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Ghanaian Mangrove Wetland Endophytic Fungus, *Penicillium herquei* strain BRS2A-AR produces (9Z, 11E)-13-oxooctadeca-9,11-dienoic acid with activity against *Trichomonas mobilensis*

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ABSTRACT

Sub-Sahara Africa is burdened with a high incidence of parasitic infections, including schistosomiasis, trypanosomiasis, trichomoniasis, and leishmaniasis. Currently, there is a rapid widespread development of resistance to prescription drugs for these neglected diseases. Microbes provide the largest chemical and biological diversity in drug discovery screening programs; therefore, our project seeks to explore microbes in the sub-region for new drugs. The oxylipin (*9Z,11E*)-13-oxooctadeca-9,11-dienoic acid (**1**) was isolated from the Ghanaian endophytic fungus, *Penicillium herquei* strain BRS2A-AR obtained from the leaves of a *Laguncularia racemosa* tree growing on the banks of the River Butre in the Western Regional wetlands of Ghana. Compound **1** was isolated on reverse phase HPLC at t_R of 34.3 minutes. The structure of compound **1** was confirmed by a combination of mass spectrometry, 1D and 2D-NMR techniques. Compound **1** showed minimal antiparasitic activity when tested against *Plasmodium falciparum* 3d7 (IC_{50}>100µM; aretesunate, IC_{50} 36nm), *Trypanosoma brucei brucei* (IC_{50}>100µM; *Coptis japonica*, IC_{50} 8.20µM), *Leishmani donovani* (IC_{50}>100µM; amphotericin B, IC_{50} 0.32µM) and *Leishmania major* (IC_{50}>100µM; amphotericin B, IC_{50} 0.31µM). Compound **1** produced IC_{50} of 44.47µM when tested against *Trichomonas mobilensis* with metronidazole (IC_{50} 5.20µM) as standard. These results show the potential to further engineer the structure of compound **1** into a potent anti-Trichomonas scaffold.

Keywords: Oxylipin, Antiprasitic, Plasmodium, Trypanosome, Leishmania, Trichomonas.

INTRODUCTION

Compound **1**, (*9Z,11E*)-13-oxooctadeca-9,11-dienoic acid (13-oxo-ODA) belongs to a family of oxygenated fatty acid derived compounds known as oxylipins. Oxylipins are highly diverse in the biology of source, chemical diversity and metabolic functions. They are biosynthesized from the enzymatic or non-enzymatic oxidative transformation of polyunsaturated fatty acids by various lipoxygenases, cyclooxygenases and certain cytochrome P450 enzymes through diverse metabolic pathways (Blée, 2002; Shea et al., 2006; Tsitsigiannis et al.,

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2007; Andrade et al., 2016). Oxylipins are normally classified based on their origin which includes mammals, plants, algae, microbes and fungi. Mammalian and fungal oxylipins are best characterized in terms of their structure and bioactivity (Van et al., 2004; Tsitsigiannis et al., 2007; Brodhun et al., 2011; Keller et al., 2016). In plants, jasmonic acid and its derivatives function as phytohormones (Li et al., 2004; Wasternack et al., 2009; Heilmann et al., 2009; Dave and Graham, 2012) while other plant derived oxylipins are also known to be antibacterial (Trapp et al., 2015). Algae have been found to produce several bioactive oxylipins with anti-inflammatory (Zubía et al., 2014) and antibacterial (Pohnert et al., 2018) properties while microbes like *Pseudomonas aeruginosa* promote biofilm formation and virulence with several structurally diverse oxylipins (Martı´nez and Campos-Go´mez, 2016). A large number of oxylipins generally exist as hydroxyl, oxo, ketone and aldehyde metabolites that execute several interesting biological roles (Muller et al., 2004; Mosblech et al., 2009). The most notable functions of oxylipins include their role in plant defense stimulation mechanisms (Blée, 2002), signaling functions in fungi, algae and mammals (Göbel and Feussner, 2009; Andreou et al., 2009), antimicrobial activities and regulation of the organisms developmental processes (Granér et al., 2003; Prost et al., 2005).

The oxylipin 13-oxo-ODA (**1**) is a linoleic acid derived oxylipin that was first reported by Brooks et al., 1977 as the ketone that resulted from the oxidation of linoleic acid hydroxyperoxides. The endophytic fungal strains *Ripartites tricholoma* and *Ripartites metrodii* isolated from the spores and tissue plugs of fruiting bodies produced mycelia from which compound **1** was isolated and found to possess antimicrobial and cytotoxic activities (Weber et al., 2006). In 2012, compound **1** was isolated from tomato juice and found to be a potent peroxisome proliferator-activated receptor α (PPARα) agonist. PPARα is a ligand-activated transcription factor which regulates energy metabolism and decreases plasma and hepatic triglyceride in obese diabetic mice (Kim et al., 2011; Kim et al. 2012; Gudbrandsen et al., 2009).

Herein we report for the first time ever the isolation of compound **1** from the endophytic fungus *Penicillium herquei* strain BRS2A-AR which was obtained from the leaves of a *Laguncularia racemosa* tree growing on the banks of the River Butre in the Western Regional wetlands of Ghana. Culture of this strain at 28 ºC, 220 rpm, in malt extract liquid broth for 28 days produced extracts which upon Sephadex LH-20 size exclusion chromatography and HPLC yielded compound **1**. Bioactivity tests conducted for compound **1** showed promising antiparasitic effects against *Trichomonas mobilensis*. To the best of our knowledge, this is the first report on the antiprasitic effects of compound **1**. Furthermore, using high resolution electrospray ionization tandem mass spectrometry, two new derivatives of compound **1** were detected and their structures proposed by interpretation of their respective fragmentation patterns.

MATERIALS AND METHODS

General experimental procedures

1D and 2D NMR data were recorded on a Bruker Avance Spectrometer at 500 MHz ^{1}H and 125 MHz for ^{13}C. The ^{1}H and ^{13}C chemical shifts were referenced to the solvent signals (δ_H 3.31 ppm and δ_C 49.00 ppm in CD_3OD). High-resolution electrospray ionization tandem mass spectrometry data were measured using a ThermoScientific LTQXL-Discovery Orbitrap coupled to an Accela UPLC-DAD system. The following conditions were used for mass spectrometric analysis: capillary voltage 45 V, capillary temperature 320 °C, auxiliary gas flow rate 10−20 arbitrary units, sheath gas flow rate 40−50 arbitrary units, spray voltage 4.5 kV, mass range 100−2000 amu (maximum resolution 30 000). Semi-preparative HPLC purifications were carried out using a Phenomenex Luna reverse-phase (C18 250 × 10 mm, L × i.d.) column connected to a Waters 1525 Binary HPLC pump Chromatograph with a 2998 photodiode array

detector (PDA), column heater and in-line degasser. Detection was achieved on-line through a scan of wavelengths from 200 to 400 nm. This system was also used to record the UV profile for the compounds. All solvents were of HPLC grade. Sephadex LH-20 and HP-20 resin were obtained from Sigma Aldrich.

Sample collection

Three mangrove plants *Conocarpus erectus*, *Rhizophora racemosa* and *Laguncularia racemosa* were sampled along the banks of the Butre River in the Western Region of Ghana, noted for its biodiversity rich indigenous mangrove plants. Five sampling sites were chosen 100 m apart, from the shore to where the river meets the sea. Different plant parts were sampled at different sections of the river. The plant parts sampled were leaves, buds, submerged roots, aerial roots, aerial stems, fruit shoots and flowers. The endophytic fungus *Penicillium herquei* strain BRS2A-AR was isolated from the leaves of a *Laguncularia racemosa* tree.

Sterilization of plant part and culture of endophytic fungus

In the laboratory, pieces of the leaves of *Laguncularia racemosa* were surface sterilized under sterile conditions by first rinsing them with sterile artificial sea water (SASW) and then immersing them in 70% ethanol for 1 minute. The leaves were then cut transversely at all sides into smaller pieces with a pair of flame sterilized scissors in a bio-safety cabinet. The pieces were again sterilized under sterile conditions by first rinsing them with SASW and then immersing them in 70% ethanol for 1 minute. Each piece was again cut at all sides into a much smaller piece with a flame sterilized scalpel. These pieces were again sterilized by rinsing them with SASW, then immersing in 2% sodium hypochlorite for 1 minute and then rinsed again with SASW 3 times. The treated plant parts were afterwards placed on malt extract agar plates (15 g each of malt extract and agar) and incubated at 28 °C for three weeks with daily observations to detect the rise of new fungal colonies.

Isolation of pure endophytic fungus

Between days 1-21, several different species of endophytic fungi grew on the parent plates that were set-up. Single colonies on the parent plates were selected and sub-cultured onto fresh malt extract agar plates and incubated at a temperature of 28 ^{o}C. All the colonies initially sub-cultured from the parent or master plates were subsequently sub-cultured until very pure strains were obtained one of which was the *Penicillium herquei* strain BRS2A-AR.

Identification of *Penicillium herquei* strain BRS2A-AR

Penicillium herquei strain BRS2A-AR was identified based on the 16S rRNA gene. The genomic DNA of isolates was extracted with a DNA bacterial/fungal extraction kit according to the manufacturer's protocol (Zymo Research, USA), and the 16S rRNA gene was amplified by using universal bacterial 16S rRNA primers 27F and 1525R (Lane, 1991). The PCR product was purified using a PCR purification kit, and direct sequence determination of the purified 16S rRNA gene was performed using universal sequencing primers 518F and 800R with an Applied Biosystems automated sequencer. Identification of the closest phylogenetic neighbors and calculation of pairwise 16S rRNA gene sequence similarities were achieved using the EzTaxon-e server-http://eztaxon-e.ezbiocloud.net (Kim et al., 2012). The MEGA6 program was used for phylogenetic analysis, and the Clustal-W option in the same program was used for alignment (Tamura et al., 2013). Phylogenetic trees were inferred using the neighbor-joining (Saitou and Nei, 1987), maximum-parsimony (Kluge and Farris, 1969) and maximum-likelihood (Felsenstein, 1981) algorithms. Evolutionary distances were calculated using the Jukes & Cantor model. Topologies of the resultant trees were evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 re-samplings.

Small and large scale fermentation of strain BRS2A-AR

Autoclaved Erlenmeyer flask (250 ml) plugged with non-absorbent cotton wool containing 50 ml of malt extract (15 g of malt extract) fermentation media in distilled water was directly inoculated with spores of strain BRS2A-AR and incubated at 28 ºC at 220 rpm for 3 days. This seed culture was subsequently used to inoculate nine Autoclaved 1 L Erlenmeyer flasks each containing 200 ml malt extract media and plugged with non-absorbent cotton wool. The 1 L flasks were incubated at 28 ºC at 220 rpm for 28 days. Two days before the culture incubation period was complete, 10 g of autoclaved HP-20 resin was added to each of the flasks under sterile conditions and the flasks were returned back to the incubator.

Extraction and purification

The strain BRS2A-AR fermentation broth (1.8 L) was filtered through a piece of glass wool under suction in a Buchner funnel to separate the supernatant from the mycelia. The supernatant was extracted with EtOAc and the mycelia and HP-20 resin were placed in a 1 L flask and extracted sequentially and alternatively with MeOH and CH_2Cl_2. All extracts were combined and evaporated under reduced pressure to obtain a total crude extract (1.36 g). The total crude extract was subjected to Sephadex LH-20 size exclusion chromatography by gravity using a 50/50 mixture of MeOH/CH_3CN as eluent to obtain seven fractions that were labelled SF1-7. Thin Layer Chromatography followed by ^{1}H-NMR spectroscopy showed the compound of interest to be concentrated in the fractions SF5-7. Hence, these fractions were all combined to give BRS2A-AR-MEEA-SF57. Fraction BRS2A-AR-MEEA-SF57 was therefore subjected to semi-preparative HPLC separation and purification using a Phenomenex Luna C18 column (C18 250 × 10 mm, L × i.d.). Gradients of Solvent A: 100% H_2O and Solvent B: 100% CH_3CN (100% A to 100% B in 30 min and hold for 30 min) were used as eluent with column flow rates set at 1.5 mL/min to afford compound **1** (2.2 mg, t_R = 34.3 min), compound **2** (0.8 mg, t_R = 55.0 min) and compound **3** (0.9 mg, t_R = 59.1 min).

The structure of compound **1** was determined using a combination of 1D and 2D-NMR techniques coupled with HRESI-LC-MS data. However, the structures of the new derivatives, compound **2** and **3** were elucidated based on the analysis of the HRESI-LC-MS data.

Bioassay reagents

Fetal Bovine Serum (FBS), Roswell Park Memorial Institute; (RPMI) 1640, IMDM, M-199, HEPES, YI-S, Fetal Bovine Serum (FBS), Adult Bovine Serum (ABS) Gentamycin, Penicillin-Streptomycin-L-Glutamine (PSG), 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES), Artesunate, Alamar dye, Dimethyl sulfoxide (DMSO), Sodium citrate, Adenine, Sodium bicarbonate ($NaHCO_3$), AlbuMax II, Sodium chloride (NaCl), Potassium chloride (KCl), Sodium Phosphate Dibasic (Na_2HPO_4), Sodium Phosphate Monobasic (KH_2PO4), Sodium hydroxide (NaOH), were purchased from Sigma-Aldrich, USA. All other chemicals and reagents were of analytical grade.

Compound preparation for bioassay

A stock solution of compound **1** was prepared at a concentration of 100 mM. This was achieved by drying the compound with nitrogen gas and weighing on a balance (AND GH-120, JAPAN) to ascertain the mass of the compound. Compound **1** was dissolved in appropriate amount of DMSO to attain desired concentration. The stock solution was vortexed (MSI Minishaker. IKA, JAPAN) and filter sterilized into a vial through 0.45 µm millipore filter under sterile conditions and stored at -20 °C until use.

Cell culture

Erythrocytes were obtained from blood of consented volunteers (Blood group O+). Venous blood was drawn and collected into containers with citrate phosphate dextrose (CPD) solution and kept at 4 °C overnight. This was centrifuged at 2,000 rpm for 10

minutes to separate the serum and buffy coat (KUBOTA 5200 centrifuge, JAPAN). Packed erythrocytes were washed three times with parasite washing medium (RPMI 1640, buffered with, 50 µg/mL gentamicin and 2 mM L-glutamine). Each washing step was carried out by the addition of wash medium, pipetting up and down thrice, centrifuging at 2,000 rpm for 10 minutes and discarding suspended medium. After washing, wash medium was added to the packed erythrocytes and stored at 4 °C until ready for use. Washed red blood cells were stored and used for up to 2 weeks maximum after which new blood was collected. Erythrocytic stages of malaria parasite (*Plasmodium falciparum*-chloroquine sensitive strain 3D7) were cultured in 25 ml flasks using the method of Trager and Jensen (1976) with modifications. Erythrocytes were maintained at 2% haematocrit (v/v) cell suspension in complete malaria parasite medium (RPMI 1640, buffered with 25 mM HEPES, supplemented with 7.5% $NaHCO_3$, 25 ug/ml gentamycin, 5% heat-inactivated human O+ serum from consenting subjects) and 5 mg/ml AlbuMax II and incubated at 37 °C under gas condition of 2% O_2, 5% CO_2 and 93% N_2. Parasite growth and development were monitored with Giemsa stained thin blood smear. Parasite culture was purified by using 5% sorbitol to obtain matured erythrocyte parasitic stages (late trophozoites and schizonts) from uninfected cells. The matured erythrocyte parasitic stages (purity > 90%) obtained were used to screen the compounds for antimalarial activity.

The GUTat 3.1 strain of the bloodstream form of *T. brucei* parasites was used in this study. Parasites were cultured *in vitro* according to the conditions established previously by Yabu et al. (1998). Parasites were used when they reached a confluent concentration of 1 x 10^6 parasites/ml. Estimation of parasitemia was done with the Neubauer counting chamber. Parasites were diluted to a concentration of 3 x 10^5 parasites/ml with IMDM medium and were used for the drug assay.

The log-phase promastigotes of *L. donovani* (D10) and *L. major* (NR48815) were cultured in M-119 growth medium with a working concentration of 6 × 10^6 cells/ml. Parasites were used when they reached confluent concentration of 1 x 10^6 parasite/ml. Estimation of parasitemia was done with the Neubauer counting chamber. Parasites were diluted to a concentration of 3 x 10^5 parasites/ml with M199 medium and used for the drug assay.

Bio-assays

In vitro* susceptibility testing of *Trichomonas mobilensis

Compound **1** was prepared at a concentration of 0-100 µM in 96 well plates. Trophozoites of *Trichomonas mobilensis* were seeded in the well plates at concentration of 2 x 10^6 cells/ml. The plates were sealed with seal stickers to provide anaerobic conditions for parasites after which the plates were transferred into an Anaero Pack jar with gas generators. The jar was incubated at 28 °C for 48 hours. Parasites were transferred into black well plates and 100 µl ATP bioluminescent was added before incubating for 20 minutes followed by luminescence readings using the GloMAX multi-detection system (Promega Corp, Madison, WI).

Screening for antimalarial activity using the SYBR Green I assay

Screening for antimalarial activity was achieved using the SYBR Green I fluorescence assay as described by Smilktein et al. (2004) with some modifications. Serial dilution of the standard (artesunate) which served as an experimental control and stock solution of compounds to yield final concentrations ranging from 15.63 nM to 500 nM and 1.95 µg/ml to 250 µg/ml respectively were prepared. The matured erythrocyte parasitic stages were treated with the compound and washed erythrocytes in 96 well plates and incubated with complete malaria parasite medium until harvested after 48 hours. Slides were prepared and percent parasitaemia was determined by counting the number of infected cells in a total of 500 erythrocytes in the Giemsa-stained thin blood smear. Briefly an aliquot of 5 µL per each concentration of the standard drug and compound was dispensed into test wells. About 95 µL of complete malaria parasite medium with washed erythrocytes at 2%

haematocrit and the purified matured erythrocyte parasitic stages (1% parasitaemia) were added, and incubated at 37 °C under gaseous conditions as stated above and untreated erythrocytes were used as control. Wells containing erythrocytes at 2% haematocrit, infected erythrocytes at 2% haematocrit and complete parasite medium alone served as negative controls, positive controls and blank controls respectively. Furthermore, wells containing infected parasites and 0.1% DMSO served as reference controls. Final volume per well was 100 µL. Plates were then incubated for 24 hours as described above in the cultivation of malaria parasites. 100 µL aliquot of 2.5x buffered SYBR Green I (0.25 µL of SYBR Green I/mL of phosphate buffer saline) was added to each well after the incubation.

In vitro viability test for trypanosome parasites

The Alamar Blue assay was carried out on treated and untreated trypanosome parasites to ascertain their viability. The assay was performed in a 96-well plate following the manufacturer's instructions with modification. Briefly, 1.5 x 10^4 parasites were seeded with varied concentrations of the compound ranging from 0 µM to 100 µM. Final concentrations of DMSO were kept at 0.1%, respectively. After incubation of parasites with or without the compound for 24 hours at 37 °C in 5% CO_2, 10% Alamar Blue dye was added, and the parasites were incubated another 24 hours in darkness. After a total of 48 hours, the plate was read for absorbance at 540 nm using the Tecan Sunrise Wako spectrophotometer, AUSTRIA GmbH. The trend curve was drawn to obtain a 50% inhibitory concentration (IC_{50}) of the compound.

In vitro viability test for leishmania parasites

The Alamar Blue assay was carried out on treated and untreated leishmania parasites to ascertain their viability. The assay was performed in a 96-well plate following the manufacturer's instructions with modification. Concentration of 3 x 10^5 parasites were seeded with varied concentrations of the compound ranging from 0 µM to 100 µM. Final concentrations of DMSO were kept at 0.1%. After incubation of parasites with or without the compound for 24 hours at 28 °C, 10% Alamar Blue dye was added, and the parasites were incubated for another 24 hours in darkness. After a total of 48 hours, the plate was read for absorbance at 540 nm using the Tecan Sunrise Wako spectrophotometer AUSTRIA GmbH. The trend curve was drawn to obtain a 50% inhibitory concentration (IC_{50}) of the compound.

RESULTS

Sediment sample collection sites

The Ghanaian endophytic fungus *Penicillium herquei* strain BRS2A-AR (Figure 3) was isolated from the leaves of *Laguncularia racemosa* collected from the banks of the Butre River in the Western Region of Ghana (4°49′43.73′N and 1°54′50.84′W). This area is noted for its biodiversity rich indigenous mangrove plants.

Taxonomy of *Penicillium herquei* strain BRS2A-AR

The organism exhibited a range of chemotaxonomic and phenotypic properties typical of members of the genus *Penicillium*. An almost complete 16S rRNA gene sequence (1455 nt) was determined for the organism. Primary sequence analysis with the sequences of representatives of the family *Trichocomaceae* confirmed that the unknown isolate was closely related to the species of the genus *Penicillium*.

Isolation and identification of compounds

The HPLC analysis (Figure 4) of the BRS2A-AR-MEEA-SF57 fraction showed the presence of three compounds **1**, **2** and **3**. About 2.2 mg of compound **1** was obtained and this was enough for the full structure determination process which was followed by bioactivity tests. For compounds **2** (0.8 mg) and **3** (0.9 mg) the amounts obtained were too small for the full structure determination and bioactivity tests. The structures of **2** and **3** were therefore elucidated by the information obtained for compound **1** and the corresponding fragmentation patterns of **2** and

3. Analysis of the 1D and 2D-NMR coupled with HRESI-LC-MS data identified compound **1** as (*9Z,11E*)-13-oxooctadeca-9,11-dienoic acid (13-oxo-ODA). The two new derivatives, compound **2** and **3** were also identified as (*9Z, 11E*)-13-hydroxypentacosa-9,11-dienoic acid and (*9Z, 11E, 13E*)-pentacosa-9,11,13-trienoic acid.

Compound **1**; (*9Z,11E*)-13-oxooctadeca-9,11-dienoic acid; brightly coloured yellowish oil; UV (H_2O:CH_3CN) λ_{max} 226, 278 nm; HRESI-LC-MS *m/z* 295.2271 $[M+H]^+$; *m/z* 294.2195, $C_{18}H_{30}O_3$. ^{13}C NMR (125 MHz, CD_3OD): δ_C 181.2 (C-1), 39.3 (C-2), 27.7 (C-3), 30.7 (C-4), 30.3 (C-5), 30.4 (C-6), 29.9 (C-7), 34.1 (C-8), 147.5 (C-9), 130.2 (C-10), 145.4 (C-11), 128.8 (C-12), 204.0 (C-13), 40.9 (C-14), 25.4 (C-15), 32.6 (C-16), 23.5 (C-17), 14.2 (C-18). ^1H NMR (500 MHz, CD_3OD): δ_H 2.18 (2H, t, *J* = 6.8 Hz, H-2), 1.63 (2H, m, H-3), 1.32 (2H, m, H-4), 1.37 (2H, m, H-5), 1.37 (2H, m, H-6), 1.49 (2H, m, H-7), 2.24 (2H, m, H-8), 6.30 (1H, d, *J* = 15.5 Hz, H-9), 6.30 (1H, dd, *J* = 9.5, 15.0 Hz, H-10), 7.27 (1H, dd, *J* = 9.5, 15.0 Hz, H-11), 6.15 (1H, d, *J* = 15.5 Hz, H-12), 2.62 (2H, t, *J* = 6.7 Hz, H-14), 1.63 (2H, m, H-15), 1.35 (2H, m, H-16), 1.36 (2H, m, H-17), 0.91 (3H, t, *J* = 6.9 Hz, H-18). Bioactivity data of compound **1** is summarized in Table 1, summarized NMR data can be found in Table 2 and raw data in Figures 11-17.

Compound **2**: (*9Z,11E*)-13-hydroxypentacosa-9,11-dienoic acid; brightly coloured yellowish oil; UV (H_2O:CH_3CN) λ_{max} 226, 278 nm ; HRESI-LC-MS *m/z* 395.3307 $[M+H]^+$; *m/z* 394.3447, $C_{25}H_{46}O_3$.

Compound **3**: (9Z,11E,13E)-pentacosa-9,11,13-trienoic acid; brightly coloured yellowish oil; UV (H_2O:CH_3CN) λ_{max} 226, 278 nm ; HRESI-LC-MS *m/z* 377.3207 $[M+H]^+$; *m/z* 376.3341, $C_{25}H_{44}O_2$.

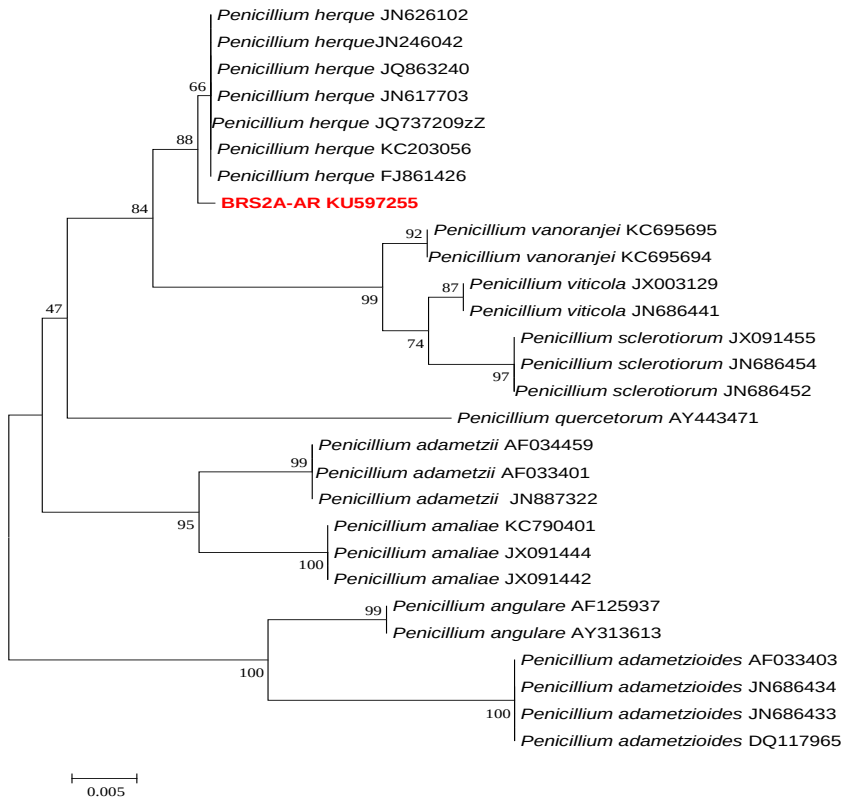
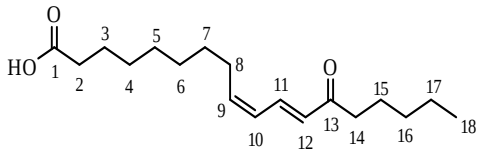
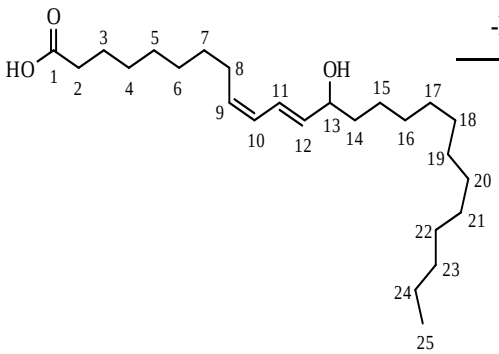


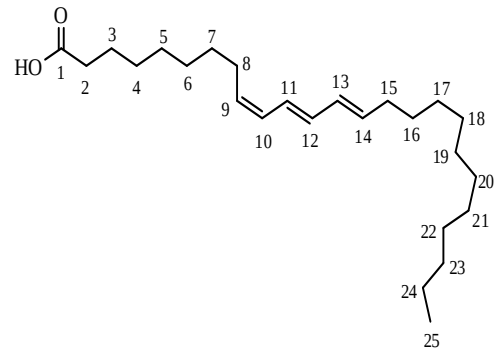
Figure 1: Phylogenetic tree based on 16S rRNA gene sequences showing the relationships of *Penicillium herquei* strain BRS2A-AR and related taxa. The dendrogram was reconstructed by using the neighbor-joining method. Bootstrap values (based on 1000 replicates) greater than 50% are shown at nodes. Bar, 0.001 sequence dissimilarity per nucleotide position. GenBank ID: KU597255.



Compound **1**
(*9Z,11E*)-13-oxooctadeca-9,11-dienoic acid
(13-oxo-ODA)



Compound **2**
(*9Z,11E*)-13-hydroxypentacosa-9,11-dienoic acid



Compound **3**
(*9Z,11E,13E*)-pentacosa-9,11,13-trienoic acid

Figure 2: Structure of compounds isolated from the endophytic fungus strain BRS2A-AR

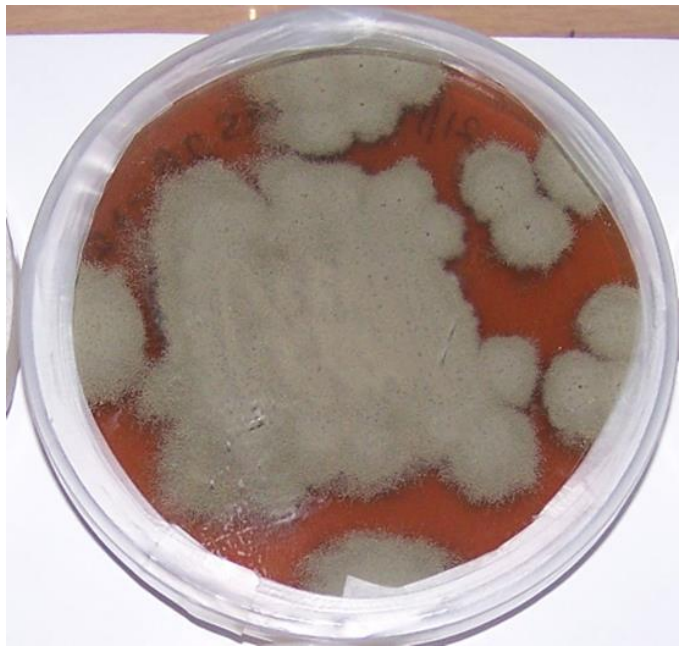


Figure 3: Endophytic fungus *Penicillium herquei* strain BRS2A-AR growing on Malt extract agar plate.

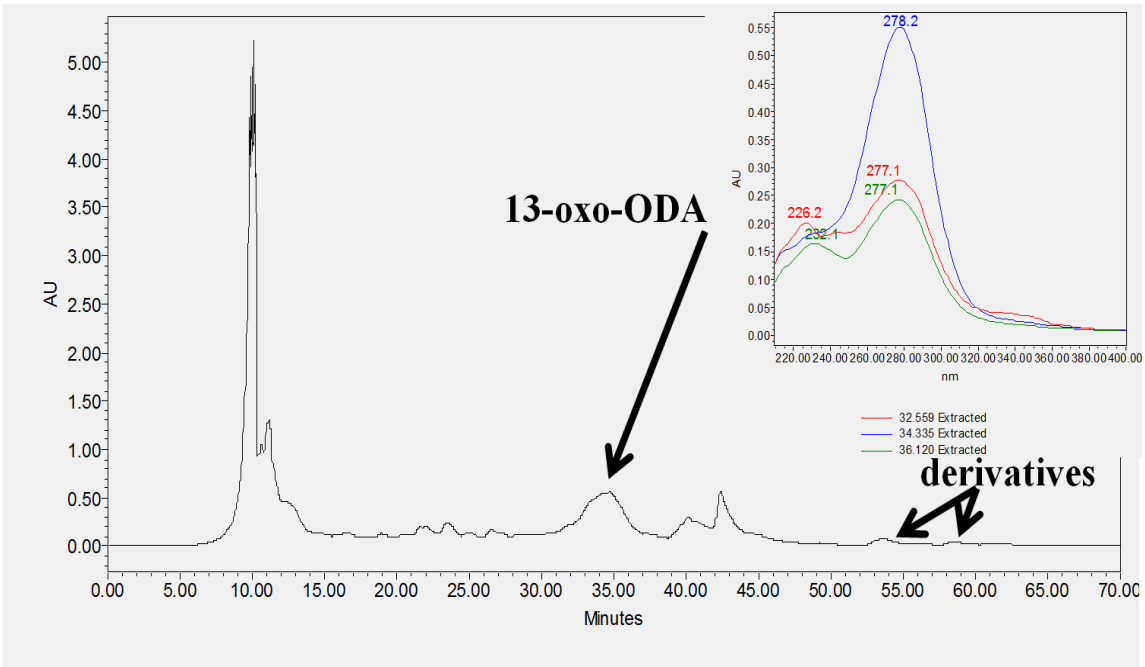


Figure 4: HPLC and UV profile of BRS2A-AR-MEEA-SF57 fraction showing the retention times of compound **1**, **2** and **3**.

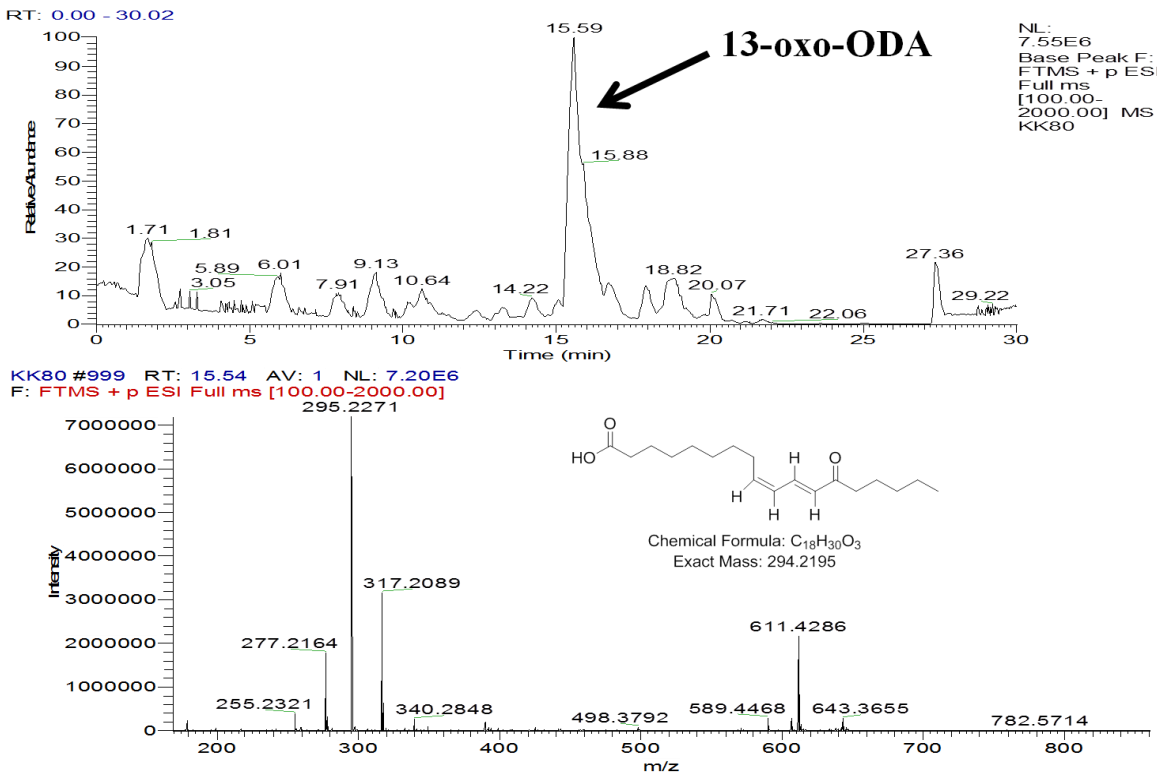


Figure 5: HRESI-LCMS chromatogram of compound **1**.

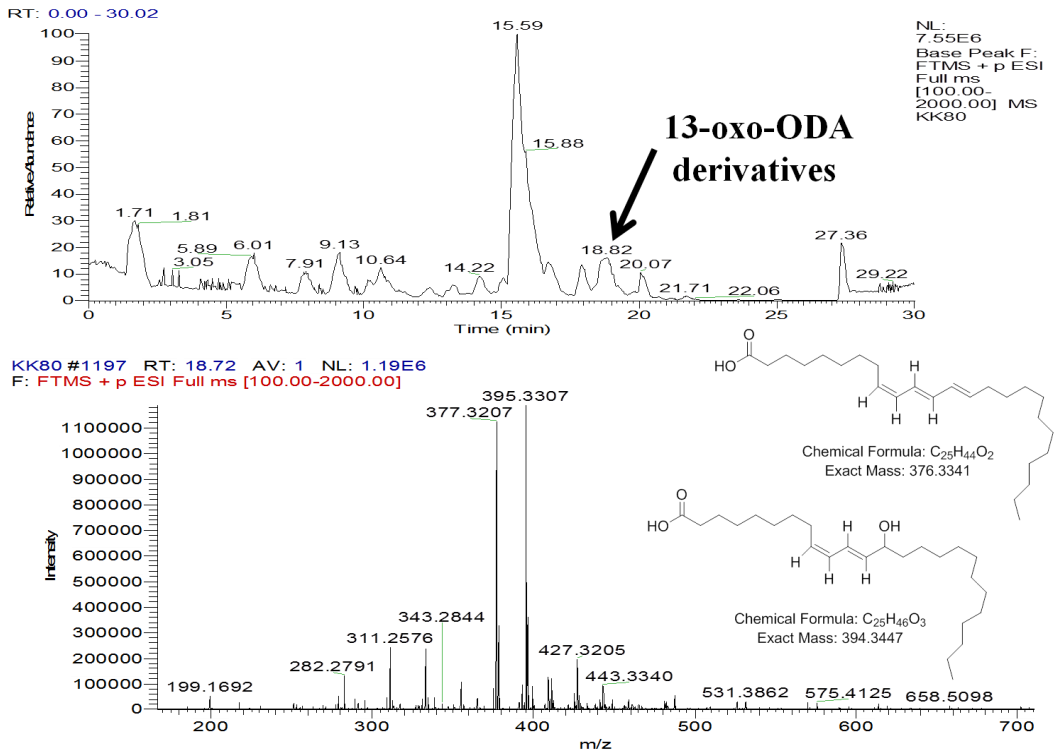


Figure 6: HRESI-LCMS chromatogram of compound **2** and **3**.

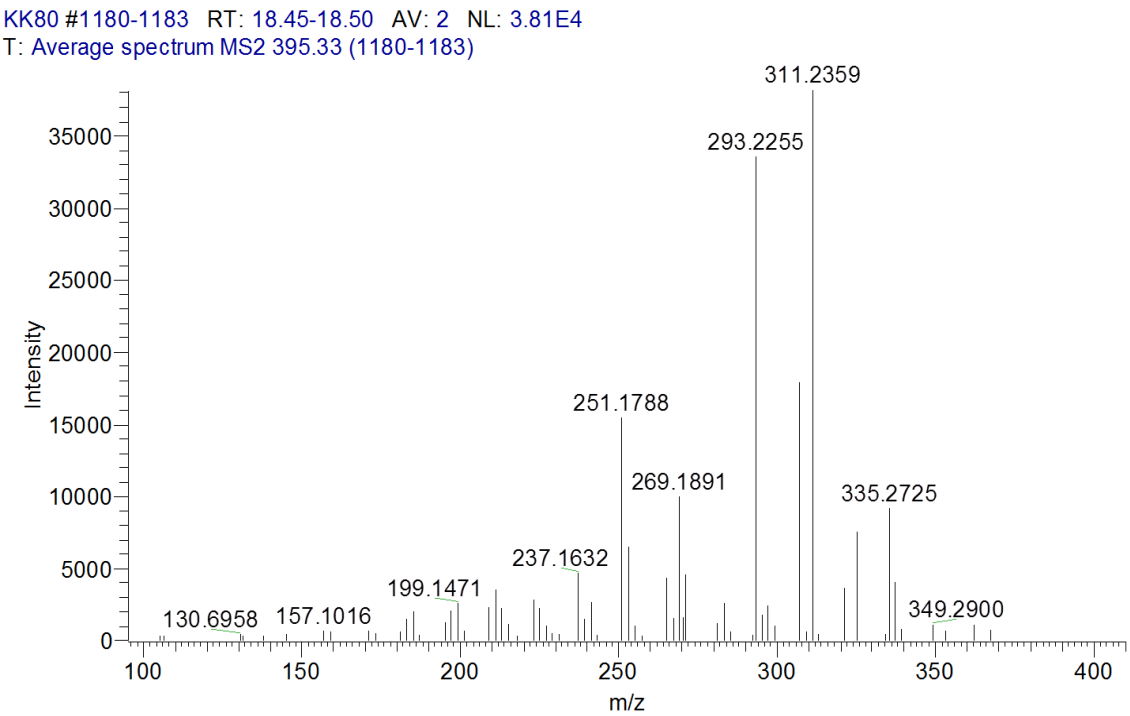


Figure 7: HRESI-LCMS fragmentation pattern of compound **2.**

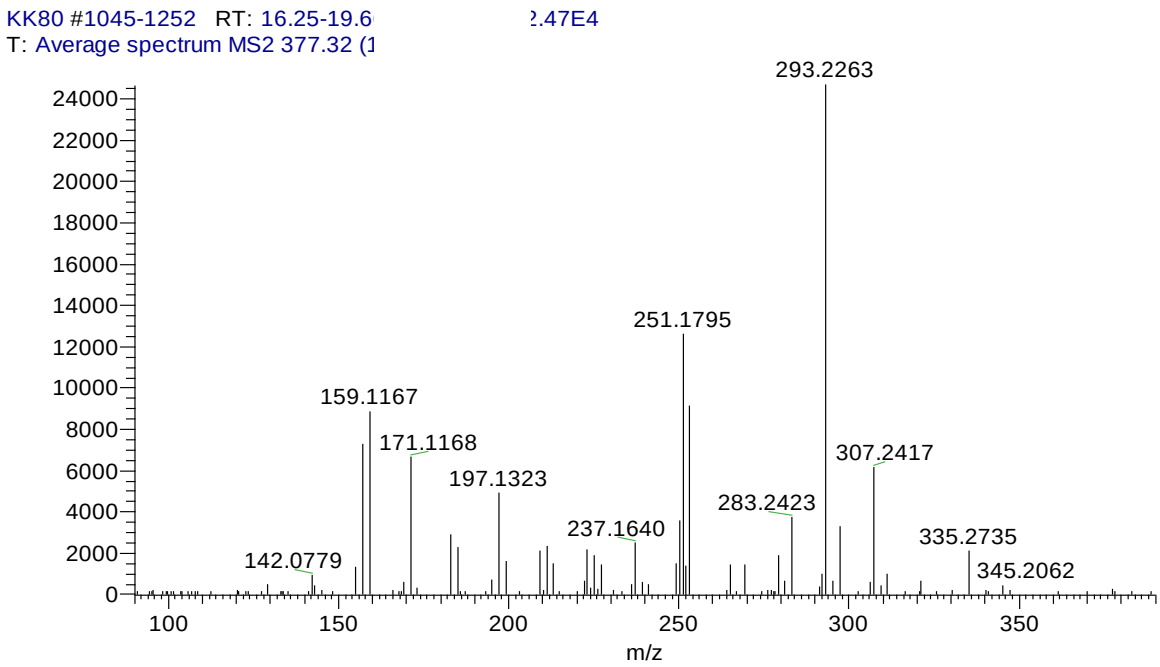


Figure 8: HRESI-LCMS fragmentation pattern of compound **3**.

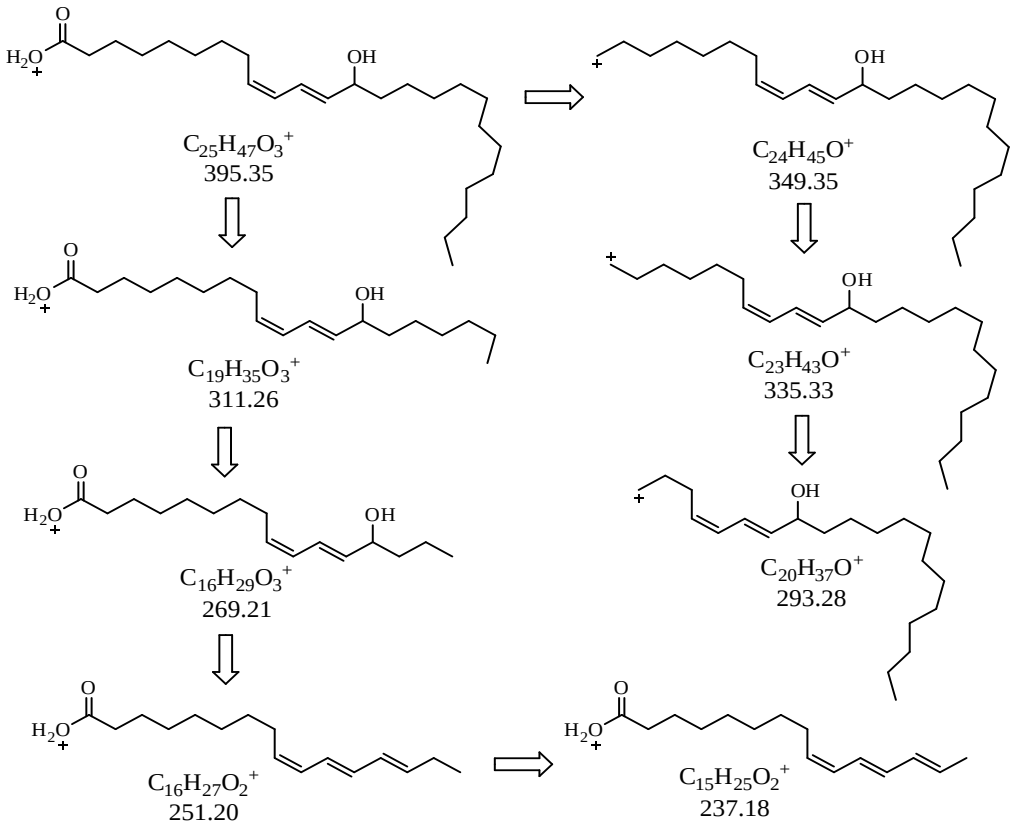


Figure 9: Proposed structures for mass fragments of compound **2** corresponding to mass peaks found in HRESI-LCMS.

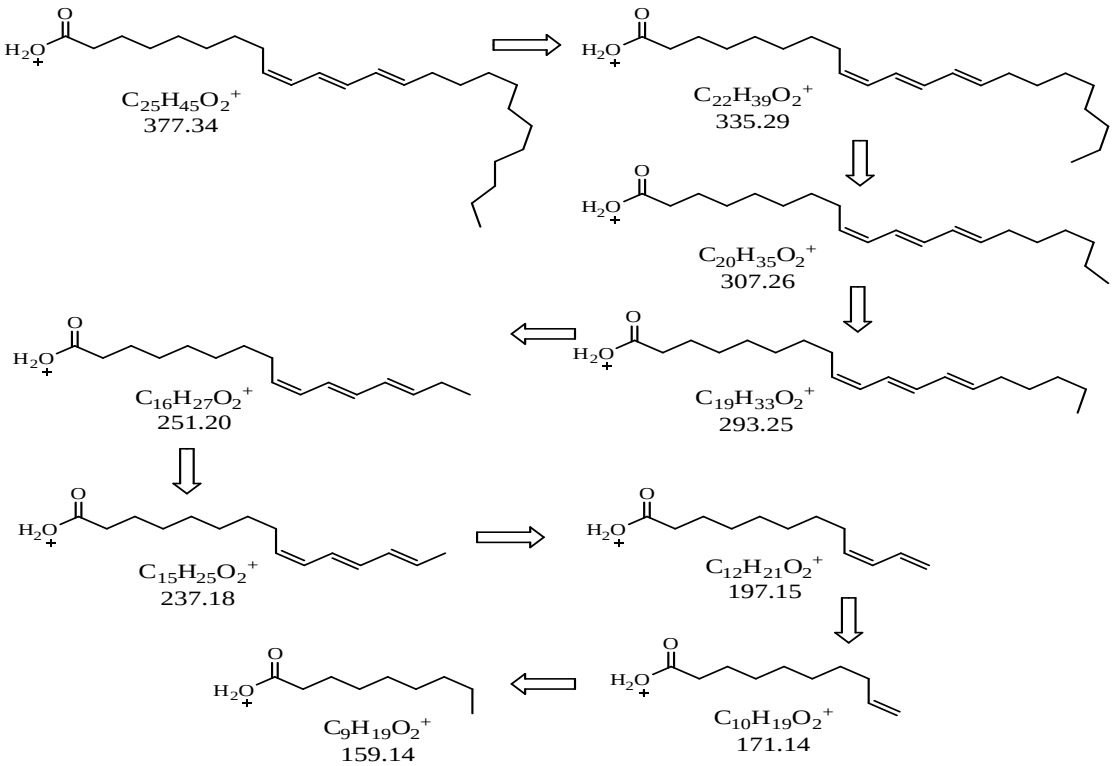


Figure 10: Proposed structures for mass fragments of compound **3** corresponding to mass peaks found in HRESI-LCMS.

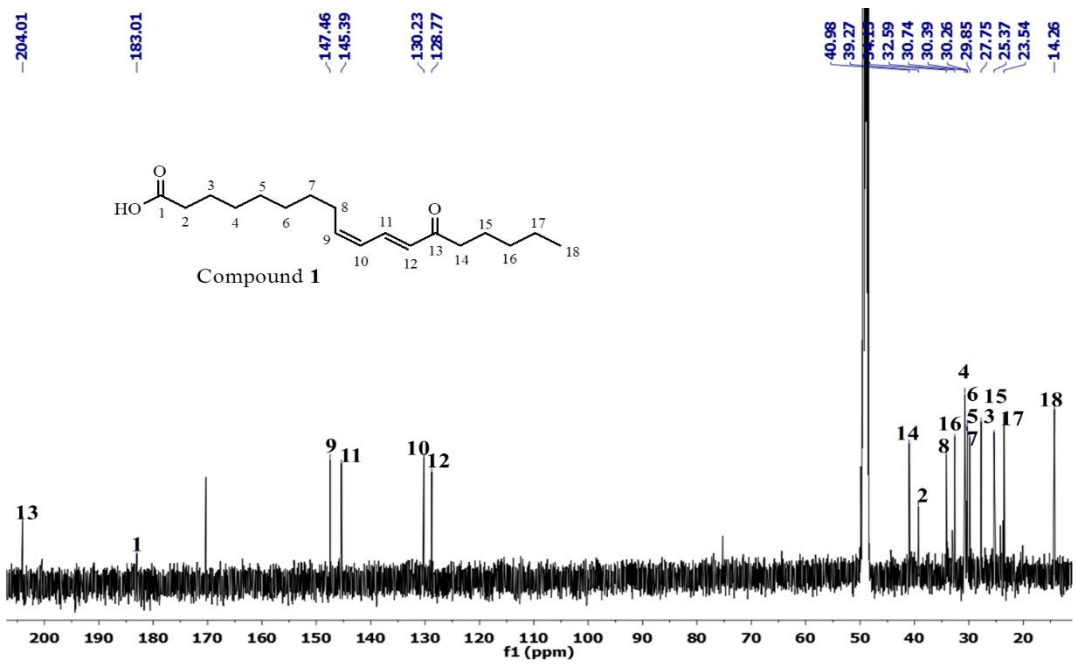


Figure 11: ^{13}C NMR (150 MHz, CD_3OD) spectrum of compound **1**.

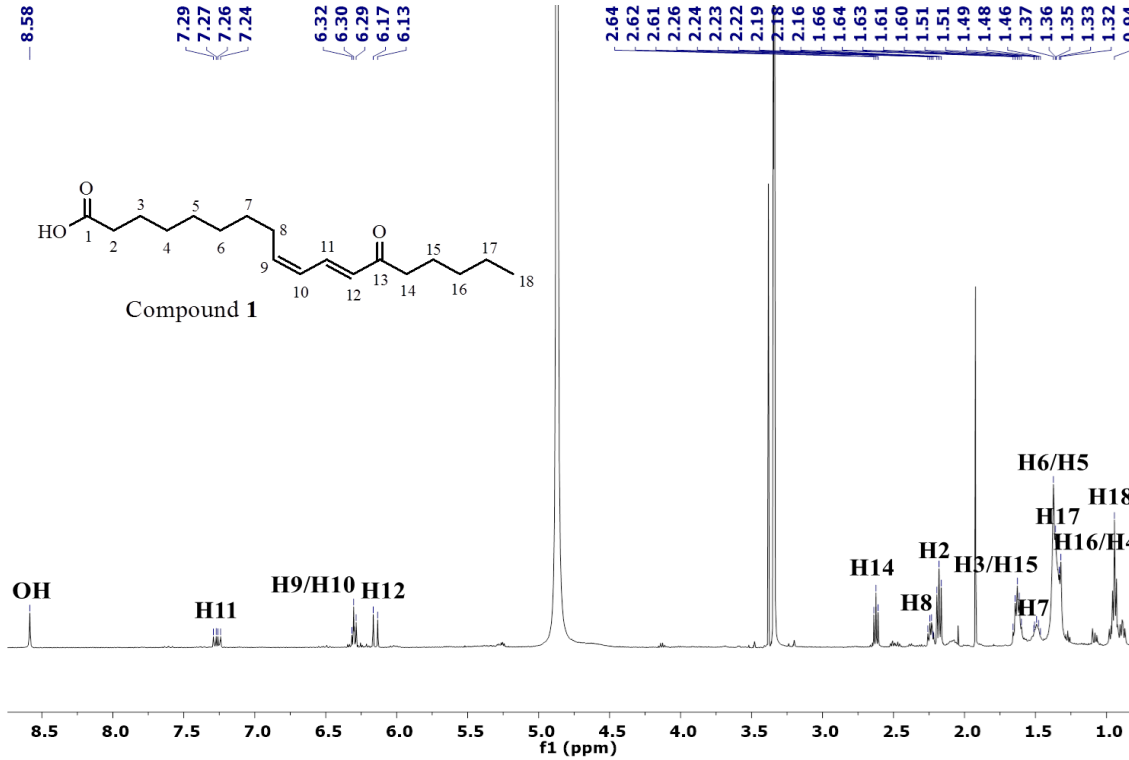


Figure 12: ^{1}H NMR (500 MHz, CD_3OD) spectrum of compound **1**.

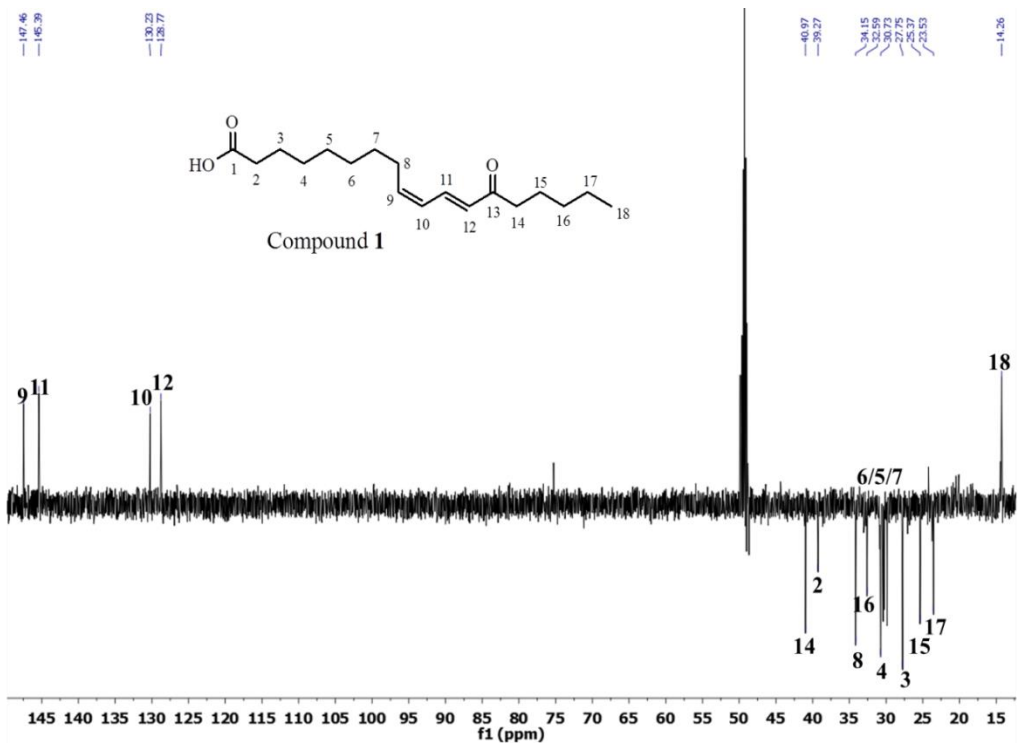


Figure 13: DEPT135 NMR (500 MHz, CD_3OD) spectrum of compound **1**.

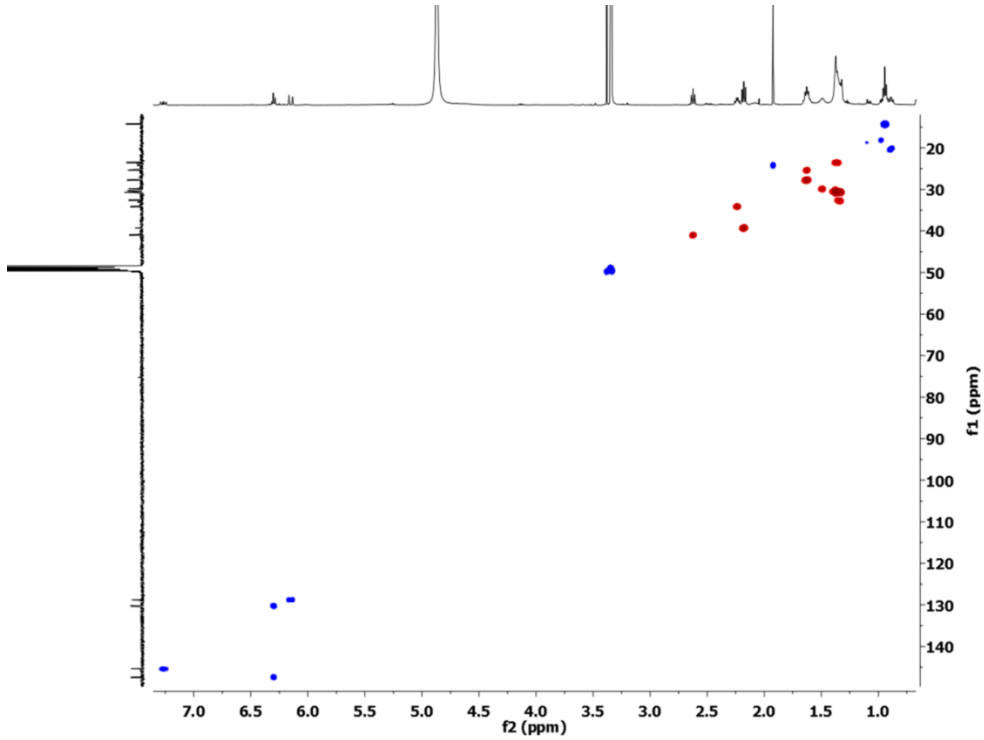


Figure 14: HSQC NMR (500 MHz, CD_3OD) spectrum of compound **1**.

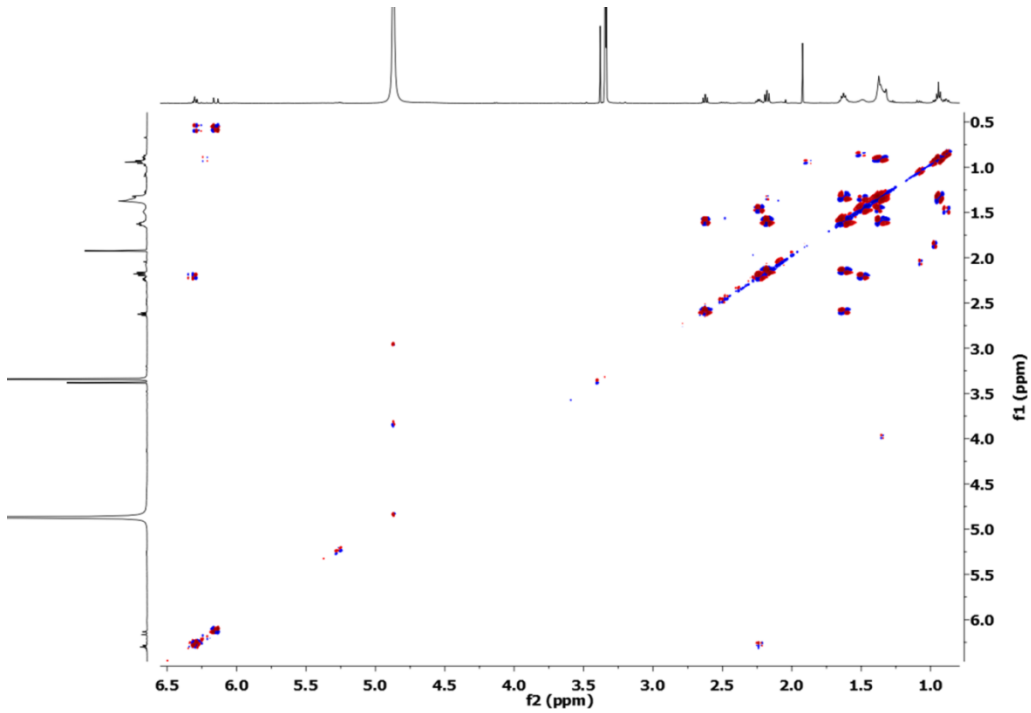


Figure 15: ^{1}H-^{1}H COSY NMR (500 MHz, CD_3OD) spectrum of compound **1**.

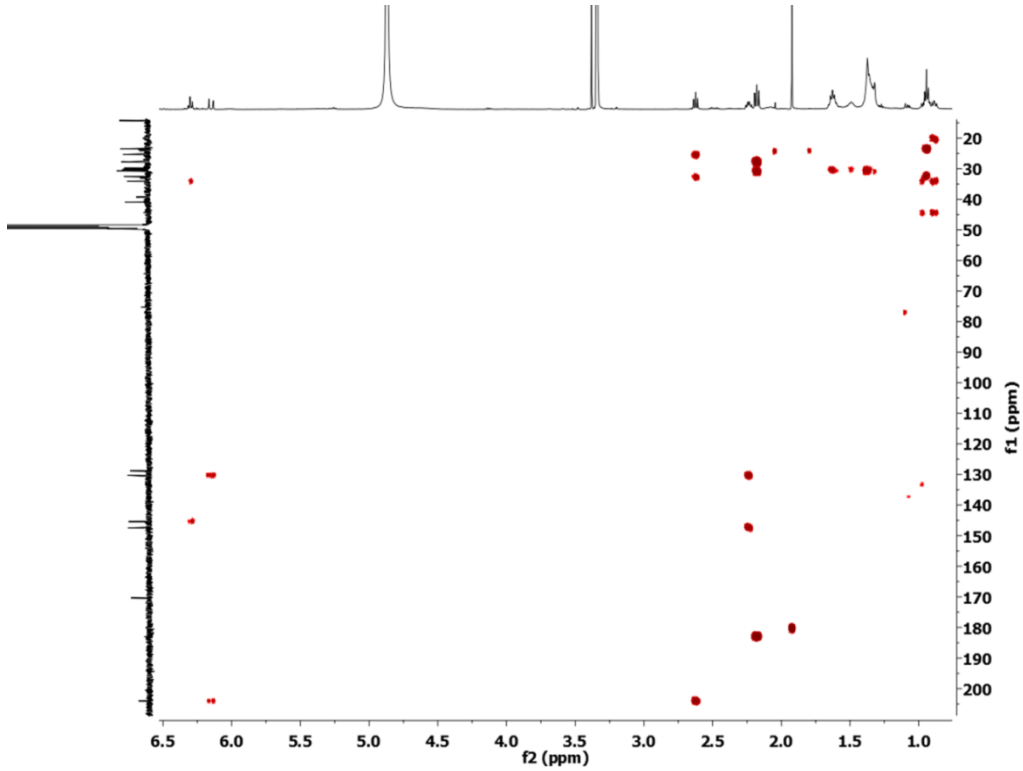


Figure 16: HMBC NMR (500 MHz, CD_3OD) spectrum of compound **1**.

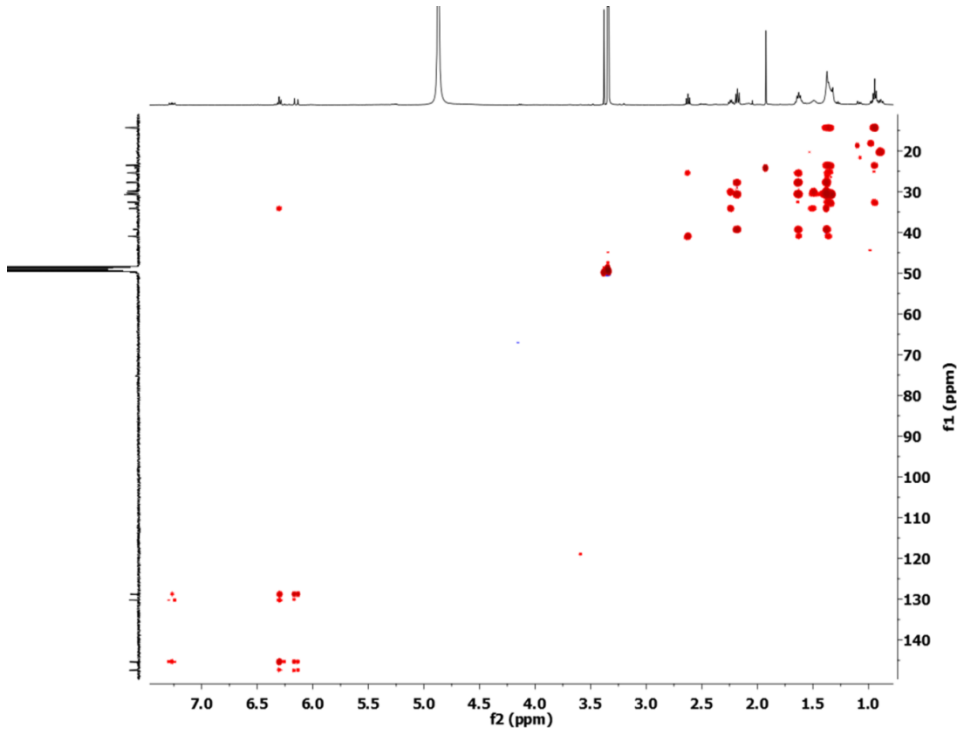


Figure 17: ^{13}C-^{1}H 2D-TOCSY NMR (500 MHz, CD_3OD) spectrum of compound **1**.

Table 1: Antiparasitic bioacivity data for compound **1**.

Compound 1	Parasite	IC_{50} (µM)	Positive Control	IC_{50}
13-oxo-ODA	*T. mobilensis*	44.47	Metronidazole	5.2 µM
	P. falciparum 3d7	> 100	Artesunate	36 nm
	T. brucei brucei	> 100	*Coptis japonica*	8.20 µM
	L. donovani	> 100	Amphotericin B	0.32 µM
	L. major	> 100	Amphotericin B	0.31 µM

Table 2: Full 1D and 2D NMR Spectroscopic Data for compound **1** in CD_3OD, δ in ppm.

#	δc mult	δ_H mult (J Hz)	1H-1H COSY	HMBC
1	181.2, C			2, 3
2	39.3, CH_2	2.18, t (6.8)	3	
3	27.7, CH_2	1.63, m	2, 4	2
4	30.7, CH_2	1.32, m	3	2
5	30.3, CH_2	1.37, m	6	7, 3
6	30.4, CH_2	1.37, m	7	5, 7
7	29.9, CH_2	1.49, m	8, 6	
8	34.1, CH_2	2.24, m	7	9, 10
9	147.5, CH	6.30, d (15.5)	8	8
10	130.2, CH	6.30, dd (9.5, 15.0)	9, 11	12, 8
11	145.4, CH	7.27, dd (9.5, 15.0)	10, 12	9, 10
12	128.8, CH	6.15, d (15.5)	11	
13	204.0, C			14, 12, 15
14	40.9, CH_2	2.62, t (6.7)	15	
15	25.4, CH_2	1.63, m	14, 16	14
16	32.6, CH_2	1.35, m	18	14, 18
17	23.5, CH_2	1.36, m	18	18
18	14.2, CH_3	0.91, t (6.9)	17	

DISCUSSION

Compound **1** was obtained at t_R of 34 min on semi-preparative reverse phase HPLC as a brightly coloured yellowish oil. The HRESI-LC-MS of compound **1**, gave a *m/z* of 295.2271 Δ = 0.01 ppm (Figure 5). The HRESI-LC-MS data showed the elemental composition of this compound to be $C_{18}H_{30}O_3$ (unsaturation number of 4) which was in agreement with the ^1H and ^{13}C-NMR data. Analysis of the ^1H, ^{13}C and multiplicity edited gHSQCAD spectra of compound **1**, suggested the presence of 2 quaternary (2C), 4 methine (4CH), 11 methylene ($11CH_2$) and 1 methyl ($1CH_3$) carbons. The comparison of the UV, 1D and 2D-NMR and HRESI-LC-MS spectra data with reported values led to the identification of compound **1** as (*9Z,11E*)-13-oxooctadeca-9,11-dienoic acid (13-oxo-ODA) (Brook et al., 1977; Kim et al., 2012). Compound **1** showed weak antiparasitic activity when tested against *Plasmodium falciparum* 3d7, *Trypanosoma brucei brucei*, *Leishmani donovani* and *Leishmania major* with IC_{50} values higher than 100 µM. However, when tested against *Trichomonas mobilensis* compound **1** gave IC_{50} 44.47 µM which was eight times less active than metronidazole (IC_{50} 5.20 µM).

The completed structure of compound **1** made it possible therefore to determine the structures of the two new derivatives, compound **2** (*9Z,11E*)-13-hydroxypentacosa-9,11-dienoic acid and compound **3** (*9Z,11E,13E*)-pentacosa-9,11,13-trienoic acid which were isolated from the extracts of strain BRS2A-AR for the first time ever. Detailed analysis of the HRESI-LC-MS data with predicted structures using ChemDraw showed that compound **3** was obtained from compound **2** through loss of water (Figures 6-8). A comparison of the fragmentation patterns detected in the HRESI-LC-MS data for compound **2** and **3** were similar and most of the predicted structures were consistent with the peaks seen in the HRESI-LC-MS data (Figures 9-10).

Compound **1** (13-oxo-ODA) and two new derivatives, compound **2** and **3** were isolated from the endophytic fungus strain BRS2A-AR isolated from the leaves of *Laguncularia racemosa* collected from the banks of the Butre River in the Western Region of Ghana. Compound **1** was found to exhibit moderate antiparasitic activity against *Trichomonas mobilensis*. This represents the first report of the isolation of 13-oxo-ODA and the two new derivatives from endophytic fungus *Penicillium herquei* strain BRS2A-AR.

The current hospital treatment for trichomoniasis involves the use of only three main drugs, metronidazole, tinidazole and benznidazole. These drugs belong to the same scaffold, which is the nitroimidazole class of compounds. Therefore, it is obvious that, the world is currently dependent on a very narrow spectrum of treatment drugs for trichomoniasis. Furthermore, these drugs have been prescribed for more than three decades in the treatment of both parasitic and bacterial infections across different organisms that include humans and animals. There is a current widespread development of resistance to metronidazole, tinidazole and benznidazole (Crowell et al., 2003; Kirkcaldy et al., 2012; Smith et al., 2018; Savoldi et al., 2018) and the discovery of new drug scaffolds from microbial natural products in sub-Saharan Africa that is known to have a high prevalence of the infection represents a very important research goal.

COMPETING INTERESTS

The authors declare that, they have no competing interests.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to this article.

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REFERENCES

Andreou A, Feussner I. 2009. Lipoxygenases–structure and reaction mechanism. *Phytochemistry*, **70**(13-14): 1504-1510. DOI: https://doi.org/10.1016/j.phytochem.2009.05.008

Barbosa M, Valentão P, Andrade P. 2016. Biologically active oxylipins from enzymatic and nonenzymatic routes in macroalgae. *Marine drugs*, **14**(1): 23. DOI: https://doi.org/10.3390/md14010023

Blée E. 2002. Impact of phyto-oxylipins in plant defense. *Trends in Plant Science*, **7**(7): 315-322. DOI: https://doi.org/10.1016/S1360-1385(02)02290-2

Brodhun F, Feussner I. 2011. Oxylipins in fungi. *The FEBS Journal*, **278**(7): 1047-1063. DOI: https://doi.org/10.1111/j.1742-4658.2011.08027.x

Brooks GT. 1977. Epoxide hydratase as a modifier of biotransformation and biological activity. *General Pharmacology: The Vascular System*, **8**(4): 221-226. DOI: https://doi.org/10.1016/0306-3623(77)90016-7

Crowell AL, Sanders-Lewis KA, Secor WE. 2003. *In vitro* metronidazole and tinidazole activities against metronidazole-resistant strains of *Trichomonas vaginalis*. *Antimicrobial Agents and Chemotherapy*, **47**(4): 1407-1409. DOI: https://doi.org/10.1128/AAC.47.4.1407-1409.2003

Dave A, Graham IA. 2012. Oxylipin signaling: a distinct role for the jasmonic acid precursor cis-(+)-12-oxo-phytodienoic acid (cis-OPDA). *Front Plant Sci*, **3**: 42. DOI: https://doi.org/10.3389/fpls.2012.00042

Felsenstein J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol*, **17**(6): 368–376. DOI: https://doi.org/10.1007/BF01734359

Felsenstein J. 1985. Phylogenies and the comparative method. *Am Nat*, **125**(1): 1–15. DOI: https://www.jstor.org/stable/2461605

Field CJ, Schley PD. 2004. Evidence for potential mechanisms for the effect of conjugated linoleic acid on tumor metabolism and immune function: lessons from n− 3 fatty acids. *Am J Clin Nutr*, **79**(6): 1190S-1198S. DOI: https://doi.org/10.1093/ajcn/79.6.1190S

Fischer GJ, Keller NP. 2016. Production of cross-kingdom oxylipins by pathogenic fungi: an update on their role in development and pathogenicity. *Journal of Microbiology*, **54**(3): 254-264. DOI: https://doi:10.1007/s12275-016-5620-z

Gabbs M, Leng S, Devassy JG, Monirujjaman M, Aukema HM. 2015. Advances in our understanding of oxylipins derived from dietary PUFAs. *Adv Nutr*, **6**(5): 513-540. DOI: https://doi.org/10.3945/an.114.007732

Granér G, Hamberg M, Meijer J. 2003. Screening of oxylipins for control of oilseed rape (Brassica napus) fungal pathogens. *Phytochemistry*, **63**(1): 89-95. DOI: https://doi.org/10.1016/S0031-9422(02)00724-0

Göbel C, Feussner I. 2009. Methods for the analysis of oxylipins in plants. *Phytochemistry*, **70**(13-14): 1485-1503. DOI: https://doi.org/10.1016/j.phytochem.2009.07.040

Gudbrandsen OA, Rodrı´guez E, Wergedahl H, Mørk S, Reseland JE, et al. 2009. Trans-10, cis-12-conjugated linoleic acid reduces the hepatic triacylglycerol

content and the leptin mRNA level in adipose tissue in obese Zucker fa/fa rats. *Br J Nutr*, **102**: 803–815. DOI: https://doi.org/10.1017/S0007114509297200

Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H, Park SC, Jeon YS, Lee JH, Yi H, Won S, Chun J. 2012. Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol*, **62**: 716–721. https://doi 10.1099/ijs.0.038075-0

Kim YI, Hirai S, Goto T, Ohyane C, Takahashi H, Tsugane T, Aoki K. 2012. Potent PPARα activator derived from tomato juice, 13-oxo-9, 11-octadecadienoic acid, decreases plasma and hepatic triglyceride in obese diabetic mice. *PLoS One*, **7**(2): e31317. https://doi.org/10.1371/journal.pone.0031317

Kim YI, Hirai S, Takahashi H, Goto T, Ohyane C, Tsugane T, et al. 2011. 9-oxo-10 (E), 12 (E)-octadecadienoic acid derived from tomato is a potent PPARα agonist to decrease triglyceride accumulation in mouse primary hepatocytes. *Molecular Nutrition & Food Research*, **55**(4): 585-593. DOI: https://doi.org/10.1002/mnfr.201000264

Kirkcaldy RD, Augostini P, Asbel LE, Bernstein KT, Kerani RP, Mettenbrink CJ, et al. 2012. Trichomonas vaginalis antimicrobial drug resistance in 6 US cities, STD Surveillance Network, 2009–2010. *Emerging Infectious Diseases*, **18**(6): 939. DOI: https://doi:10.3201/eid1806.111590

Kluge AG, Farris JS. 1969. Quantitative phyletics and the evolution of anurans. *Syst Zool*, **18**: 1–32. DOI: https://doi:10.2307/2412407

Kupchan MS, Britton RW, Zeigler MF, Sigel CW. 1973. Bruceantin, a new potent antileukemic simaroubolide from Brucea antidysenterica. *J. Org. Chem.*, **38**: 178–179. DOI: https://doi:10.1021/jo00941a049

Lane DJ. 1991. 16S/23S rRNA sequencing. Nucleic acid techniques in bacterial systematics. Wiley: Chichester.

Li L, Zhao Y, McCaig BC, Wingerd BA, Wang J, Whalon ME, Howe GA. 2004. The tomato homolog of CORONATINE-INSENSITIVE is required for the maternal control of seed maturation, jasmonate-signaled defense responses, and glandular trichome development. *The Plant Cell*, **16**(1): 126-143. DOI: https://doi: 10.1105/tpc.017954

Martínez E, Campos-Gómez J. 2016. Oxylipins produced by Pseudomonas aeruginosa promote biofilm formation and virulence. *Nat. Commun.*, **7**: 13823. DOI: https://doi: 10.1038/ncomms13823

Meyer N, Rettner J, Werner M, Werz O, Pohnert G. 2018. Algal Oxylipins Mediate the Resistance of Diatoms against Algicidal Bacteria. *Marine Drugs*, **16**(12): 486. DOI: https://doi.org/10.3390/md16120486

Mosblech A, Feussner I, Heilmann I. 2009. Oxylipins: structurally diverse metabolites from fatty acid oxidation. *Plant Physiology and Biochemistry*, **47**(6): 511-517. DOI: https://doi.org/10.1016/j.plaphy.2008.12.011

Mueller MJ. 2004. Archetype signals in plants: the phytoprostanes. *Curr Opin Plant Biol*, **7**(4): 441-448. DOI: https://doi.org/10.1016/j.pbi.2004.04.001

Prost I, Dhondt S, Rothe G, Vicente J, Rodriguez MJ, Kift N, Castresana C. 2005. Evaluation of the antimicrobial activities of plant oxylipins supports their involvement in defense against pathogens. *Plant Physiol*, **139**(4): 1902-1913. DOI: https://doi:10.1104/pp.105.066274

Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol*, **4**(4): 406–425. DOI: https://doi.org/10.1093/oxfordjournals.molbev.a040454

Savoldi A, Carrara E, Graham DY, Conti M, Tacconelli E. 2018. Prevalence of

antibiotic resistance in Helicobacter pylori: a systematic review and meta-analysis in World Health Organization regions. *Gastroenterology*, **155**(5): 1372-1382. DOI: https://doi.org/10.1053/j.gastro.2018.07.007

Shea JM, Del Poeta M. 2006 Lipid signaling in pathogenic fungi. *Curr. Opin. Microbiol.*, **9**: 352–358. DOI: https://doi.org/10.1016/j.mib.2006.06.003

Smilkstein M, Sriwilaijaroen N, Kelly JX, Wilairat P, Riscoe M. 2004. Simple and inexpensive fluorescence-based technique for high-throughput antimalarial drug screening. *Antimicrobial Agents and Chemotherapy*, **48**(5): 1803-1806. DOI: https://doi:10.1128/AAC.48.5.1803-1806.2004

Smith A. 2018. Metronidazole resistance: a hidden epidemic. *British Dental Journal*, **1**. DOI: https://doi.org/10.1038/sj.bdj.2018.221

Trager W, Jensen JB. 1976. Human malaria parasites in continuous culture. *Science*, **193**(4254): 673-675. DOI: https://DOI:10.1126/science.781840

Vianna JS, Ramis IB, Ramos DF, Von Groll A, Silva PEAD. 2016. Drug Resistance in Helicobacter pylori. *Arquivos De Gastroenterologia*, **53**(4): 215-23.http://dx.doi.org/10.1590/S0004-28032016000400002

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol*, **30**(12): 2725–2729. DOI: https://doi:10.1093/molbev/mst197

Trapp MA, Kai M, Mithöfer A, Rodrigues-Filho E. 2015. Antibiotic oxylipins from Alternanthera brasiliana and its endophytic bacteria. *Phytochemistry*, 110: 72-82. DOI: https://doi.org/10.1016/j.phytochem.2014.11.005.

Tsitsigiannis DI, Keller NP. 2007. Oxylipins as developmental and host–fungal communication signals. *Trends Microbiol*, **15**(3): 109-118. DOI: https://doi.org/10.1016/j.tim.2007.01.005

Van Leeuwen W. Ökrész L, Bögre L, and Munnik T. 2004. Learning the lipid language of plant signalling. *Trends Plant Sci*, **9**(8): 378-384. DOI: https://doi.org/10.1016/j.tplants.2004.06.008

Wasternack C, Kombrink E. 2009. Jasmonates: structural requirements for lipid-derived signals active in plant stress responses and development. *ACS Chemical Biology*, **5**(1): 63-77. DOI: https://doi:10.1021/cb900269u

Yabu Y, Minagawa N, Kita K, Nagai K, Honma M, Sakajo S, Yoshimoto A. 1998. Oral and intraperitoneal treatment of Trypanosoma brucei brucei with a combination of ascofuranone and glycerol in mice. *Parasitol. Int.*, **47**(2): 131-137. DOI: https://doi.org/10.1016/S1383-5769(98)00011-7.