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Antifungal Activities of *Cymbopogon citratus* Essential Oil against *Aspergillus* Species Isolated from Fermented Fish Products of Southern Benin

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HIGHLIGHTS

- The major components of *Cymbopogon citratus* Essential Oil (EO) were geranial (41.3%), neral (33.0%), myrcene (10.4%), and geraniol (6.6%).
- *C. citratus* EO exhibited considerable antifungal activity against *Aspergillus* spp.
- *C. citratus* EO may be a practical application in controlling fungal contamination in fermented fish.

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Acronyms and abbreviations
EO= Essential Oil
GC-MS= Gas Chromatography-Mass Spectrometry
MIC=Minimal Inhibitory Concentration
MFC=Minimal Fungicidal Concentration

ABSTRACT

Background: In Benin Republic, the conservation of fermented fishes for a long time is difficult due to the contamination of fungi, which lead to its rapid degradation. This experiment was conducted to evaluate the effect of *Cymbopogon citratus* Essential Oil (EO) against *Aspergillus* species isolated from fermented fish samples.

Methods: Gas chromatography-mass spectrometry was used to determinate the chemical composition of the *C. citratus* EO. The agar dilution method was used to evaluate the antifungal activity of the *C. citratus* EO against *Aspergillus* species. Data were analyzed using SPSS, Chicago, IL, USA, version 10.0.

Results: The major components of *C. citratus* EO were geranial (41.3%), neral (33.0%), myrcene (10.4%), and geraniol (6.6%). The dominant *Aspergillus* fungi isolated from the fermented fish samples were *A. ochraceus*, *A. oryzae*, *A. fumigatus*, and *A. parasiticus*. *C. citratus* EO exhibited considerable antifungal activity against the growth of fungi isolated from fermented fish samples. There was no significant difference between minimal inhibitory concentrations and minimal fungicidal concentrations of *A. ochraceus* and *A. parasiticus* ($p>0.05$).

Conclusion: The findings of this research clearly indicate that the *C. citratus* EO may be a practical application in controlling the growth of *Aspergillus* species in fermented fish.

Introduction

Nowadays, the people sometimes are concerned about consumption of unsafe and contaminated foods. Fungal deterioration is a common problem in food storage systems. *Aspergillus* spp. are able to colonize food products, leading to deterioration and mycotoxin production (da Rocha et al., 2014; Probst et al., 2007). Also, the fungal contamination can result in spoilage of food and reduction of its quality (Candlish et al., 2001). On the other

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hands, some fungi may induce immunologic or allergic responses in human beings (Samadi-Foroushani et al., 2011; Simon-Nobbe et al., 2008; Taghavi et al., 2017).

In Benin Republic, fishing plays a significant role in national socio-economic balance. However, rapid degradation of fish is of concern due to the lack of adequate conservation system and climatic or environmental conditions. Several artisanal treatments reduce these post-capture losses of fish. However, nowadays, the fermentation of fish became one of the mostly conservation methods. Despite the social importance and the nutritious nature of fermented fish, several problems are remained related to their hygienic quality as well as their suitability for conservations (Adjou et al., 2017).

Increasing of public awareness of the toxic effects of many synthetic fungicides leads to the focusing on the alternative indigenous products to control fungal deterioration of food (Soumanou and Adjou, 2016; Tatsadjieu et al., 2009). From many decades ago, herbs and spices are as aromatic plants in many parts of the world, both as flavoring agents and as preservatives of food. They may be effective sources of biodegradable fungitoxicants without harmful side effects. Essential Oils (EOs) extracted from herbs and spices are also reported to possess some antifungal activities (Hyldgaard et al., 2012). *Cymbopogon citratus* plant (Poaceae) which is popularly known as citronella grass or lemongrass, has about 55 species of grasses with a large distribution in tropical and sub-tropical regions of the world (Matasyoh et al., 2011). Thus, this study aims to evaluate the antifungal potential of the EO of *C. citratus* against *Aspergillus* spp. isolated from fermented fish in southern Benin.

Materials and methods

Plant leaves collection and EO extraction

Fresh leaves of *C. citratus* were collected from Abomey-Calavi (Southern Benin) and verified at University of Abomey-Calavi. The EO was extracted by hydro-distillation method using Clevenger-type apparatus. The anhydrous sodium sulfate was used for drying the recovered the oil, which was stored at 4 °C until used (de Billerbeck et al., 2001).

Gas Chromatography–Mass Spectrometry (GC-MS) analysis

Gas chromatograph (Perkin Elmer Auto XL GC; Waltham, MA, USA) equipped with a flame ionization detector was applied for analysis of the EO components, and the GC conditions were EQUITY-5 column (60 mx0.32 mmx0.25 µm); H_2 as the carrier gas; column head pressure 10 psi; oven temperature program isotherm 2 min at 70 °C, 3 °C/min gradient 250 °C, isotherm 10 min; injection temperature, 250 °C; detector temperature 280 °C. GC-MS analysis was performed using a Perkin Elmer Turbomass GC-MS (Perkin Elmer; Waltham, MA, USA). The GC column was EQUITY-5 (60 mx0.32 mmx0.25 µm); fused silica capillary column. The GC conditions were injection temperature, 250 °C; column temperature, isothermal at 70 °C for 2 min, then programmed to 250 °C at 37 °C/min and held at this temperature for 10 min; ion source temperature, 250 °C. Helium was the carrier gas. The effluent of GC column was introduced directly into the source of MS and spectra obtained in the EI mode with 70 eV ionization energy. The sector mass analyzer was set to scan from 40 to 500 amu for 22 s. The identification of individual compounds is based on their retention times, retention indices relative to C_5–C_{18} n-alkanes, and matching spectral peaks available in the published data (Adams, 2007).

Collection of fermented fish samples

Fifty samples of fermented fish were collected from the main markets of localities located along the South Benin fisheries road. A total of 10 different markets were investigated, and samples were purchased from five different sellers in each market. The obtained samples were mixed together to give a composite samples from each market which were used for the analysis.

Fungal isolation and identification

The fungi isolation was performed by using dilution-plating method. Fermented fish sample (10 g) was added separately to 90 ml of sterile water containing 0.1% peptone water and thoroughly mixed to obtain 10 dilutions. Further, 10 fold serial dilutions up to 10 were made. One ml volume of each dilution was separately placed in petri dishes, over which, 15 ml of Potato Dextrose Agar (PDA; Difco, USA) amended with 60 µg/ml of chloramphenicol was poured. After that, the plates were incubated at 28±2 °C for 7 days. Fungal isolates from PDA were subcultured on Malt Extract Agar (MEA; Difco, USA), and identification was carried out by using a taxonomic scheme primarily based on morphological characters, using the methods described by Singh et al. (1991).

Antifungal assay

Antifungal assay was performed by the agar medium assay (Adjou et al., 2013). Different concentrations of EO (1.0, 2.5, 5.0, and 7.5 µl/ml) were prepared by adding appropriate quantity of EO to melted medium, followed by manual rotation of Erlenmeyer to disperse the oil in the medium. About 20 ml of the medium were poured into 9-cm glass Petri-dishes. Each Petri-dish was inocu-

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lated at the center with a mycelial disc (6 mm diameter) taken at the periphery of fungi strains isolated from samples of fermented fish grown on MEA for 48 h. Plates without EO as control groups were inoculated following the same procedure. Plates were incubated at 25 °C for 8 days and the colony diameter was recorded each day. The Minimal Inhibitory Concentration (MIC) and also Minimal Fungicidal Concentration (MFC) were determined according Robinson (2014).

Statistical analyses

Experiments were performed in triplicate, and data obtained as mean±SE were analyzed by ANOVA test using SPSS, Chicago, IL, USA, version 10.0. Means are separated by the Tukey's multiple range test when ANOVA was significant ($p<0.05$).

Results

Chemical analysis of *C. citratus* EO by GC and GC-MS analysis of EO enabled the identification of 20 components (Table 1), representing 98.1% of the EO. The major components of *C. citratus* EO were geranial (41.3%), neral (33.0%), myrcene (10.4%), and geraniol (6.6%).

The dominant *Aspergillus* fungi isolated from the fermented fish samples were *A. ochraceus*, *A. oryzae*, *A. fumigatus*, and *A. parasiticus*. Taking into account the number of case of 11 *Aspergillus* strains isolated from fermented fish samples collected in the current study, the obtained occurrences were 27.27% for *A. ochraceus*, 27.27% for *A. oryzae*, 18.18% for *A. fumigatus*, 9.09% for *A. parasiticus*, and 18.18% for *Aspergillus* spp.

C. citratus EO exhibited considerable antifungal activity against the growth of fungi isolated from fermented fish samples (data not shown). The MIC and MFC for *A. oryzae*, *A. fumigatus*, and *Aspergillus* spp. were found to be respectively 1.0 and 2.5 µl/ml. Statistical analysis indicated that there was not any significant difference between MICs and MFCs of *A. ochraceus* and *A. parasiticus* ($p>0.05$). However, significant difference was found between MICs and MFCs of *A. parasiticus* and those of *A. oryzae*, *A. fumigates*, and *Aspergillus* spp. ($p<0.05$).

Table 1: Chemical composition of the tested essential oil of *Cymbopogon citratus*

Compound	Retention index	%
6-méthyl-hep-5-en-2-one	985	1.2
Myrcene	991	10.4
(Z)-β-ocimene	1036	0.2
(E)-β-ocimene	1047	0.2
6,7-epoxymyrcene	1091	0.2
Pirillene	1098	0.1
Linallol	1100	0.5
2,2-octa-3,4-dienal	1106	0.1
Cis-vervenol	1140	0.1
Menth-3-en-9-ol	1150	0.1
Citronella	1153	0.4
Cis-chrysanthenol	1162	0.5
Epoxy rose furane	1170	0.2
Nerol	1231	0.3
Neral	1245	33.0
Geraniol	1256	6.6
Geranial	1276	41.3
Formate of neryle	1285	0.1
Acetate of geranyle	1378	2.4
Oxyde of caryophyllene	1587	0.1

Discussion

EOs are natural mixtures of hydrocarbons and oxygen such as alcohols, aldehydes, ketones, carboxylic acids, esters, and lactones containing organic substances of plants. They have a long history of application as antimicrobial agents in food preservation (Soumanou and Adjou, 2016). EO activities depends on the qualitative and quantitative characteristics of their components affected by some parameters such as the plant genotype, plant geographical origin, harvesting season, agronomic conditions, extraction method, and storage condition of

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EOs. The present study focuses on the antifungal activities of *C. citratus* EO as the promising plant-based antimicrobials against fermented fish fungi. The EO is effective against isolated fungi; depend on the fungi species and the concentration of EO. The bioactivity of the EO may be because of the presence of some highly fungitoxic components in the oil. Indeed, *C. citratus* EO has chemical composition characterized by the presence of oxygenated monoterpenes (85.5%), oxygenated sesquiterpenes (0.2%), and oxygenated aliphatic compounds (1.3%). This result differs from those indicated in early reports on the EO extracted from *C. citratus* plant collected in Kenya (Matasyoh et al., 2011), and also during the investigation on the chemical composition of Egyptian lemongrass EO (Soliman et al., 2017). This controversy indicated that EOs might have a heterogenic chemical composition depending on the geographic location of harvesting sites.

Microbiological assays revealed that our analyzed fermented fish samples were contaminated with the *Aspergillus* fungi. Generally, most of the deterioration of food is caused by several species of *Aspergillus* which are responsible for many cases of food and feed contamination (Oguz et al., 2003). These results indicate that the preservation methods used by traders promote the growth of fungi which may finally lead to production if hazardous mycotoxins in fermented fish distributed in South Benin. Therefore, certain critical points should pay to special attention in the chain of processing, including the solar drying. Indeed, according to Adjou et al. (2017), during the traditional fish fermented process, fish are set to solar drying for 6 days. This open-air exposure for solar drying, during a long time, can greatly serve as source of contamination. Hout-Kasef, a traditional fermented fish product in Saudi Arabia, recently reported similar fungal contamination (Gassem, 2019). This research also explores the efficacy of *C. citratus* EO from Southern Benin as the promising plant-based antimicrobial compound against fungal contamination. *C. citratus* EO is effective against fungi species isolated from fermented fish samples. The MIC of our tested EO was lower than the earlier reported antimicrobial effect of EOs such as *Lippia alba* (Shukla et al., 2009), *Cymbopogon flexuosus (*Kumar et al., 2007), and *Lantana indica (*Kumar et al., 2010) tested against *Aspergillus* spp. This may be because of the presence of components with highly fungitoxic potential in the mentioned EOs. Indeed, *C. citratus* EO has monoterpenes as the major components which belong to a group of high antimicrobial components (Soumanou and Adjou, 2016). The inhibitory action of natural products on mould cells involves major alterations in the cytoplasm and the inhibition of intercellular and extracellular enzymes (Cowan, 1999; Souza et al., 2005). The findings of the present investigation clearly showed that *C. citratus* EO would be acting as inhibitor of fungal growth.

Conclusion

This survey underlined the bioactivity of EO of fresh leaves of *C. citratus* from Benin as preservative of stored fermented fish products against *Aspergillus* species contamination. The findings clearly indicate that the *C. citratus* EO may be a practical application in controlling the growth of *Aspergillus* spp. in fermented fish. Further researches need to done on the mode of action of the EO on the ultrastructure of the *Aspergillus* spp. In addition, sensorial tests are needed in the next researches in order to study the probable undesirable impact of this EO on favor and odor of the fish products.

Author contributions

E.S.A. and R.G.D. designed the study; A.C.A., E.S.A., and R.G.D conducted the experimental work; E.S.A., E.D-A., and R.G.D analyzed the data; E.S.A., E.D-A., and R.G.D wrote the manuscript. All authors revised and approved the final manuscript.

Conflicts of interest

There is no conflict of interest in the study.

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