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An investigation of chemical composition and antimicrobial activity of essential oils extracted from *Aeollanthus and Plectranthus* species

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ABSTRACT

Essential oils from plants represent a rich source of new antimicrobials that are able to overcome the microbial virulence. The present study was designed to determine the chemical compositions and assess antimicrobial potentials of essential oils derived from *Aeollanthus cucullathus* (Ryding), *Aeollanthus heliotropioides* (Oliv.) and *Plectranthus glandulosus* (Hook. F.) against *Candida* species biofilm formation. Essential oils were extracted using hydrodistillation followed by the chemical composition determination using gas chromatography coupled with mass spectrometry (GC-MS). The minimum inhibitory concentration (MIC) was determined using a microdilution method. Then, the antibiofilm assay was performed in two stages; germ tubes and mature biofilm formation by a micro biofilm inhibition assay. Extraction yields were 0.0001%; 0.07% and 0.04%, respectively for *Aeollanthhus cucullathus* (ACap), *Aeollanthus heliotropioides* (AHap) and *Plectranthus glandulosus* (PGl) essential oils (EOs). The chemical analysis revealed the presence of naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylet henyl)-, [2R-(2.alpha.,4a.alpha.,8 a.beta.)]- (6.71%), caryophyllene oxide (5.12%), 2-Isopropyl-5-methyl-9-methylenebicyclo[4.4.0]dec-1-ene (4.95%) in ACap EOs; linalool (25.67%), farnesene (13.20%), caryophyllene (6.02%) in AHap EOs and germacrene D (9.90%), (E)-3-hexenyl butyrate (9.30%), L-fenchone (8.75%) in PGl EOs. MIC values ranged from 0.31 mg/mL to 5 mg/mL respectively for AHap and PGl EOs. The inhibition of pseudo-hyphae formation revealed an activity up to 0.03 mg/mL for AHap EO on *C. albicans*. All the EOs inhibited the mature biofilm formation at subinhibitory concentrations. This study highlighted the possible uses of *A. heliotropioides, A. cucculathus* and *P. glandulosus* for therapeutic agent's development.

1. Introduction

The impact of fungal infections is increasing with the number of immunocompromised people, especially those living with HIV (Binder and Lass-Flörl, 2011; Ortega et al., 2011; Vandeputte et al., 2012). The rate of HIV/AIDS infected people is higher in Sub-Saharan Africa than the other world regions, which makes such population more vulnerable to opportunistic candidiasis (Unaids and Who, 2009). *Candida albicans* (*C. albicans*) induces more than 60% of infections, which leads to 40% of death despite the use of conventional drugs (Mensa et

Abbreviations: ACap, Aeollanthhus cucullathus, AHap, Aeollanthus heliotropioides, PGl, Plectranthus glandulosus, EOs, Essential oils, MIC, Minimum Inhibitory Concentration

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al., 2008; Richardson and Medicine, 2008; Zaoutis et al., 2005). Among the limited classes of antifungal drugs available, most of them have produced antimicrobial resistance (Sardi et al., 2013; Spampinato and Leonardi, 2013), which is mediated by the expression of virulence factors, especially with biofilm formation (Fanning and Mitchell, 2012). Biofilms are sets of microbial communities, which constitute a complex three-dimensional structure, helping the microorganisms to strengthen their pathogenicity (Ramage et al., 2006). The expression of pathogenic microorganisms as biofilms is a kind of microbial protection allowing their development, a symbiotic relationship and survival in hostile environments (Davey and O'toole, 2000). Biofilms maintain the pathogenicity of fungi by evading host immune mechanisms, resisting antifungal treatment which leads to a high level of antifungal resistance of organisms (Ozkan et al., 2005). The rise in the prevalence of anticandidal resistance has increased the need for new and effective alternative treatments, which specifically target the resistance factors implicated in *Candida* drug tolerance.

The World Health Organization (WHO) has stated that 80% of the world's population depend on traditional medicine for primary healthcare (WHO, 2002). There is significant knowledge about the use of medicinal plants in Cameroon. More than two hundred plants are used to treat infectious diseases in the country (Nkongmeneck et al., 2007; Jiofack et al., 2010). In general, EOs from traditional plants and those from *Lamiaceae* family in Cameroon have been widely studied for their antifungal activities (Dongmo et al., 2008; Hzounda Fokou et al., 2014; Ngo Mback et al., 2016). *Plectranthus* and *Aeollanthus* plants are commonly used for medicinal purposes to treat infectious diseases. Also, these plants have not been explored fully (Bouquet, 1969; Gradé et al., 2009). Thus, the present study has hypothesized that the EOs from *Aeollanthus cucullathus* (*A. cucullathus*), *Aeollanthus heliotropioides* (*A. heliotropioides*) *and Plectranthus glandulosus* (*P. glandulosus*) which are rich in multiple bioactive compounds that act synergistically to produce the desired activity and fight against the resistance phenomena could constitute a viable alternative. The main objective of the present study was to determine the chemical compositions and antimicrobial potentials of *A. cucullathus, A. heliotropioides and P. glandulosus* EOs. The specific objectives were to determine the chemical composition of the EOs by GC-MS and to determine the antibiofilm activities of EOs on *Candida* species via microplate biofilm assay.

2. Materials and methods

2.1. Plant material and yeasts

Vegetal material from Cameroon was harvested in Fongo Tongo (Ouest Region), Obala (Centre Region) and Mayo-Darle (Adamaoua Region) respectively for *A. Cucullathus* (24622SRFCAM), *A. heliotropioides* (42756HNC) and *P. Glandulosus* (7656/SRF/Cam). These plants have been identified at the National Herbarium of Cameroon (NHC/HNC) by comparison with the specimens recorded previously. The two yeast microorganisms used were obtained from Yaoundé Central hospital (*Candida glabrata* (*C. glabrata*) *44B* and *C. albicans 141S*), which were isolated from HIV/AIDS patients.

2.2. Extraction of EOs

Fresh leaves of *P. glandulosus* (PGl) and aerial parts of *A. heliotropioides* (AHap) and *A. cucullathus* (ACap) were subjected to a hydrodistillation extraction for 3–4 h using a Clevenger type apparatus as described in previous studies (Ndoye, 2001). The plant material was boiled in water. During steam distillation, the volatile plant constituents were vaporized and then, condensed on cooling to produce an immiscible mixture of an oil phase and an aqueous phase. Then, the EO (oil phase) mixture was removed by decantation. The oil phase obtained was purified over an anhydrous sodium sulphate column. The dried EOs were then kept in opaque bottles at 4 °C until use. The yield of EOs was calculated as a percentage (w/w (%)) according to the total weight of the initial, fresh plant material.

2.3. Chemical analysis (GC/MS)

GC-MS analysis was performed on an Agilent 5977A MSD and 7890B GC System, Chemetrix (pty) Ltd; Agilent Technologies, DE (Germany) with a Zebron-5MS column (ZB-5MS 30 m × 0.25 mm x 0.25 μm) (5%-phenylmethylpolysiloxane). The following column and temperature conditions were used: GC grade helium at a flow rate of 2 mL/min and splitless 1 ml injections were used. The injector, source and oven temperatures were set at 280 °C and 70 °C, respectively. The ramp settings were 15 °C/min to 120 °C, then 10 °C/min to180 °C, then 20 °C/min to 270 °C and held for 3 min. The identification of the EO compounds was performed using GC-MS-HP 6890 (GC-MS- HP 6890) and a mass selective detector (HP5973). The interpretation of data was performed using NIST online library and Kovats index was also calculated (Adams, 2007).

2.4. Microdilution for determination of MIC

A 96 well-plate microdilution method was used with sabouraud dextrose broth (SDB) medium as described by CLSI (2008) and Omoruyi et al. (2014) with some modifications. Different concentrations of EOs, ranging from 0.005 to 5 mg/mL, were poured into the wells. A solution of 0.5 McFarland fungal cells was prepared to reach the final inoculated concentration of 2.5×10^3 CFC/mL in the wells except those with sterile medium (blank). All the treatments were performed in triplicates. The growth of the fungi was determined by measuring the absorbance at 600 nm and visual observation was performed. The plates were incubated at 37 °C for 48 h. The lowest concentration at which no visible growth was observed and was considered as the minimum inhibitory concentration (MIC). For performing the inhibition of germ tubes and biofilm formation assay, subinhibitory concentrations were prepared from visual MIC values obtained.

2.5. Inhibition of pseudohyphae development

The effects of EOs at different subinhibitory concentrations on pseudohyphae development ability of test microorganisms were studied using sterile fetal bovine serum (FBS). A solution of 100 μl of *C. albicans* suspension (2.5×10^3 CFU/mL) was introduced into FBS in the absence and presence EOs, respectively. The as-prepared mixtures were incubated at 37 °C for 4 h; and pseudohyphae formation and morphological changes were subsequently observed using an optical microscope (Kretschmar et al., 1999).

2.6. Inhibition of mature biofilm formation

The effects of EOs at subinhibitory concentrations on biofilm forming ability of test microorganisms were investigated using a microplate biofilm assay as described by Ngo-Mback et al. (2019) with some modifications. Briefly, 100 μL of overnight cultures of isolates was added into 100 μL of fresh SDB supplemented with 6% glucose and cultivated in the presence and absence of EOs, and, then incubated for 48 h at 37 °C. The wells containing SDB + cells served as control. After incubation, the wells were washed with distilled water to remove the planktonic cells. The remaining yeasts were subsequently stained with 0.1% crystal violet solution for 20 min at room temperature. The wells were washed once again to remove the crystal violet solution. The plates were dried at room temperature for 2 h followed by the addition of 250 μL of 33% glacial acetic acid into the wells. After 20 min, the optical density (OD) of each well was mea-

sured at 590 nm (Thermo Scientific Multiskan FC, Vantaa, Finland). Then, the proportional optical densities were recorded at 590 nm and were plotted against the corresponding subfraction concentrations.

3. Results

3.1. Extraction of EOs

The results from Table 1 have shown that the yield of EOs ranged from 0.0001% to 0.07% for 100 g of vegetable material used. AHap EO (0.07%), has shown a higher extraction yield than PGl EO (0.004%) followed by ACap EO (0.0001%) which showed the lowest yield.

3.2. Chemical compounds

From the GC-MS analysis of the EOs, 88, 74 and 76 compounds were identified in ACap, PGl and AHap EOs respectively. The major compounds are presented in Table 2. The secondary metabolites identified in these samples are aromatic compounds mainly monoterpenes and sesquiterpenes. The presence of linalool (25.67%), farnesene (13.20%), caryo phyllene (6.02%), terpineol (4.88) and germacrène D (4.14%) as main compounds in AHap EO was observed. Concerning the second sample (ACap EO) from the same genus as the AHap sample, five major compounds were highlighted. They included naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylet henyl)-, [2R-(2.alpha.,4a.alpha.,8 a.beta.)]- (6.71%), caryophyllene oxide (5.12%), 2-Isopropyl-5-methyl-9-methylenebicyclo[4.4.0]dec-1-ene (4.95%), 2-methyl-3-(3-methylbut-2-enyl)-2-(4-methylpent-3-enyl) oxetane (4.88%) and caryophyllene (4.71%). For PGl EO, the main compounds obtained were germacrene D (9.90%), (E)-3-hexenyl butyrate (9.30%), L-fenchone (8.75%), caryophyllene (8.39%) and Z-ocimene (7.55%). These three EOs from the *Lamiaceae* family plants shared two hydrocarbonated sesquiterpenes stereoisomers ($C_{15}H_{24}$) as caryophyllene for all the analysed EOs and germacrene D for AHap and PGl EOs. Hence, AHap, ACap and PGl EOs from the same family had some chemical similarities with respect to their secondary metabolite content. Moreover, it was observed that ACap EO was constituted of terpenes with higher molecular masses (Triacontyl acetate $C_{32}H_{64}O_2$) than the other EOs. ACap EO also highlighted a great complexity because of its higher number of compounds (88 compounds) followed by AHap EOs (76 compounds). These results showed that the *Aeollanthus* EOs were very complex compared to *P. glandulosus* species studied.

3.3. Minimum inhibitory concentration (MIC)

The obtained results shown in Table 3, reveal the anticandidal activity of the tested EOs. The minimum inhibitory concentration (MIC) assay was performed using the microplate reader spectrophotometer and with the observations of visible growth. The MIC values ranged from 0.31 ± 0.00 mg/mL to >5 mg/mL. AHap EO was the most active with a MIC value of 0.31 ± 0,00 mg/mL for *C. albicans* and 0.62 mg/mL for *C. glabrata*. PGl EO exhibited a moderate activity from

Table 1
EOs extraction yield.

Plant parts	EO Extraction Yield (%)
AHap	0.07
ACap	0.0001
PGl	0.004

AHap: *Aeollanthus héliotropioides* aerial part; ACap: *Aeollanthus cucullathus* aerial part; PGl: *Plectranthus glandulosus* leaves; EO: Essential oil.

Table 2
Major compounds of EOs determined by GC-MS analysis.

S·NO	Compound	Chemical Formula	KI	Percentage of composition (EOs) (%)		
				AHap	ACap	PGl
1	Bicyclo[3.1.0]hexane, 6-methylene-	C_7H_{10}	–	–	–	4.16
2	3,4-Dimethyl-3-cyclohexene-1-carboxaldehyde	$C_9H_{14}O$	1256	–	3.93	–
3	(R)-α-pinene	$C_{10}H_{16}$	920	2.69	–	–
4	Z-Ocimene	$C_{10}H_{16}$	979	–	–	**7.55**
5	β-Ocimene	$C_{10}H_{16}$	984	–	–	7.00
6	beta.-Myrcene	$C_{10}H_{16}$	961	–	–	3.82
7	L-Fenchone	$C_{10}H_{16}O$	1005	–	–	**8.75**
8	Citral	$C_{10}H_{16}O$	1075	2.28	–	–
9	Linalool	$C_{10}H_{18}O$	1006	**25.67**	–	–
10	alpha.-Terpineol	$C_{10}H_{18}O$	1061	**4.88**	–	–
11	Geraniol	$C_{10}H_{18}O$	1086	3.05	–	–
12	(E)-3-hexenyl butyrate	$C_{10}H_{18}O_2$	1045	–	–	**9.30**
13	(E)-.beta.-Famesene	$C_{15}H_{24}$	1106	**13.20**	–	–
14	Humulene	$C_{15}H_{24}$	1112	3.12	–	–
15	Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene	$C_{15}H_{24}$	1160	2.98	–	–
16	Alloaromadendrene	$C_{15}H_{24}$	1114	–	3.01	–
17	2-Isopropyl-5-methyl-9-methylenebicyclo[4.4.0]dec-1-ene	$C_{15}H_{24}$	1161	–	**4.95**	–
18	Caryophyllene	$C_{15}H_{24}$	1102	**6.02**	**4.71**	**8.39**
19	Germacrene D	$C_{15}H_{24}$	1119	**4.14**	–	**9.90**
20	β-Bisabolene	$C_{15}H_{24}$	1122	–	–	6.62
21	Dodecane, 2,6,11-trimethyl-	$C_{15}H_{32}$	1169	–	2.99	–
22	Caryophyllene oxide	$C_{15}H_{24}O$	1148	–	**5.12**	–
23	2-methyl-3-(3-methylbut-2-enyl)-2-(4-methylpent-3-enyl)oxetane	$C_{15}H_{26}O$	1165	–	**4.88**	–
24	β-Dihydroagarofuran	$C_{15}H_{26}O$	1127	–	4.37	–
25	4-*epi*-cubedol	$C_{15}H_{26}O$	1128	–	–	3.91
26	Triacontyl acetate	$C_{32}H_{64}O_2$	–	–	3.38	–
27	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylet henyl)-, [2R-(2.alpha.,4a.alpha.,8 a.beta.)]-	–	–	–	**6.71**	–

AHap: *Aeollanthus héliotropioides*aerial part; ACap: *Aeollanthus cucullathus* aerial part; PGl: *Plectranthus glandulosus* leaves; KI: Kovat Index; EOs: Essential oils-: Undetermined.

Table 3
Minimum Inhibitory Concentration (MIC) values (mg/mL) of EOs.

	MIC Visual observation (mg/mL)		MIC Microplate Reader (mg/mL)	
	V *C. glabrata*	V *C. albicans*	DO *C.albicans*	DO *C. glabrata*
ACap	$>5^{c,d}$	$>5^{c,d}$	$>5^{c,d}$	$>5^{c,d}$
PGl	$5 \pm 0.00^{a,d}$	$3.75 \pm 1.77^{a,d}$	$5 \pm 0.00^{a,d}$	$5 \pm 0.00^{a,d}$
AHap	$0.62 \pm 0.00^{a,d}$	$0.31 \pm 0.00^{a,d}$	$1.25 \pm 0.39^{a,d}$	$0.62 \pm 0.24^{a,d}$
NYST*	$0.06 \pm 0.00^{b,d}$	$0.03 \pm 0.00^{b,d}$	$0.03 \pm 0.00^{b,d}$	$0.06 \pm 0.00^{b,d}$

V: Visual observation; DO: Optical density read with a microplate reader at 600 nmwavelenght; (a, b, c, d) the same letter mean there is no significant difference $P^{>}0.05$; NYST*: Nystatin.

3.75 ± 1.77 mg/mL on *C. albicans* to 5 mg/mL on *C. glabrata*; while ACap EO was the less active one with values greater than 5 mg/mL on both pathogens. There were significant differences (P-value$^{<}$0.05) between the tested antimicrobial agents. However, there were no significant differences between the two MIC revelation methods (Micro reader plate and visual observation) used (P′-value$^{>}$0.05).

3.4. Inhibition of pseudohyphae development

The starting inhibitory concentration for ACap EO to inhibit germ tubes and biofilms formation was set at 30 mg/mL; because its MIC was greater than 5 mg/mL. The pseudohyphae formation inhibition was assessed by the reduction of germ tube formation by EOs at different concentrations. The EOs from the *Aeollanthus* genus were more potent on pseudohyphae inhibition up to 4 times reduced MIC values. *Aeollanthus* species EOs have shown the best inhibition potentials on the formation of pseudohyphae. According to the previous micrograph (Fig. 1), it was observed that *A. heliotropioides* and *A. cuccullathus* EOs respectively at 0.078 mg/mL and 1.25 mg/mL led to the inhibition of biofilm formation. For *P. glandulosus* EOs, the minimum inhibitory pseudohyphae concentration was the same as the MIC concentration (3.75 mg/mL) at which no filaments were visible.

3.5. Inhibition of mature biofilm

The inhibition of mature biofilms formation is recorded in Figs. 2–4. *A. heliotropioides* EO has shown the best antibiofilm activity. The results obtained also confirmed the higher virulence and resistance of *C. glabrata* isolated from HIV + patients than *C. albicans*. *C. glabrata* exhibited greater growth in comparison with *C. albicans* as indicated by the higher optical density (OD) values greater than 1 (Figs. 3 and 4). However, the inhibition of its biofilm formation was effective up to 0.06 mg/mL by using AHap EO as shown in Fig. 2. It was observed that *C. albicans* required more stress conditions and produced a well-developed biofilm network. Further, PGl and ACap EOs also exhibited inhibitions of *C. glabrata* biofilm formation respectively at concentrations up to 0.5 mg/mL and 3 mg/mL.

4. Discussion

It has been shown in the literature that the dried leaves of *Aeollanthus* genus produced more EOs (Maia et al., 2003). The leaves of another *Aeollanthus* species (*A. pubescens*) exhibited a great extraction yield of EOs up to 1.5% (Alitonou et al., 2013). The EO extraction yield from the dried leaves of *P. glandulosus* from Cameroon was 0.46% (Nukenine et al., 2010) while *Plectranthus incanus* has shown 0.6% of EO yield (Pal et al., 2011). Some authors have highlighted that the quantity of EOs in a plant could be influenced by the species, the harvesting time and site, cultivation conditions, morphological parameters of the plant and the composition of the soil where the materials have been harvested (Aminzadeh et al., 2010). However, to the best of our knowledge, there are no available studies on the extraction of EOs from *A. cucullathus*. Alternatively, this low yield could be due to the delay in the time of extraction. There was no available hydrodistillation apparatus in the harvesting site and the plants have been sent to another town for extraction leading to a release of a greater quantity of volatile compounds. The fingerprints of AHap EOs obtained were the same as those of the review; linalool (38.5%) and

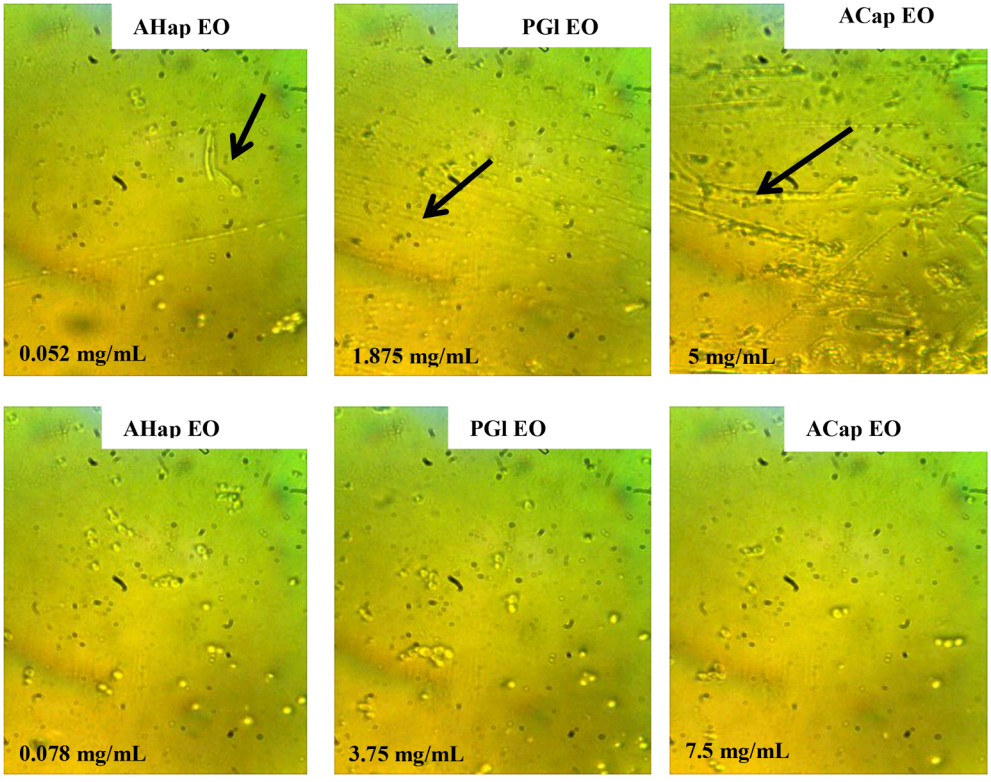
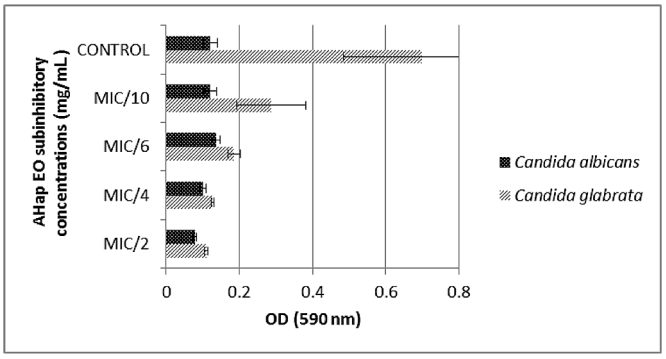
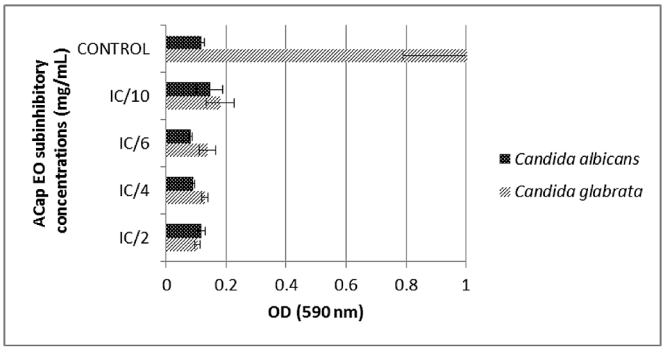


Fig. 1. Effect of EOs on pseudo-hyphae formation on *C. albicans*.



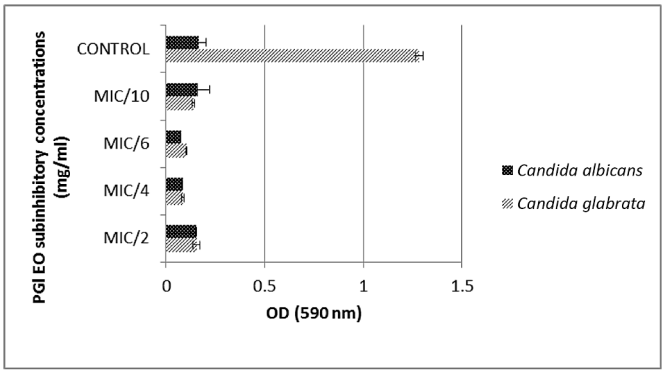
MIC=0.312 mg/mL (*C. albicans*); MIC=0.625 mg/mL (*C. glabrata*)

Fig. 2. Effect of AHap EO on *C. albicans* and *C. glabrata* biofilm formation.



IC=30 mg/mL (both pathogens)

Fig. 3. Effect of ACap EO on *C. albicans* and *C. glabrata* biofilm formation.



MIC=3.75 mg/mL (*C. albicans*); MIC=5 mg/mL (*C. glabrata*)

Fig. 4. Effect of PGl EO on *C. albicans* and *C. glabrata* biofilm formation.

Farnesene (25.1%) were the major compounds (Ngo Mback et al., 2016). There were some differences in terms of minor compounds. These differences could be due to the period of harvesting, which was not the same. It has been shown that the chemical compositions of EOs could vary according to the period in which the vegetal plant has been harvested (Goudoum et al., 2012). For PGl EOs, the major compounds were similar to those obtained by others from a species harvested in Cameroon. The authors have demonstrated fenchone (29.81%), alpha-terpinolene (28.29%), piperitenone oxide (11.08%) and beta-Myrcene (5.13%) as major compounds. The fingerprints were also conserved with similarities on two compounds that have been also identified in this study (fenchone (8.75%) and beta-myrcene (3.82%)). However, there were some compounds such as germacrene D (9.90%) which are found in a small amount (1.62%) in previous publications. Globally, the chemical content would be quite similar as reported in the literature. Among the major compounds of ACap EOs, the main one was naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2.alpha.,4a.alpha.,8 a.beta)]- (6.71%),a naphthalene derivative compound, which did not match with any of the standard library compounds.

Naphthalene is a compound, which is found in some plants and used as a positive control or reference product for repellent activity. Therefore, EOs from plants could constitute a greater source of naphthalene or its derivatives (Yuan et al., 2003). EOs from *Citrus grandis* at Cameroon has been characterised by the presence of *cis*-decahydronaphthalene (16.09%) a major compound that would inhibit the growth of *Anopheles gambiae* at a low concentration of 400 ppm (Akono et al., 2016). Some research activities on Chinese plants have also mentioned a high content of naphthalene derivatives in *Meconopsis punicea* (49.4%) and *Meconopsis delavayi* (42.6%) (Yuan et al., 2003). Because of the presence of naphthalene derivative as the major compound in ACap EOs, it could be possible for it to possess a considerable repellent activity.

Some scientific works have reported the antifungal activities of AHap EOs on 19 yeast pathogens and the MIC values ranged from 0.6 to 5 mg/mL (Ngo Mback et al., 2016). This MIC interval was almost similar to the one obtained in the present study (0.62 mg/mL to 1.25 mg/mL). It was observed that the AHap EOs exhibited better activities on *C. albicans* (MIC = 0.31 mg/mL) than the others. The antimicrobial activities of AHap EOs could be attributed to the presence of its major compounds namely, linalool and farnesene. Some other papers have highlighted the antifungal activities of linalool and farnesene against *C. albicans* ATCC10231 strains (Thakre et al., 2016). Linalool, particularly, acts at the level of membrane transport by inhibiting H+ extrusion from proton pumps. However, it is also demonstrated that the activity of EOs is not only due to their major compounds. It is the reason why some pure compounds tested alone exhibited less antifungal activity compared to their corresponding EOs. From the observation of MIC values, PGl EOs have also exhibited anticandidal activity (3.75 mg/mL-5 mg/mL) against *C. Albicans* and *C. glabrata*. Some studies have reported almost the same MIC values ranging from 0.8 mg/mL to 2 mg/mL (Aoudou et al., 2010). Rice et al. (2011) have also mentioned plants from *Plectranthus* genus with huge ethnobotanical usage (Rice et al., 2011). Concerning ACap EOs, to the best of our knowledge, there are no reported scientific studies on the antimicrobial activities of ACap EOs. The major compound of ACap EOs was a naphthalene derivative; naphthalene,1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R (2.alpha.,4a.alpha.,8 a.beta.)] (Simões et al., 2010). Some studies have reported that the naphthalene derivatives have repellent activities against *Anophele gambiae*, the vector for malaria (Akono et al., 2016). There are many antimicrobials manufactured with naphthalene as active compounds such as terbinafine, which is active against fungi (Rokade and Sayyed, 2009). However, in the present study, the major compound of ACap EOs was a naphthalene derivative, which did not exhibit a strong antifungal activity compared to the others. This could be due to an antagonistic interaction between all the components of EOs. Hence, in further studies, this main compound could be extracted and tested alone to screen its anticandidal potential. However, some researchers have investigated the solvent extract of *A. cucullathus* and found its anticandidal biofilm activity up to 0.06 mg/mL (Ngo-Mback et al., 2019).

The results obtained highlighted the antibiofilm activities of *Lamiaceae* plants through the inhibitions of virulence factors. Especially, AHap EO exhibited the greatest antibiofilm potential (0.06 mg/mL) followed by PGl EO (0.5 mg/mL) and then, *A. cucullathus* (3 mg/mL) on *C. glabrata*. In the same way, several plants have shown antibiofilm activities and some of them are traditionally used to treat resistant candidiasis. *Mentha piperica* exhibited minimum antibiofilm property at the concentration of 0.38–2.5 mg/mL for *C. albicans* and *C. dublien-*

sis while *Rosmarinus officinalis* exhibited antibiofilm potential at 1 mg/mL and *Moringa olifeira* at 0.42 mg/mL, respectively (Onsare and Arora, 2015; Saharkhiz et al., 2012; Sandasi et al., 2011). In comparison to these previous results, the activities obtained with the tested plants were more effective with lower antibiofilm inhibitory concentrations up to 0.0625 mg/mL. Moreover, the antibiofilm activities of the tested EOs could be attributed to their terpene content. Some researches in the same field have illustrated the effects of terpenic compounds involved in the biofilm inhibition process. Previously, casbane, a diterpene, was isolated from *Croton nepetaefolius* and its antiyeast biofilm potential was revealed (Carneiro et al., 2010). More specifically, Z. *multiflora* is a plant which inhibited biofilms at the low concentrations of 1.5 mg/mL and 0.84 mg/mL. It was discovered that their major compounds were linalool, thymol and carvacrol, which could be involved in the biofilm inhibition process (Rahimi et al., 2014). It is known that the terpenes from EOs can target phospholipids of the fungal membrane leading to its damage, which disturbs cellular growth (Bakkali et al., 2008). On the other hand, the respective hydroxyl and oxo-groups of linalool and fenchone could be responsible for the anticandidal activity (Kim et al., 2007; Koroch et al., 2007). Particularly, the hydroxyl group of linalool could interact with the amino acid residues and increase the antibiofilm activity of terpenes (Takaishi et al., 2014; Ultee et al., 2002). It is also worth to note the antibiofilm activity of *Plectranthus amboinicus* which showed activity against Staphylococcal biofilms up to 0.25 mg/mL suggesting that its EOs acted by attaching to biofilms and reducing the expression of quorum sensing activation of genes (Vasconcelos et al., 2017).

5. Conclusion

The present study highlighted the anticandidal and antibiofilm activities of *Aeollanthus* and *Plectranthus* EOs. The data obtained in this study could represent perspectives for antifungal drugs discovery by extracting lead compounds on a bioassay basis and/or performing a combination approach to look for more synergistic antifungal effects.

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