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***Corresponding Author**
Towett Kipngetich Erick
E-mail
joycebett01@gmail.com

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Two New Aliphatic Alkenol Geometric Isomers and a Phenolic Derivate from Endophytic Fungus *Diaporthe* sp. Host to *Syzygium cordatum* (Myrtaceae)

Towett Kipngetich Erick[1]*, Joyce Kiplimo[1], Josphat Matasyoh[2]

[1] Department of Physical Sciences, School of Science and Technology, University of Kabianga, 20200, Kericho, Kenya
[2] Department of Chemistry, Faculty of Science, Egerton University, PO Box 536-20115, Njoro, Kenya

Abstract

Fungal endophytes are regarded as a prolific source of secondary metabolites with desirable antibacterial, anticancer, antifungal and antidiarrheal properties. In this study, the fungal endophyte *Diaporthe* sp. host to the plant *Syzygium cordatum* yielded three novel antibacterial compounds after extraction with ethyl acetate and purification using column chromatography and preparative high-performance liquid chromatography. The eluted fraction that yielded the three compounds showed significant antibacterial activity against selected common bean bacterial pathogens; *Pseudomonas syringae* and *Xanthomonas axonopodis* with their corresponding zones of inhibition of 10.7 mm and 14 mm, respectively. The structural determination of three compounds was achieved using spectral information from 1D and 2D NMR as well as mass spectrometry. Two enol geometric isomers that were accorded trivial names as *Z*-cordatenol, *E*-cordatenol, and a phenolic derivative was accorded a trivial name as α-cordenol. Bioactivity of *Z*-cordatenol and *E*-cordatenol was attributed to hydroxylation on the aliphatic alkene chains, whereas, in α-cordenol, hydroxylation of the benzene ring and side chain aziridine ring enhanced its activity. These results are evident that endophytic fungi are rich sources of secondary metabolites that can be utilized to control phytopathogens.

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Introduction

Common bean (*Phaseolus vulgaris L.)* as a cheap alternative source of proteins and other essential minerals, is the most consumed leguminous crop among the Kenyans, who cannot afford other sources like meat and fish. Kenyans consume approximately 450,000 tons of beans against the local production between 150,000 and 200,000 tons harvested from about 800,000 hectares [1]. The declining level of bean's productivity is attributed to bacterial pathogens *Pseudomonas syringae* pv. *phaseolicola* and *Xanthomonas axonopodis* pv. *phaseoli*, which causes halo blight and common bacterial blight (CBB), respectively. These bacterial diseases in beans can be easily noticed by water-soaked coloration and red-brown scratches on the leaves, which subsequently affects the beans hence reducing their productivity [2, 3]. Currently, chemical control methods have been used to manage these bacterial pathogenic diseases in beans. This involves the use of copper based foliar spray and antibiotics like streptomycin sulphate for controlling the effects on the leaves and seeds, respectively. The usage of these pesticides has been reported to cause environmental pollution, which includes the adverse effects on water bodies, soil and food [4]. These bacterial pathogens have also developed resistance towards the synthetic agrochemicals hence reducing their effectiveness in plant applications [5]. Therefore, there is a need to look for an alternative source of anti-phytopathogenic compounds from natural sources, including the use of extracts isolated from plants and endophytic fungi.

The tropical ecosystem is home to most plant and animal species worldwide. The ecosystem by extension, is the major source of bioactive compounds whereby most of the medicinal plants are found in it [6]. Currently, due to the extinction of some plant species and abiotic stress, plants' potential of producing secondary metabolites is not to the expected level [7]. Researchers then shifted their concentrations to exploring secondary metabolites isolated from microorganisms (fungal or bacterial) that reside inside tissues of healthy plants, generally referred to as endophytes. The endophytic fungi are known to produce secondary metabolites that help them to defend their host plant territories against epiphytes and other plant pathogens [8]. Some of the isolated secondary metabolites from endophytic fungi of higher plants have desirable anti-bacterial, anti-cancer, anti-inflammatory, anti-diabetic and anti-viral activities [8]. *Syzygium cordatum* (water berry) of Myrtaceae family is among these plants that host important endophytes [9]. This plant grows to a maximum of 20 m and native to high altitude areas of Zimbabwe and Kenya highlands [6]. The concoction of leaves has been traditionally used in the treatment of stomach ache and diarrhea [10]. The hydro-distillates from the leaves have also been used to treat airborne related diseases like tuberculosis [11]. Due to long term co-hesitant of the fungal endophytes and the plant in a mutualistic relationship, the exchange of genetic materials is therefore possible; hence can synthesize similar secondary metabolites [8]. This study was aimed at evaluating the antibacterial activities of endophytic fungi isolated from *S. cordatum* against selected bacterial pathogens of beans.

Materials and Methods

Collection of plant materials

A research permit was sorted from the National Commission for Science, Technology and Innovation (NACOSTI). Later, fresh leaves and stem bark of *S. cordatum* plant were collected from Mt. Elgon forest (01^{0} 08'00'' N 34 ^{0} 35'00'' E or 1.13333 ° N, 34.583333 ° E). The samples were taken to the Botany Department of Egerton University for scientific identification. Isolation of fungal endophytes was thereafter done within eight hours after the collection of plant materials to avoid drying.

Isolation of endophytic fungi

The endophytic fungi were isolated from internal plant tissues using the method of Tian et al. [12] with some modifications. The leaves and stem bark of the selected healthy plants were washed in running tap water to remove any soil or other foreign material and blot dry. The leaves and the stem were then sectioned to approximately 1-4 mm size. The sectioned materials were surface sterilized for 5 minutes in 10 ml of 1% sodium hypochlorite followed by 20 ml of 70% ethanol. Thereafter, the materials were adequately rinsed with sterile distilled water to wash off disinfectants. The materials were then placed in petri plates containing potato dextrose agar (PDA) medium amended with 200 mg/l of streptomycin sulphate. The plates were sealed using Parafilm and incubated at 25±2ºC in a light chamber. The growth of mycelia was monitored, which thereafter, were used to generate pure cultures of the fungal endophytes on PDA medium.

Screening and identification of fungal endophytes against bacterial pathogens

Antimicrobial activity of isolated endophytic fungi was determined against common bean bacterial pathogens using the method of Arya and Sati [13] with slight modifications. The pathogens *P. syringae and X. axonopodis* were inoculated in 50 ml conical flask containing nutrient broth medium and incubated at 37±2°C. After 24 hours, pathogen cultures were transferred separately to Erlenmeyer flasks containing sterilized water and shaken until the turbidity of bacterial suspension was comparable to the turbidity of McFarland's standard solution (0.05 ml of 1.175% barium chloride ($BaCl_2.2H_20$) in 9.95 ml of 1 % sulphuric acid). Around 100 µl of suspended bacteria (1.5×10^8 CFU/ml) were inoculated in Petri dishes containing Muller Hinton agar (MHA) using a sterile micro-dispenser. The six-millimeter diameter plugs of actively growing mycelia of fungal endophytes from PDA plates were cut using a sterile cork-borer and placed on the surface of the Muller Hinton agar medium containing the bacterial pathogens. These plates were sealed with Parafilm and incubated at 37±2°C. The zones of inhibition were then measured in mm using a ruler scale after 24 hours of incubation. The experiment was performed in triplicates.

The fungal endophyte that showed the highest antibacterial activity against selected bacterial pathogens was selected and coded as SC-S-11. The molecular method of identification was employed for SC-S-11, where the genomic DNA was extracted and the ITS (Internal Transcriptase Spacer) region was amplified using PCR method. The ITS region was sequenced by Sanger sequencing protocol and compared to known sequences in NCBI GeneBank using BLAST at >98% similarity. The ITS region sequence was submitted to GenBank with accession number: JF773672.1.

Fermentation and extraction of antibacterial metabolites

The fungal endophyte that showed the highest activity against selected bacterial pathogens was selected for solid fermentation. In the method, ten 500 ml Erlenmeyer flask containing 90g of parboiled rice in 90 ml distilled water per flask were autoclaved at 121±2ºC for 40 minutes. Agar plugs (six-millimeter diameter) were cut from the 7-day-old fungal culture on PDA agar then placed on the surface of sterile rice media. One flask without inoculum was used as a control. After 21 days of incubation, 200 ml of methanol was added to each flask and the contents were ultra-sonicated for 90 minutes at 30±2ºC to enhance extractions of secondary metabolites from the endophytic fungi. The methanol extracts were filtered and evaporated under reduced pressure to yield their respective crude extracts. The methanol crude extract was then suspended in water, followed by liquid-liquid partitioning between hexane and ethyl acetate. Around 200 ml of resulting organic layers was evaporated separately, under reduced pressure to yield hexane and ethyl acetate crude extracts. Hexane crude extracts were discarded, whereas the ethyl acetate crude extract was used for subsequent procedures.

Column chromatography

A series of thin layer chromatography analysis (TLC) was done to ascertain the best solvent mixture to be used in eluding the columns, after which hexane: ethyl acetate: methanol (3:5:2) as mobile phase was reached on due to good separation pattern. Dry ethyl acetate crude extract of *Diaporthe* sp. metabolites was reconstituted in the little amount of distilled ethyl acetate and then loaded on the surface of an evenly packed silica gel column using a clean micropipette. Silica gel (70-230 ASTM) supplied by Scharlau Lab supplies limited was used. Columns of 50 cm length and 20 mm diameter were used. The collected fractions that showed similar patterns on TLC analysis were pooled together. Ethyl acetate crude yielded four fractions, named as F1-F4. F2 of this fungal extract was further purified using preparative high-pressure liquid chromatography (HPLC) due to its significant antibacterial activity against the test organisms.

High performance liquid chromatography

Preparative high-performance liquid chromatography system (Shimadzu-UFLC prominence), fitted with an auto sampler (Model- SIL 20AC HT) and UV-visible detector (Model-SPD 20A) was used to separate the compounds. Dry samples obtained from column chromatography were re-dissolved in HPLC grade methanol each to make a concentration of 20 mg/ml. The prepared solutions were centrifuged using Bio-Cote centrifuge, to enhance the sedimentation of solids that may block the column. 150 µl of samples were loaded onto an auto-sampler. This separation was performed on the Kromasil reverse phase C18 5 µm column (4.6 × 250 mm). Gradient separation was performed using mobile phase A (100 % Milli pore water) and mobile phase B (100 % HPLC grade methanol). Both Milli pore water and methanol were of analytical grade supplied by Scharlau Lab supplies

limited. The separation conditions were set as follows: 10% of B in A at injection time, 80% of B in A at 30th minute, 100% of B at 31st to 37th minute then normalized to 10% of B in A at 38th minute to 45th minute where the separation process was stopped. Chromatographic separations were monitored at the absorbance range of 220-420 nm. The collected fractions were concentrated under a reduced pressure in a rotary evaporator to yield pure compounds. Oven temperature at 40°C and a flow rate of 3 ml/minute were maintained. F2 of SC-S-11 yielded three pure compounds, recorded as compounds 1, 2 and 3, respectively.

Antibacterial assay for crude extract, fractions and pure compounds

The paper disc diffusion assay was used to screen for anti-bacterial activities of crude ethyl acetate extracts, fractions from column chromatography and the purified compounds. 100 µl of bacterial pathogen suspensions (1.5×10^8 CFU/ml) was homogeneously spread on sterile Mueller Hinton agar (38 g/l) in Petri dishes. The ethyl acetate fungal extracts, fractions from column chromatography analysis and pure compounds were prepared by dissolving them in 1% DMSO in distilled sterile water. The sterile paper disc was soaked in 5 mg/ml concentration of the prepared extracts, then placed at the center of MHA plates containing the bean bacterial pathogens. A sterile disc dipped in 1% DMSO was used as a negative control, while standard chloramphenicol antibiotic was used as a positive control. The plates were sealed using Parafilm and inoculated at 37± 2°C for 24 hours, after which the zones of inhibitions were measured in mm using a ruler scale. This experiment was done in triplicates.

^{1}H, ^{13}C and ^{2}D NMR spectroscopy and mass spectroscopy

The ^{1}H, ^{13}C and all 2D NMR spectroscopy were recorded on advance Bruker 500 MHz NMR spectrometer. The spectra were referenced according to the deuterochloroform signal at δH 7.24 (for 1H NMR spectra) and δC 77.0 (for ^{13}C NMR spectra). The off-diagonal elements were used to identify the spin-spin coupling interactions in the ^{1}H-^{1}H COSY (Correlation spectroscopy). The proton-carbon connectivity, up to three bonds away, was identified using ^{1}H-^{13}C HMBC (Heteronuclear Multiple bond Correlation). HSQC spectrum (Heteronuclear Single Quantum Coherence) was used to determine the connectivity of hydrogen to their respective carbon atoms. The compound's mass spectra were recorded on Finnigan Tripple Stage Quadrupol Spectrometer (TSQ-70) with an electron spray ionization (ESI) method in the analysis. Thermo X Calibur Qual computer software was used in the analysis of the mass chromatograms. Merck silica gel 60 (0.040-0.063 mm) was used for column chromatography and Merck 20 × 20 cm silica gel 60 F254 aluminum sheets were used for thin-layer chromatography. The TLC plates were analyzed under UV (254 and 366 nm) before being sprayed and developed with a [1:2:97] anisaldehyde: concentrated sulphuric acid: methanol spray reagent and then heated.

Data analysis

The comparison of means was done using SPSS version 21.0 and the most bioactive secondary metabolites were selected based on the antibacterial activity as shown by the size of inhibition zones. The difference in the mean inhibitory effect of each fungal extract was determined using one-way ANOVA. Tukey's Honestly Significant Difference (HSD) and a Post-Hoc analysis were used to determine if there was any significant difference between the means of the isolates and the positive control.

Results and Discussion

The endophytic fungus was isolated from the stem bark tissues of *S. cordatum* plant and given the code as SC-S-11. Molecular information reveals that SC-S-11 belongs to the genus *Diaporthe* of phylum *Ascomycota*, Kingdom Fungi. It showed a close correlation with *Diaporthe sophorae* according to BLAST (Basic Local Alignment Search Tool) information. Genus *Diaporthe* is the most encountered genera of fungal endophytes in several host plants. The genus is known to be a source of enzymes and bioactive secondary metabolites having anti-bacterial, anti-cancer and anti-fungal activities. In the past, plants of genus *Diaporthe* have been known to produce biochemical that deter herbivores, hence can be used as a biocontrol agent [14-16]. As compared to this study, endophytic fungi of genus *Diaporthe* have also been isolated from plants such as acacia, *Maytenus ilicifolia, Berberis vulgaris* having palpable anti-bacterial and antifungal activities [8].

Dual culture assay

Diaporthe sp. SC-S-11 showed a good antagonism against selected common bean bacterial pathogens *X. axonopodis and P. syringae* with corresponding zones of inhibition of 17.67 mm and 1.67 mm, respectively. These results were not significantly

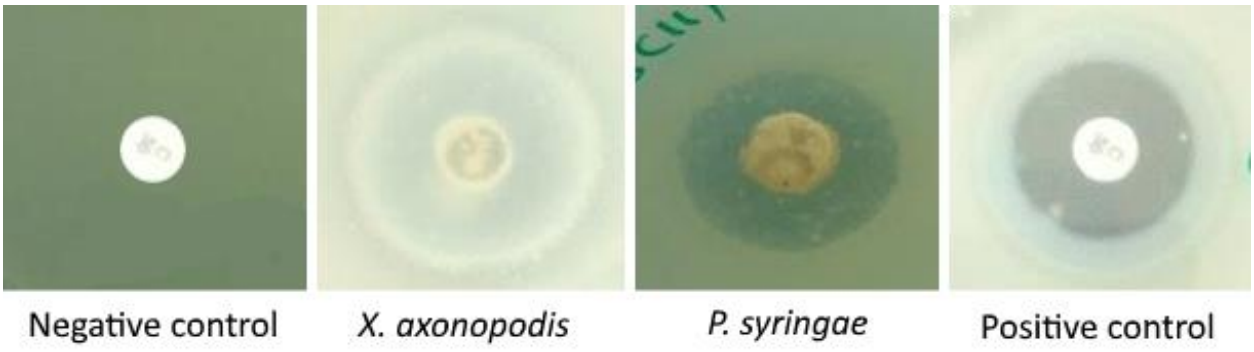

Fig. 1 Antibacterial activity of *Diaporthe* sp. SC-S-11 against *Pseudomonas syringae* pv. *phaseolicola* and *Xanthomonas axonopodis* pv. *phaseoli.* The standard antibiotic chloramphenicol was used as a positive control.

different at $P>0.05$ with that of standard antibiotic chloramphenicol having zones of inhibitions as 25 mm and 20 mm against *X. axonopodis and P. syringae*, respectively (Fig. 1). The antibacterial activity is attributed to the production of secondary metabolites by strain SC-S-11. *Diaporthe* fungal species have been known to possess good antagonism with bacterial pathogens. For instance, Li et al. [16] showed an appreciable antibacterial efficacy of *Diaporthe* sp. LG23 against human pathogens *Streptococcus pyogenes* and *Pseudomonas aeruginosa*. These results were also in agreement with that of Tong et al. [17] where endophytic fungus *Diaporthe* sp. isolated from traditional herb *Orthosiphon stamieus* was found to have good anti-candida activity against *Candida albican*, an opportunistic pathogen that causes nosocomial infections, especially in the USA. This shows that endophytic fungi of genus *Diaporthe* are a good source of bioactive secondary metabolites that can be used in the formulation of antibiotics or agrochemicals.

Extraction and purification of secondary metabolites

The secondary metabolites from the liquid culture of endophytic fungus SC-S-11 were extracted using methanol. SC-S-11 methanol after portioning between hexane and ethyl acetate solvents yielded 2.03 g hexane extract and 1.92 g ethyl acetate extract. Antibacterial compounds possess mid to high polarity; in this case, the ethyl acetate portion was taken for fractionation using column chromatography, which yielded four fractions named; F1 (10.12 mg), F2 (80.43 mg), F3 (42.14 mg) and F4 (30.02 mg). The fraction F2 was selected for further purification due to its appreciable antibacterial activity against *P. syringae and X. axonopodis*.

MIC determination of active metabolites

MIC assay was performed for ethyl acetate extract and its fractions obtained after column chromatography. In this method, a stock solution of 5 mg/ml (100%) was prepared. Later, three serially diluted concentrations of 3.75 mg/ml (75%), 2.50 mg/ml (50%) and 1.25 mg/ml (25%) were prepared. The agar disc diffusion assay was then performed for all the solutions against selected bean bacterial pathogens and the results are tabulated in Table 1. The secondary metabolites present in all the solutions showed activity against *X. axonopodis* but poor anti-bacterial activity was noticed against *P. syringae.* The fraction F2 showed palpable MIC values of 2.50 mg/ml against *X. axonopodis* and 1.25 mg/ml against *P. syringae*. On the other hand, ethyl acetate crude extract was also active against the test organisms with MIC values of 2.50 mg/ml against *X. axonopodis* and 1.25 mg/ml against *P. syringae* (Table 1). The results of this study correlate with a study done by Wanga et al. [18] where the ethyl acetate of fungal endophyte *Fusarium solani* isolated from *Markhamia lutea* showed antibacterial activity against *X. axonopodis* with an inhibition zone of 14 mm, while dismal activity was recorded against *P. syringae.* The resistivity of *P. syringae* is associated with action modes under which the bacterium can easily mutate. Secondly, the presence of an e-flux pump; an internally developed mechanism, which is a substrate specific and functions to prevent the accumulations of antibacterial drugs within their system and prevents them from reaching the target sites [19].

Characterization of antibacterial compounds

Three new compounds were isolated from the ethyl acetate extract of SC-S-11 and named as compounds 1, 2 and 3. Compound 1 was obtained as a brown solid at room temperature. Its molecular mass was

Table 1 Inhibition zones (mm) of serially diluted ethyl acetate extracts and collected fractions against common bean bacterial pathogens.

Treatments	Extracts	Serial dilution	*X. axonopodis* pv. *phaseoli*	*P. syringae* pv. *phaseolicola*
	Ethyl acetate	100%	11.67±0.88 ^{b,c}	12.67±0.67 ^{c}
		75%	10.00 ±0.58 ^{b,c}	10.67±0.67 ^{b,c}
		50%	8.00±0.58 ^{b}	10.00±0.58 ^{b,c}
		25%	0.00±0.00 ^{a}	7.33±0.33 ^{b}
	F1	100%	9.00±0.58 ^{b}	13.33±1.20 ^{c}
		75%	8.33±0.88 ^{b}	11.67±0.33 ^{b,c}
		50%	7.67±0.67 ^{b}	10.33±0.88 ^{b,c}
		25%	0.00 ±0.00 ^{a}	9.33±0.67 ^{b}
Diaporthe sp.	F2	100%	14.00±0.88 ^{c}	10.67±0.33 ^{b,c}
SC-S-11		75%	12.00±0.58 ^{b,c}	10.33±0.68 ^{b,c}
		50%	10.67±0.58 ^{b,c}	7.00±0.00 ^{b}
		25%	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
	F3	100%	12.33±0.88 ^{b,c}	0.00±0.00 ^{a}
		75%	10.00±1.73 ^{b,c}	0.00±0.00 ^{a}
		50%	9.00±0.58 ^{b}	0.00±0.00 ^{a}
		25%	7.33±0.33 ^{b}	0.00±0.00 ^{a}
	F4	100%	10.67±0.88 ^{b,c}	0.00 ±0.00 ^{a}
		75%	9.67±0.67 ^{b}	0.00±0.00 ^{a}
		50%	8.33±0.88 ^{b}	0.00±0.00 ^{a}
		25%	7.33±0.33^{b}	0.00±0.00 ^{a}
Chloramphenicol	-	-	23.33±0.88 ^{d}	20.67±0.33 ^{d}
Negative control	-	-	0.00±0.00 ^{a}	0.00±0.00 ^{a}

The values given are the mean of the three replicates ± standard error (S.E). Values sharing the same letter (s) within the columns are not significantly different in their anti-bacterial activities (P<0.05, Turkey's test).

established to be 198.13 amu from MS data, corresponding to molecular ion at *m/z* 221.26 $(M+Na)^+$ (Fig 2) and a molecular formula of $C_{11}H_{18}O_3$, which indicates double bond equivalence of 3, corresponding to the three double bonds presence in the aliphatic chain. This compound was identified as an aliphatic enol compound based on its characteristic absorption on both 1D and 2D NMR data. ^{1}HNMR spectrum showed the presence of methylene and methyl protons with different multiplicities resonating at δ_H 4.00 and 4.13 (H-1), δ_H 5.66 (H-2), δ_H 6.40 (H-3), δ_H 6.00 (H-4), δ_H 3.98 (H-6), δ_H 4.29 (H-7), δ_H 5.34 (H-8), δ_H 5.45 (H-9), δ_H 1.57 (H-10) and δ_H 1.75 (H-5'), which corresponds to carbon signals at δ_C 61.4 (C-1), δ_C 129.4 (C-2), δ_C 127.5 (C-3), δ_C 128.0 (C-4), δ_C 76.7 (C-6), δ_C 68.4 (C-7), δ_C 131.5 (C-8), δ_C 125.3 (C-9), δ_C 18.2 (C-10) and δ_C 13.4(C-5'), respectively. The coupling constant of protons at δ_H 5.66 (H-2) and δ_H 6.40 (H-3) was found to be 7.24 and 1.74 while those at δ_H 5.34 (H-8), δ_H 5.45 (H-9) were found to be 2.14 and 7.06 Hz. This implies that the $\Delta^{2,8}$ in compound 1 is a *Z* (*cis*) isomer (Table 2). This, therefore, is evidence that compound 1 has a small dihedral angle between protons found at the stilbene alkenes at C-2 and C-3, C-8 and C-9. ^{13}C NMR and DEPT spectral information showed presence of hydroxylated aliphatic alkene carbon system with a total of 11 carbon signals having one methylene carbon at δ_C 61.4 (C-1), seven methine carbon at δ_C 68.4 (C-7), δ_C 76.7 (C-6), δ_C 125.3 (C-9), δ_C 127.5 (C-3), δ_C 128.0 (C-4), δ_C 129.4 (C-2) and δ_C 131.5 (C-8), two methyl carbons at δ_C 13.4 (C-11) and δ_C 18.2 (C-10), and a quaternary carbon at δ_C 138.9 (C-5).

The COSY spectrum showed the correlation of H-2 (δ_H 5.66) with H-3 (δ_H 6.40), H-3 (δ_H 6.40) with H-4 (δ_H 6.00), H-7 (δ_H 4.29) with H-6 (δ_H 3.98) and H-8 (δ_H 5.34) while H-9 (δ_H 5.45) with H-10 (δ_H 1.57). While the HMBC spectrum showed that proton resonating at δ_H 6.00 (H-4) correlates with C-2 (δ_C 129.4), C-3 (δ_C 127.5), C-5 (δ_C 138.9) and C-6 (δ_C 76.7), while that resonating at δ 3.98 correlates with C-4 (128.0), C-5 (δ_C 138.9), C-7 (δ_C 68.4) and C-8 (δ_C 131.5). Other COSY and HMBC spectral information is summarized in Fig 3 and Table 2. Compound 1 was assigned an IUPAC name as (*2Z, 4Z, 8Z*)-5-methyldec-2, 4, 8-triene-1, 6, 7-triol and trivial name as *Z*- cordatenol coined from the species name of the host plant and enol functional group.

Compound 2 was similar to compound 1, it was as a brown solid at room temperature, having close absorptions on NMR data. The two compounds are geometric isomers having a difference in the spatial arrangement of atoms at the alkene sections. Its mass was established to be 198.13 amu from MS data, corresponding to molecular ion at *m/z* 221.26 $(M+Na)^+$ (Fig 2) and a molecular formula of $C_{11}H_{18}O_3$, which indicates double bond equivalence of 3,

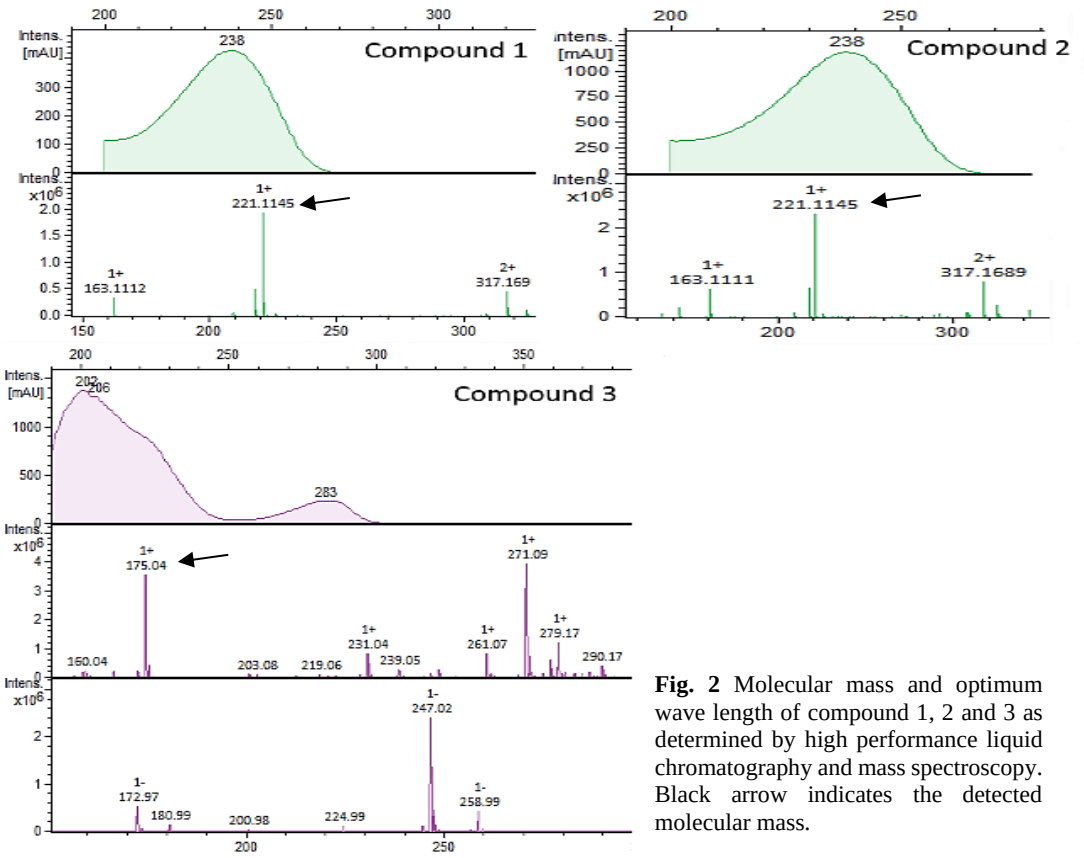

Fig. 2 Molecular mass and optimum wave length of compound 1, 2 and 3 as determined by high performance liquid chromatography and mass spectroscopy. Black arrow indicates the detected molecular mass.

corresponding to the three double bonds presence in the aliphatic chain. The compound 2 was identified as an *E* isomer of compound 1, and both belong to an enol group of compounds. ^{1}H NMR spectrum showed the presence of methylene and methyl protons with different multiplicities resonating at δ_H 4.02 and 4.06 (H-1), δ_H 6.04 (H-2), δ_H 6.34 (H-3), δ_H 6.34 (H-4), δ_H 3.90 (H-6), δ_H 3.87(H-7), δ_H 5.49 (H-8), δ_H 5.53 (H-9) , δ_H 1.62 (H-10) and δ_H 1.71 (H-11), which corresponds to carbon signals resonating at δ_C 56.6 (C-1), δ_C 125.2 (C-2), δ_C 124.8 (C-3), δ_C 122.5 (C-4), δ_C 77.3 (C-6), δ_C 73.7(C-7), δ_C 132.2 (C-8), δ_C 125.1 (C-9), δ_C 17.6 (C-10) and δ_C 12.8 (C-11), respectively. In addition, ^{1}H NMR showed *trans* vicinal correlations of protons attached to C-2 and C-3, C-8 and C-9 with $^{3}J_{H\text{-}H}$ coupling constants 11.88, 11.00, 11.24 and 11.36 Hz, respectively. These higher coupling constants prequalifies the double bond in compound 2, as a *trans* or an *E*-isomer. ^{13}C NMR and DEPT spectral information showed the presence of hydroxylated aliphatic alkene carbon system with a total of 11 carbon signals having one methylene carbon at δ_C 56.6 (C-1), seven methine carbon at δ_C 125.2 (C-2), δ_C 124.8 (C-3), δ_C 122.5 (C-4), δ_C 77.3 (C-6), δ_C 73.7(C-7), δ_C 132.2 (C-8) and δ_C 125.1 (C-9), two methyl carbons at δ_C 12.8 (C-11) and δ_C 17.6 (C-10) and a quaternary carbon at δ_C 126.2 (C-5).

The COSY spectrum showed the correlation proton at δ_H 5.53 (H-9) and δ_H 1.62 (H-10). HMBC spectrum shows a correlation of proton and carbons that are 2-3 bonds away, it revealed that proton at δ_H 4.02 and 3.06 (H-1) correlates with C-2 (δ_C 125.2), proton at δ_H 6.34 correlates with C-5 (δ_C 126.2), C-6 (δ_C 77.3) and C-11 (δ_C 12.8), while that resonating at δ_H 5.49 (H-8) correlates with C-6 (δ_C 77.3), C-7 (δ_C 73.7), C-9 (δ_C 125.1) and C-10 (δ_C 17.6). Other COSY and HMBC spectral information is summarized in Table 3 and Fig 3. Compound 2 was assigned an IUPAC name (*2E, 4E, 8E*)-5-methyldec-2, 4, 8-triene-1, 6, 7-triol and a trivial name as *E*-cordatenol a geometric isomer of compound 1.

Compounds 1 and 2 are geometric isomers with different orientations of atom attachments in C-2, C-3, C-8 and C-9. Generally, *cis* isomers have lower $^{3}J_{HH}$ coupling constants, which typically ranges between 6-10 Hz, while that of *trans* isomers have higher $^{3}J_{HH}$ coupling values, which ranges between 11-18 Hz. Basically, the higher coupling values in

Table 2 Nuclear magnetic resonance spectroscopy analysis of compound 1.

No.	^{13}C NMR δ_C ppm	Type	HSQC δ_H ppm	Coupling constant	COSY(^{1}H/^{1}H)	HMBC
1	61.4	CH_2	4.00, 4.13	-	-	2,3
2	129.4	CH	5.66	7.24	3	3,4
3	127.5	CH	6.40	1.76	2,4	2,4,5
4	128.0	CH	6.00	6.08	3	2,3,5,6
5	138.9	C	-	-	-	-
6	76.7	CH	3.98	-	7	4,5,7,8
7	68.4	CH	4.29	-	6,8	5,6,8
8	131.5	CH	5.34	2.14	7,9	7,9,10
9	125.3	CH	5.45	7.06	8	7,8,10
10	13.4	CH_3	1.57	-	9	7,8,9
11	18.2	CH_3	1.75	-	-	5

Table 3 Nuclear magnetic resonance spectroscopy analysis of compound 2.

No.	^{13}C NMR δ_C ppm	TYPE	HSQC δ_H ppm	$^3J_{HH}$ Hz	COSY(^{1}H/^{1}H)	HMBC
1	56.6	CH_2	4.02, 4.06	-	-	2
2	125.2	CH	6.04	11.88	-	1,3
3	124.8	CH	6.34	11.00	-	4,5
4	122.5	CH	6.34	11.00	-	5,6,11
5	126.2	C	-	-	-	-
6	77.3	CH	3.90	-	-	7,8
7	73.7	CH	3.87	-	-	8,9
8	132.2	CH	5.49	11.24	-	6,7,9,10
9	125.1	CH	5.53	11.36	10	7,8,10
10	17.6	CH_3	1.62	-	9	8,9
11	12.8	CH_3	1.71	-	-	5

Table 4 Nuclear magnetic resonance spectroscopy analysis of compound 3.

	13C	Type	HSQC	COSY(^{1}H/^{1}H)	HMBC
1	152.9	C	-	-	-
1'	32.0	CH	2.67	5'	-
2	121.2	C	-	-	-
2'	38.7	CH	3.07		
3	129.1	CH	6.90		1,4,5,7
3'	43.7	CH	2.77	4'	
4	112.7	CH	6.59		1,2,3
4'	12.1	CH_3	0.76	3'	2',3'
5	140.6	C	-	-	-
5'	23.2	CH_3	1.15	-	1,2',5
6	113.8	CH	6.50	-	1,1',2,3
7	15.9	CH_3	2.03	-	1,2,3

trans isomers are generally attributed to a large dihedral angle, which in most cases is 180º, while that in *cis* is due to a small dihedral angle which is about 0-60º [20]. The two compounds belong to an enol group of compounds formally referred to as alkenols [21]. Enols are compounds represented with the hydroxylation of olefin's carbon chain; they are regarded as reactive compounds or intermediates. In plant biological systems, they are synthesized via a substrate level phosphorylation with the help of the enzyme amylase. These compounds are known to undergo tautomerism involving auto-conversion of enol compounds to ketones; which helps in their stability [21]. The activity of the two compounds 1 and 2 can be attributed to the presence of sp^2 hybridized carbon atoms and hydroxylation on the olefin carbon chain, which imparts more nucleophilicity to the compounds. The two parameters render the compounds more reactive sites within the molecule.

Compound 3 was isolated from fraction F2 as a cream yellow solid at room temperature. Its mass was established to be 191.0 amu based on MS data, corresponding to a molecular ion at 175.0 *m/z* ([M–NH_3]+H)$^+$ (Fig 2) and a molecular formula of $C_{12}H_{17}NO$, which indicates a double bond

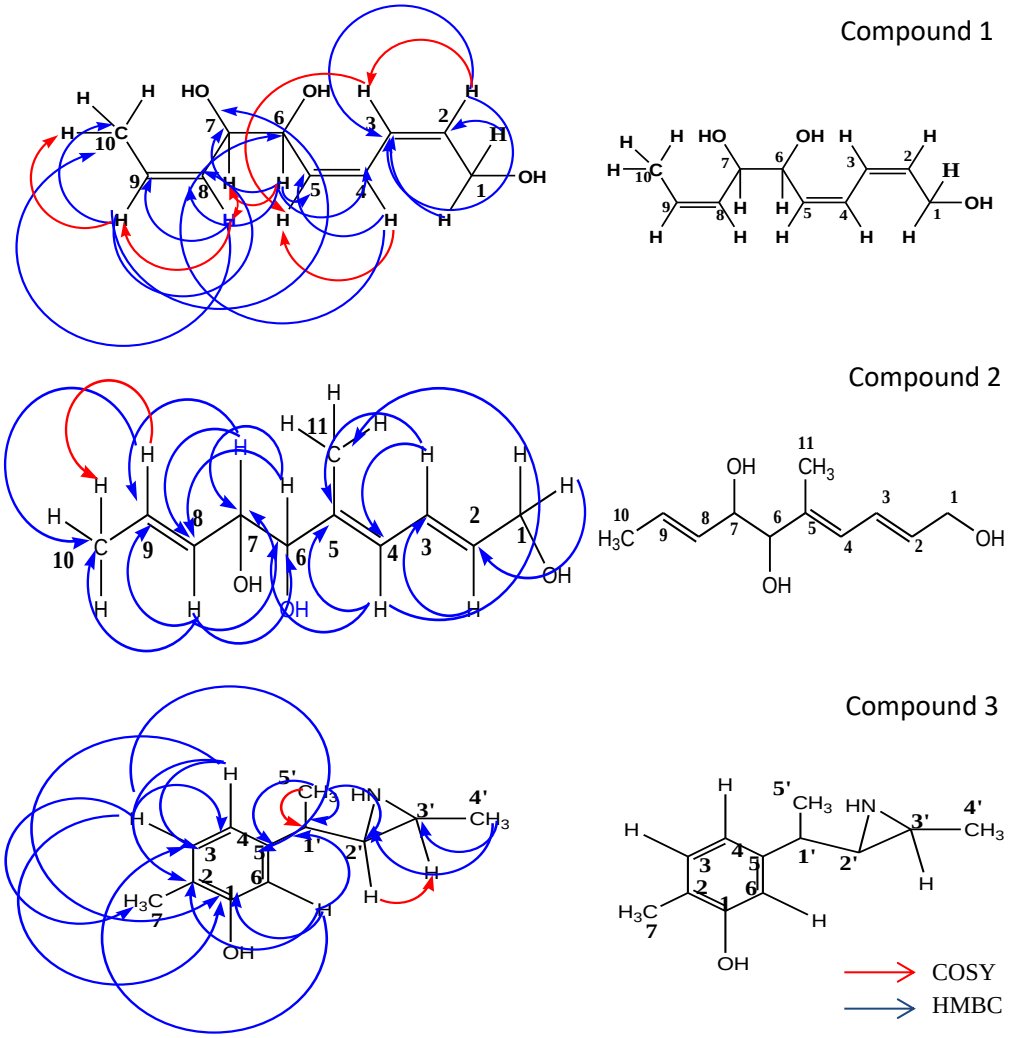

Fig. 3 Molecular structure and HMBC-COSY correlations of compound 1, 2 and 3.

equivalence of 5; one aromatic ring, three double bonds within the aromatic ring and one aziridine ring at the side substituent. ^{1}H NMR spectrum reveals that compound 3 contains three aromatic protons resonating at δ_H 6.90 (H-3), δ_H 6.59 (H-4) and δ_H 6.50 (H-6), which corresponds to carbon signals resonating at δ_C 129.1 (C-3), δ_C 112.7 (C-4) and δ_C 113.8 (C-6), respectively. Five methyl protons resonating at δ_H 2.67 (H-1'), δ_H 3.07 (H-2'), δ_H 2.77 (H-3'), δ_H 0.76 (H-4') and δ_H 1.15 (H-5'), which corresponds to carbon signals resonating at δ_C 32.0 (C-1'), δ_C 38.7 (C-2'), δ_C 43.7(C-3'), δ_C 12.1 (C-4') and δ_C 23.2 (C-5'), respectively, 1 benzylic proton resonating at δ_H 2.03 (H-7) which corresponded to carbon signal resonating at δ_C 15.9 (C-7). ^{13}C NMR and DEPT spectral information were used in identifying the number and the type of carbon atoms present in the compound. Compound 3 had at a total of 12 carbon atoms, with, six methine carbon at δ_C 129.1 (C-3), δ_C 112.7 (C-4), δ_C 113.8 (C-6), δ_C 32.0 (C-1'), δ_C 38.7 (C-2'), and δ_C 43.7(C-3'), three methyl carbons at δ_C 15.9 (C-7), δ_C 12.1 (C-4') and δ_C 23.2 (C-5') and three quaternary carbons at δ_C 152.9 (C-1), δ_C 121.2 (C-2) and δ_C 140.6 (C-5).

The COSY spectrum showed the correlation of neighboring protons that proton resonating at δ_H 2.67 (H-1') correlates with δ_H 1.15 (H-5'), while a proton resonating at δ_H 2.77 (H-3') correlates with δ_H 0.76 (H-4'). HMBC spectrum showed a correlation of proton and carbons that are 2-3 bonds away; this spectral information reveals that protons resonating at H-3 (δ_H 6.90) correlate with C-1, C-4, C-5 and C-7, at H-4 (δ_H 6.59) correlate with C-1, C-2 and C-3, while at H-6 (δ_H 6.50) correlate with C-1, C-1', C-2 and C-3. The other COSY and HMBC correlations are summarized in Table 4 and Fig 3. The IUPAC

name assigned to compound 3 was 2-methyl-5-(1-(3-methylaziridin-2-yl) ethyl) phenol and a trivial name as α-cordenol coined from the species name of the host plant and "ol" functional group representing a phenol group of compounds. Compound 3 is among the phenolic derivatives, which comprise the second largest group of secondary metabolites isolated from plant and their endophytes [22]. Phenol and phenol derivatives are biosynthesized through condensation of acetic acid (acetic acid pathway) or metabolism of phosphorylated sugars through skimmic acid and aromatic amino acid (skimmate pathway) [23, 24]. Phenol derivatives, such as carvacrol (5-isopropyl-2-methylphenol) isolated from the essential oil of thyme leaves, thymol (2-isoprophyl-5-methylphenol) isolated from the essential oil of thyme and aregona plants and eugenol (4-allyl-2-methoxyphenol) were isolated from the essential oils of clove and rose plants. These compounds are known for their aromatherapy, antioxidant, antifungal and antibacterial activities because of hydroxylation within their aromatic rings [25]. Compound 3 is among these phenolic derivatives, having an aziridine ring as part of a substitute. Aziridine is a nitrogen-containing functional group in a 3-membered strained ring, their biosynthetic pathways are not straightforward due to regiospecificity and stereospecificity [26]. The presence of aziridine within the ring offers molecule a useful property that can be utilized as an active intermediate in the synthesis of drugs or agrochemicals [27]. The significant antibacterial activity of compounds produced by SC-S-11 against bean bacterial pathogens *P. syringae* and *X. axonopodis* is chiefly attributed to the presence of these three bioactive secondary metabolites. This then shows a route of sourcing agrochemicals from natural sources like endophytic fungi.

Conclusions

The study reveals that *S. cordatum* plant hosts an important endophytic fungus of genus *Diaporthe*. *Diaporthe* sp. isolated from the stem bark tissues of *S. cordatum* showed appreciable anti-bacterial activity against bean bacterial pathogens, *P. syringae* and *X. axonopodis*, which is attributed to the mixture of secondary metabolites. The extraction and purification of active metabolites showed three new compounds, two geometric isomers named as **(***2Z, 4Z, 8Z*)-(5-methyldec-2, 4, 8-triene-1, 6, 7-triol) and **(***2E, 4E, 8E*)-(5-methyldec-2, 4, 8-triene-1, 6, 7-triol) and one phenolic derivative with an aziridine side chain, named as (2-methyl-5-(1-(3-methylaziridin-2-yl) ethyl phenol). The identification of new antibacterial compounds showed the potential of using endophytic fungi as a biocontrol agent to control bean pathogens and its active compounds as antibacterial agents for other bacterial diseases.

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Conflict of interest

The authors have no conflict of interest.

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