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Mycotoxins Content of Smoked Fishes Sampled on Market Stalls of Ngaoundere and Antifungal Activity of Essential Oils of *Cymbopogon citratus* and *Ocimum basilicum* against Mycotoxin Producing Strains

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Authors' contributions

This work was carried out in collaboration among all authors. All members contributed in designing the study. Authors NDN, EST, ATS wrote the protocol and managed the analyses of the study. Author ATS performed the statistical analysis together with authors NDN, FKN, SFN, MTNB who wrote the first draft of the manuscript. Author LNT supervised the work. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study was to evaluate the sanitary quality of smoked fish by determining their mycotoxin content, and also the chemical composition, antifungal activity of *Cymbopogon citratus* and *Ocimum basilicum* essential oils from Cameroon against some mycotoxigenic fungal strains

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responsible of the smoked fish biodegradation.
Place and Duration of Study: Laboratory of Food Microbiology and Biotechnology, National School of Agro-Industrial Sciences, University of Ngaoundere, Cameroon, from August 2019 to April 2020.
Methodology: Fifteen samples of smoked fishes have been collected in August 2019 from "petit marché" market (Ngaoundere, Cameroon). Physico-chemical parameters of smoked fishes were evaluated. Mycotoxin (AFB1, CIT, and OTA) contents have been determined with HPLC. Isolation and identification of molds were done using their macroscopic and microscopic characteristics, and the identity of the strains was done by PCR sequencing methods. For plants, 15 kg of *Cymbopogon citratus* leaves and 5 kg of *Ocimum basilicum* leaves have been harvested on 10 September 2019 in Ngaoundere and used for the essential oil's extraction. Essential oil extraction has been done through hydrodistillation and the determination of its chemical composition done with GC/MS.
Results: Major part of samples contains at least one mycotoxin, in quantities which are beyond the safe dose. Eleven species of molds have been identified: *Aspergillus spp*., *Penicillium citrinum*, and *Mucor hiemalis*. Amongst the isolated species, those which can produce mycotoxins are: *Penicillium citrinum*, *Aspergillus versicolor*, *Aspergillus fumigatus*, *Aspergillus carbonarius*, *Aspergillus niger,* and *Fusarium moniliforme*. Geranial (42.4%), Neral (33.5%) and Myrcene (10.8%) are major compounds found in the essential oil of *C. citratus* while monoterpens (60.8%), Eugenol (30.7%), Linalol (29.4%) and 1,8-Cineol (14.3%) are the major compounds found in the essential oil of *O. basilicum*.
Conclusion: Essential oils of *C. citratus* and *O. basilicum* are efficient against isolated toxigenic species. *C. citratus* being more efficient than *O. basilicum*.

Keywords: Toxigenic molds; mycotoxins; essential oils; antifungal activity.

1. INTRODUCTION

There is an increasing consumption of fishing products, mainly due to food crisis which has been observed with other animal products (avian flu, Bovine Spongiform Encephalopathy, etc.). According to FAO [1], aquaculture and fishing have supplied 167.2 millions of tons in 2014, 93.4 millions of tons of this production being obtained from fishing and 73.8 millions of tons obtained from aquaculture. Africa fishing production represents 6% of the world production and is mainly made of fishes. The latter are mainly liked and consumed on smoked form in Africa [2]. Smoking allows flavoring the fishes while increasing its shelf life, and is an important source of incomes in African countries [3]. However, the consumption of smoked fishes can lead to health issues since they sometimes contain pathogenic microorganisms as well as chemical contaminants [4]. Moreover, since they are stored for many days while trading, molds, which are known to produce mycotoxins, are generally observed at their surface after few days [5]. Amongst these molds found on smoked fishes, *Aspergillus spp* are the most dominant, followed by *Penicillium* [6]. The presence of these molds on smoked fishes is an important source of financial losses [7]. Since mycotoxins of different mold species don't have the same toxicity, it is therefore important to assess those found on smoked fishes of different market stalls. It has been shown that some essential oils are toxic for some specific molds [8]. Essential oils of some plants (*O. gratissimum, S. aromaticum,* and *C. citratus*) have been shown to be efficient on some mold species (*B. oryzea, A. padwickii,* and *E. coli*) [9,10]. It is in the same framework that the present work has been carried out to identify some toxigenic molds found in smoked fishes and to determine the anti-fungus activity of essential oils of *Cymbopogon citratus* and *Ocimum basilicum.*

2. MATERIALS AND METHODS

2.1 Sampling and Extraction of Essential Oils

2.1.1 Sampling

Smoked fishes used in the present study have been sampled on the 15th of August 2019 on market stalls of "Petit marché" market, Ngaoundere (town), Cameroon. Fifteen samples have been randomly collected from smoked fish traders; three samples of the same fish species being sampled from the same trader. These smoked fishes have been aseptically sampled in sterile and waterproof containers and once done, they have been brought to the laboratory and stored at 4°C for further analyses.

For plants, 15 kg of *C. citratus* leaves and 5 kg of *O. basilicum* leaves have been harvested on 10

September 2019 in Ngaoundere and used for the extraction of essential oils.

2.1.2 Extraction of essential oils

The extraction of essential oils has been done with a Clevenger through hydrodistillation. In this respect, a 15-liter reactor has been used. The leaves/water ratio was 1/3. The mixture was boiled in the reactor up to the completion of the extraction (5 hours). At the end of each distillation cycle, essential oil was separated from water by decantation after addition of a pinch of sodium chloride, and the extraction yield determined. Once obtained, essential oil was weighed, stored in a hermetically closed brown glass container.

2.2 Physico-Chemical Analysis of Smoked Fishes

On the smoked fishes, the determination of the temperature as well as of the humidity has been done with a thermo-hygrometer (THERMO-HYGRO). Water content has been done using AOAC method. Water activity has been determined using an electronic hygrometer.

2.3 Microbiological Analysis of Smoked Fishes

This has been done using standard analysis reported by Joffin and Joffin [11].

2.3.1 Stock solution preparation

Twenty-five g of each species of smoked fish has been sampled, aseptically crushed and added in an Erlenmeyer. Two hundred and twenty-five milliliters of buffered peptone water has been added into the container and the mixture homogenized on stomacher. From this prepared suspension, decimal dilutions have been done.

2.3.2 Mold numeration

0.1 mL aliquot of the main suspension as well as of its decimal dilutions have been taken and inoculated in Petri dishes (9 cm of diameter) containing Sabouraud agar + chloramphenicol. and Dichloran 18% Glycerol agar (DG18) medium. Since it is done at the surface, this method allows a uniform development of molds because they have the same access to oxygen [12]. Enumeration of mold colonies has been done after 5 and 7 days of incubation at 25 °C. An estimation of the population (N) has been computed according to ISO norm (NF ISO 7218/A1) [13].

2.4 Morphological Identification

Obtained mold cultures have been examined with naked eyes and a magnifying glass. Colony color (on both sides) and their variation with time, medium color and its variation with time, surface texture and flagrance were studied [14]. Microscopic observations have been made on freshly prepared suspensions at objectives x10 and x40. The identification of isolated molds has been done according to morphological criteria using following characters: morphology and structure of spores and conidia, mycelium structure, characteristics and type of conidiogenesis [14].

2.5 DNA Extraction of Molds, Amplification by PCR and Sequencing

Mold spores have been aseptically taken from Petri dishes using sterile distilled water containing 0.1% of Tween 80. It is on these spores that the extraction of DNA has been done according to protocol described by El Sheikha and Nganou [15]. DNA was quantified with spectrophotometer (NanoDrop ND-1000, NanoDrop Technologies, Wilmington, USA) and kept at 20 °C until their use as a template for PCR amplification. The amplification and identification of fungi species have been done in unique PCR step using eukaryotic universal primers (forward, U1f GC [5' - CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GTG AAA TTG TTG AAA GGG AA – 3'] and backward, U2r [5' - GAC TCC TTG GTC CGT GTT – 3']).

In the present study, after the addition a 30 bp GC clamp (Sigma) on the forward universal eukaryotic primer procured from Sigma, PCR was carried out in a final volume of 50 µL containing 1.5 mM of $MgCl_2$, 5 µL of $MgCl_2$-free 10xTaq reaction buffer (Promega), all the deoxyribonucleotide triphosphate (dNTPs) at 200 µM, 0.2 µM of each primer, 2 µL of extracted DNA (≈ 30 ng) and 1.25 Units of Taq DNA polymerase (Promega). The amplification conditions were: an initial denaturation of 3 min at 94ºC, 30 cycles of heating at 94 ºC during 45 sec, cooling at 50 ºC during 50 sec, heating at 72ºC during 90 sec and a final extension of 5 min at 72°C.

Once obtained, PCR products were verified by electrophoresis by adding 5 µL of the final

amplified solution on 2% TAE agarose gels with a 100 pb molecular weight ladder. Gels were colored and photographed. PCR products were purified of DNA from low melting agarose gel, with the Wizard PCR temperature Preps DNA purification system. These PCR products were then re-amplified under the same initial amplification conditions but using primers without GC-clamp. They were then sent for sequencing at GATC Biotech (Germany) were the sequences of the 28S rDNA obtained were compared with those of the database available at NCBI GenBank (National Center for Biotechnology Information databases) using the BLAST program to determine the closest known sequences.

2.6 Gas Chromatography–Mass Spectrometry (GC/MS) Analysis of Essential Oils

The GC/MS analysis of essential oil was carried out according to the method described by Tchinda [16], using a Hewlett-Packardchromatograph equipped with an automatic injector and an HP1column (30 mm 0.25 mm d.i, film thickness 0.25 lm), coupled toa mass detector (GC-quadrupole MS system, model QP5000, Shimadzu). The programming of temperature involves raising from 70 to 200 °C at a speed of 10 °C/min. Helium was used as carrier gas with a flow rate of 0.6 ml/min. The injection is carried out by the split mode with an injection temperature set at 200°C. The molecules are bombarded by an electron beam of 70 eV and the detection is performed by an analyzer quadrupole filter. The mass spectra obtained by electron impact were acquired over the mass range of 40–400 m/z. The ion source temperature is 200°C.

The identification of chemical components of these essential oils has been done by comparison of their retention index and mass spectra with those found in NIST08 bank [17].

2.7 *In vitro* Antifungal Activity of Essential Oil on Toxigenic Strains

In vitro antifungal activity of essential oils on mycelial growth has been assessed using the incorporation method as described by Tchinda [16]. All the tests have been carried out on triplicate. The mycelial growth has been daily monitored (for 7 days) by measuring the growth diameter [18] as well as the inhibition percentage of the growth using Lahlou [19] formula (1).

$$\%I = \frac{(Dt-De)}{Dt} X\ 100 \quad (1)$$

%I = Inhibition percentage; Dt (mm) = Average diameter of mycelial growth in the negative control container; De (mm) = Average diameter of mycelial growth in the trial container.

The method of Djeddi et al. [20] has been used in order to assess the sensitivity of isolated strains to different concentrations of *C. citratus* and *O. basilicum*. According to this method which classifies the sensibility of strains to essential oils using the inhibition diameter, sensible strains are those with inhibition diameter varying between 15 mm and 19 mm, and non-sensible strains are those with inhibition diameter lower than 8 mm. Molds presenting a sensibility to different concentrations of essential oils have been selected for the determination of Minimal inhibitory concentration (MIC). For each strain, MIC has been determined using the micro-atmosphere as described by Neri et al. (2006). Essential oil concentrations used were varying from 250 ppm to 400 ppm for C. citratus, and for 650 ppm to 800 ppm for *O. basilicum*. After inoculation and incubation, observations have been done in order to determine the lowest concentration where there is not a mold growth.

2.8 Statistical Analysis

The analysis of variance (for significant differences) followed with DUNCAN test (for classification of samples with similarities has been done using IBM SPSS statistics (version 1.0.0-2482) package software. All these tests were done at 95% of confidence intervals.

3. RESULTS AND DISCUSSION

3.1 Physico-Chemical Parameters of Sampled Smoked Fishes

In general, temperature of samples varies between 25°C and 28°C and hygrometry between 65% and 86% (Table 1). It is well known that a relative humidity greater than 70% as well as storage temperature less than 25°C improve the growth of molds and might modify the chemical composition of the products [21]. It has been showed in Morocco [22] that the relative humidity of the storage area is dependent on the weather. Since sampling has been done in rainy season, it becomes obvious to understand obtained values. With respect to water content

and water activity, there is not a significant difference ($P<0.05$) between samples which were collected in the same area (Table 1). Water content varies from 8% to 15%, *Airus africanus* and *Coryphaena hippurus* being fish species with highest water content. Oxidation of fats is known to occur at very low water content (around 9%) and this favors the development of off-products [23]. Moreover, oxidation rate increases with increasing storage temperature. Since water activity of 0.6 minimizes the growth of fungi [21], the data obtained in this work are more important (0.75-0.81), it is obvious that fungi can be found in sampled smoked fishes.

3.2 Microbiological Characteristics of Sampled Smoked Fishes

All the samples are contaminated by fungi (Table 2). The fungal contamination rate varies from 1.10^1 to $5.4.10^3$ CFU/g. According to microbiological criteria of AFNOR [13], fishes which are good for consumption must not have a fungus contamination rate greater than 10 CFU/g. On this basis, only one sample (Ch3, *Coryphaena hippurus*) is conforming. This observation seems to be general since it has been shown in Morocco that smoked fishes sold on market stalls are not good ($3.2.10^2$ CFU/g) for consumption [22].

Table 1. Physico-chemical parameters of samples smoked fishes

		Temperature (°C)	Hygrometry (%)	Water activity	Water content (%)
Cyprinus carpio	C_1	25.87	70	0.76	8.02
	C_2	25.74	65	0.75	9.85
	C_3	25.87	72	0.78	9.92
Arius africanus	A_1	25.90	78	0.79	14.58
	A_2	26.32	73	0.77	15.03
	A_3	25.67	84	0.77	15.01
Silurus sp.	S_1	26.03	83	0.77	12.66
	S_2	25.85	81	0.76	11.89
	S_3	26.32	80	0.79	11.95
Dicentrarchus labrax	D_1	28.05	86	0.77	11.33
	D_2	27.85	82	0.78	10.94
	D_3	27.58	85	0.79	11.04
Coryphaena hippurus	Ch_1	26.08	75	0.80	15.33
	Ch_2	25.42	72	0.80	14.52
	Ch_3	26.75	77	0.81	15.25

Table 2. Contamination rate of smoked fishes

Smoked fish species	Samples code	Total Molds (CFU/g)
Arius africanus	A_1	$(3.05 \pm 1.40) \times 10^2$
	A_2	$(2.68 \pm 1.91) \times 10^2$
	A_3	$(3.65 \pm 1.72) \times 10^2$
Cyprinus carpio	C_1	$(1.60 \pm 0.55) \times 10^1$
	C_2	$(1.05 \pm 0.40) \times 10^1$
	C_3	$(2.30 \pm 0.57) \times 10^1$
Coryphaena hippurus	Ch_1	$(2.05 \pm 0.65) \times 10^1$
	Ch_2	$(1.00 \pm 0.34) \times 10^1$
	Ch_3	$(1.40 \pm 0.40) \times 10^1$
Dicentrarchus labrax	D_1	$(1.53 \pm 1.02) \times 10^2$
	D_2	$(5.00 \pm 1.24) \times 10^2$
	D_3	$(1.56 \pm 1.64) \times 10^2$
Silurus sp.	S_1	$(5.40 \pm 0.58) \times 10^3$
	S_2	$(4.00 \pm 0.56) \times 10^3$
	S_3	$(94.6 \pm 0.71) \times 10^3$
Microbiological norm (AFNOR, 2002)	/	<10

Altogether, 11 strains of molds have been isolated (Table 3). The most found on smoked fishes are *Aspergillus spp*., *Penicillium citrinum* and *Mucor hiemalis*. They have been incriminated as being the main microorganisms involved in the alteration of smoked fishes [24]. In fact, it has been shown that *Aspergillus spp*., *Fusarium spp*., *Rhizopus spp*. and *Penicillium spp*. are the major fungi found on fishes, either smoked or not, *Aspergillus* spp. being the major mold [25,26]. Mold spores can also be found on fishes because they can be transported by insects which infest smoked fishes [27]. Amongst the identified species, those which are known as mycotoxin producers are: *Penicillium citrinum, Aspergillus versicolor, Aspergillus fumigatus, Aspergillus carbonarius, Aspergillus niger* and *Fusarium moniliforme*. *A. versicolor* which has been identified in smoked fishes of *Arius africanus* and *Silurus sp*. is known as being a producer of sterigmatocystin, a cancerogenic toxin for the liver [12]. But this toxin is lessening toxic (150 times) than Aflatoxin B1 (AFB1), despite the fact that it is its precursor (Pitt and Hocking, 2009). Thus, the simultaneous presence of *A. versicolor* and *A. fumigatus*, which have been found in fishes of species *Arius africanus*, is a supplementary risk of contamination to AFB1. *P. citrinum* is known as being a strain which can produce CIT while *A. carbonarius*, as well as *A. Niger* are molds which can produce many mycotoxins, amongst which Ochratoxin A (OTA).

However, it is well establish that the presence of toxigenic strains doesn't mean that the product in which they are found contained mycotoxins since the latter are produced under specific conditions [28,29]. Many factors including intrinsic and extrinsic are known as influencing the production of mycotoxins. For instance, the toxicity of mycotoxins which are produced by *Fusarium* decreases with successive regeneration from more concentrated solutions [27]. Extrinsic factors like pH, humidity, aeration, temperature, microbial concurrence and chemical nature of the product are also important in the production of mycotoxins [27].

3.3 Mycotoxin Contents of Sampled Smoked Fishes

Out of sample C3 and D3 which don't contain any of the sought mycotoxins (OTA, AFB1, and CIT), the remaining samples contain at least one type of mycotoxin (Table 3). Although some samples are co-contaminated with two types of mycotoxins, any of them is contaminated with the three mycotoxins (Table 3). OTA is the most found mycotoxin since it is found in 8 out of 15 samples, followed by AFB1 which occurs in 7 out of 15 samples, and finally CIT, which occurs in 40% of samples. Six samples (A1, A3, S1, S2, Ch1 and Ch3) have AFB1 and OTA contents beyond the authorized quantity according to European Union regulation (No 1881/2006) and should not be consumed.

In order to understand the relationship between measured parameters, fish species and mycotoxin contents, Principal Component Analysis (PCA) and Hierarchical Ascendant Classification (HAC) have been carried out. It comes out (Fig. 1) that there are three distinct groups of species. The first one is made of fishes of *Cyprinus carpio* (C1, C2, and C3) species which mainly contain CIT as the main mycotoxin. The second group is made of *Arius africanus*, *Silurus sp*. and *Coryphaena hippurus* (A1, A2, A3, S1, S2, S3, Ch1, Ch2, and Ch3) which contain AFB1 and OTA and have higher water content and water activity content. The third group is made of fishes of *Dicentrarchus labrax* (D1, D2, and D3) species and these smoked fishes have higher hygrometry and higher storage temperature.

3.4 Chemical Composition of Essential Oils

Essential oil yields were 0.68% for *Cymbopogon citratus* and 0.56% for *Ocimum basilicum*. The yield for *C. citratus* seems to be constant when using the same extraction method since the same values have been reported in other areas [30,31]. For *O. basilicum*, the reported yields vary from 0.17% to 1.26% and might be dependent to seasons and areas [16,32].

The essential oils of *C. citratus* and *O. basilicum* were analyzed by gas chromatography coupled to mass spectrometry (GC/MS) with the aim to identify chemical compounds responsible for their biological properties. Fig. 2 shows the chromatograms of the chemical compounds of the essential oils of the different samples studied.The chemical composition of essential oil of *C. citratus* shows (Table 5) that it contains 15 components which are mainly monoterpens (98.5%) and particularly, the oxygenated compounds (86.8%). The major compounds are Geranial (42.4%), Neral (33.5%) and Myrcen (10.8%). No sesquiterpene has been found in this essential oil. Geranial and Neral have

already been reported as been the major component of essential oil of *C. citratus*, the first one always being the major of both components [30,33]. With respect to *Ocimum basilicum*, 15 components have also been found (Table 5). Monoterpen (60.8%) and aromatic components (30.7%) are the major ones. Amongst monoterpens, the oxygenated ones are more important in terms of quantities (54.7%) than the ones which are only made of carbon and hydrogen (6.1%). With respect to aromatic components, the major ones are Eugenol (30.7%), Linalol (29.4%), and 1.8-Cineol (14.3%). The importance of monoterpen in essential oil of *O. basilicum* has already been reported [31].

Table 3. Microorganisms found in smoked fishes

Fish species	Morphological identification	Molecular identification	
		Strain's name	Identification frequency (%)
Cyprinus carpio	*Penicillium citrinum*	*Penicillium citrinum*	100
	Fusarium sp.	*Fusarium moniliforme*	100
	Mucor sp.	*Mucor hiemalis*	100
	NI	*Absidia sp*.	96
Arius africanus	*Rhizopus sp.*	*Rhizopus nigricans*	100
	penicillium citrinum	*penicillium citrinum*	100
	Fusarium sp.	*Fusarium moniliforme*	100
	Mucor sp.	*Mucor hiemalis*	100
	Aspergillus versicolor	*Aspergillus versicolor*	100
	Aspergillus fumigatus	*Aspergillus fumigatus*	99
Silurus sp.	*Aspergillus carbonarius*	*Aspergillus carbonarius*	100
	Aspergillus versicolor	*Aspergillus versicolor*	100
	Mucor sp	*Mucor hiemalis*	100
	NI	*Wallemia muriae*	98
Dicentrarchus labrax	*Aspergillus niger*	*Aspergillus niger*	99
	Rhizopus sp.	*Rhizopus nigricans*	100
	NI	*Acremonium murorum*	99
Coryphaena hippurus	*Aspergillus carbonarius*	*Aspergillus carbonarius*	100
	Mucor sp,	*Mucor hiemalis*	100
	penicillium citrinum	*penicillium citrinum*	100

NI : Not identified

Table 4. Aflatoxin B1 (AFB1), Ochratoxin A (OTA) and Citrinin (CIT) contents of sampled smoked fishes

Fish species		AFB1 content (µg/Kg)	CIT content (µg/kg)	OTA content (µg/Kg)
Cyprinus carpio	C_1	ND	3.6 ± 1.93	ND
	C_2	ND	0.6 ± 0.97	ND
	C_3	ND	ND	ND
Arius africanus	A_1	5.80 ± 5.72	ND	ND
	A_2	0.90 ± 4.82	0.30 ± 0.15	ND
	A_3	12.30 ± 5.02	0.10 ± 0.05	ND
Silurus sp.	S_1	1.40 ± 0.70	ND	11.40 ± 5.61
	S_2	ND	ND	3.10 ± 4.62
	S_3	0.70 ± 0.70	ND	0.70 ± 4.01
Dicentrarchus labrax	D_1	ND	0.80 ± 0.46	0.10 ± 0.11
	D_2	ND	ND	0.40 ± 0.21
	D_3	ND	ND	ND
Coryphaena hippurus	Ch_1	5.10 ± 2.86	ND	4.20 ± 2.35
	Ch_2	ND	0.70 ± 0.40	5.30 ± 0.33
	Ch_3	0.30 ± 1.06	ND	0.80 ± 1.05

ND: Not detected;

Table 5. Chemical composition of essential oils of *C. citratus* and *O. basilicum*

Retention index	Component	*Cymbopogon citratus*	*Ocimum basilicum*
	Monoterpene	98.5	60.8
	Hydrocarbon monoterpene	11.7	6.1
979	β-pinene	-	1.4
992	myrcene	10.8	-
1030	Limonene	-	2.4
1037	(Z)-β-Ocimene	0.6	2.3
1047	(E)-β-Ocimene	0.3	-
	Oxygenated monoterpene	86.8	54.7
1033	1,8-cineol	-	14.3
1092	fenchon	-	1.7
1102	Linalol	0.8	29.4
1146	Isocitral « exo »	0.2	-
1148	Camphor	-	1.8
1154	Citronellal	0.3	-
1166	Thujanol « 3 »	1.0	-
1181	Terpinen-4-ol	-	5.5
1184	Isocitral « z »	1.6	-
1194	α-terpineol	-	2.0
1231	Nerol	0.6	-
1246	Neral	33.5	-
1257	Geraniol	5.2	-
1276	geranial	42.4	-
1386	Geranyle acetate	1.2	-
	Hydrocarbon sesquiterpene	-	8.5
1439	2-norpinene	-	4.0
1546	α-humulene	-	1.3
1624	γ.-Cadinen	-	3.2
	Aromatic components	-	30.7
1365	eugenol	-	30.7
	Aliphatic components	1.5	-
987	6-methylhept-5-en-2-one	1.0	-
1685	Benylate	0.5	-
Total	*/*	**100**	**100**

-: Not detected;

Table 6. Inhibition (%) of the mycelial growth of toxigenic strains by essential oils of *C. citratus* and *O. basilicum*

Concentration (ppm)	*A. carbonarius*	*A. versicolor*	*A. fumigatus*	*A. niger*	*P. citrinum*
	Inhibition (%) with *C. citratus*				
400	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0
375	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	85.32 ± 2.80
350	100 ± 0.00	100 ± 0.00	100 ± 0.00	95.30 ± 1.28	58.86 ± 3.75
300	100 ± 0.00	100 ± 0.00	96.41 ± 1.02	81.35 ± 2.21	27.76 ± 0.99
275	100 ± 0.00	91.36 ± 2.88	91.05 ± 0.45	72.33 ± 1.27	/
250	84.38 ± 1.55	61.98 ± 1.84	77.31 ± 2.56	60.77 ± 2.39	/
/	**Inhibition (%) with *O. basilicum***				
800	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
775	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	73.05 ± 0.45
750	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	43.36 ± 0.82
700	100 ± 0.00	94.56 ± 2.15	100 ± 0.00	100 ± 0.00	/
675	75.21 ± 1.56	83.58 ± 2.04	97.65 ± 2.47	97.04 ± 2.43	/
650	41.36 ± 0.92	69.55 ± 1.88	87.55 ± 1.95	85.05 ± 1.52	/

3.5 Antifungal Activities of Essential Oils of *C. citratus* and *O. basilicum*

Regardless of the essential oil used as well as the type of microorganism, there is an increase of inhibition percentage with increasing concentration of essential oil used (Table 6). However, essential oil efficiency is dependent on the germ. In this respect, *A. carbonarius* is the most sensible of tested strains for *C. citratus* essential oil since this mold displays the lowest Minimal Inhibition Concentration (MIC) while *P. citrinum* is the most resistant since it presents the highest MIC (Table 6). For *O. basilicum*, there are many sensible strains: *A. carbonarius, A. fumigatus*, and *A. niger*. It has already been shown that essential oil of *C. citratus* was inhibiting the growth of fungi [16].

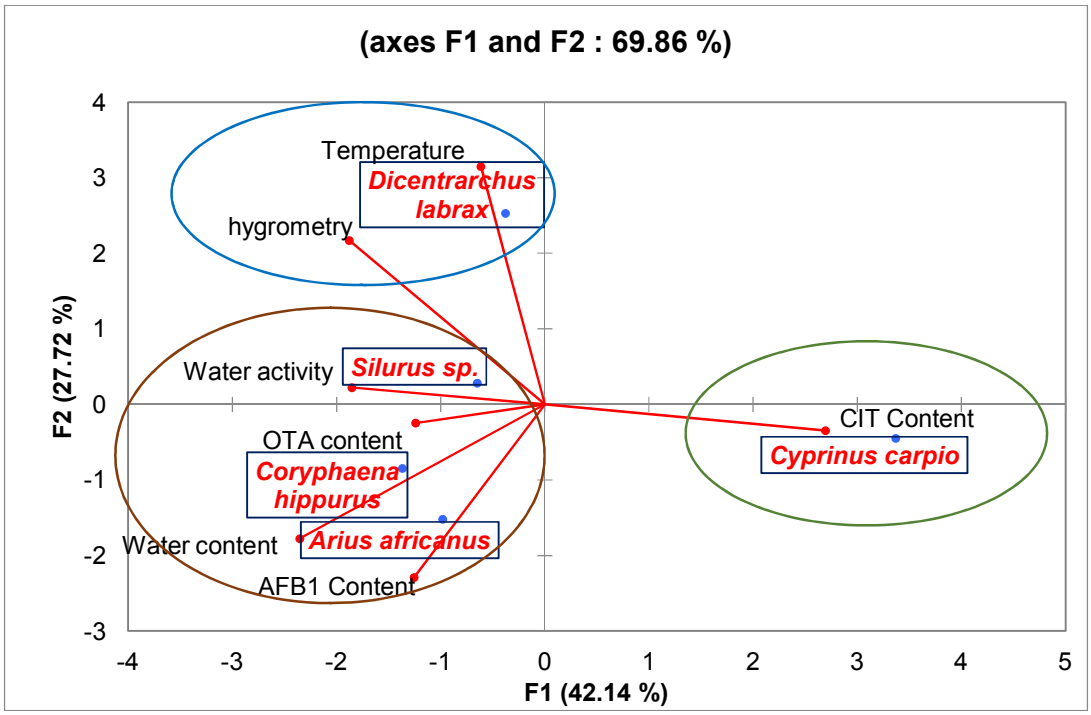
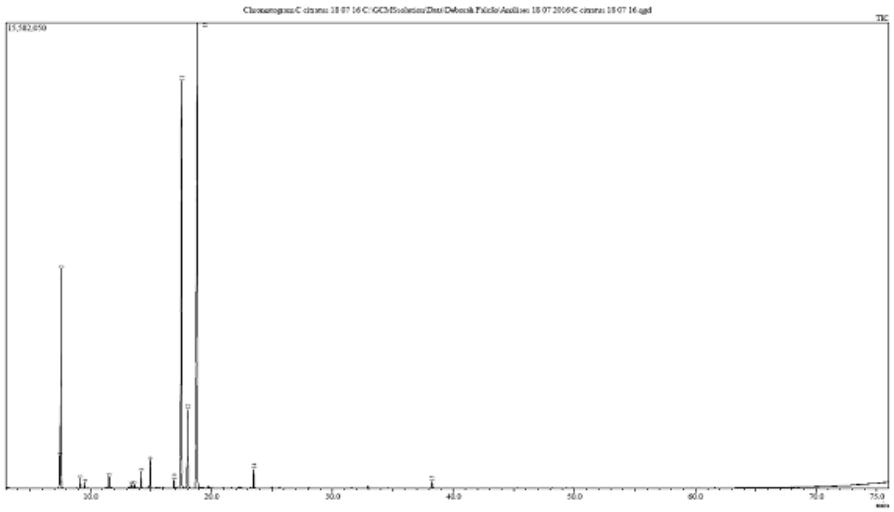


Fig. 1. Principal Component Analysis (PCA) of smoked fishes with measured physicochemical parameters of these samples



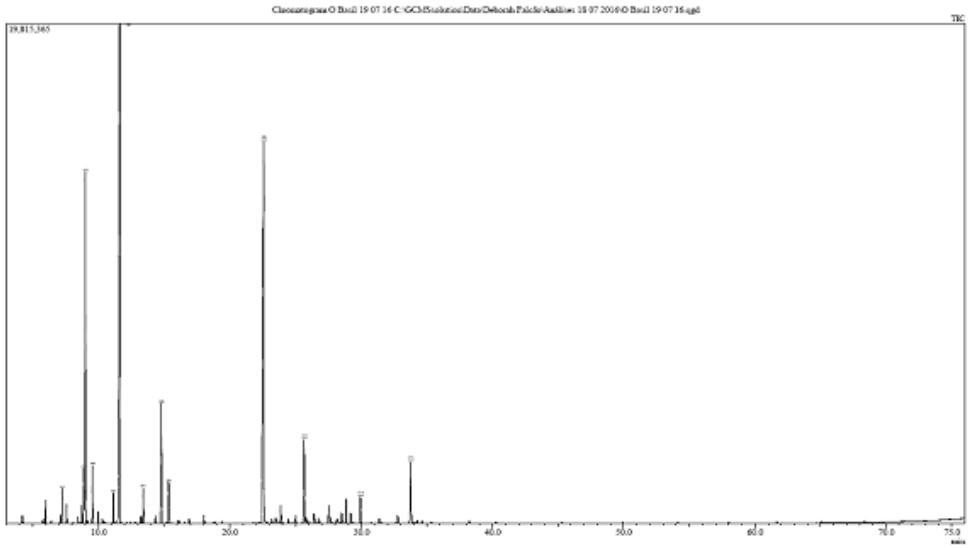


Fig. 2.Total ion chromatogram of volatile compounds from essential oils of different *C. citratus* and *O. basilicum*, respectively

4. CONCLUSION

The aim of this work was to determine the fungi profile as well as mycotoxin (OTA, AFB1, and CIT) contents of smoked fishes sold on market stalls of Ngaoundere, and to assess the efficiency of essential oils of *C. citratus* and *O. basilicum* on these microorganisms. It comes out that many samples have one or two tested mycotoxins, at quantities which are beyond the European Union norm. Altogether, 11 fungus strains, which are known to be mycotoxin producers, have been identified and the most abundant are Aspergillus sp., *P. citrinum* and *Mucor hiemalis*. Essential oil of *C. citratus* mainly contains Geranial (42.4%), Neral (33.5%) and Myrcen (10.8%), while essential oil of *O. basilicum* mainly contain monoterpen (60.8%) and aromatic components (Eugenol, Linalol and 1,8-Cineol) as major components. These essential oils are efficient against *A. carbonarius, A. versicolor, A. fumigatus, A. niger* and *P. citrinum.* Therefore, these essential oils can be used as a bio-fungicide for smoked fishes.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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