooms. As phenolic compounds, all these 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.

### DPPH scavenging activity

DPPH scavenging activity is a rapid method to characterize the antioxidant capacity of extracts against oxidation caused by free radicals. Several authors have employed this method in assessment of mushroom antioxidant properties [7, 25, 41, 44, 45,]). 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 *C. platyphyllus* exhibited the highest DPPH scavenging activity with 77.42 %, followed by that of *A. rubescens* with 68.19 % (Fig. 3). As for *L. subsericatus*, methanolic extract displayed scavenging activity of % (Fig. 3). From these results, we can postulate that methanolic extracts of our three mushroom species displayed a noticeable effect on scavenging free radical.

Methanolic extracts of several wild edible mushroom species were also successfully tested for their DPPH scavenging activity [3, 4, 10, 46, 47, 48]. Additionally, these results were in the range of those from wild mushrooms recognized as endowed of high antioxidant capacity [3, 7, 25, 41, 49]



**Figure 3:** DPPH radical scavenging (%) of extracts of three ectomycorrhizal mushrooms from Côte d'Ivoire

## CONCLUSION

The results presented in this study are the first information on phenolic compounds contents, profiles of phenolic compounds and organic acids, and antioxidant properties of the antioxidant activities of samples of mushroom of Côte d'Ivoire. Findings from this work allowed to emphasize the high contents of total phenolic, flavonoids and tannins in the methanolic extracts from three edible ectomycorrhizal mushrooms identified as *L. subsericatus*, *C. platyphyllus* and *A. rubescens*. This constitutes interesting data since these compounds are included in the antioxidant compounds of mushrooms. In the other hand, HPLC analysis of phenolic extract showed the presence in relatively high amounts of individual 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 analysis of organic acids in the extracts revealed the presence of fumaric and citric acids which are well known to possess a positive role in the organoleptic properties as well as in antioxidant properties of food. In addition, the methanolic extracts from these three ectomycorrhizal mushrooms displayed significant antioxidant properties as demonstrated by their high capacity to scavenge DPPH free radical. Ultimately, these mushroom species could constitute the potential of easily accessible sources of natural antioxidants and other bioactive compounds for local population nutrition.

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