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Two Widely Consumed Wild Mushrooms from Central Côte d'Ivoire: Phytochemical Compounds and Radical Scavenging Abilities

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Abstract

This study presents data on phenolic compounds contents, HPLC-profiles of individual phenolic compound and organic acids and DPPH Scavenging Abilities of widely consumed wild mushrooms identified as *Volvariella volvacea* and *Psathyrella tubercula* from the three the regions of central Côte d'Ivoire. Contents of total phenolic, flavonoids and tannins in samples of *V. volvacea* were estimated by colorimetric assays in ranges of 302.15-345.60mg (GAE)/100 g DW, 73.86-86.86 mg (QE)/100 g DW and 167.40-177.82 mg (TAE)/100 g DW, respectively; regarding *P. tuberculata*, these parameters were assessed in ranges 392.31-406.73 (GAE)/100 g DW, 55.07-63.77 (QE)/100 g DW and 200.02-214.38 (TAE)/100 g DW, respectively. In terms of individual phenolic compounds, catechin, ellagic acid, protocatechuic acid and cinnamic acid were found with significant amounts in samples of *V. volvacea* from the three regions. As for samples of *P. tuberculata*, the major phenolic compounds detected in the three regions were gallic acid, catechin and ellagic acid. With respect to organic acids, the results showed that citric acid was the major organic acid in all the samples of both mushrooms species. Shikimic acid and fumaric acid were also found with significant amounts in samples of *V. volvacea* from Bélier and N'Zi regions and in samples of *P. tuberculata* from Gbêkê and Bélier regions, respectively. The methanolic extracts from all mushrooms samples exhibited the DPPH radical scavenging activities ranging from 50.88 to 64.24%. Ultimately, both mushroom species highly consumed in central Côte d'Ivoire could be regarded as good source of natural antioxidant for local population.

1. Introduction

Wild mushrooms have always constituted a part of human diet in many countries in the world for centuries due to their organoleptic characteristics as well as the nutritional values [1, 2]. In general, mushrooms are referred as precious healthy foods, low in calories, fat and rich in vegetable protein, vitamins and minerals [3, 4].

Although nowadays, many species of edible wild mushrooms are able to be cultivated, collecting wild mushrooms in the wild for the food and commercial purposes remains a significant activity in many developing countries [5- 8].

In Côte d'Ivoire and in most countries of Humid Tropical Africa, in the rain season, wild mushrooms are picked in the wild by the rural population for their own consumption as well as commercial purposes. In central area of this country, two wild mushrooms identified as *Volvariella volvacea* and *Psathyrella tubercula* are among well appreciated and highly consumed mushroom species by the local communities [9]. *V. volvacea* and *P. tuberculata* are known in local language (Baoulé) as “Boyéfè” and “Ndré blé”, respectively. Generally, these mushrooms are harvested on oil palm frustums decaying for *V. volvacea* and on the trunks and roots of dead trees for *P. tuberculata*, respectively.

Moreover, nowadays, it appears advisable to have a better knowledge of biological potential of wild edible mushrooms species. For this reason, currently, mushrooms are become a focus of interest of many researchers as a source of bioactive compounds such as organic acids and antioxidants compounds [10-12]. It is well-established that antioxidant activity is mainly related to their phenolic content [13, 14] and organic acids influence the organoleptic properties of food matrices, and have also been used for their quality control [15]. Relative to this, several reports focusing on total phenols and antioxidant activities of wild and cultivated mushrooms have been published [8, 10, 16-19, 20].

To our knowledge, there is no literature data on levels of phenolic compounds and organic acids as well on antioxidant activities concerning both wild edible mushrooms species from Côte d'Ivoire. Therefore, in this study, phenolic compounds, organic acids and antioxidant activity in these widely consumed wild edible mushrooms from the three administrative regions of central Côte d'Ivoire were investigated. Thus, apparently for the first time, the analysis of phenolic compounds content by colorimetric assay and the identification and quantification of individual phenolic compounds and organic acids by HPLC, in *V. volvacea* and *P. tuberculata* from Côte d'Ivoire were carried out. Their antioxidant activity via their ability to scavenge DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical were also explored.

2. Materials and Methods

2.1. Standards and Reagents

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

2.2. Sample Collection

The species of mushrooms used in this work were picked

in the wild in the three administrative regions of central Côte d'Ivoire which were region of Gbêkê Béliér and N'Zi. Taxonomic identification was carried out by Dr Souleymane Yorou Nourou (Abomey Calavy University of Benin/ Munich University of Germany), as *Volvariella volvacea* and *Psathyrella tubercula*. Samples of *V. volvacea* and *P. tubercula* were harvested on oil palm frustums decaying and on the trunks and roots of dead trees, respectively. After picking, the mushrooms were immediately transferred to the laboratory and cleaned.

2.3. Preparation of Phenolic Compound Extract

The different samples of mushrooms were dried at 25°C for ten days, until constant weight, as described previously with slight modifications [15]. Then, each mushroom sample was ground into a fine-dried powder (mill IKA, Germany/Deutschland) and 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.

2.4. Preparation of Organic Acid Extract

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.

2.5. Determination of Total Phenolic Compound Content

Contents of total phenolic compounds were determined using Folin-Ciocalteu method [21]. Briefly, 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.

2.6. Determination of Flavonoid Content

Total flavonoid content was determined according method used by [22], 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.

2.7. Determination of Tannin Content

Tannins content was estimated using the method described by Bainbridge *et al.* [23]. 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.

2.8. Identification and Quantification of Phenolic Compounds by HPLC Analysis

The phenolic extracts previously prepared (50 ml) were diluted in 100 ml of distilled water and 20 µl 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 NaH₂HPO₄ to pH 2.6 (eluent A), a solution of acetonitrile/NaH₂HPO₄ (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 quantitative purposes, using external calibration with standards.

2.9. Identification and Quantification of Organic Acids by HPLC Analysis

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 µm, Interchrom, 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 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 DPPH Radical Scavenging Abilities

The DPPH scavenging activity was determined using the method described by Shimada *et al.* [24]. 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:

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

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. General Phenolic composition of mushroom samples

The contents of total phenolic, total flavonoids and tannins of methanolic extracts of *V. volvacea* and *P. tuberculata* collected in the three administrative regions of central Côte d'Ivoire are presented in Table 1

Table 1. Total phenolic compounds, flavonoids and tannins of sample *V. volvacea* and *P. tuberculata* from central Côte d'Ivoire.

Species	Regions	Total phenolics (mg/100g)	Total flavonoids (mg/100g)	Total tannins (mg/100g)
<i>Volvariella</i>	Gbêkê	302.15±1.11 ^b	86.86± 0.61 ^c	177.82± 0.70 ^c
<i>volvacea</i>	Bélier	342.27± 0.86 ^a	73.86± 0.65 ^a	167.40± 1.03 ^a

Species	Regions	Total phenolics (mg/100g)	Total flavonoids (mg/100g)	Total tannins (mg/100g)
<i>Psathyrella tuberculata</i>	N'zi	34560± 1.70 ^a	81.77± 0.89 ^b	174.23± 1.06 ^b
	Gbêkê	40673± 0.97 ^c	55.07± 0.60 ^a	214.38± 0.56 ^b
	Bélier	392.31± 0.73 ^a	55.20± 0.40 ^a	200.37± 0.22 ^a
	N'zi	397.19± 1.25 ^b	63.77± 0.69 ^b	200.02± 0.79 ^a

Each value is an average of three replicate.

Values are mean ± standard deviation.

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

With respect to samples of *V. volvacea*, the highest levels of total phenolic were detected in N'Zi and Bélier regions (342-345 mg (GAE)/100g DW) whereas samples of Gbêkê region displayed a content of 302 mg (GAE)/100g DW. Regarding *P. tuberculata*, total phenolic contents were in the range of 392.31 mg (GAE)/100g DW (Bélier region) to 406.73 mg (GAE)/100g DW (Gbêkê region) with 397.19 mg GAE/100g DW for N'Zi region. To get more information on the nature of these phenolic compounds, the levels of total flavonoids and tannins were also determined. For levels of total flavonoids, results indicated that the methanolic extracts of samples from *V. volvacea* exhibited total contents of 73.86 (Bélier Region), 81.77 (N'Zi Region) and 86.86 (Gbêkê Region) mg (QE)/100g DW. As for tannins, it was observed that their contents were ranged between 167.40 and 177.82 mg TAE/100g DW with the higher value in Gbêkê Region. With respect to *P. tuberculata*, concentrations of flavonoids and tannins ranged from 55.07 to 63.77 mg (QE) /100g DW and 200.02 to 214.38 mg (TAE) /100g DW, respectively. Overall, for total phenolic, flavonoids and tannins contents, in some cases, there were significant differences ($p < 0.05$) between the samples of the same species from the three regions and in others cases, there were no significant differences ($p < 0.05$). From our results, it follows that *V. volvacea* and *P. tuberculata* collected in three administrative regions of central Côte d'Ivoire had relatively high contents of total phenolic compounds, since several reports indicated total phenolic contents from others wild mushrooms in the range of our results [19, 20, 25-28]. These relatively high contents of phenolic compounds obtained in this work could be explained in part by the nature of the extraction solvent used. Indeed, it is known that phenolic compounds extraction from their natural environment depends on their solubility in the solvent used [29]. Thus, methanol which is a polar solvent has generated high extraction yield. Additionally, many reports have showed that methanol constitute one of the best solvents for extraction of total phenolic compounds in mushrooms [1, 30-32]. These relatively high contents of total phenolic compounds observed in samples of *V. volvacea* and *P. tuberculata* from the three administrative regions of central area of Côte d'Ivoire could constitute an interesting data for local population nutrition, since it had been reported that the antioxidant activity of plant materials was well correlated with the content of their phenolic compounds [1, 33]. Additionally, phenolic compounds are involved in stabilizing lipid peroxidation [34]. Concerning flavonoids, they are among the most interesting groups of natural

phenolic compounds. Indeed, flavonoids are known to be endowed antioxidant properties by breaking the radical chains and more stable products in the membranes of the hepatic microsomes [35], and also to have an important role in the instinctive protection against oxidative stress [36]. The amounts of flavonoids in methanolic extracts of our mushroom samples were in the range of those reported for others wild mushrooms studied elsewhere [37, 38]. Nevertheless, these flavonoid levels were well below than some values of literature [19, 39, 40]. However, Hussein *et al.* [20] reported levels of flavonoids lower (between 20.86 and 2.49 mg (QE) 100g⁻¹ DW) in wild mushrooms collected in Tanzania. But also, it is necessary to emphasize that the determination of flavonoids is highly selective with respect to their structure since the isoflavone derivatives not give color with aluminum chloride [41]. This could influence the flavonoid content. Tannins constitute one class of natural phenolic compounds which contribute in part to the antioxidant properties of plants and mushrooms [27, 34, 42]. But, when the tannins are available with content above 10% of the total dry weight, they can act as an antinutrient by affecting the nutritional potentials of the mushroom (protein digestibility and metal ions availability) [43]. Although seemingly high, tannin levels of our samples are well below 10% of total dry weight. So, these tannins could act as antioxidant compounds within our samples of mushrooms.

3.2. Identification and Quantification of Phenolic Compounds

The analysis by HPLC of the methanolic extract of our *V. volvacea* samples (Figure 1) showed the presence of five phenolic acids (protocatechuic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, ellagic acid and Cinnamic acid) and one flavonoid (catechin) in samples of Gbêkê region; four phenolic acids (gallic acid, protocatechuic acid, ellagic acid and Cinnamic acid) and one flavonoid (catechin) in samples of Bélier region; six phenolic acids (gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, ellagic acid and Cinnamic acid) and one flavonoid (catechin) in samples of N'Zi region. With regard to samples of *P. tuberculata*, the analysis of their methanolic extract by HPLC (Figure 2) allowed to identify four phenolic acids (gallic acid, protocatechuic acid, *p*-coumaric acid, and ellagic acid) and two flavonoids (catechin and naringenin) in samples of Gbêkê region, also four phenolic acids (gallic acid, protocatechuic acid, *p*-hydroxybenzoic and ellagic acid) and two flavonoids (catechin and naringenin); three phenolic acids (gallic acid, *p*-hydroxybenzoic and ellagic acid) and

two flavonoids (catechin and naringenin) in samples of N^oZi region. For both kinds of samples, many peaks corresponding to others phenolic compounds were observed on the chromatograms. But, we had not been able to identify them

and this, due to lack of corresponding standards in our laboratory. The tannins whose levels were not negligible in all samples could be among the unidentified phenolic compounds.

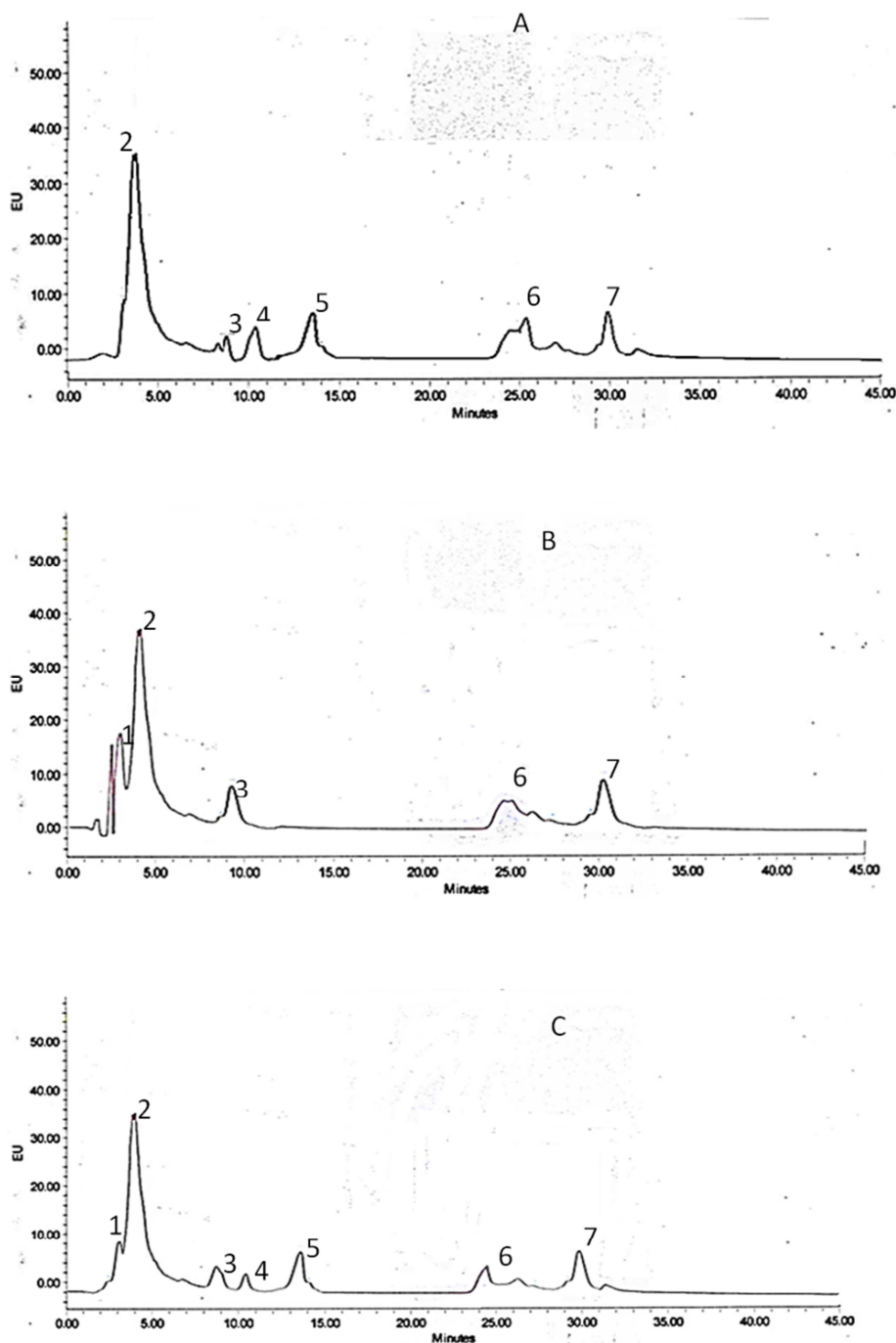


Figure 1. HPLC-profiles of phenolic compounds in *V. volvacea* from central Côte d'Ivoire.

(A: *V. volvacea* from Gbêkê, B: *V. volvacea* from Béliér, C: *V. volvacea* from N^oZi)

Detection at 280 nm: 1 (Gallic acid), 2 (Catechin), 3 (protocatechuic acid), 4 (*p*-Hydroxybenzoic acid), 5 (*p*-Coumaric acid), 6 (ellagic acid), 7 (cinamic acid), 8 (naringenin)

All these phenolic compounds are reported for the first time in both species of mushrooms (*V. volvacea* and *P. tubercula* from Côte d'Ivoire. Furthermore, to our knowledge, this is also the first time that individual phenolic

compounds are identified from these mushroom species, even if contents of total phenolic compounds and total flavonoids and antioxidant properties were widely documented amongst samples of *V. volvacea* from several countries [1, 27, 44, 46].

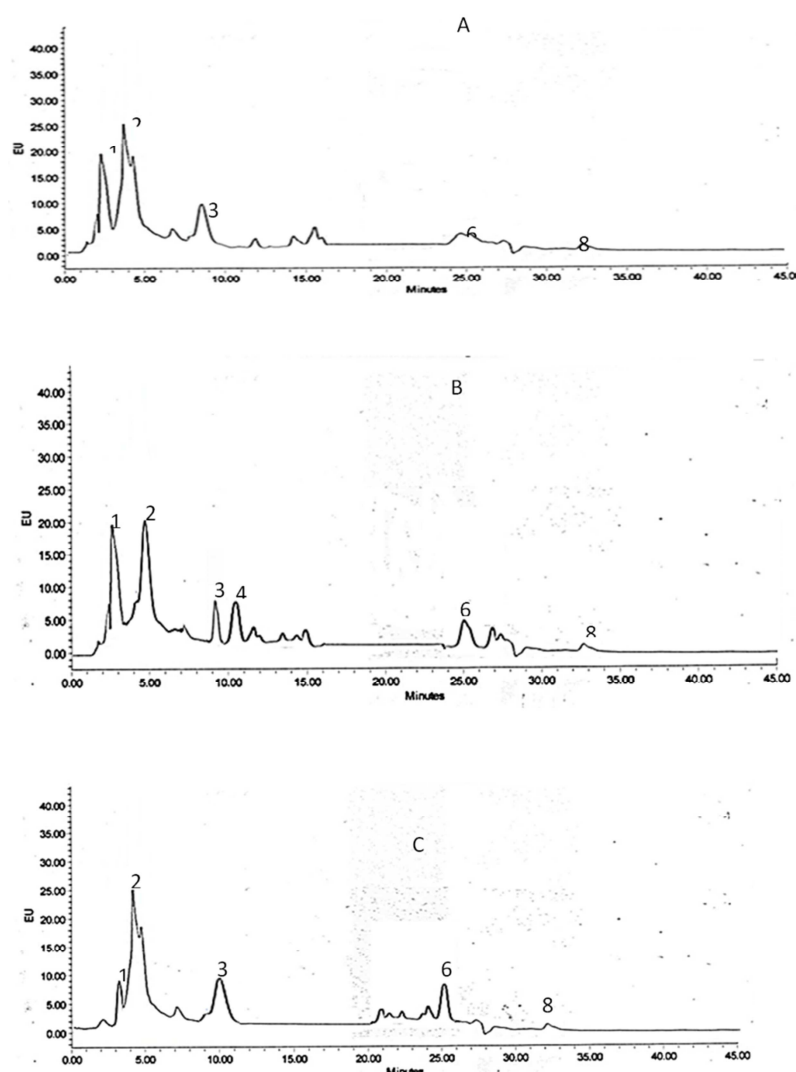


Figure 2. HPLC-profiles of phenolics compounds in *P. tuberculata* from central Côte d'Ivoire.

(A: *P. tuberculata* from Gbêkê, B: *P. tuberculata* from Béliér, C: *P. tuberculata* from N'Zi)

Detection at 280 nm: 1 (Gallic acid), 2 (Catechin), 3 (protocatechuic acid), 4 (*p*-Hydroxybenzoic acid), 6 (ellagic acid), 7 (cinamic acid), 8 (naringenin)

However, generally most of these phenolic compounds identified in our samples were detected in numerous species of wild mushrooms investigated by several authors in many countries [3, 8, 15, 47, 48]

In terms of levels of each individual phenolic compound, for samples of *V. volvacea*, as indicated in table 2, catechin showed the highest concentration in the three administrative regions with statistically identical values ($p < 0.05$) around 101-102 mg/kg DW. Kim *et al.* [49] and Palacios *et al.* [50] had also reported important concentrations of catechin in wild mushrooms from Korea and Spain, respectively. Cinnamic acid was detected with interesting concentration in samples of the three administrative regions (around 30 mg/kg DW, for the three regions). This phenolic acid was also identified in many wild or cultivated mushrooms such as *Agaricus bisporus* [10], *Lactarius bertillonii* and *L. vellereus* [51] *Boletus badius* [52]. Ellagic and protocatechuic acids were found in *V. volvacea* samples from the three regions

with significant levels ranging 20.00 ± 1.41 to 28.00 ± 10.41 and 10.50 ± 2.12 to 20.60 ± 0.14 mg/kg DW, respectively. Findings of Ribeiro *et al.* [15] had previously reported the presence of ellagic acid in mushroom *Fistulina hepatica* from Portugal. As for protocatechuic acid, Barros *et al.* [48] have already identified this phenolic acid as the main phenolic compound in *Lepista. nuda* and *Ramaria botrytis* also collected in Portugal. Gallic acid which constituted one of the major phenolic compounds in samples of *V. volvacea* from region of Béliér (65.40 ± 6.50 mg/kg DW) and N'Zi (40.60 ± 0.14 mg/kg DW), was not detected in sample of Gbêkê region. This phenolic acid was successfully investigated in many wild mushrooms such as *Lactarius deliciosus* [47], *Russula senecis* [53] and *Boletus badius* [52]. *p*-hydroxybenzoic and *p*-coumaric acids were available in *V. volvacea* samples of Gbêkê and N'Zi regions, but not observed in sample of Béliér region. Levels of *p*-hydroxybenzoic acid were 30.65 ± 0.21 and 21.50 ± 0.70 mg/kg

DW in Gbêkê and N'Zi samples, respectively, whereas regarding *p*-coumaric acid, levels were 20.50 ± 0.14 and 3.10 ± 0.57 mg/kg DW, respectively. Both phenolic

compounds were already identified in several wild mushrooms [3, 15, 54, 55].

Table 2. Phenolic compound contents (mg/kg DW) of sample *V. volvacea* from central Côte d'Ivoire.

Phenolic compound mg/Kg	Retention time (min)	<i>V. volvacea</i>		
		Gbêkê	Bélier	N'Zi
Gallic acid	2.8	nd	65.40 ± 6.50^b	40.60 ± 0.14^a
Catechin	5.00	102.10 ± 0.28^a	101.95 ± 0.21^a	102.90 ± 0.28^b
Protocatechuic acid	9.5	10.50 ± 2.12^a	20.60 ± 0.14^c	15.75 ± 0.07^b
<i>p</i> -hydroxybenzoic acid	10.50	30.65 ± 0.21^b	nd	21.50 ± 0.70^a
<i>p</i> -coumaric acid	12	20.50 ± 0.14^b	nd	3.10 ± 0.57^a
Ellagic acid	25	28.00 ± 10.41^a	26.00 ± 0.42^a	20.00 ± 1.41^b
Cinnamic acid	30	30.00 ± 0.21^a	30.55 ± 0.21^a	30.55 ± 0.07^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. nd: not detected

With regard to *P. tuberculata*, in terms of individual phenolic compound quantification (Table 3), we noted that catechin was the major compound in samples of the three regions statistically identical values ($p < 0.05$) around 101 mg/kg DW for Gbêkê and N'Zi region and 99.85 ± 0.35 mg/kg DW for Bélier region. Gallic acid was also preponderant in samples of the three regions with concentrations ranging between 30.45 ± 0.07 and 80.8 ± 0.14 mg/kg DW. It was the same for ellagic acid that displayed contents ranging from 20.65 ± 0.70 to 28.50 ± 0.70 mg/kg DW. Protocatechuic acid displayed statistically identical rates ($p < 0.05$) around 20 mg/kg DW, in samples from regions of

Gbêkê and Bélier, but it was not detected in sample of N'Zi region. As for *p*-hydroxybenzoic acid, it was present with statistically identical contents ($p < 0.05$) around 39-40 mg/kg DW in samples from Bélier and N'Zi regions whereas it was not observed in sample from Gbêkê region. *p*-coumaric acid was not identified in samples from Bélier and N'Zi regions while it had a low rate in the sample from Gbêkê region. The samples of the three regions displayed statistically identical low contents of naringenin of around 3.50 mg/kg DW. This flavonoid was previously identified in mushroom species *Lentinus lepideus* [56] and *L. elodes* [57] from Korea.

Table 3. Phenolic compounds contents (mg/kg DW) of sample *P. tuberculata* from central Côte d'Ivoire.

Phenolic compound (mg/Kg)	Retention time (min)	<i>P. tuberculata</i>		
		Gbêkê	Bélier	N'Zi
Gallic acid	2.8	70.70 ± 0.14^b	80.8 ± 0.14^c	30.45 ± 0.07^a
Catechin	5.00	101.05 ± 0.21^a	99.85 ± 0.35^b	101.4 ± 0.28^a
protocatechuic acid	9.5	30.70 ± 0.42^a	30.00 ± 1.41^a	nd
<i>p</i> -hydroxybenzoic acid	10.50	nd	39.05 ± 1.48^a	40.45 ± 0.21^a
<i>p</i> -coumaric acid	12	4.50 ± 0.70	nd	nd
Ellagic acid	25	26.50 ± 0.70^b	28.50 ± 0.70^c	20.65 ± 0.70^a
Naringennin	32	3.50 ± 0.70^a	3.50 ± 0.70^a	3.50 ± 0.70^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.

nd: not detected

From these results focusing on contents of individual phenolic compounds, we can point out that overall; there were some significant differences between the three administrative regions of central area of Côte d'Ivoire. This could be explained by two reasons. Firstly, it is well-known that phenolic compound content in mushroom is instable over time after collection due to enzymatic and oxidative decomposition [55]. Secondly, phenolic compound content depend on the different stress conditions at which mushrooms were submitted within their natural ecological environment [29, 48].

3.3. Identification and Quantification of Organic Acids

The organic acids profiles of *V. volvacea* showed that all

the samples of the three administrative regions contained, citric, malic and fumaric acids (Figure 3). The main organic acid found in samples of the three regions was citric acid. In addition to these three organic acids, the different samples contained other organic such as oxalic and ascorbic acids (sample of Gbêkê region); oxalic and shikimic acids (sample of Bélier region); shikimic acid (N'Zi region). As for *P. tuberculata*, figure 4 revealed that fumaric acid was the preponderant organic acid in samples of Gbêkê and Bélier whereas sample of N'Zi displayed citric as the major organic acid. Samples of Gbêkê and N'Zi contained all organic acids tested in this work (apart from succinic acid for Gbêkê sample). In contrast, ascorbic, shikimic and malic acids were missing in the sample of Bélier.

In terms of content of each organic acid (Tables 4 and 5), it

was noted that citric acid had the highest levels in samples of *V. volvacea* from the three regions. Indeed, content of this organic acid was 3238.20 ± 9.62 ; 3341.52 ± 2.51 and 2944.59 ± 2.32 mg/kg DW in samples of Gbêkê, Béliér and N'Zi regions, respectively. Citric acid was also quantified in samples of *P. tuberculata* from the three regions with

relatively high amounts in the range of $[875.64 \pm 7.69 - 1072.94 \pm 2.98$ mg/kg DW]. Due its antibacterial and antioxidant properties, the citric acid is known to be very important in the prevention of mushroom browning and to extend its shelf life [58, 59].

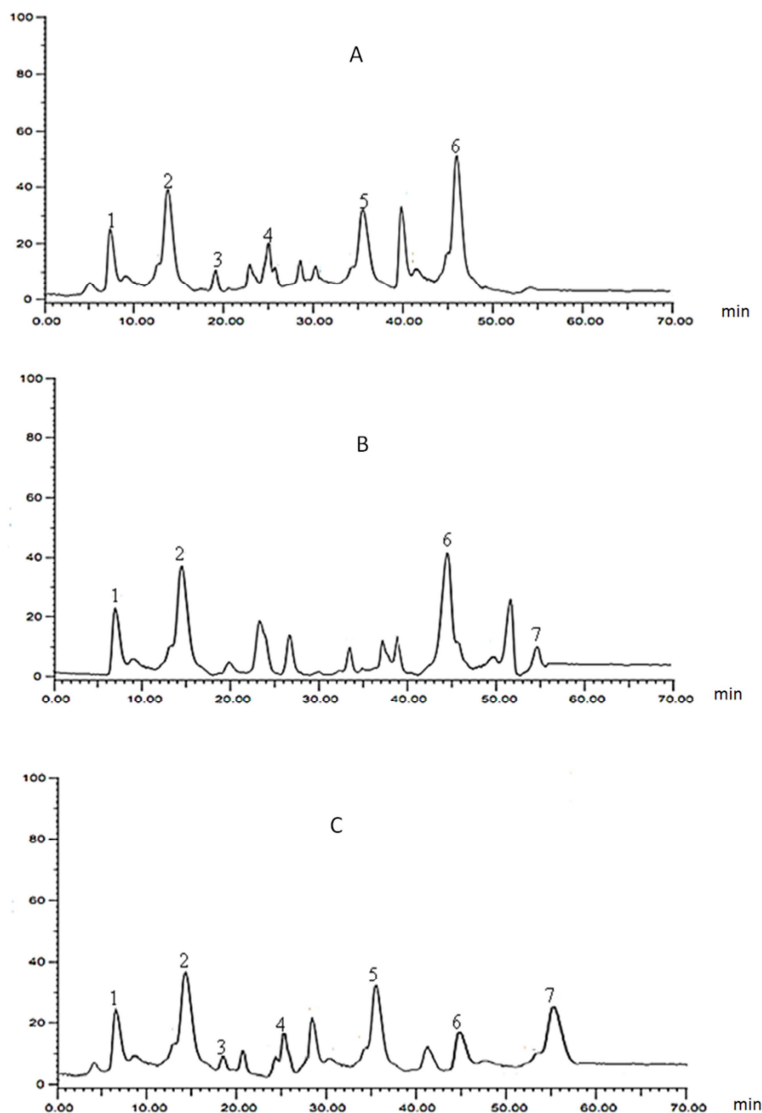


Figure 3. HPLC chromatograms of organic acids in *V. volvacea* from central Côte d'Ivoire.

(A: *V. volvacea* from Gbêkê, B: *V. volvacea* from Béliér, C: *V. volvacea* from N'zi)

1: Oxalic acid; 2: Citric acid; 3: Ascorbic acid; 4: shikimic acid; 5: Malic acid; 6: Fumaric acid

Shikimic acid had moderate concentrations in *V. volvacea* sample from Béliér (484.41 ± 8.97 mg/kg DW) and N'Zi (398.67 ± 1.93 mg/kg DW), but, it was not detected in sample from Gbêkê. Samples of *P. tuberculata* displayed also moderate concentrations of Shikimic acid in samples of Gbêkê and N'Zi (211.68 ± 3.35 and 224.59 ± 3.09 mg/kg DW, respectively). Other reports had previously indicated that Shikimic acid was identified in wild mushrooms with low contents [58, 60]. Overall, ascorbic acid was present in all samples with low contents or was missing except in in *V. volvacea* from Gbêkê (316.66 ± 2.17 mg/kg DW). Fumaric

acid was detected in important amounts in samples of *P. tuberculata* from Gbêkê (1791.36 ± 16.77 mg/kg DW) and Béliér (1897.86 ± 7.43 mg/kg DW) while its level was enough moderate in sample of *P. tuberculata* from N'Zi and in all the samples of *V. volvacea*. Fumaric acid is an important organic acid because of its antioxidant, antimicrobial and acidifying properties [58, 61]. Contents of malic acid were ranged from 278.18 ± 4.41 to 345.81 ± 5.04 mg/kg DW in all the samples, apart from in sample of *P. tuberculata* from Béliér which did not contain this organic acid. Malic acid which is well-known to be used as a food additive was also described in other wild

mushrooms [61, 62]. Overall, in all the samples investigated, oxalic and succinic acids showed small levels or they were not detected. 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 samples.

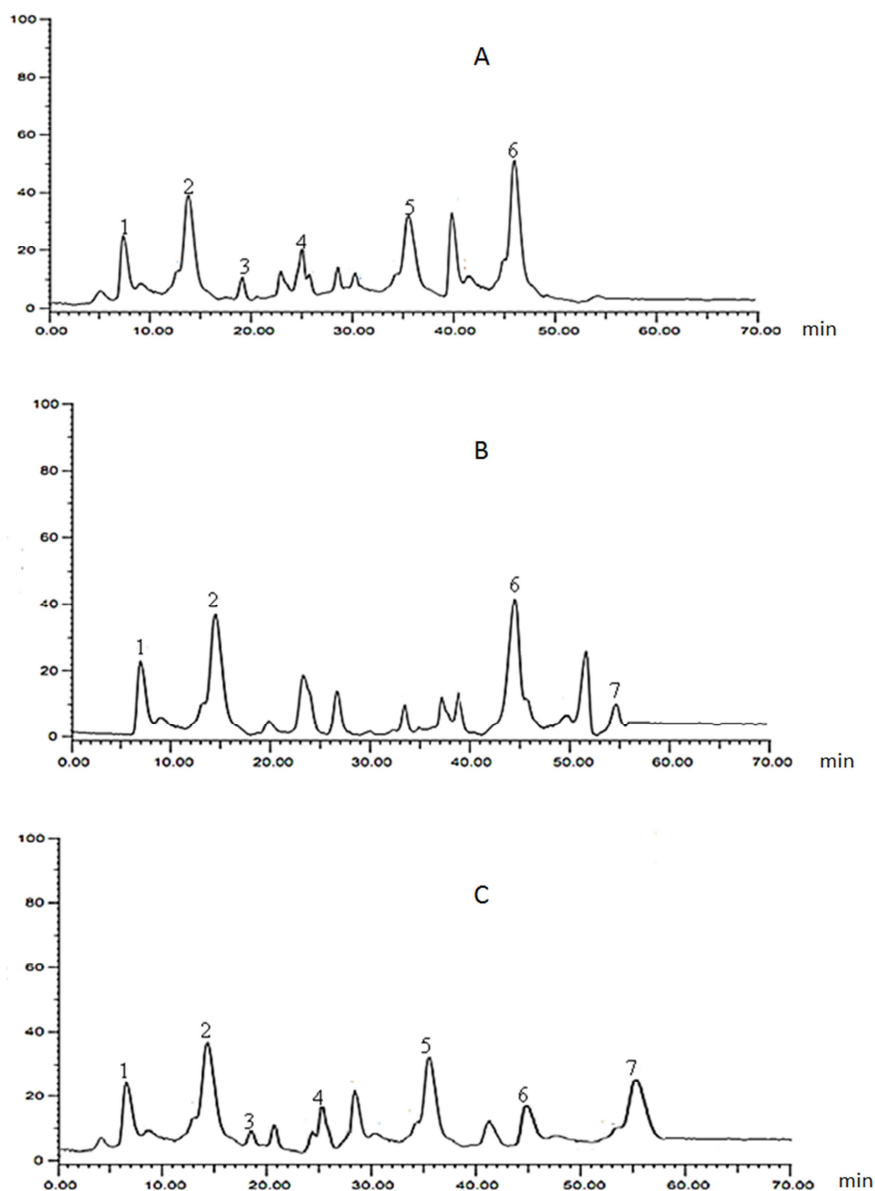


Figure 4. HPLC chromatogram of organic acids, in *P. tuberculata* from central Côte d'Ivoire

(A: *P. tuberculata* from Gbêkê, B: *P. tuberculata* from Bélier, C: *P. tuberculata* from N'zi)

1: Oxalic acid; 2: Citric acid; 3: Ascorbic acid; 4: shikimic acid; 5: Malic acid; 6: Fumaric acid; 7: Succinic acid

Table 4. Contents (mg/kg DW) of organic acids of sample *V. volvacea* from central Côte d'Ivoire.

Organic acid (mg/kg DW)	Retention times (mn)	<i>V. volvacea</i>		
		Gbêkê	Bélier	N'zi
Oxalic acid	7.5	80.22±1.16 ^b	73.17±1.45 ^a	nd
Citric acid	14	3238.20±9.62 ^b	3341.52±2.51 ^c	2944.59±2.32 ^a
Ascorbic acid	18	316.66±2.17	nd	nd
Shikimic acid	25	nd	484.41±8.97 ^b	398.67±1.93 ^a
Malic acid	35	286.77±3.78 ^a	329.12±4.49 ^b	278.18±4.41 ^a
Fumaric acid	45	61.4±1.69 ^a	132.70±8.49 ^c	103.52±5.13 ^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 $p < 0.05$ as assessed by the test of Duncan

nd: not detected

Table 5. Contents (mg/kg DW) of organic acids of sample of *P. tuberculata* from central Côte d'Ivoire.

Organic acids (mg/kg DW)	Retention times (mn)	Gbêkê	<i>P. tuberculata</i>	
			Bélier	N'zi
Oxalic acid	7.5	109.78±3.84 ^a	120.86±2.02 ^b	112.1±3.28 ^a
Citric acid	14	997.9±5.79 ^b	1072.94±2.98 ^c	875.64±7.69 ^a
Ascorbic acid	18	52.61±3.51 ^b	nd	41.52±1.24 ^a
Shikimic acid	25	211.68±3.35 ^a	nd	224.59±3.09 ^b
Malic acid	35	321.87±3.25 ^a	nd	345.81±5.04 ^b
Fumaric acid	45	1791.36±16.77 ^b	1897.86±7.43 ^c	100.99±3.01 ^a
Succinic acid	55	nd	68.92±1.72 ^a	96.855±4.74 ^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 $p < 0.05$ as assessed by the test of Duncan

nd: not detected

From these results, it appears that the geographical origin could influence the organic acid profile of mushrooms analyzed as described for dried wild mushrooms from Portugal [54], since some significant differences were observed in the samples of the same mushrooms from one region to another. However, our findings were contrary to those previously reported by Valentão *et al.* [60] about wild mushrooms also from Portugal. According to these authors, this factor may not interfere with the organic acids composition.

3.4. DPPH Radical Scavenging Abilities

The antioxidant capacities of methanolic extracts of different mushroom samples were evaluated in terms of DPPH radical scavenging ability. Results were expressed as the ratio percentage of sample absorbance decrease and the absorbance of DPPH solution in the absence of extract at 517 nm. Overall in this study, methanolic extracts of mushroom samples had DPPH scavenging activity values ranging beyond 50% (Figure 5). Therefore, we could conclude that methanolic extracts of our samples of *V. volvacea* and *P. tuberculata* displayed a significant effect on scavenging of free radicals. Thus, methanolic extracts of mushroom samples of the three regions were free radical scavengers, acting possibly as primary antioxidants [63]. It was observed that for each region, sample of *P. tuberculata* exhibited the better DPPH scavenging activity compared with that of *V. volvacea*. For example, in Gbêkê region, sample of *P. tuberculata* displayed a value of 64.24% while the value for *V. volvacea* was 50.88%.

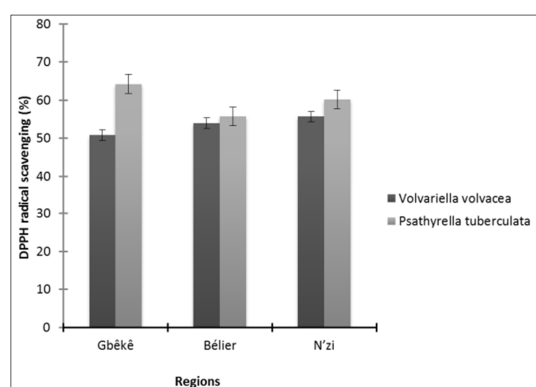


Figure 5. DPPH radical scavenging (%) of extracts of samples of *V. volvacea* and *P. tubercula* from central Côte d'Ivoire.

Methanolic extracts of several wild edible mushroom species were also successfully tested for their DPPH scavenging activity [8, 17, 19, 20, 64].

Interestingly, this is the first time that DPPH scavenging abilities were reported in these species of wild mushrooms from Côte d'Ivoire and our findings could be of the added value since both species of wild mushrooms are highly consumed in this area of Côte d'Ivoire.

4. Conclusion

This is the first time that samples of *V. volvacea* and *P. tuberculata* from the three regions of central area of Côte d'Ivoire were submitted to studies of phenolic compounds and organic acids determination and assessment of antioxidant abilities. According to the obtained results of this study, it is clearly indicated that the methanolic extracts of samples of these mushroom species had significant contents of total phenolic compounds, total flavonoids and tannins. This constitutes interesting data since phenolic compounds are included in the antioxidant compounds of mushrooms. In addition, HPLC analysis of phenolic compounds and organic acids in the different samples of mushroom revealed the presence of components such as phenolic acids, flavonoids, citric, fumaric and malic acids which are well known to possess antioxidant properties as well as positive role in the organoleptic properties (organic acids). Overall, some significant differences assigned generally to the influence of natural ecological environment, were observed within samples of *V. volvacea* and *P. tuberculata* from the three regions of central Côte d'Ivoire. In the other hand, the methanolic extracts of samples of these highly consumed mushrooms of central Côte d'Ivoire exhibited significant antioxidant properties demonstrated by their significant capacity to scavenge DPPH free radical. Therefore, *V. volvacea* and *P. tuberculata* could be regarded as a potential of easily accessible sources of natural antioxidants and other bioactive compounds for local population.

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