Turkish Journal of Agriculture - Food Science and Technology, 9(10): 1931-1937, 2021
DOI: https://doi.org/10.24925/turjaf.v9i10.1931-1937.4454

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

Phenolic compound and antioxidant activity of two slightly consumed wild mushrooms (*Lentinus squarrosulus* and *Auricularia politrich*) in three regions from center Ivory Coast

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ARTICLE INFO

Research Article

Received : 17/05/2021
Accepted : 13/06/2021

Keywords:
Antioxidant activity
Saprophitic mushrooms
Phenolic compounds
Côte d'Ivoire
Auricularia politrich

ABSTRACT

Mushrooms contain a variety of secondary metabolites, including various phenolic compounds, which have been shown to act as excellent antioxidants. In this study, the contents of total phenolic, flavonoids and tannins of mushrooms methanolic extracts were evaluated by colorimetric assays to ranges of 277.36±0.66 to 420.86±0.90 mg (GAE)/100g DW; 31.99±0.90 to 90.90±0.07mg (QE)/100g DW and 150.61±0.16 to 220.47±1.01mg (TAE)/100g DW respectively. HPLC-profiles of methanolic extracts indicated that the individual phenolic compounds found to the samples of the species of mushrooms that are *Lentinus squarrosulus* and *Auricularia politrich* revealed us that gallic acid, catechin, acid ρ-hydroxybenzoïc, acid ellagique and naringerin were presented to the level of the two species. With respect to organic acids, the results showed that citric acid and fumaric acid was the major organic acid in all the samples of both mushrooms species. The methanolic extracts of the two mushrooms exhibited the high DPPH radical scavenging activities ranging from 34.10±1.12 to 58.95±0.52%. These data indicated that these mushrooms could constitute a potential good source of natural antioxidant for the local population.

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Introduction

Fungi, constituting the most diverse and abundant group on the planet, are an excellent candidate for the production of bioactive metabolites due to their resemblance to the animal system (Vieira Gomes et al., 2019). Like plants, certain fungi are appearing favorably in the emerging industry, which represents non-wood forest products (Wills and Lipsey, 1999; Vieira Gomes et al., 2019). Once considered a food of low nutritional value, mushrooms are of particular interest today. Indeed, research on wild edible fungi of nutritional and medicinal interest has developed significantly in recent years and is now oriented towards the discovery of new sources beneficial to human health and having therapeutic effects on certain infectious diseases (Erbiai et al., 2021). Now, more than 80% of people consider them a "healthy" food and do not hesitate to include them in a diet aimed at good cardiovascular health (Stanton, 2006; Yuwa-Amornpitak et al., 2020). In recent decades, interest in fungi has jumped considerably due to their richness in phenolic compounds, tocopherols and carotenoids, considered to be the most responsible for antioxidant activity. In addition to antioxidant capacity, this diversity of biomolecular compounds in wild edible fungi is also responsible for other biological activities; namely, antibacterial, antifungal, anti-inflammatory, antitumor and antiviral properties (Aprotosoaie et al., 2017; Liaotrakoon and

Liaotrakoon, 2018). These compounds can be involved in the protection against various diseases, due to their antioxidant potential (Silva et al., 2004). Polyphenols are very good anti mutagens and anti carcinogens with respect to one of their properties which is to be a very good powerful antioxidant (Middleton et al., 2000; Yuwa Amornpitak et al., 2020). Thus, anti-cancer drugs, cholesterol-lowering drugs, immunostimulants and antioxidants give fungi several therapeutic properties (Mizuno et al., 1995; Ferreira et al., 2007). In Côte d'Ivoire, several species of fungi have been identified (Avit et al., 1999). Among these are species like Lentinus squarrosulus and Auricularia politrich which are edible wild saprophytic fungi, which grow on decaying organic matter and perform different ecological functions. In this study, we were therefore interested in determining the phytochemical parameters and in evaluating the antioxidant activity of two edible saprophitic fungi in three regions of central Côte d'Ivoire identified as Lentinus squarrosulus and Auricularia politrich. In addition, we will study their individual organic acid.

Materials and Methods

Sample Collection

The harvest of the mushrooms has been made to the hand and only the aerial part has been harvested. In every locality (Gbeke, Belier, and N'Zi regions), a quantity (800 g; 2 times) of cool mushrooms has been harvested then conditioned in aired baskets. After picking, the samples of mushroom were immediately transferred to the laboratory for ulterior analysis. Taxonomic identification was achieved by Dr Souleymane Yorou Nourou (Abomé Calavy University of Benin/ Munich University of Germany), as *Lentinus squarrosulus* and *Auricularia politrich*.

Extraction of Phenolic compounds

The mushrooms have been dried to 25 °C for ten days, until constant weight, as described previously with slight modifications (Ribeiro et al., 2007). Then, every sample of the mushroom has been ground in a fine-dried (cast IKA, Germany / Deutschland) powder. A sample (10 g) of every powder of the mushroom fine-dried has been extracted while moving with 50 mL of methanol 80% (v/v) to 25°C for 24 hours. 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.

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 no 4 paper, then through 0.45 µm filter (Millipore; Sartorius AG, Goettingen, Germany) until 25 ml, prior to phenolic compound contents determination and HPLC analysis.

Determination of Total Phenolic Compounds Content

Contents of total phenolic compounds were estimated according Folin-Ciocalteu method (Singleton et al., 1999), 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. All experiments were performed in triplicate.

Determination of flavonoid content

Total flavonoids content was determined as described previously with slight modifications (Meda et al., 2005). 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% (w/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 Tannin Content

The content of the tannins has been determined to use the method describes by Bainbridge et al. (1996). 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)/100g DW. All experiments were performed in triplicate.

HPLC analysis of Phenolic Compounds

The phenolic extracts prepared (50 mL) previously has been diluted in 100 mL of distilled water and 20 µL of every sample has been analyzed to use an unit analytic HPLC equipped with a binary pump (LC-6A) (HPLC (Corporation Shimadzu, Japan) associated to a detector UV-LIVE (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.

Evaluation of Activity of the Antioxidant by DPPH Radical Scavenging

The antioxydant activity of every mushroom sample has been estimated by the method of trapping of the radical 1,1-diphenyl-2-picrylhydrazyle (DPPH.) according to the method described by Bidié et al. (2011). Thus, in every tube to test, 2,5 mL of every excerpt methanolic has been added to 1 mL of solution of DPPH. (1,1-diphenyl-2-picrylhydrazyle, 3 mM in the methanol). The tubes have been left to rest during 30 min to the obscurity and the reading of the absorbance has been done to the spectrophotometer to 517 nm against a white. Absorbance was converted to the DPPH radical-scavenging rate according to the equation:

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

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 (statistica version 7.1) statistical software. Significance of differences was defined at the 5% level ($P<0.05$).

Results and Discussion

Contents of Phenolic Compounds, Flavonoids and Tannins

Table 1 show the content of total phenolic, flavonoids and tannins of methanolic extracts of *Lentinus. squarrosulus* and *Auricularia. politrich* collected in the three administrative regions of center from Côte d' Ivoire. Overall, for total phenolic, flavonoids and tannins contents, there were significant ($P<0.05$) differences between the two species in each region. The specie *Auricularia polirtich* possesses the most elevated contents of total phenolic (419.54±1.73 to 420.86±0.90 mg (GAE)/100g DW), total flavonoids (88.31±1.73 to 91.34±1.01 mg (QE)/100g DW) and total tannins (180.16±1.06 to 220.47±1.01 mg (TAE)/100g DW) whatever is the region. But, the specie *Lentinus squarrosulus* possesses the weakest contents in these compounds. These relatively high contents of phenolic compound obtained in this work could be explained in part by the nature of the extraction solvent used. Also, depend the biotic conditions (species, body and the physiological stage) and abiotic (temperature, climat) (Ksouri et al., 2008). The contents in compounds total phenolic, total flavonoid and total tannin of the mushrooms of the Center from Côte d'Ivoire are comparable to data of the literature on edible wild mushrooms (Wong et al., 2013; Tibuhwa and Mwanga, 2014; Hussein et al., 2015; Tripathy et al., 2014). In addition, in the present study, the total phenolic and flavonoid contents were lower than recently reported values (Siangu et al. 2019).

HPLC-profiles of Phenolic Compounds

The analysis by HPLC of the methanolic extract of L*entinus squarrosulus* samples and *Auricularia politrich* samples (Figure 1 and 2) showed the presence of four phenolic acids (gallic acid, protocatechiuc acid, ellagic acid, *ρ*-Hydroxybenzoic acid and ρ-coumaric acid), and two flavonoid (catechin and naringininn). Our results corroborate with those of Valentão et al. (2005); Ribeiro et al. (2007); Barros et al. (2009); Kouassi et al. (2016a; b) that mentioned the presence of the phenolic acid as the gallic acid, protocatechiuc acid, p-hydroxybenzoïc acid, cinnamic acid and of the flavonoïd as the catechin, quercetin in the excerpts of mushrooms. According to Ferreira et al. (2009), the phenolic acid constitutes the major part of the compound phenolic of the mushrooms.

One notes globally that the gallic acid and the ρ-hydroxybenzoïc acid are the most major with contents understood between 100.85±0.07 and 105.70±6.50 mg/kg (DW) for the gallic acid and between 17.56±1.41 and 50.20±0.14mg/kg (DW) for the ρ-hydroxybenzoïc acid. Otherwise, the ellagic acid is present in the mushrooms with weak contents around of 14.00±1.41 mg/kg (DW) (Table 2). The content in the ρ-hydroxybenzoïc acid is superior to those returned by Muszyńska et al. (2013) on six species of mushrooms of whose values are consisted between 1.28 ± 0.20 and 3.60 ± 0.05 mg / kg (DW). The presence of the gallic acid, protocatechiuc acid, ρ-hydroxybenzoïc acid, and naringenin were also observed by many researchers (Bożena et al., 2013; Barros et al., 2009; Puttaraju et al., 2006).

DPPH Radical Scavenging Abilities

Overall in this study. methanolic extracts of mushroom samples had DPPH scavenging activity values around 50% (Figure 3). Several species of edible wild mushrooms have also been tested with success for their activity of inhibition of the DPPH• (Mau et al., 2002; Ferreira et al., 2007; Obodai et al., 2014; Tibuhwa and Mwanga, 2014; Hussein et al., 2015).

Table 1. Total phenolic, flavonoids and tannins of two mushrooms from center of Côte d'Ivoire: *Lentinus squarrosulus* and *Auricularia politrich*

Especies	Regions	Total phenolic (mg (GAE)/100g)	Total flavonoid (mg (QE)/100)	Total tannin (mg (TAE)/100g)
Lentinus squarrosulus	Gbêkê	277.36±0.66^{a}	31.99±0.90^{a}	158.16±0.12^{b}
	Bélier	305.53±1.50^{b}	42.73±0.64^{b}	150.61±0.16^{a}
	N'zi	305.53±1.50^{b}	42.73±0.64^{b}	163.09±0.08^{c}
Auricularia politrich	Gbêkê	419.54±1,73^{a}	91.34±1,01^{b}	219.21±0.90^{b}
	Bélier	420.86±0,90^{a}	90.90±0,07^{b}	220.47±1.01^{b}
	N'zi	419.90±0,94^{a}	88.31±1,73^{a}	180.16±1.06^{a}

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

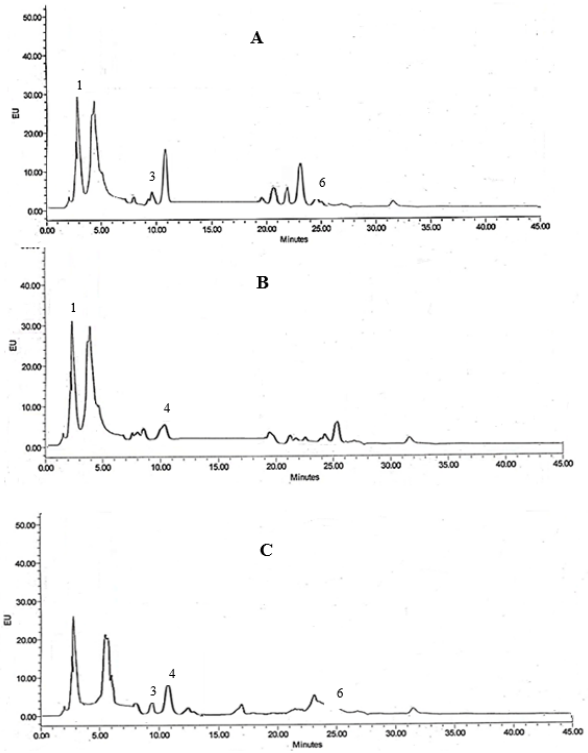

Figure 1. HPLC-profiles of phenolic compounds in *Lentinus squarrosulus* of three regions from central côte d'Ivoire

A: *L. squarrosulus* Gbêkê, B: *L. squarrosulus* Bélier, C: *L. squarrosulus* N'zi) Detection at 280 nm: 1 (gallic acid: TR= 2,8mn), 3 (protocatechiuc acid: TR= 9,50mn), 4(*ρ*-Hydroxybenzoic acid: TR= 10,50mn), 6(ellagic acid: TR= 25mn)

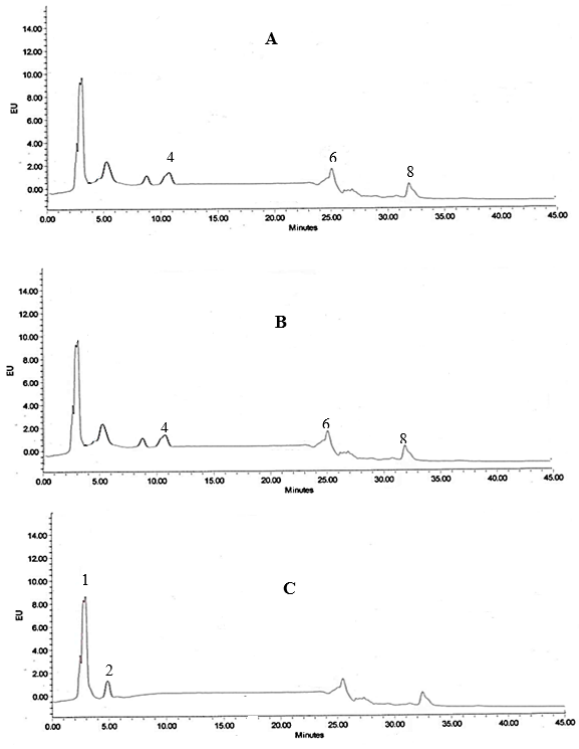

Figure 2. HPLC-profiles of phenolic compounds in *Auricularia polirich* of three regions from central côte d'Ivoire

A: *A. polytrich* Gbêkê B: *A. polytrich* Bélier, C: *A. polytrich* N'zi Detection at 280 nm: 1(gallic acid : TR= 2,80mn), 2(Catechin: TR= 5min), 4 (*ρ*-Hydroxybenzoic acid: TR= 10,50mn), 6(ellagic acid: TR= 25mn), 8(naringenin: TR= 32mn)

Table 2. Phenolic compounds contents (mg/kg DW) of samples *L. squarrosulus* and *A. politrich* of three regions from centre Côte d'Ivoire

Phenolic compounds (mg/Kg)	Regions	Retention Times (TR) (min)	L. squarrosulus	A. politrich
Gallic acid	Gbêkê	2.8	100.85±0.07^{a}	nd
	Bélier		105.4±6.64^{b}	nd
	N'zi		nd	105.70±6.50a
Catechin	Gbêkê	5	nd	nd
	Bélier		nd	nd
	N'zi		nd	28.50±2.12^{a}
protocatechiuc acid	Gbêkê	9.5	7.00±1.41^{b}	nd
	Bélier		nd	nd
	N'zi		5.50±0.70^{a}	nd
ρ-hydroxybenzoïc acid	Gbêkê	10.5	50.20±0.14^{c}	17.56±1.41^{a}
	Bélier		25.50±2.12^{a}	20.20±0.14^{b}
	N'zi		37.50±0.70^{b}	nd
ρ-coumaric acid	Gbêkê	13.5	nd	nd
	Bélier		nd	nd
	N'zi		nd	nd
Ellagic acid	Gbêkê	25	nd	27.34±0.14^{a}
	Bélier		nd	27.00±1.41^{a}
	N'zi		14.00±1.41^{a}	nd
Naringenin	Gbêkê	32	nd	18.43±0.56^{a}
	Bélier		nd	18.50±0.70^{a}
	N'zi		nd	nd

Means not sharing a similar letter in a line are significantly different P<0.05as assessed by the test of Duncan. nd: Not Detected, nd: no detected

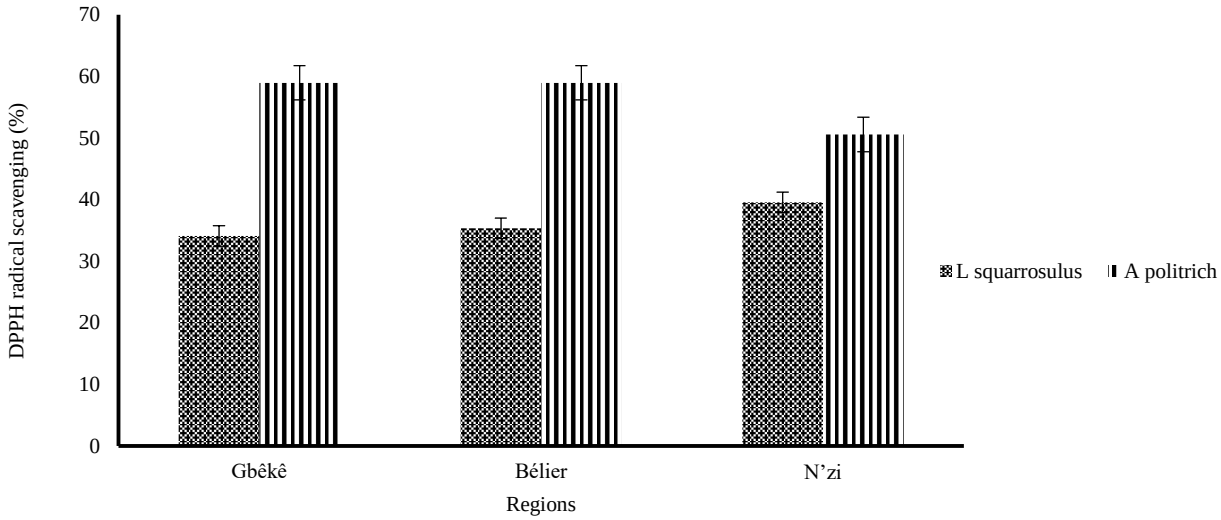

Figure 3: DPPH radical scavenging (%) of extracts of samples of V.volvacea and P. tubercula from central Cote d'Ivoire

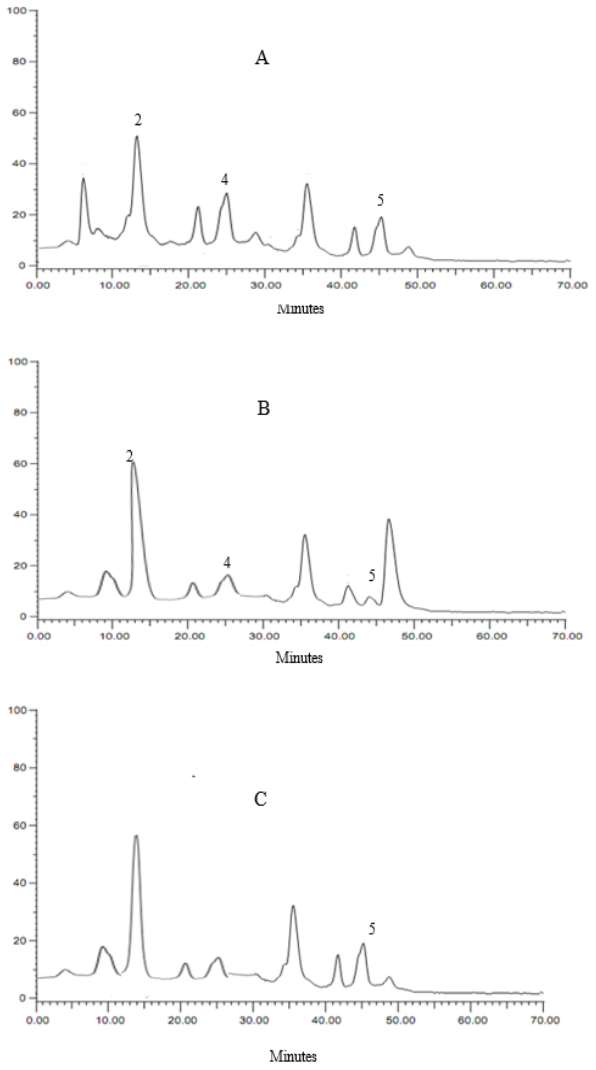

Figure 4. HPLC chromatograms of organic acids in *Lentinus squarrosulus* from center Côte d'Ivoire
A: *L. squarrosulus* Gbêkê. B: *L. squarrosulus* Bélier. C: *L. squarrosulus* N'zi
2: citric acid TR= 14; 4: shikimic acid TR= 25; 5: fumaric acid TR= 45

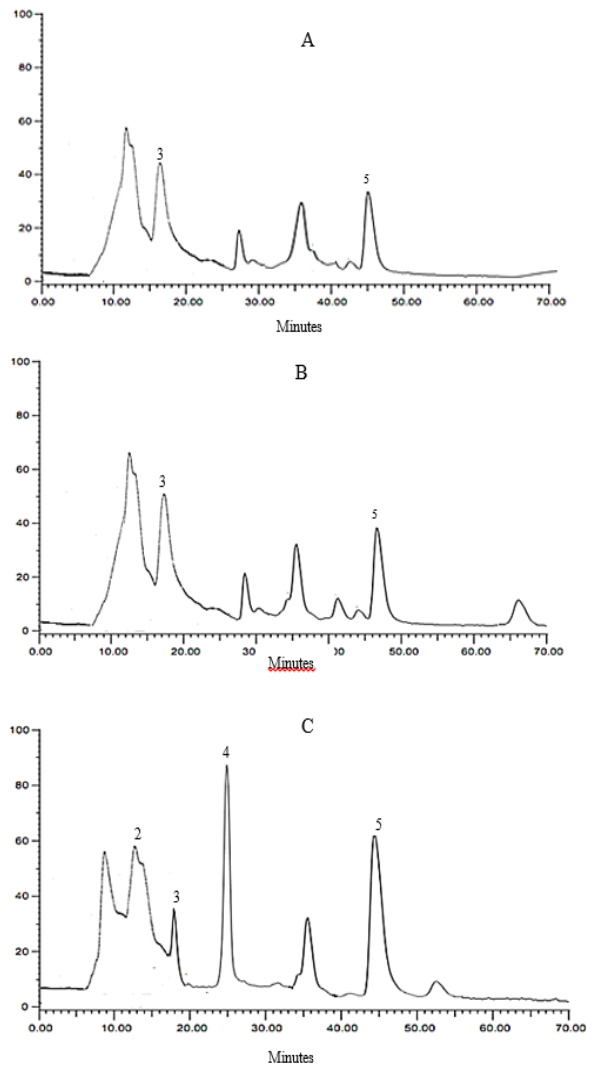

Figure 5. HPLC chromatograms of organic acids in Auricularia politrich from center Côte d'Ivoire
2: citric acid TR= 14; 3: ascorbic acid TR= 18;
4: shikimic acid TR= 25; 5: fumaric acid TR= 45

Identification and Quantification of Organic Acids

The organic acid profiles of *Lentinus squarrosulus* and *Auricularia politrich* showed that all the samples of the three administrative regions from center Côte d'Ivoire contained citric acid, shikimic acid, fumaric acid, ascorbic acid (Figure 4 and 5). The main organic acid found in samples of the three regions was fumaric acid. But citric acid was found in samples of *Auricularia politrich* in the three administrative regions (Table 3).

The profil in organic acid of the samples of *Lentinus squarrosulus* and *Auricularia politrich* showed that the citric acid and the fumaric acid are the majority compounds in these species. The contents in citric acid ranging from 1952.55±6.00 to 2508.56±2.63 and the one of the fumaric acid of 63.98±4.20 to 2714.61±13.98 whatever is the region (Table 3). Of these results one can deduct that the geographical origin have influenced the profile of organic acids of the mushrooms analysis as describes for the wild mushrooms dried of Portugal (Ribeiro et al., 2006).

The Bélier region record the strongest content in citric acid at the specie *Lentinus squarrosulus*. According to some authors, the citric acid is known to be very important in the prevention of mushroom browning and to extend its shelf life Due its antibacterial and antioxidant properties (Ribeiro et al., 2008; Kouassi et al., 2016). *Auricularia politrich* possesses the most elevated content fumaric acid in the region of N'zi. Fumaric acid is an important organic acid because of its antioxidant, antimicrobial and acidifying properties (Barros et al., 2013 ; Ribeiro et al., 2008).

Table 3. Contents (mg/kg DW) of organic acids of sample *Lentinus squarrosulus* and *Auricularia politrich* from center Côte d'Ivoire

Organic acid (mg/Kg)	Regions	Retentions times (TR) (min)	L. squarrosulus	A. polytrich
Citric acid	Gbêkê	14	2460.71±9.31^{a}	nd
	Bélier		2508.56±2.63^{b}	nd
	N'zi		nd	1952.55±6.00^{a}
Ascorbic acid	Gbêkê	18	nd	nd
	Bélier		nd	396.49±7.71^{b}
	N'zi		nd	183.37±4.70^{a}
Shikimic acid	Gbêkê	25	285.73±4.70^{b}	nd
	Bélier		125.56±5.35^{a}	nd
	N'zi		nd	463.59±5.13^{a}
Fumaric acid	Gbêkê	45	227.43±4.20^{a}	63.98±4.20^{a}
	Bélier		nd	nd
	N'zi		262.52±7.09^{b}	2714.61±13.98^{b}

Means not sharing a similar letter in a line are significantly different P<0.05as assessed by the test of Duncan. nd: Not Detected

Conclusion

According to the results of this study. mushroom species contains the phenolic compound, flavonoid, tanin and the antioxidant activities of samples of mushroom from center of Côte d'Ivoire. Total phenolic compound could make a significant contribution to the antioxidant activity in these sample. The mushroom species can be used as an easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry. Consumption of dishes prepared from edible mushrooms is safe and beneficial due to good assimilability of their nutrients that are protective against civilization diseases and have vitalizing potential for human organism.

Acknowledgement

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 advice and his contribution on taxonomic identification of the mushroom samples.

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