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Chemical Composition and Antifungal Potential of the Essential Oils of *Ocimum gratissimum* L, *Ocimum suave* L, *Aframomum alboviolaceum Ridley* and *Zingiber officinale Roscoe* against Two Molds associated with to the Alteration of Smoked Fish *Ethmalosa fimbriata* Bowdich

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A B S T R A C T

This study aimed at analyzing the chemical composition of the essential oils of *Ocimum gratissimum*, *Ocimum suave*, *Aframomum alboviolaceum Ridley* and *Zingiber officinale Roscoe*, and evaluate their antifungal activities. Hydrodistillation with a Clevenger type apparatus was used for the extraction of essential oils from plant materials. Gas chromatography and gas chromatography coupled with Mass Spectrometry were utilized to identify the chemicals. The antifungal activity of the essential oils was tested *in vitro* against *Aspergillus flavus* and *Aspergillus niger*. For *O. gratissimum* the major components identified were thymol (31.71%), p-cymene (17.86%) and γ-Terpinene (5.90%). For *A. alboviolaceum*, 1.8-Cineole (58.90%), α-Terpinyl acetate (9.58%) and α-pinene (4.86%) were found, while geranial (34%), neral (23.79%) and borneol (9.04%) were identified in the volatile extract of *Z. officinale*. The essence of *O. suave* had benzene, 1.2.4-trimethoxy-5- (1-propenyl) - (Z) (22.79%); β-bisabolene (19.10%) and spathulenol (18.69%) as the major compounds. Total inhibition of *Aspergillus flavus* and *Aspergillus niger* by the essential oil of *O. gratissimum L*. was observed at 5500 ppm and 4750 ppm, respectively. The presence of compounds such as methyl eugenol, thymol and carvacrol, which have previously demonstrated antimicrobial properties could explainthe effect of *O. gratissimum L*. The essential oil of *Z. officinale* totally inhibited *Aspergillus flavus* and *Aspergillus niger* at 5000ppm and 4500ppm. *Z. officinale's* action could be linked with the presence of compounds such as geranial, neral, α-pinene and 1.8-cineole. The antifungal activities of the essential oils of *O. gratissimum* and *Z. officinale* stronger than those of *O. suave* and *A. alboviolaceum*. The inhibition of mycelia growth of the fungus was significantly ($p < 0.05$) positively correlated with the essential oil concentrations. These essential oils could be a promising alternative as biofungicides for the post-smoking conservation of fish.

Introduction

Ethmalosa fimbriata, known as "Bonga" is a clupeid fish and one of the best sources of protein, vitamins, essential fatty acids and minerals. It contains essential nutrients for the supplementation of infant and adult diets (Abdullahi *et al.*, 2001) Coastal and riparian populations commonly fish for this species. Fresh bongo is processed to lengthen its shelf life and diversify its consumption pattern. Salting, drying, frying, and smoking are the most common processing procedures. During smoking, the heat from the fire dries the fish, while the volatile compounds in the smoke infuse the fish. However, food preservation methods like smoking can not always protect food from microbes like mould.

The most harmful mold species belong to the genus *Aspergillus*, especially when stored under unsuitable conditions (Falioye *et al.*, 2012). Chemicals are often used for the conservation of these smoked fishs (Degnon *et al.*, 2000). Artificial preservatives have been restricted due to their negative impact on human health and the environment, necessitating the search for other suitable alternatives such as Natural substances like essential oils which could be alternative to synthetic chemical currently used (Adjou *et al.*, 2013). The antioxidant and antimicrobial activity of essential oils against a wide range of microorganisms, including filamentous fungi has been reported (Tchabong *et al.*, 2019). The Cameroonian flora is rich in aromatic plants with various biological activities. *Ocimum gratissimum*, *Ocimum suave*, *Aframomum alboviolaceum* and *Zingiber officinale* are aromatic plants used in culinary practice and African medicine (Ndoye, 2001).

Several studies reported the antifungal, antimalarial, and antibacterial activities of the essential oils of these plants. These biological activities were associated with to the presence of compounds such as 1.8 Cineole (Alves-Silva *et al.*, 2013). However, there is no information on the antifungal activities of these essential oils against *Aspergillus flavus* and *Aspergillus niger* that we are aware of.

The aim of this study was to analyze the chemical composition of the essential oils from *Ocimum gratissimum, Ocimum suave, Aframomum alboviolaceum* and *Zingiber officinale* as well as their antifungal activities against *Aspergillus flavus* and *Aspergillus niger*, which caused smoked fish to deteriorate.

Materials and Methods

Plant material and essential oil extraction

O. gratissimum and *Z. officinale* were harvested in November 2020in the Littoral Region, Department of Moungo, Arrondissement of Manjo, more precisely between 32.0589910-32.0588532 UTM north latitude and 32.0535227-32.0534496 UTM east longitude. *A. alboviolaceum* and *O. suave* were harvested in October 2020 in the western region, specifically at Battak, between 32.0616333 - 32.0646348 UTM north latitude and 32.0571366-32.0571420 UTM east longitude respectively.

Plants were dried at room temperature (28 ± 2°C) for three days. Using a Clevenger apparatus, leaves and stems were steam-distilled for 4 hours. Each oil recovered was dried over anhydrous Na_2SO_4then stored in an amber-colored flask and kept at 4°C until used.

Essential oils analysis

Essential oils were analysed by gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC/MS).Each oil was analysed on a Varian CP-3380 GC with flame ionisation detector fitted with a fused silica capillary column (30 m x 0.25 mm coated with DB-5, film thickness 0.25 µm); temperature program 50°C-200°Cat 5°C/min, injector temperature 200°C, detector temperature 200°C, carrier gas N2 1 ml/min. The linear retention indices of the components were determined relatively to the retention times of a series of n-alkanes and the percentage compositions were obtained from electronic integration measurements without taking into account relative response factors.

GC-MS analyses were carried out using a furnace Focus GC (Thermo) apparatus equipped with a TG-5MS5MScolumn (30 m x 0.25 mm, film thickness, 0.25 µm) and interfaced with a quadruple detector (DSQ II). Column temperature was programmed from 60 to 200° C at 10° C/mn; injector temperature was 220° C. The injections were carried out in mode SPLIT (Ratio: 1/100). Helium was used as carrier gas at a flow rate of 1.2 ml/min; the mass spectrometer was operated at 70 eV. The components were identified based on the comparison of their retention indices and their mass spectra with those given in the literature (Adams, 2007).

Fungal pathogen isolation

The *Aspergillus flavus* and *Aspergillus niger* strains were isolated from smoked fish *Ethmalosa fimbriata* (bonga) in July 2020 at the biochemistry laboratory of the University of Douala. The mycelia emerging from tissues were put into fresh Sabouraud medium after 21 days of incubation at 28°C. The operation was repeated several times to obtain pure culture of each isolate. Both fungal isolates were identified using macroscopic and microscopic criteria outlined by (Rippon, 1988). The cultures were kept at 4°C for three months and sub-cultured every three month.

Antifungal assay

The agar incorporation method (Lahlou, 2004) was used to evaluate the antifungal activity of the essential oils. The test was carried out in 90 mm Petri dishes containing SDA- medium supplemented with chloramphenicol. The oils were first diluted with Dimethyl Sulphur Oxide (DMSO) (ratio1:9). These essential oils were added aseptically into the medium at an appropriate volume to achieve concentrations ranging from 1000 to 6000 ppm. SDA medium, chloramphenicol supplemented only with DMSO was used as negative control. After solidification, the media were inoculated with 5 mm discs obtained from the edge of 3-days old *A. flavus* and *A. niger* mycelia culture. Each treatment consisted of triplicate plates incubated at 28°C in the dark. Mycelia growth was monitored by measuring the growth diameter following two perpendicular lines going through the centre of the dish. These measurements were made daily for 7 days. The inhibition percentage of mycelia growth was calculated by comparing them with those in the blank dish without essential oil using the formula below: %I = (Dc - Dt) / Dc, where Dc is the diameter of microbial colony in the control and Dt the diameter of the colony in the treated plate. After incubation, the MIC (minimum inhibitory concentration) considered was the lowest concentration of essential oil that inhibited any visible growth of the germs after eight days of incubation.

Statistical analysis

Data was entered into the Microsoft Office Excel spread sheet and statistical analyses were carried out using SPSS version 20.1. The one-factor ANOVA (Variables Analysis) test was used. When the ANOVA result was significant, Duncan's post hoc test was performed to establish two-to-two comparisons. The significance threshold was set at $p\text{-value} < 0.05$.

Results and Discussion

Extraction yield of the essential oils

The yields of essential oils from *Ocimum gratissimum, Ocimum suave, Aframomum alboviolaceum* and *Zingiber officinale* were 0.71%, 0.48%, 0.79% and 0.45% respectively (Table 1). The yield obtained with the dried leaves of *O. gratissimum* (0.71%) harvested at the end of November 2020 (dry season) is higher than that reported by Tchoumbougnang *et al.,* (2009) (0.6%) when the plant was harvested in April (rainy season). The essential oil yield *Zingiber officinale* (0.45%) was higher than the 0,39% and 0.31 % reported by Ndoye (2001) in Abong-bang and Zende (0.31%), but lower than the 0.6% obtained by Hassan *et al.,* (2020).This discrepancy could be

attributable to harvesting seasons and years, as well as extraction methods The yield of *A. alboviolaceum* (0.79) was lower than that of Abondo and Amvam (1995)who reported yield ranging from 1.7% to 2.6%. The disparity in harvest time could be the cause of the variance. While we harvested the leaves of *A. alboviolaceum* during the early dry season, Abondo and Amvam (1995) harvested the leaves during the rainy season. For *O. suave*, its oil yield (0.48%) is lower than that obtained by Tchoumbougnang (1997) (0.53%).

Chemical composition of the essential oils

A representative GC map of the oils is shown in Figures 1, 2, 3 and 4. Compounds identified in essential oils are listed in Table 1 along with their percentage composition. In the oil of *O. gratissimum*, sixty-two constituents were identified and classified, sixty-five in *O. suave*, thirty-six in *A. alboviolaceum*, thirty-three in *Zingiber officinale*. Except *O. suave* which is rich in hydrocarbon sesquiterpenes, these plants are rich in oxygenated monoterpenes (MTO) The major compounds of *O. gratissimum* are Thymol (31.71%), p-cymene (17.86%) and γ-Terpinene (5.90%) followed by α-bisabolene (17.19%) and thymol (9.8%). Previous studies have found similar compounds with a lower or greater percentage of the above-mentioned chemicals. Lexa *et al.,* (2006) found that the essential oil of *Ocimum gratissimum* consists mainly of eugenol (68.81%), followed by methyl eugenol (13.21%). The main chemicals in *A. alboviolaceum* were 1,8-Cineole (58.90%), α-Terpinyl acetate (9.58%) and α-pinene (4.86%). Abondo and Anvam (1995)also reported 1,8-cineole (60%) as the mainconstituent in *A. alboviolaceum*. While Geranial (34.79%), Neral (23.79%) and 1.8-cineole (5.02%) were the most abundant compounds in the essential oil of *Zingiber officinale* in our study, Ndoye (2001) found β-curcumene (14%), Géranial (12%), Néral + Nérol (10.7%) and 1,8-cineole (11.1%) as the main compound in *Zingiber officinale*. More recently, Bagora (2015) reported 35 compounds in the essential oil of *Zingiber officinale* with Ar-curcumene (16.67%), camphene (12.69%) and 1,8-cineole (4.79%) being the most prominent. In the oil of *O. suave*, sixty-five components were identified and quantified. The major compounds being benzene, 1,2,4-trimethoxy-5- (1-propenyl) - (Z) - (22.79%); β-bisabolene (19.10%) and spathulenol (18.69%). Detected eugenol (8.2%) and one of its derivatives, 3,4,5-trimethoxyallylbenzene (33%) in *O.suave* essential oil. In previous investigation using sample from Tanzania,eugenol was shown to be the most common compound (71.5%) (Chogo and Crank, 1981). The discrepancies in the oils content could be attributed to genetic and environmental factors (Bakkali *et al.*, 2008).

Effects of essential oils on mycelia growth of *Aspergillus flavus* and *Aspergillus niger*

The inhibition of mycelia growth of the fungus increased significantly ($p < 0.05$) with the essential oils concentrations (Table 2). With the essential oil of *O. gratissimum.* L, total inhibition of *Aspergillus flavus* and *Aspergillus niger* is seenat 5500ppm and 4750 ppm, respectively, which is the Minimum Inhibitory Concentrations (MIC). The observed effect of this essential oil could be linked with the presence of compounds such as methyl eugenol, thymol and carvacrol with proven antimicrobial properties (Janine de Aquino Lemos *et al.,* 2005). The MICs reported in this case for *O. gratissimum.* L (5500ppm and 4750ppm) are lower than those found by Degnon *et al.,* (2000) (7500ppm) when assessing the antifungal activity of the essential oil of the same plant on *Aspergillus candidus* isolated from smoked *Trachurus trachurus* (horse mackerel). Differences in MIC between studies could be explained by a variety of factors, including the plant's harvest site which affect the plant chemical makeup of the plant. As previously reported, the plant chemical composition is an indicator of their antimicrobial activities (Lopez *et al.,* 2020). As for the essential oil of *O. suave* MIC of 6000 ppm and 5500 ppm were obtained against *Aspergillus flavus* and *Aspergillus niger*, respectively.

Table.1 Chemical composition of essential oils

Compounds	*O. gratissimum*	*O. suave*	*A. alboviolaceum*	*Z. officinale*
Monoterpenes	**88.13**	**11.78**	**75.12**	**90.37**
Hydrocarbonmonoterpenes	**39.13**	**1.02**	**10.65**	**1.04**
α-Thujene	2.60	/	0.15	/
α-pinène	1.49	0.07	4.86	/
Camphène	0.13	/	/	1.04
Sabinene	0.75	/	0.44	/
β-Pinène	0.38	0.04	2.03	/
Myrcène	3.44	/	1.42	/
p-mentha-1(7), 8-diène	0.02	/	/	/
α-phellandrene	0.23	/	/	/
Bicyclo[4.1.0]Hept-3-ène, 3.7.7-Trimethyl-	0.16	/	/	/
1.3-cyclohexadiène, 1-methyl-4-(1-	2.36	/	/	/
methylethyl)-	/	0.36	/	/
1.6-Octadiène	17.86	0.19	/	/
p-Cymène	1.24	0.36	/	/
Limonène	0.24	/	/	/
(E)-β-Ocimene	5.90	/	1.58	/
α-Terpinene	/	/	0.04	/
γ-Terpinene	0.14	/	0.13	/
Cyclohexene, 1-methyl-4-(1-methylethylidene)	2.16	/	/	/
Benzene, 1-methyl-4-(1-methylethenyl)-	0.03	/	/	/
Cycloheptane, 1.3.5-tri(methylene)-	/	/	/	/
Monoterpènes oxygénés	/	/	/	/
3-méthyl-2-(2-méthy-2-butenyl)-Furan	**49.00**	**10.76**	**64.47**	**89.33**
Linalool	/	0.22	/	/
Benzene, 1-Methoxy-4-(2-Propenyl)	1.16	0.27	1.37	4.07
β-Cyclocytral	/	0.16	/	/
Neral	/	0.08	/	/
2-Cyclohexen-1-one	1.20	0.09	/	23.79
Geraniol	1.00	0.13	/	/
Geranial	0.18	0.07	/	2.66
Isogeranial	/	0.16	0.07	34.79
5-Allyl-2-Méthoxyphenol	/	/	/	2.14
4-Thujanol	/	9.58	/	/
Carveol	2.92	/	0.21	/
Thujone	0.19	/	/	/
Cis-p-menth-2-en-1-ol	0.40	/	/	/
Trans-chrysanthemol	0.12	/	/	/
Trans-3(10)-caren-2-ol	0.08	/	/	/
Bicyclo[2.2.1]heptan-2-ol, 1.7.7-Trimethyl-	0.24	/	/	/
3-Cyclohexen-1-ol, 4-methyl-1-(1-	0.43	/	/	/
methylethyl)-	1.93	/	/	/

Verbenone	0.78	/	/	/
m-cymen-8-ol	0.52	/	/	/
3-cyclohexen-1-methanol	0.44	/	/	/
6-octen-1-ol, 3.7-dimethyl	0.23	/	/	/
3.7-dimethyl-2.6-octadienal	0.35	/	/	/
Thymol	36.71	/	/	/
Phenol, 2-methoxy-3-(2-propenyl)-	0.12	/	/	/
1.8-cinéole	/	/	/	/
δ-terpineol	/	/	58.90	5.02
4-terpineol	/	/	0.21	/
α-terpineol	/	/	0.45	/
Borneol	/	/	3.33	3.34
Eproxy-linalooloxide	/	/	/	9.04
Citronellol	/	/	/	1.11
Citronellal	/	/	/	1.79
Camphor	/	/	/	1.01
	/	/	/	0.57
Sesquiterpenes	**11.07**	**63.35**	**13.45**	**4.63**
Hydrocarbonsesquiterpenes	**8.87**	**33.25**	**3.42**	**0.55**
α-Cubebene	0.13	0.42	/	/
Copaene	1.03	3.37	/	/
Cyclobuta[1.2.3.4]Dicyclopentène	/	1.44	/	/
β-Copaene	/	1.02	/	/
Cis-Thujobsadiène	/	0.26	/	/
Cis-α-Bergamotène	/	0.16	/	/
Bicyclo[7.2.0]Undec-4-ène	2.95	2.84	/	/
β-Cubebene	0.44	0.56	/	/
Cis-α-Bergamotène	/	0.07	/	/
Trans-β-Bergamotène	/	1.06	/	/
9-epi-(E)-Caryophyllene	/	1.05	/	/
γ-Muurolène	/	0.10	/	/
1.6-Cyclodecadienne, 1-methyl-5-methylène-8-	0.10	0.69	/	/
(1-methylethyl)-	/	/	/	/
Cis-Muurola-4(14), 5-diène	/	0.08	/	/
(Z)-α-Bisabolene	/	/	0.53	/
β-Bisabolene	/	19.10	0.2	/
(E)-γ-Bisabolene	/	/	0.07	/
D-Cadinène	/	0.62	/	/
Sesquisabinène	/	0.26	/	/
α-Calacorene	/	0.15	/	/
Trans-α-bergamotène	0.08	/	/	/
1.4.8-Cycloundecatriène	0.42	/	/	/
Prezizaene	0.05	/	/	/
β-selinene	2.13	/	/	/
α-selinene	0.92	/	/	/
Naphtalene	0.62	/	/	/

(E)-caryophillene	/	/	/	0.22
Isocaryophillene	/	/	2.2	/
(Z)-β-Santalol	/	/	0.29	/
Bicycloelemene	/	/	0.13	/
Ar-curcumene	/	/	/	0.33
Oxygenated sesquiterpenes	**2.2**	**30.10**	**10.03**	**4.08**
Epi-Cubebol	0.18	0.80	/	/
Caryophylleneoxide	/	0.76	/	0.64
1.6.10-Dodecatrien-3-ol, 3.7.11-Triméthyl-	/	0.25	/	/
Spathulenol	/	18.69	0.11	/
Salvial-4(14)-en-1-one	/	0.87	/	/
3.7.11-Trimethyldodeca-6.10-Dien-1-yn-3-ol	/	0.37	/	/
Humuleneepoxide II	0.12	2.04	/	/
6-Isopropenyl-1,4, 8a-dimethyl-1.1.2.3.5.6.7.8.	/	/	/	/
8a-Octahydronaphthalen-2-ol	/	0.79	/	/
Cis-Lanceol	/	1.38	/	/
Di-epi-1.10-Cubenol	/	0.17	/	/
4.6.6-Trimethyl-2-(3-Methyl-Buta-1.3-Dienyl)-	/	0.14	/	/
11.11-Dimethyl-4.8-	/	0.25	/	/
Dimethylenebicyclo[7.2.0]Undecan-3-ol				/
Alloaromadendrenoxid-(1)	/	0.45	/	/
Cadin-4-en-10-ol	0.20	0.40	/	/
Caryophyllene Oxide	1.50	0.40	/	/
(1R.7S.E)-7-Isopropyl-4.10-	/	1.46	/	/
dimethylenecyclodec-5-enol	/	0.18	/	/
Curlone	/	0.13	/	/
14-Hydroxy-9-epi-(E)-Caryophyllene	/	0.58	/	/
(R)-2-methyl-5-(6-methylhepta-1.5-dien-2-		/	/	/
yl)cyclohex-2-enone	0.05	/	/	/
Sesquisabinene hydrate	0.15	/	/	/
Trans-Sesquisabinene hydrate	/	/	0.25	0.51
13-Hydroxy-Valencene	/	/	0.44	0.39
α-Elenol	/	/	/	/
Cis-nerolidol	/	/	2.1	/
Longifolol	/	/	0.63	/
α-Eudesmol	/	/	0.17	/
β-Eudesmol	/	/	0.21	/
α-Bisabolol	/	/	/	0.34
β-Bisabolol	/	/	0.45	/
Cis-mayol	/	/	3.36	/
Santalol	/	/	1.83	/
Spathulenol	/	/	0.38	/
(E)-nerolidol	/	/	0.11	/
Zingiberenol	/	/	/	0.45
Cis-carveol	/	/	/	0.58
Cedroxyde	/	/	/	0.39

Viridiflorol	/	/	/	0.41
	/	/	/	0.37
Aliphatic and linear compounds	**0.79**	**24.87**	**11.42**	**4.99**
Phenol-3.5-diméthyl-	/	0.13	/	/
1-Octen-3-ol	0.06	0.18	/	/
5-Hepten-2-one	/	0.58	/	0.73
Trans-2-(2-pentenyl) furan	/	0.21	/	/
Nonanal	/	0.08	/	/
2-Nonanone	/	/	/	0.35
Nonan-2-ol	/	/	/	0.46
Nona-1.3.7-triène <4.8- diméthyl-, (E)- >	/	0.07	/	/
Dispiro [2.6.2.5]Undecane,10-Methylen-	/	0.11	/	/
Nona (2E, 6Z)-dienal	/	0.05	/	/
Benzène, 1.2-Diméthoxy-4-(2-propényl)-	/	0.10	/	/
6.10-Diméthyl-5.9-Undecadien-2-one	/	0.13	/	/
Androstan-17-one	/	0.08	/	/
Benzène, 1.2.4-Triméthoxy-5-(1-propenyl)-	/	22.79	/	/
1-Cyclohexen-1-propanal-2.6.6-Trimethyl-	/	0.24	/	/
2-Pentadecanone-6.10.14-Trimethyl-	/	0.12	/	/
2-hydroxy-2-methyl-4-pentanone (diacetone)	0.05	/	/	/
2-hexenal	0.05	/	/	/
3-hexen-1-ol	0.05	/	/	/
Heptan-2-ol	0.02	/	0.82	1.09
3-octanone	0.43	/	/	/
Ethyl-hexanol	0.03	/	/	/
Anisole, 2-isopropyl-5-methyl-	0.10	/	/	/
Linalyl acetate	/	/	0.40	/
δ-Terpinyl acetate	/	/	0.55	/
α-Terpinyl acetate	/	/	9.58	/
2-undecanone	/	/	/	0.4
Dodecanoic acid	/	/	/	0.42
Epoxy-linalooloxide	/	/	/	0.38
Geranylacetate	/	/	/	1.04
Geranicacid TMS	/	/	/	0.12
Total	**100**	**100**	**99**	**99**
Yield(%)	**0.71**	**0.48**	**0.79**	**0.45**

Table.2 Mean values of the percentages of growth inhibition at different concentrations of essential oils.

Conc (ppm)	*O. s* on *A.f*	*O. suave*	*O. gratissimu On A.niger*	*O.gratissimum On A.niger*	*A. alboviolaceum*	*A. alboviolaceum*	*Z. officinale*	*Z. officinale*	*O. gratissimum*	*Z. officinale*
500	41.27±27.67^{d}	32.37±24.76^{c}	/	/	31.54 ±2.72^{c}	33.97±1.14^{e}	38.65 ±2.32^{c}	30.94 ±1.04^{d}	/	38.65 ±2.32^{c}
1000	62.3±5.63^{c}	73.9±5.82^{b}	/	/	63.09±1.53^{b}	39.60±1.60^{d}	45.79 ±1.03^{c}	33.37 ±1.22^{d}	/	45.79 ±1.03^{c}
2000	80.56±0.69^{b}	83.07±1.13^{ab}	/	/	84.44± 3.05^{ab}	65.06±0.91^{cd}	79.10 ±0.32^{b}	68.42±0.81^{c}	/	79.10 ±0.32^{b}
3000	88.69±1.57^{ab}	91.43±0.90^{a}	/	/	91.28±0.70^{a}	75.35±1.32^{bc}	93.25 ±3.07^{a}	80.04±1.63^{ab}	/	93.25 ±3.07^{a}
4000	93.65±1.82^{a}	92.83±1.19^{a}	91.67±2.38^{c}	96.81±1.82^{b}	94.63±0.55^{a}	81.48±1.50^{b}	96.63±0.71^{a}	93.74±1.17^{a}	91.67±2.38^{c}	96.63±0.71^{a}
4250	/	/	/	98.2±0.61^{ab}	/	/	/	/	/	/
4500	/	/	/	99±0.92^{a}	/	/	/	100±0.00^{a}	/	/
4750	/	/	/	100±0.00^{a}	/	/	/	/	/	/
5000	93.85±0.34^{a}	96.22±0.33^{a}	95.83±0.60^{b}	/	96.69±0.14^{a}	96.25±1.93^{a}	100 ±0.00^{a}	/	95.83±0.60^{b}	100 ±0.00^{a}
5250	/	97.41±0.36^{a}	98.8±1.19^{a}	/	/	100±0.00^{a}	/	/	98.8±1.19^{a}	/
5500	95.83±0.60^{a}	100±0.00^{a}	100±0.00^{a}	/	/	/	/	/	100±0.00^{a}	/
5750	97.22±0.69^{a}	/	/	/	100±0.00^{a}	/	/	/	/	/
6000	100±0.00^{a}	/	/	/	/	/	/		/	/

The results are presented in the form of mean ± standard deviation. Values with different letters differ significantly at p<0.05

Fig.1 Chromatogram of chemical analysis of *O. gratissimum. L* essential oil.

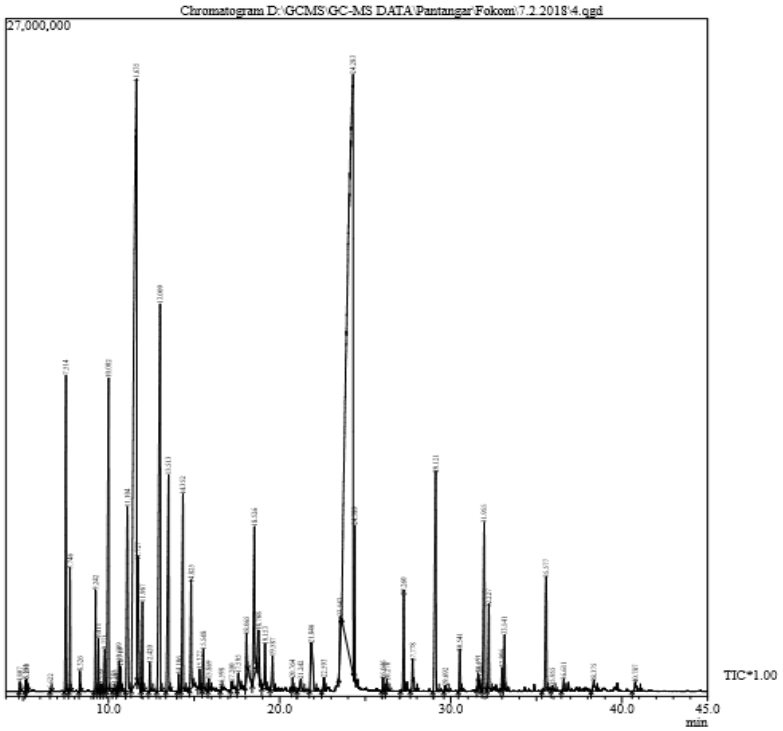


Fig.2 Chromatogram of chemical analysis of *O. suave. L* essential oil.

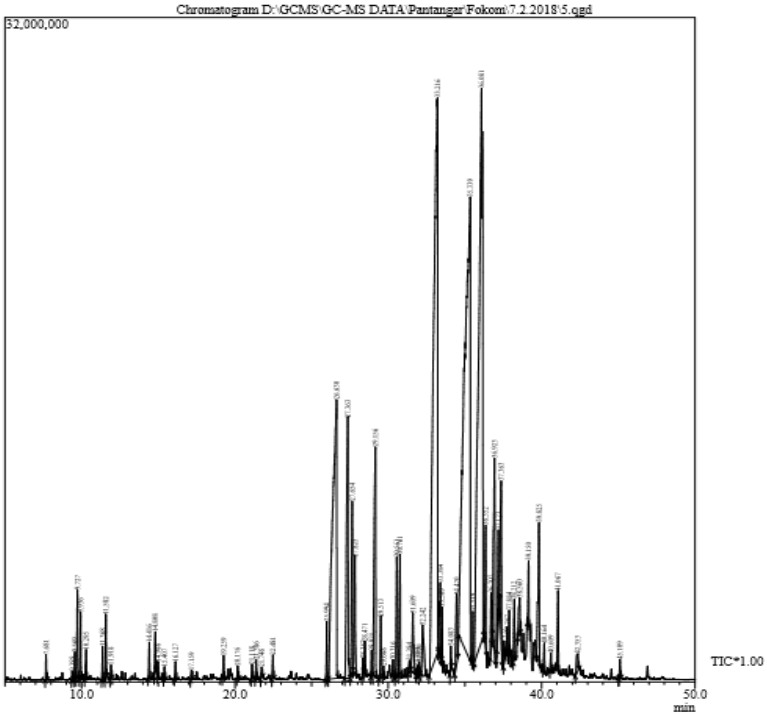


Fig.3 Chromatogram of chemical analysis of *A. alboviolaceum* essential oil.

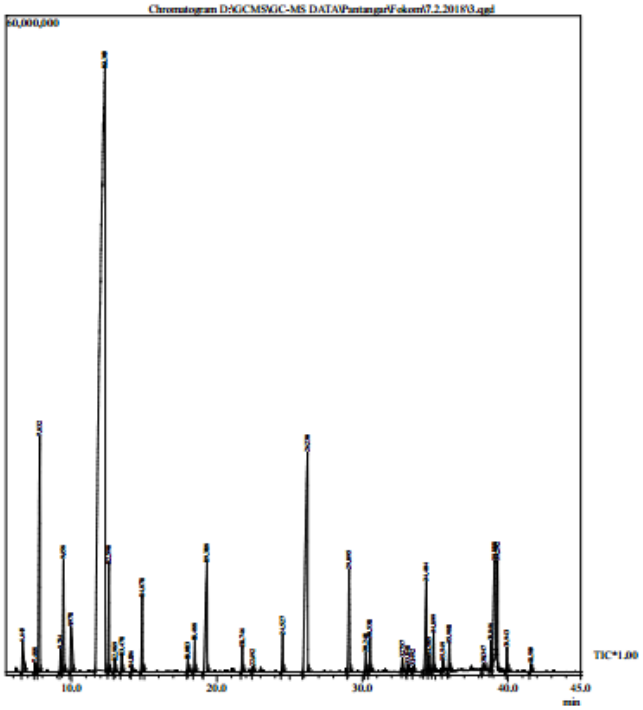
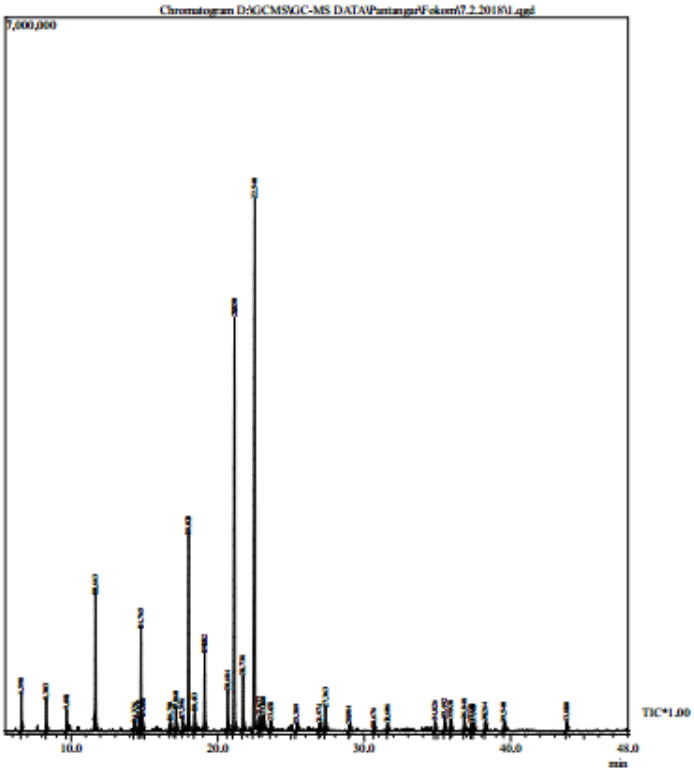


Fig.4 Chromatogram of chemical analysis of *Z. officinale* essential oil.



The antifugal activity of the essential oil of *O. suave. L* could be linked to the presence of spathulenol, bisabolene, benzene, 1.2.4-trimethoxy-5- (1-propenyl) - (Z) - (eugenol derivative) as well as the synergy between all volatile constituents.**)**

The synergistic reactions between the different compounds can lead to a substantial higher level of activity than predicted by individual compound (Gueldener *et al.*, 1985).

These obtained results corroborate those of Ekoue (2011) who investigated the activity of an *Ocimum* species (*O. canum*) essential oil on *A. flavus* isolated from *Ethmalosa fimbriata* (Bonga) and obtained a MIC of 4000ppm, which is not substantially different ($p > 0.05$) from those obtained in this study or *Aspergillus flavus* and *Aspergillus niger*, a complete inhibition was observed at the concentrations of 5750ppm and 5250ppm, for the essential oil of *A. alboviolaceum*. The essential oil of *Z. officinale* totally inhibits *Aspergillus flavus* and *Aspergillus niger* at 5000ppm and 4500ppm, respectively. The antifungal activity of these oils could be associated with the presence of compounds such as geranial, neral, α-pinene and 1.8-cineole with demonstrated antifungal properties (Singh *et al.*, 2008).

The essential oil of *O. suave*, *O. gratissimum*, *A. alboviolaceum* and *Z. officinale* were extracted, their chemical composition characterized and their antifungal properties tested against *Aspergillus flavus* and *Aspergillus niger*. After seven days of incubation at 25°C, minimums Inhibitory Concentrations (MIC) were found to be between 4500ppm and 6000ppm.

These results obtained support the use of these essential oils to preserve smoking fish from moulds alterations. However, further studies should be conducted to evaluate their efficacy *in situ*.

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