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Research Article

A New Unsaturated Aliphatic Anhydride from *Aspergillus candidus* $T_{12}19W1$, an Endophytic Fungus, from *Pittosporum mannii* Hook f.

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A new unsaturated aliphatic anhydride derivative (*Z*)-(12*Z*)-heptadec-12-enoic-2′-hydroxypropanoic anhydride (**1**) and ten known compounds, three flavonoids **(2–4),** two terphenyllins **(5-6)**, four triterpenoids **(7-8, 10-11)**, and one *n*-fatty acid **(9)**, were isolated from the EtOAc extract of *Aspergillus candidus* $T_{12}19W1$, an endophytic fungus, inhabiting *Pittosporum mannii*. All the isolated compounds were characterized using 1D- and 2D-NMR and HR-EI experiments together with the reported literature. *p*-terphenyls are suggested to be the chemophenetic marker of the genus *Aspergillus*. The ethyl acetate crude extract as well as some isolated compounds of *A. candidus* was assayed for antibiofilm activity, anti-inflammatory (ROS) activity, and cytotoxicity on brine shrimps and the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cytotoxicity assay on 3T3 cell lines. The tested sample showed good antibiofilm activity with the lowest $MBEC_{50}$ obtained at 64 μg/mL. Compounds showed low anti-inflammatory activity even at high concentration (250 $\mu g{\cdot}mL^{-1}$ with an IC_{50} of $59.6 \pm 0.1\ \mu g{\cdot}mL^{-1}$) with moderate cytotoxicity on brine shrimps at high concentration (1000 $\mu g{\cdot}mL^{-1}$, with 46.67% mortality). However, no cytotoxic activity was observed against 3T3 cell lines.

1. Introduction

Endophytes have been of increasing prominence as hopeful sources of new biologically active natural products including anti-infective, cytotoxic, anti-parasitic, radical scavenging, and anti-inflammatory activities [1, 2]. Fungal endophytes show a wide diversity and host specificity [3, 4]. The endophytic fungal genus *Aspergillus* is a significant reservoir of promising biologically active chemical leads including butenolides, alkaloids, terpenoids, cytochalasins,

phenalenones, *p*-terphenyls, xanthones, sterols, diphenyl ether, and anthraquinone derivatives and consequently diverse biological activities [5, 6]. *Aspergillus candidus* had been shown to be rich in flavonoid secondary metabolites [7] amongst which are antibiotic chlorflavonin [8] and cytotoxic terphenyls [9, 10]. In our ongoing search for new bioactive compounds from endophytic fungi isolated from plants [2, 11, 12], we isolated *Aspergillus candidus* from the fresh twigs of *Pittosporum mannii*, a medicinal plant in Cameroon known for its ethnomedicinal virtues. The investigation of the ethyl acetate crude extract of *A. candidus* led to the isolation of an unsaturated *n*-aliphatic anhydride derivative, (*Z*)-(12*Z*)-heptadec-12-enoic-2′-hydroxypropanoic anhydride (**1**), described here for the first time, together with 10 previously described compounds. The crude extract was evaluated for anti-inflammatory (ROS), cytotoxicity using brine shrimps (*Artemia salina*), and cytotoxicity on the 3T3 cancer cell line.

2. Materials and Methods

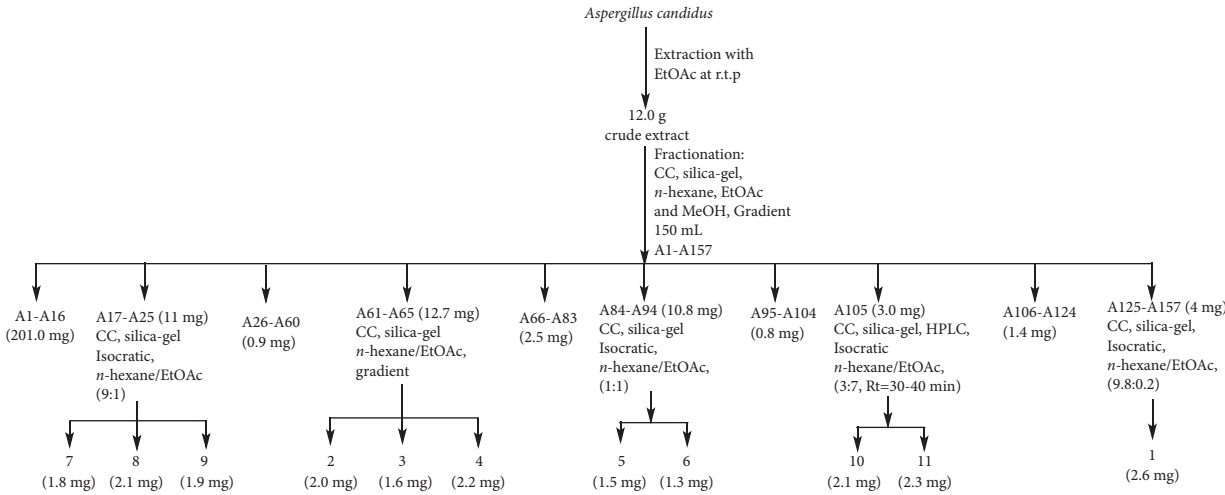
2.1. General Experimental Procedures. IR and optical rotation were recorded on a Bruker vector 22 spectrophotometer and a JASCO P-2000 polarimeter, respectively. The UV spectrophotometer (Thermo Scientific) was used to record the UV spectra. Low-resolution FAB and EI mass spectrometry spectra were measured on JOEL 600H, while high-resolution electronic impact mass spectrometry (HR-EI-MS) spectrum was recorded on Thermo Finnigan MAT 95XP. 1D (^{1}H & ^{13}C) NMR and 2D (homonuclear and heteronuclear) NMR spectra were measured on Bruker Ascend-800, AMX-600, and AMX-500 machines. Tetramethylsilane (TMS) was used as internal for carbon and proton chemical shifts (δ in ppm), and the coupling constants (*J*) are reported in Hz. Column chromatography over normal phase silica gel 60 (0.060–0.200 mm, 70–230 mesh) was used for fractionation and purification of compounds as well as Sephadex LH-20 (GE Healthcare Europe GmbH). Precoated TLC sheets and UV light (254–354 nm) were used to check the purity of compounds and the monitoring of fractions, while detection was accomplished by visualizing the heated TLC cart after spraying with a solution of ceric sulfate or sulfuric acid (10%).

2.2. Fungal Material. *Aspergillus candidus* (Trichocomaceae) fungus was isolated from the freshly harvested leaves and twigs of *Pittosporum mannii* Hook f. (Pittosporaceae) collected in Bali Nyonga, North-West Region of Cameroon, in November 2016. The plant material was identified in comparison with a specimen available in the National Herbarium of Cameroon in Yaoundé with 32235/HNC as a voucher number. The isolation of fungi from the plant material was achieved by following the methodology previously reported [13]. The plant twigs and leaves were cut into small sections and then washed with running tap water for 15 min before being serially surface sterilized with 70% ethanol for 1 min, 1% sodium hypochlorite for about 1 min, and three times wash with autoclaved distilled water. Following surface sterilization, the plant material was sliced into small pieces (1 cm). The pieces were placed on plates of PDA containing chloramphenicol (100 mg/L) to inhibit bacterial growth. These Petri dishes were incubated at 28°C till the fungal mycelia began to grow from the samples. The morphologically different colonies were subcultured repeatedly till pure cultures were obtained. The endophytic fungi studied were identified using microscopic and macroscopic analysis. The strain was identified as *Aspergillus candidus* following GenBank research for DNA sequence similarity of the isolate with *Aspergillus candidus* reference strain 18sRNA sequence (GenBank accession number. AB008396.2). The fungal sample is deposited in the EPLANAPROLAB of the University of Yaoundé I, Cameroon.

The large-scale fermentation of *Aspergillus candidus* was carried out in a solid medium according to a previously described technique [14] with certain modifications. Briefly, 50 g of rice mixed with 100 mL of water was poured into 12 conical flasks of 500 mL each and then autoclaved at 121°C for 30 minutes. Discs, 6 mm in diameter, containing the spores of the endophyte cultured previously on PDA for 10 days at 28°C, were inoculated into these flasks containing rice after cooling. All flasks were then incubated at 25–27°C for 21 days with periodic shaking.

2.3. Extraction and Isolation. At the end of the fermentation period in a solid medium, the crude extracts were obtained using a mixture of 3 solvents: ethyl acetate, dichloromethane (drops), and *n*-hexane. The equal amount of solvent mixture was added into the vessels containing the fermented rice culture. The organic phase was collected in Petri dishes by filtration using a separating funnel and was evaporated to dryness at room temperature. Combined organic extracts were concentrated under reduced pressure to afford a brown gum (12 g). The crude extract was chromatographed on a silica gel column with a gradient of elution consisting of *n*-hexane-EtOAc-MeOH (0 : 10-0 : 10, each 150 mL), to obtain fractions A1 to A157, which were grouped according to their TLC profiles (Scheme 1).

So compounds (**7**; 1.8 mg), (**8;** 2.1 mg), and (**9**; 1.9 mg) were obtained after further purification of fractions A17–A25 (11 mg) over silica-gel column chromatography (CC) eluted with an isocratic system of *n*-hexane/EtOAc (9 : 1; v : v). Further purification of fractions A61–A65 (12.7 mg) over silica-gel CC eluted stepwise with a system of *n*-hexane/EtOAc (10–0 : 0–10; v : v) was performed to obtain compounds (**2**; 2.0 mg), (**3**; 1.6 mg), and (**4**; 2.2 mg). On the other hand, the purification of fractions A84–A94 (10.8 mg) using isocratic silica-gel CC, (*n*-hexane/EtOAc; 1 : 1; v:v) led to compounds (**5**; 1.5 mg) and (**6**; 1.3 mg). Furthermore, fraction A105 (3 mg) was purified using normal phase preparative HPLC (isocratic, *n*-hexane-EtOAc, 3 : 7, Rt = 30–40 min) to obtain compounds (**10**; 2.1 mg) and (**11** 2.3 mg). Fractions A125–A157 were run using an isocratic system of EtOAc/MeOH (9.8–0.2; v:v) in silica-gel column chromatography to obtain the compound (**1**; 2.6 mg).



Scheme 1: Flow diagram for extraction and isolation of pure compounds from *A. candidus*.

*2.4. Spectroscopic Data of Compound (**1**). (Z)-(12Z)-heptadec-12-enoic-2′-hydroxypropanoic anhydride (**1**)*: white amorphous powder; $[a]_D^{24}$ = +0.8905 (*c* 0.01; *MeOH*); UV (MeOH): 231, 246, 270, and 291 nm; FT-IR (MeOH): 3435, 2250, 2125, 1635, 1015, 1010, 821, and 758 cm^{-1}; LR-FAB-MS $[M-H]^-$ at *m/z* 339.2 (calcd. *m/z* 339.2 for $C_{20}H_{35}O_4$); HR-EI-MS: M^+ at *m/z* 340.2628 (calcd. *m/z* 340.2614 for $C_{20}H_{36}O_4$); ^{1}H-NMR (800 MHz, DMSO-d_6); and ^{13}C-NMR (200 MHz, DMSO-d_6) (see Table 1).

2.5. Biofilm Inhibition Assay. Two bacterial strains obtained from the American Type Culture Collection were used: *Staphylococcus aureus* ATCC1026 and *Escherichia coli* ATCC 10536. They were maintained on Muller–Hinton agar slant at 4°C and subcultured on fresh appropriate agar plates 24 hours prior to the antibiofilm assay. The capacity of the isolated compounds to alter mature biofilm was determined using the microtiter plate method as previously described [15]. Briefly, 100 μL of a bacterial suspension at 1.5×10^6 CFU/mL was added to 100 μL of MHB supplemented with 2% glucose into 96-well flat-bottomed sterile polystyrene microplates and incubated at 37°C for 48 h. After incubation, planktonic cells in the well of the microplates were removed by washing with phosphate-buffered saline (PBS). Then, 100 μL of compounds or reference antibiotic, respectively at 256 μg/mL and 32 μg/mL, in Mueller–Hinton broth supplemented with 2% glucose was applied on mature biofilm biomass embedded on the surface of the wells of the microtiter plate. The plates were further incubated at 37°C for 24 h. Following incubation, the plates were washed with PBS, and the remaining biofilm biomass was fixed with methanol, then stained with 150 μL of safranin (1%), and incubated for 15 min. Thereafter, the excess of safranin was removed, the dye bound to the cells was solubilized with 150 μL of ethanol 95%, and the optical density (OD) was measured at 570 nm using a microplate reader (Spectramax 190, Molecular Devices). The wells containing only MHB supplemented with 2% glucose were used as a negative control. The percentage of biofilm inhibited was determined using the following formula: % inhibition = $[(OD_{\text{Negative control}} - OD_{\text{Sample}})/OD_{\text{Negative control}}] \times 100$. Compounds showing more than 50% inhibition were further serially two-fold diluted in MHB, and the minimum biofilm eradication concentration (MBEC50) was determined as the lowest concentration of compounds, which reduces biofilm biomass by 50%. All experiments were performed in triplicate.

Table 1: ^{13}C (200 MHz, DMSO-d_6) and ^{1}H-NMR (800 MHz, DMSO-d_6) data for compound **1**.

1 ((*Z*)-(12*Z*)-heptadec-12-enoic-2′-hydroxypropanoic anhydride (1))				
Pos	δ_C	δ_H (mult., *J* (Hz))	HMBC	TOCSY
1	174.45			
2	35.15	2.00 (*m*)	1, 3, 4	
3	25.15	1.45 (*m*)	1, 2, 5	H_2-H_3-H_4
4	28.76	1.23 (*brs*)		
5	28.72	1.23 (*brs*)		
6	28.85	1.23 (*brs*)		
7	28.88	1.23 (*brs*)		
8	29.00	1.23 (*brs*)		
9	29.05	1.23 (*brs*)		
10	29.10	1.23 (*brs*)		
11	28.60	1.23 (*brs*)		
12	129.70	5.38 (*brs*)		
13	129.70	5.31 (*dt*, 5.6, 4.2)	14	H_{15}-H_{13}-H_{14}
14	26.58	1.97 (*m*)		
15	31.31	1.28 (*brs*)	13	
16	22.13	1.26 (*brs*)	14	
17	13.98	0.85 (*t*, 6.7)	15, 16	H_{17}-H_{16}-H_{15}
1′	176.84			
2′	67.08	3.45 (*obs*)		
3′	21.57	1.07 (*d*, 6.7)	1', 2'	H_2'-H_3'

Obscured by overlapping resonances (obs).

2.6. Anti-Inflammatory (Reactive Oxygen Species) Assay. The luminol-enhanced chemiluminescence assay was performed [16] with minor modifications. The experimental goal was to detect intracellular reactive oxygen species (ROS)

produced by blood phagocytes and with ibuprofen (with $IC_{50} = 11.2 \pm 1.9$) used as the standard drug. In summary, 25 μL of diluted whole blood $HBSS^{++}$ (Hanks balanced salt solution containing calcium chloride and magnesium chloride) (Sigma, St. Louis, USA) was incubated with three different concentrations of samples (10, 50, and 250 μg/mL), each in triplicate. Manipulation was carried out by following the previously reported study [17].

2.7. Brine Shrimp (Artemia salina) Lethality. *Artemia salina* (leach) shrimp larvae are often affected by bioactive extract and pure compounds. Their eggs are readily available as fish food in pet shops. The numbers of larvae produced by egg hatch is significant when placed in artificial seawater for 48 hrs. In this study, the ethyl acetate crude extract of *A. candidus* was evaluated on brine shrimp (*Artemia salina* L.) larvae by following an existing method [18]. The reference drug used here was etoposide. The ethyl acetate crude extract of *A. candidus* was initially dissolved in DMSO, then subjected to 10, 100, and 1000 μg/mL ten brine shrimp nauplii sea water in triplicates, and incubated at 25–28°C under illumination for 24 hours. For surviving shrimps, the determination of the mortality percentage and the calculation of LC_{50} as well as LD_{50} were carried out as previously reported [19, 20].

2.8. Cytotoxicity Assay against 3T3 Cell Lines. The crude extract was tested for cytotoxic activity using 96-well flat-bottomed microplates by the standard MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) colorimetric assay [21, 22]. Dulbecco's modified Eagle medium, supplemented with 5% of fetal bovine serum (FBS), 100 IU/mL of penicillin, and 100 µg/mL of streptomycin in 75 cm^2 flasks and kept in a 5% CO_2 incubator at 37°C, was used to culture 3T3 (mouse fibroblast) cells. Practically, the growing cells were collected, counted with a haemocytometer, and diluted with a particular medium. The cell culture, incubation, extent of MTT reduction to formazan within cells calculated by measuring the absorbance at 540 nm using a microplate reader, and the determination of IC_{50} for 3T3 cells were determined by following the previously reported studies [20, 23].

3. Results and Discussion

Aspergillus candidus, an endophyte, was isolated from the leaves of *Pittosporum mannii* and cultured with PDA and unpolished rice. Successive normal phase column chromatography used to fractionate and purify the ethyl acetate crude extract of *A. candidus* led to the isolation of eleven compounds (**1–11**), among which an unparalleled unsaturated aliphatic anhydride derivative (*Z*)-(12*Z*)-heptadec-12-enoic-2′-hydroxypropanoic anhydride (**1**) as well as ten known compounds (**2–11**) (Figure 1).

Compound (**1**) was obtained as a dextrorotatory ($[a]_D^{24} = +0.89$ (*c* 0.01; *MeOH*)) white amorphous powder. The molecular formula, $C_{20}H_{36}O_4$, was deduced from its LR and HR-EI-MS spectrum (Figures S1 and S1′) with a molecular ion peak M^+ at *m/z* 340.2628 (calcd. *m/z* 340.2614), indicating three double-bond equivalents. Its UV spectrum (Figure S10) revealed maxima at 231, 246, and 270 nm typical of a conjugated chromophore [24, 25]. Its IR spectrum (Figure S11) exhibited absorption bands at 3435 (-OH), 1635 (-C=O), and 1010 (-C-O) cm^{-1}, respectively. The ^{1}H-NMR spectrum (Figure S2, Table 1) of compound (**1**) exhibited spectral features closely related to that reported for a known polyoxygenated fatty acid: δ_H 1.23 (*brs,* 20H), 2.00–1.97 (*m*, 4H), and 0.85 (*t*, $J = 6.7$, 3H) [26–28]. In addition, signals attributable to olefinic protons and a secondary methyl group were observed at δ_H 5.38 (*brs,* 1H), 5.31 (*dt*, $J = 5.6$–4.2, 1H), and 1.07 (*d*, 6.7, 3H), respectively, indicative of compound (**1**) to be an unsaturated aliphatic chain to which is attached an additional methyl group. The presence of a secondary methyl group (δ_H 1.07, 3H) as well as a proton was attached to a carbinol at δ_H 3.45 (*m,* 1H) overlapping with the signal of water (D_2O). This was further supported by the COSY (Figure S3) and TOCSY (Figure 2S4 and S9) experiments that revealed homonuclear cross-peak correlations between proton H-3′ (δ_H 1.07) and H-2′ (δ_H 3.45) and HSQC (Figure S5) heteronuclear correlations between H-2′ (δ_H 3.45) and C-2′′ (δ_C 67.1). The interpretation of ^{13}C-NMR spectrum (Figure S6, Table 1), together with DEPT-135 (Figure S7), HSQC (Figure S5), and HMBC (Figure 2S8, and S9) spectra, culminated to a total of twenty carbon atoms. These carbon atoms were sorted into two methyls [δ_C 21.6 (C-3′) and 14.0 (C-17)], fourteen methylenes [δ_C 35.2–22.1 (C2–C11; C14–C17)], three methines, among which are two olefinic [δ_C 129.7 (C-12 and C-13)] and an oxygenated [δ_C 67.1 (C-2′)], and two carboxylated quaternary carbons [δ_C 176.8 (C-1′) and 174.5 (C-1)], which are in agreement with the three double-bond equivalents inferred by HREIMS (Figure S1′), suggesting compound **1** to be an anhydride derivative [28]. The two carboxyl groups as well as the alcohol and the secondary methyl group were assigned by following the HMBC (Figure S8) experiment in which correlations were noticed between H-3′ (δ_H 1.07) and carbons C-1′ (δ_C 176.8) and C-2′ (δ_C 67.1) and between H-3 (δ_H 1.45) and carbons C-1 (δ_C 174.5), C-2 (δ_C 35.2), and C-5 (δ_C 28.7). The positions of the double bond found in the aliphatic moiety was first suggested by the TOCSY (Figure S4) experiment which revealed long-range homonuclear cross-correlations between H-13 (δ_H 5.31), H-14 (δ_H 1.97), and H-15 (δ_H 1.28) and between H-15 (δ_H 1.28), H-16 (δ_H 1.26), and H-17 (δ_H 0.85), suggesting the olefinic bond at Δ^{12} alongside the HMBC data (Figure S8). The suggested position of Δ^{12} as well as its configuration was further supported by the reported ^{13}C-NMR (Figure S6) data of (*Z*)-6-methyl-12-heptadecenoic acid counting the same number of terminal carbons with the same chemical shifts [28], while the *cis* configuration of the double bond was evidenced based on ^{13}C-NMR (δ_C 28.6/C-11 and 26.6/C-14) and FT-IR data (Figure S11). The absence of absorption bands at 980–960 cm^{-1} on the FT-IR spectrum is indicative of *Z* geometry, while on the ^{13}C-NMR spectrum, the chemical shifts of allylic carbons ranging from 26 to 28 ppm are indicative of a *Z*-configuration and those from 32 to 34 ppm suggestive of

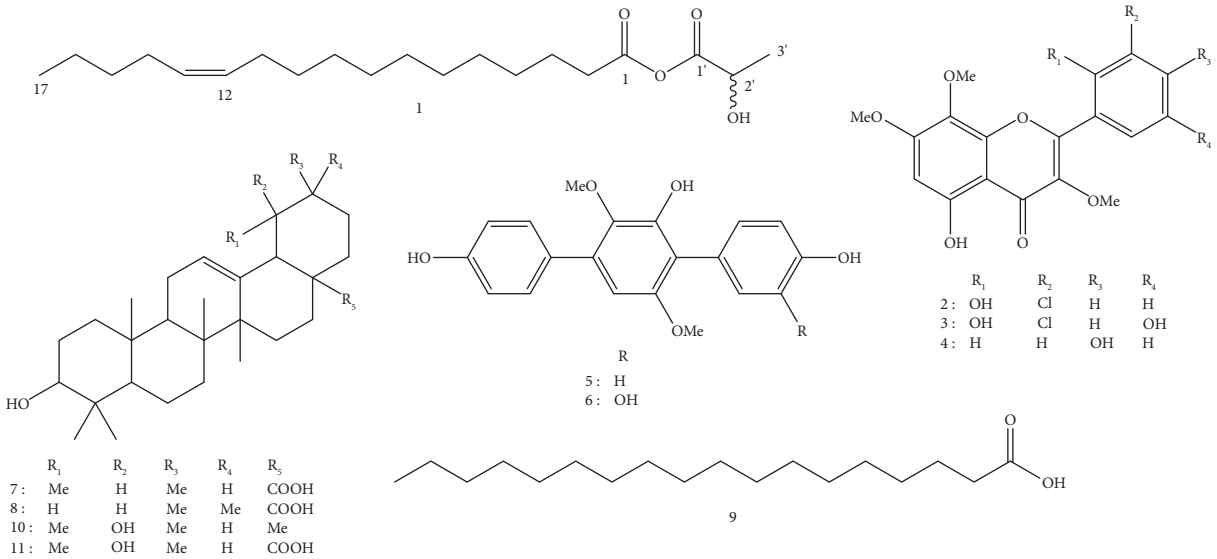


Figure 1: Chemical structures of compounds **1** to **11**.

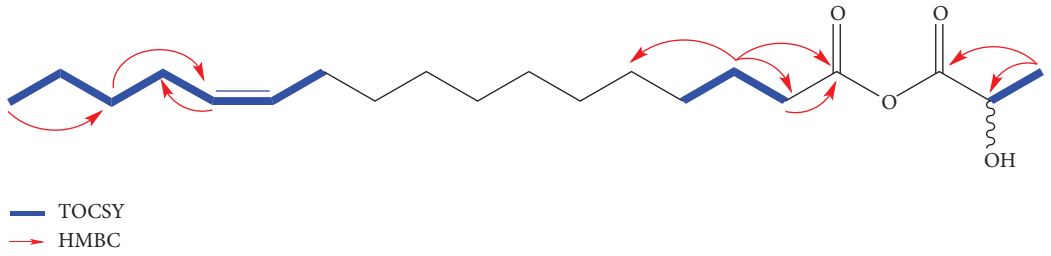


Figure 2: TOCSY and HMBC correlations of compound (**1**).

an E-configuration [26, 29, 30]. Compound (**1**) was therefore identified as a new unsaturated aliphatic anhydride derivative named (*Z*)-(12*Z*)-heptadec-12-enoic-2′-hydroxypropanoic anhydride (**1**).

The structures of all known compounds were deduced from their NMR data in comparison with the previously reported literature as chlorflavonin (**2**) [8, 31], chlorflavonin A (**3**) [31], 5,4′-dihydroxy-7,8-dimethoxyflavone (**4**) and 1,4-dimethoxy-2,4′,4′-trihydroxy-*p*-terphenyllin (**5**) [8], 3-hydroxyterphenyllin (**6**) [32], ursolic acid (**7**) and oleanolic acid (**8**) [33], stearic acid (**9**) [34], pomolic acid (**10**) [35, 36], and (3*β*)-urs-12-ene-3,19-diol (**11**) [37].

Among the isolated compounds **1–11**, **4, 7-8,** and **10-11** have been previously reported from the host plant *Pittosporum mannii* [38], indicating a chemophenetic relationship between the endophyte and its host. Equally, compounds **2-3** and **5-6** have been reported from *Aspergillus candidus* [8, 31, 32]. *p*-terphenyl derivatives are suggested to be the chemophenetic marker of the genus *Aspergillus* since they are reported from the very first chemical study of this species about fifty years ago [8, 39] and also in almost all other numerous chemical studies undertaken on *Aspergillus* sp. [40–45].

The ethyl acetate crude extract of *Aspergillus candidus* was assayed for its anti-inflammatory (ROS), brine shrimp (*Artemia Salina*) lethality, and cytotoxic (3T3 cell line) activities. The anti-inflammatory activity was evaluated by following a previously reported procedure [17], and the tested sample showed a slow inhibition of reactive oxygen species (ROS) only at a high concentration of 250 $\mu g \cdot mL^{-1}$ with an IC_{50} value of $59.6 \pm 0.1\ \mu g \cdot mL^{-1}$. On the other hand, the tested sample revealed a moderate cytotoxicity at the highest concentration of 1 000 $\mu g \cdot mL^{-1}$ on brine shrimps (*Artemia salina*) with 46.67% mortality. The MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cytotoxicity assay on 3T3 cell lines showed no activity.

S. aureus and *E. coli* are among the leading pathogens that cause nosocomial infections throughout the world [46]. With the reemergence of antibiotic resistance, there is an urgent need for a new approach to antimicrobial therapy, since common antibiotics do not generally prevent biofilm formation at MIC levels. In this study, some isolated compounds were tested for their capacity to interfere with biofilm in *S. aureus* and *E. coli* strains. All tested compounds inhibited the biofilm of both bacterial strains (Table 2). With exception to compound **1**, compounds **2** and **3** had percentage of inhibition greater than 77% on average over gentamicin (59%), the reference drug, while their $MBEC_{50}$ values varied between 64 $\mu g/mL$ and >256 $\mu g/mL$ for all tested compounds. Out of the flavone derivatives tested, chlorflavonin A (**3**) showed better antibiofilm activity against the Gram-negative bacteria

Table 2: Percentage of biofilm inhibition (%) and the minimum biofilm eradication concentration ($MBEC_{50}$) values of some isolated compounds against *S. aureus* and *E. coli* strains.

Compounds	*S. aureus*		*E. coli*	
	Inhibition (%)	$MBEC_{50}$	Inhibition (%)	$MBEC_{50}$
1	29.28	-	36.09	-
2	72.15	256	80.12	128
3	75.32	256	81.22	128
Gentamicin	62.34	8	57.05	16

(-): >256 μg/mL.

E. coli, with a biofilm inhibition percentage of 81.22% and an $MBEC_{50}$ value of 128 μg/mL. Some flavonoid compounds were previously reported to have potential for controlling biofilm formation by the virulence of *Acinetobacter baumannii* [47], in support of our work. The structure-activity relationship (SAR) studied flavonoids for antibiofilm properties against bacterial pathogens that have not yet been reported. However, from the chemical structure view of the flavonoid compounds studied here, the structure-activity relationship analysis suggests that, for a good inhibitory effect, the hydroxyl group substitution at C-5 in the A ring and C-4′ in the B ring and the methoxyl group substitution at C-3, C-7, and C-8 in the A ring appear to be involved in the antibiofilm activity, while the presence of the hydroxyl group at C-2′ in the B ring seems to reduce the biofilm inhibition in the tested bacteria.

4. Conclusion

Endophytes are of increasing importance as optimistic leads of new drugs from natural sources for both infective and noninfective diseases. *Aspergillus candidus* was isolated from the leaves of *Pittosporum mannii,* cultured and extracted with ethyl acetate. A chemical study of the crude extract led to the isolation of eleven compounds, among which an unparalleled unsaturated aliphatic anhydride derivative (*Z*)-(12*Z*)-heptadec-12-enoic-2′-hydroxypropanoic anhydride (**1**).

The ethyl acetate crude extract showed slow ROS and moderate cytotoxicity on brine shrimps only at high concentration. This study revealed that chlorflavonin (**2**) and chlorflavonin A (**3**) exhibited interesting antibiofilm potential and could be considered promising drug candidates for the development of therapeutic molecules to overcome biofilm-associated infections caused by *S. aureus* and *E. coli*.

Data Availability

In the supplementary information file, the findings of this study are included within the spectroscopic spectra 1D (^{1}H-NMR, ^{13}C-NMR, and DEPT-135), 2D (COSY, TOCSY, HSCQ, and HMBC) (see data Table 1), UV and IR spectra, and spectrometric spectra (LR-EI-MS and HR-EI-MS) of the new compound (**1**).

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

All authors contributed to study conception and design. Alain Meli Lannang, Denis Kehdinga Sema, and Gaëlle Talle Juidzou conceptualized the study. Gaëlle Talle Juidzou, Elodie Gisèle Mouafo Anoumedem, Peron Bosco Leutcha, and Larissa Yetendje Chimi handled the methodology. Peron Bosco Leutcha and Denis Kehdinga Sema were in charge of original draft preparation. Gaëlle Talle Juidzou, Elodie Gisèle Mouafo Anoumedem, and Virginie Flaure Tsague Tankeu were in charge of writing reviews and editing. Alain Meli Lannang, Simeon Kouam Fogue, Jean Paul Dzoyem, Norbert Sewald, and M. Iqbal Choudhary were in charge of supervision. All authors have read and agreed to the published version of the manuscript.

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Supplementary Materials

Figure S1: LR-EI-MS spectrum and HR-EI-MS of (*Z*)-(12*Z*)-heptadec-12-enoic-2′-hydroxypropanoic anhydride (**1**). Figure S2–S8: ^{1}H-NMR, COSY ^{1}H-^{1}H, TOCSY ^{1}H-^{1}H, HSQC, ^{13}C-NMR, DEPT-135, and HMBC spectra for (*Z*)-heptadec-12-enoic-2′-hydroxypropanoic anhydride (**1**), respectively. Figure S10 and S11: UV and IR of compound 1. Figure S12-S13: ^{1}H-NMR and ^{13}C-NMR spectra of chlorflavonin A (**3**). Figure S14-S15: ^{1}H-NMR and ^{13}C-NMR spectra of chlorflavonin (**2**). Figure S16-S17: ^{1}H-NMR and HMBC spectra of 5,4‟-dihydroxy-7,8-dimethoxyflavone (**4**). Figure S18-S19: ^{1}H-NMR and ^{13}C-NMR spectra of 3-hydroxyterphenyllin (**6**). Figure S20-S21: ^{1}H-NMR and ^{13}C-NMR spectra of 1,4-dimethoxy-2,4,”4″-trihydroxy-p-terphenyllin (**5**). Figure S22-S23: ^{1}H-NMR and ^{13}C-NMR spectra of ursolic acid (**7**). Figure S24-S25: ^{1}H-NMR and ^{13}C-NMR spectra of oleanolic acid (**8**). Figure S26-S27: ^{1}H-NMR and HMBC spectra of stearic acid (**9**). Figure S28-S29: ^{1}H-NMR and HMBC spectra of (3β)-urs-12-ene-3,19-diol (**11**). Figure S30: ^{1}H-NMR spectrum of pomolic acid (**10**). (*Supplementary Materials*)

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