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Metabolites of *Colletrotricum* species, an endophytic fungus isolated from *Vernonia amygdalina Del* possess antimicrobial and antioxidant activities

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Abstract

The endophytic fungus, *colletrotricum* species, isolated from the leaves of *Vernonia amygdalina* was investigated for its chemical constituents and biological activity. The pure fungus was grown using solid fermentation on rice medium and the metabolites were extracted using ethyl acetate. Further purification of the extract was carried out using vacuum liquid chromatography (VLC) and gel chromatography on Sephadex LH-20. The chemical constituents were detected by dereplication using HPLC-DAD. Antimicrobial assay of the Sephadex fractions EC3-1 and EC3-3 were evaluated using agar well diffusion assay against seven pathogenic microorganisms (*Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Aspergillus niger* and *Candida albicans*). EC3-3 was also subjected to antioxidant assay using DPPH free radical scavenging model. HPLC-DAD analysis of the VLC fractions revealed the presence of compounds like Hydroxybenzaldehyde, Cladosporin, Desmethyldichlorodiaportin and p-Hydroxybenzoic acid. EC3-1 and EC3-3 showed mild antimicrobial activity against *S. aureus*, *S. typhi* and *A. niger* strains, with minimal inhibitory concentrations ranging from 0.19 to 0.46 mg/mL. EC3-3 was found to exhibit very high ability to scavenge DPPH radicals with IC_{50} of 3.16 µg/mL compared to Ascorbic acid (IC_{50} of 50.72 µg/mL). HPLC analysis of EC3-3 revealed the presence of Palitantin, which has been shown to possess antimicrobial and antioxidant activity, and two unidentified major peaks. The endophytic fungus, *Colletotrichum* species isolated from *Vernonia amydalina* produced bioactive metabolites which exhibited antimicrobial and antioxidant activities thus projecting *Vernonia amydalina* as a potential source of fungal endophytes that can be further studied for generation of novel bioactive compounds.

Keywords: Antioxidant activity; Antimicrobial assay; Gel chromatography; HPLC-DAD; Endophyte; Fungi

1. Introduction

Cancer and many infectious diseases have been a major threat to health worldwide [1]. As a result, there have been relentless research efforts aimed at discovering new chemical entities for development into novel therapeutic agents for the management of these ailments. One area that has attracted major attention within the past decades is natural product research. Many plants have been specifically screened for the presence of bioactive compounds which are known to possess antioxidant, antimicrobial and other therapeutic properties [2, 3, 4]. In the recent time, the focus has been on the search for discovery of novel lead compounds from microorganisms for use in development of drugs for the

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management of threatening human ailments [5, 6, 7]. Microorganisms have also been reported to be an important source of bioactive natural products for industrial use and agricultural applications [8, 9].

Vernonia amygdalina, Del (family, Asteraceae) is a major vegetable in Nigeria with the common name as bitter leaf and serves as an important diet among the several ethnic groups in the country [10]. It is widely distributed in tropical Africa and Asia and most common in natural forest. It has long history in traditional medicine and the plant parts: leaves stem and roots have been exploited in the treatment of various ailments.

The leaves are particularly utilized in the treatment of malaria, diabetes mellitus, venereal diseases, wounds, hepatitis and cancer [11, 12, 13]. The anthelminthic properties of the plants amongst the diverse uses of the plant in combating diseases have also been reported [14]. The roots and the leaves are used in ethno-medicine to treat fever, hiccups, kidney problems and stomach discomfort. Extract of bitter leaf has been reported to exert antimicrobial action against drug resistant microorganisms and possess antioxidant, anticancer, antiviral, anti-helminthic and anti-inflammatory activities[15, 16]. Over the past few years, research for discovery of new drugs has been on course and there has been increasing interest in the investigation of endophytic fungi producing antimicrobial substances [17, 18, 19, 20]. Fungal Endophytes are organisms that live in a symbiotic biological association within the living plant [21] and have advantage over total plant due to its ability to preserve biodiversity of plants. Endophytic fungi which harbor internal tissues of plants have been shown to possess unprecedented chemo-diversity and represent a reliable source of lead compounds needed for development of antioxidant, antimicrobial, anti-inflammatory, anticancer agents [22, 23]. In Nigeria, there is a serious need to search for new drugs; hence, this study aimed to verify the antibacterial and antioxidant activities of the secondary metabolites from *Colletotrichum species,* an endophytic fungus isolated from *Vernonia amygdalina so as to* find alternatives for the common therapeutic drugs.

2. Material and methods

2.1. Plant Material

Fresh and healthy leaves of *Vernonia amygdalina* were collected in June, 2015 from Agulu, Anambra State, Nigeria. The plant was authenticated by Mrs. Anthonia Emezie of the Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka with voucher Number PCG/474/A/020.

2.2. Isolation of endophytic fungi, fermentation, and extraction of metabolites

The isolation of endophytic fungi from a fresh leaf of *V. amygdalina Del* was carried out using standardized methods[24]. The isolated pure fungal strains were maintained in malt extract agar. Solid-state fermentation was carried out in 1 Litre Erlenmeyer flasks containing autoclaved rice medium (100 g of rice and 110 mL of distilled water). The flasks were inoculated with 3 mm diameter agar blocks cut out from malt extract agar plates containing pure cultures of the fungus. The inoculated flasks were incubated at 22 °C for 21 days. At the completion of fermentation, the secondary metabolites were extracted with ethyl acetate and then concentrated using a rotary evaporator at 50 °C.

2.3. Vacuum Liquid Chromatography (VLC) of the extract

The crude endophytic fungal extract (1.5 g) was dissolved in 1 mL of methanol and adsorbed on about 20 g silica gel (200-400 mesh size) by carefully triturating in a mortar to form a homogenous mixture. The glass column (diameter 2.5 cm×30 cm heights) was packed with silica gel (200- 400 mesh size) to the bed size of 14 cm height. The adsorbed fungi extract admixture was introduced from the top of the column and a small amount of silica gel was added on top of the adsorbed fungi extract admixture (in place of sea sand). Cotton wool was used to cover the silica gel to prevent distortion of the silica gel bed when the solvent system was been introduced and a vacuum pump was connected to the column. The column was developed with a gradient mixture of 500 mL of n-hexane and ethyl acetate solvent system in the ratios of 10:0, 9:1, 7:3, 5:5, 3:7, 0:10 using negative pressure created by the vacuum pump to obtain fractions EC1 to EC6. Thereafter, the column was further developed with a gradient mixture of 500 mL of dichloromethane and methanol solvent system in the ratio of 10:0, 9:1, 8:2, 6:4, 4:6, 2:8, 0:10 to give fractions EC7 to EC11. The column was allowed to run dry after each fraction was collected before the addition of the next eluent. The eluate was collected separately with a round bottom flask, evaporated using a rotary evaporator and labeled appropriately.

2.4. Analytical High Pressure Liquid Chromatography (HPLC)

The crude extract and each of the VLC fractions (2 mg) respectively was reconstituted respectively with 2 ml of HPLC grade methanol. The mixture was sonicated for 10 min and thereafter centrifuged at 300 rpm for 5 min. About 100 µl of the dissolved sample was mixed with 500 µL of HPLC grade methanol. HPLC analysis was carried out using Dionex

p580 HPLC system coupled to a photodiode array detector (UVD340S). Detection was carried out at 235, 254, 280 and 340 nm. The separation column (125 × 4 mm; length × internal diameter) was prefilled with Eurospher C-18 and a linear gradient of nanopure water (adjusted to pH 2 by addition of formic acid) and methanol was used as eluent. Compounds were detected using diode array detector and identified based on similarity with data in the inbuilt library. The Chromeleon 6.30 software was used to create results for the HPLC chromatograms and UV spectra of the secondary metabolites.

2.5. Sephadex LH-20 Separation of Fraction EC3

About 10 g of Sephadex LH-20 (0.25-0.1 mm mesh size), the stationary phase was dispersed in a 100 ml of dichloromethane and methanol (1:1 (v/v)) solvent system which serve as a mobile phase. The mixture was shaken for 10 min and the slurry transferred into a glass column (30 cm × 1.5 cm; length × internal diameter). The gel was allowed to stabilize for about 5 hours before use. Approximately 78 mg of EC3 was reconstituted in 2 mL of dichloromethane and methanol [1:1]. The solution was sonicated for 5 min and centrifuged at 1000 revolution per minute (rpm) for 10 min to remove undissolved particle. The supernatant was collected using a pipette and was carefully introduced into Sephadex LH-20 column adjusted to the flow rate of approximately 1.1 ml/min. About 90 fractions were collected with the aid of test tube and monitored with TLC on silica gel G_{254} developed with n-Hexane: Ethyl acetate, (9:1). Similar fractions were combined and concentrated with rotary evaporator to obtain fraction EC3-1, EC3-2, EC3-3, EC3-4 and EC3-5. These Sephadex fractions were also subjected to HPLC-DAD using the same procedure described earlier in section 2.4.

2.6. Antimicrobial Assay

Antimicrobial assay for the Sephadex LH-20 fractions (EC3-1 and EC3-3) were carried out using the agar well diffusion assay as previously described [25] with little modification. The antimicrobial activities of the fractions were tested against five standard clinical bacteria isolates namely: *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and two fungi isolates namely *Aspergillus niger* and *Candida albicans*.

A concentration of 1 mg/mL of each of EC3-1 and EC3-3 were prepared by dissolving each of the fractions in DMSO. Twenty (20) mL of molten Mueller Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) (for bacterial and fungal isolates respectively) were poured into sterile Petri plates (90 mm) and allowed to set. Standardized concentrations (McFarland 0.5) of overnight cultures of test isolates were swabbed aseptically on the agar plates and holes (8 mm) were made in the agar plates using a sterile metal cork-borer. Aliquots of 80 µL of each extract dilutions, reconstituted in DMSO at concentration of 1, 0.5, 0.25, 0.125 and 0.0625 mg/mL for each of the fractions were applied in each of the wells in the culture plates previously seeded with the test organisms. Gentamicin (10 µg/mL) and Miconazole (50 µg/mL) served as positive control for bacteria and fungi respectively, while DMSO was used as the negative control. The MHA plates were then incubated at 37 °C for 24 h, and the SDA plates were incubated at 25-27 °C for 2-3 days. The inhibition zones diameters (IZDs) were measured and recorded. The size of the cork borer (8 mm) was deducted from the values recorded for the IZDs to get the actual diameter. This procedure was conducted in triplicate and the mean IZDs calculated and recorded.

2.6.1. Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations (MIC) of the fungal fractions were determined using the agar dilution method as previously described [25]. A stock solution of 1 mg/mL was prepared for each fungal fraction and these were further diluted in a 2-fold serial dilution. Agar plates were prepared by pouring 4 mL of the molten double strength MHA and SDA, for bacterial and fungal isolates respectively, into the sterile Petri dish containing 1mL of the various dilutions of the fungal fractions to give the final plate concentration of 1, 0.5, 0.25, 0.125 and 0.0625 mg/mL. The test isolates which were grown overnight in broth were adjusted to McFarland 0.5 standard and streaked onto the surface of the agar plates containing dilutions of the fungal fractions. The plates were then incubated at 37 °C for 24 h and 25± 2 °C for 3 days, after which the plates were visually observed for growth. The minimum dilution (concentration) of the fractions that completely inhibits the growth of each test organisms was taken as the MIC.

2.7. Antioxidant Assay

The free radical scavenging capacity of the Sephadex fraction EC3-3 was determined using the DPPH (1, 1-dipheny1-2 picrylhydrazy1) assay as previously described [26] using ascorbic acid as a reference antioxidant. Here, the free radical scavenging properties of extracts against DPPH radical were measured at 490 nm, as an index to their antioxidant activity. The concentrations of the extracts used were 31.25, 62.50, 120.00, 250.00, and 500.00 µg/mL and that of ascorbic acid used were 6.25, 12.50, 25.00, 50.00 and 100.00 µg/mL. The use of DPPH assay provides an easy and rapid way to evaluate antioxidant activity by spectrophotometry, so it can be useful to access various products at a time. The

samples were reacted with the stable DPPH radical in a methanol solution. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The change in colour (from deep violet to light yellow) was measured at 490 nm using a UV-visible spectrometer. A solution of DPPH in methanol (0.1 Milimole) was prepared and 25 µl of its solution was added to the reaction mixture containing 25 µL of the fraction and methanol was used to make the volume up to 250 µL. The concentrations of the fraction used were 500, 250, 125, 62.5, and 31.25 µg/mL. The reaction mixtures were incubated at room temperature for 30 min and the absorbance was measure at 490 nm. Ascorbic acid was also used as positive control in this test. The experiments were performed in triplicates and values are expressed as mean±SD. The free radical scavenging activity of the Gel fraction was obtained using the relationship;

$$\text{DPPH scavenging activity} = [AC - AS)/AC] * 100$$

Where;
AC = Absorbance of Control
AS =Absorbance of Sample [27].

2.7.1. Determination *of inhibitory concentration (IC_{50})*

IC_{50} is simply the minimum inhibition concentration at 50%. The effective concentration of the fungal extract required to scavenge DPPH radical by 50% (IC_{50} value) and was obtained by linear regression analysis of dose-response curve plotting between percentage (%)inhibition and concentration [27].

2.8. Statistical Analysis

The antimicrobial results in triplicate were analyzed with Statistical Package for Social Sciences (SPSS) version 16.0 and presented as mean ± standard error of mean (SEM) inhibition zone diameters (IZD) using One-Way ANOVA and further subjection to Tukey's post hoc test. Differences between means were accepted at significance of $p<0.05$.

3. Results

3.1. Percentage yield of fractions from crude extract obtained from *Colletorichum* species

Table 1 Percentage yield of the Vacuum Liquid Chromatography (VLC)

VLC Fractions	Weight (g)	Percentage Yield (%)
EC 1	0.030	2.000[b]
EC 2	0.040	2.667[b]
EC 3	0.061	4.067[b]
EC 4	0.060	4.000[b]
EC 5	0.025	1.676[b]
EC 6	0.030	2.000[b]
EC 7	0.043	2.867[b]
EC 8	0.040	2.667[b]
EC 9	0.075	5.000[b]
EC 10	0.094	6.267[b]
EC 11	0.095	6.333[b]
EC 12	0.077	5.133[b]

[b] Percentage yield calculated from 1.5g of crude extract

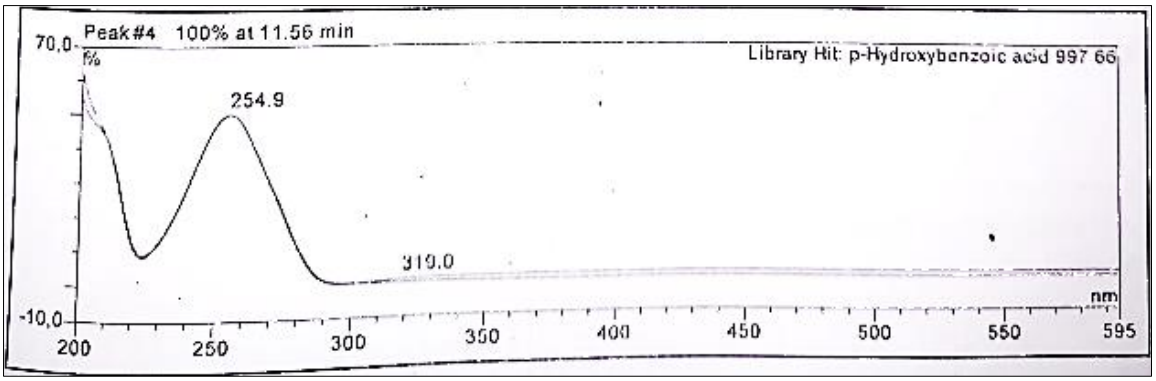
Table 2 Percentage yield of gel Chromatography (Sephadex LH-20) fraction

Sephadex LH-20 Fractions	Weight (g)	Percentage Yield (%)
EC3-1	0.00956	15.672[b]
EC3-2	0.00968	15.869[b]
EC3-3	0.00953	15.623[b]
EC3-4	0.00961	15.754[b]
EC3-5	0.00952	15.607[b]

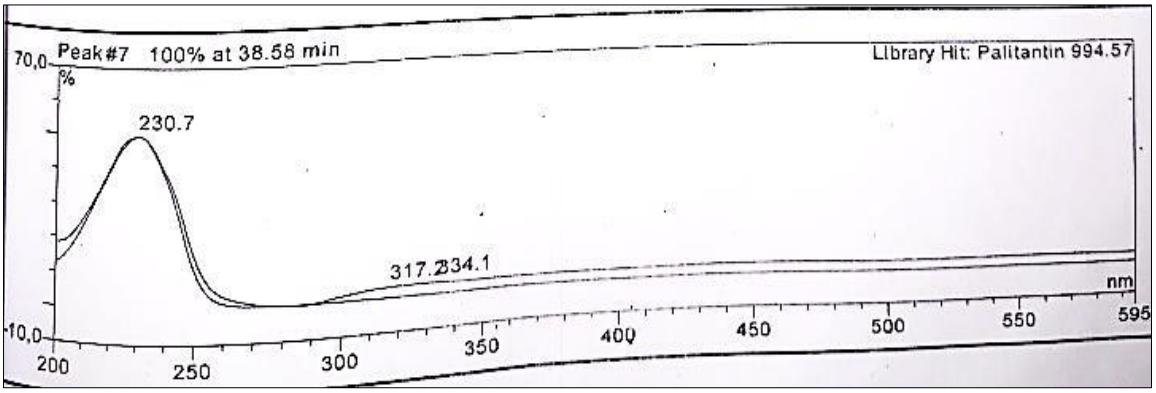
[b]Percentage yield calculated from 0.078g of Vacuum Liquid Chromatography (EC3) fraction.

3.2. High Performance Liquid Chromatography-Diode Array Detector (HPLC-DAD) analysis of the Extract and fractions.

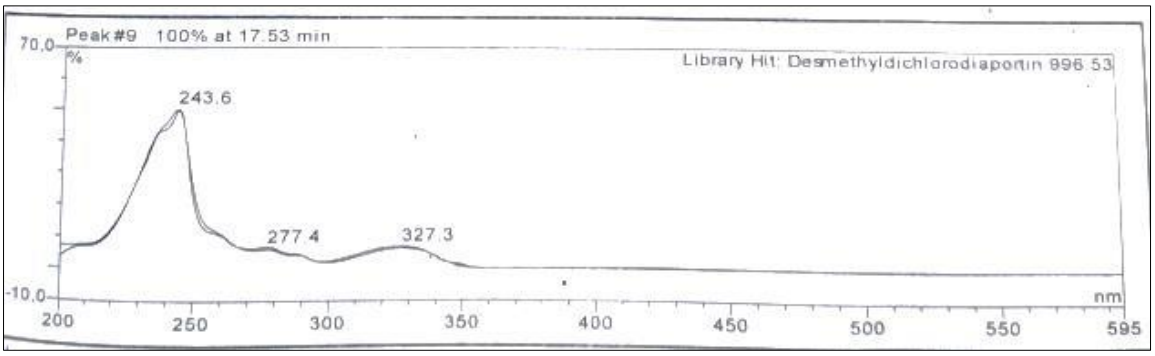
HPLC analysis was based on retention time and the UV absorption spectra at 235nm. A total of five (5) compounds were identified based on the analysis of HPLC-DAD spectra. The various constituents present in the VLC/Sephadex fractions were identified by comparing the peak area of the fractions to that of the marine libraries. Some peaks, including two major ones from EC3-3, were not identified due to lack of library hits.



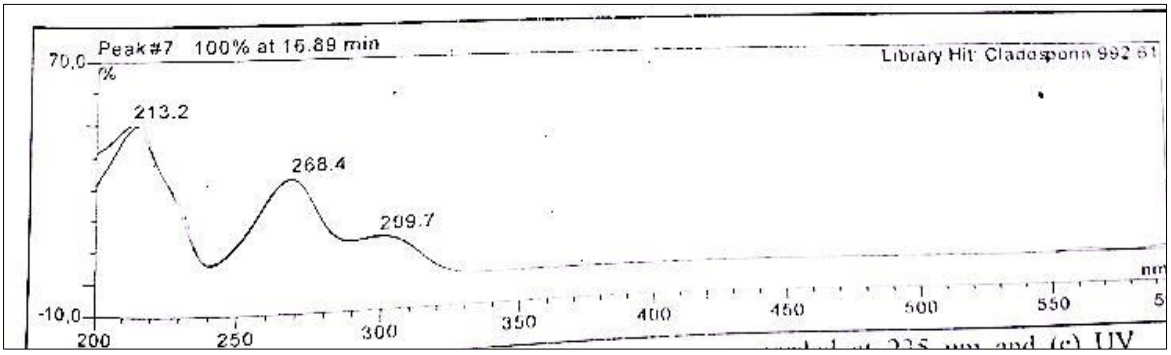
p-Hydroxybenzoic acid (A)



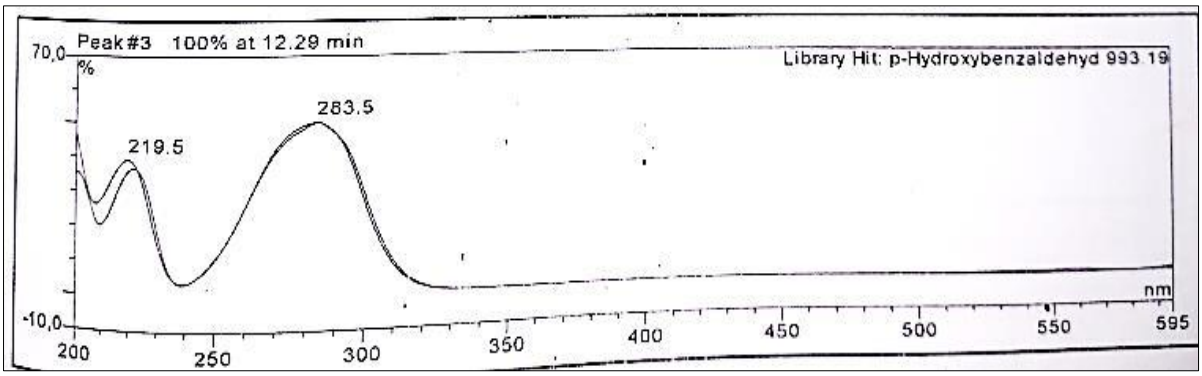
Palitantin (B)



Desmethyldichlorodiaportin (C)



Cladosporin (D)



p-Hydroxybenzaldehyde (E)

Figure 1 UV spectra of detected compounds showing (A) p-Hydroxybenzoic acid, (B) Palitantin, (C) Desmethyldichlorodiaportin,(D) Cladosporin, (E) p-Hydroxybenzaldehyde

Table 3 Bioactive compounds from the VLC fractions of the endophytic fungi isolated from *Colletorichum* sp (Anamorph) detected by HPLC analysis with their retention time

VLC Fractions	Compounds	Correlation Of Hit	Retention Time	Biological Activity	References
ECC4	p-Hydroxybenzaldehyde	993.19	12.29	Antimicrobial, Cytotoxic	Haque *et al*. [28]
ECC4	Cladosporin	992.61	16.87	Antimicrobial, Antiplasmodal, Insecticidal and Antitumor properties	Scott *et al*. [29] Eze *et al*. [30]

ECC5	Desmethyldichloro-Diaportin	996.53	17.53	Cytotoxic Antimicrobial	El-Ghazooly *et al.* [31]
ECC6	p-Hydroxybenzoic acid	997.66	11.56	Antioxidant, Antimicrobial, Anti-inflammatory, Antiviral	Khadem *et al.* [32]
EC3-3	Palitantin	994.57	38.58	Antimicrobial, Antioxidant	Thaipong *et al.* [33] Chigozie *et al.* [34]

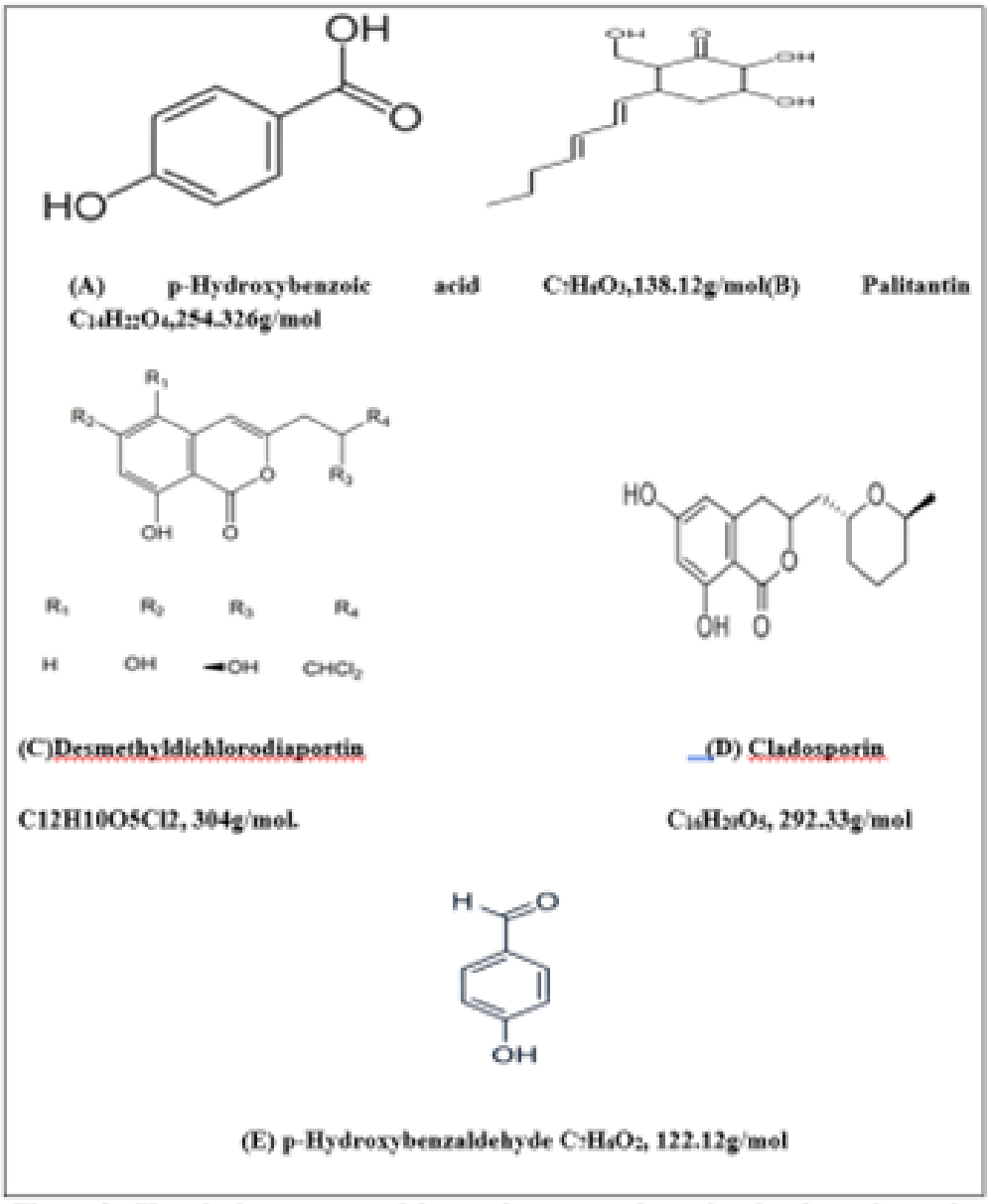


Figure 2 Chemical structures of detected compounds, molecular formular and weight : (A) p-Hydroxybenzoic acid $C_7H_6O_3$, 138.12g/mol (B) Palitantin $C_{14}H_{22}O_4$,254.326g/mol (C) Desmethyldichlorodiaportin $C_{12}H_{10}O_5Cl_2$, 304g/mol (D) Cladosporin $C_{16}H_{20}O_5$, 292.33g/mol (E) p-Hydroxybenzaldehyde $C_7H_6O_2$, 122.12g/mol

3.3. Antimicrobial Screenings

Table 4 Result of Antimicrobial Activity of EC3-1 fungal fraction against tested pathogenic microorganism

Test Organisms	**Concentration (mg/mL) / IZD (mm)**						
	1	**0.5**	**0.25**	**0.125**	**0.0625**	**Gentamycin (10 µg/mL)**	**DMSG**
Staphylococcus aureus	5.0 ±0.0	4.0±0.5	2.0±0.0	-	-	19.0±0.0	-
Escherichia coli	-	-	-	-	-	20.0±0.0	-
Salmonella typhi	8.0±0.0	6.0±0.0	3.0±0.5	-	-	12.0±0.0	-
Pseudomonas aeruginosa	-	-	-	-	-	19.0±0.0	-
Bacillus subillis	-	-	-	-	-	15.0±0.0	-
						Miconazole (50µg/ml)	**DMSG**
Aspergillus niger	6.0±0.0	2.0±0.0	-	-	-	11.0±0.0	-
Candida albicans	-	-	-	-	-	14.0±0.0	-

All Values are expressed as Mean ± SEM, µ = 3, # = $p < 0.05$ compared to negative control (One-way ANOVA, Tukey's post hoc),

- = No inhibition; IZD = Inhibition zone diameter; EC3-1 = Sephadex LH-20 sub-fraction of EC3.

Table 5 Result of antimicrobial activity of EC3-3 fungal fraction against tested pathogenic microorganism

Organisms	**Concentration (mg/mL) IZD (mm)**						
	1	**0.5**	**0.25**	**0.125**	**0.0625**	**Gentamycin (10 µg/mL)**	**DMSO**
Staphylococcus aureus	5.0 ± 0.0	-	-	-	-	19.0±0.0	-
Escherichia coli	-	-	-	-	-	20.0±0.0	-
Salmonella typhi	8.0±0.0	6.0±0.0	3.0±0.5	-	-	12.0±0.0	-
Pseudomonas aeruginosa	-	-	-	-	-	19.0±0.0	
Bacillus subillis	-	-	-	-	-	15.0±0.0	
						Miconazole (50µg/mL)	DMSO
Aspergillus niger	6.0±0.0	2.0±0.0	-	-	-	11.0±0.0	-
Candida albicans	-	-	-	-	-	14.0±0.0	-

All Values are expressed as Mean ± SEM, n = 3, # = $p<0.05$ compared to negative control (one-way ANOVA; Tukey's post hoc),

- = No. inhibition; IZD = Inhibition zone diameter; EC3-3 = Sephadex LH-20 sub-fraction of EC3

Table 6 Results of Minimum inhibitory concentrations (MIC) of fungal fractions against tested pathogenic microorganism

Test Organisms	**EC3-1 MICs (mg/mL)**	**EC3-3 MICs (mg/mL)**
Staphylococcus aureus	0.34	0.19
Escherichia coli	-	-
Salmonella typhi	-	0.2
Pseudomonas aeruginosa	0.34	-
Bacillus subillis	-	-
Aspergillus niger	0.4	0.46
Candida albicans	-	-

Table 7 Result of DPPH scavenging activity of the Sephadex fraction EC3-3 of *Colletotrichum spp (Anarmoph)* from *Vernonia amygdalina*

Extract/fractions	**IC_{50} (µg/mL)**
EC3-3	3.16
Ascorbic Acid	50.72

4. Discussion

Five bioactive compounds, namely: p-Hydroxybenzoic acid, desmethyldichlorodiaportin, Cladosporin, p-Hydroxybenzaldehyde and palitantin were detected from the VLC and Sephadex fractions of the metabolites produced by the endophytic fungus *Colletotrichum species* from *Vernonia amygdalin.* The compounds were detected by dereplication using HPLC-DAD analysis. P-Hydroxybenzaldehyde, an isomer of hydroxybenzaldehyde has been previously isolated from the epicuticular wax of seedling bicolor and also found in orchids *Gastrodia elata* and *Galeola faberi*. Previous report by Tawfik *et al*. [35] has established the antimicrobial activity of this compound. Cladosporin was previously isolated from *Colletortrichum cladosporioides* and there are reports of its antimicrobial, antifungal, antiplasmodal, anti-insecticidal and anti-tumor properties [29, 30]. Desmethyldichloro- diaportin was shown to possess cytotoxic and antimicrobial activities in previous reports [23, 36]. The data obtained from the antimicrobial screening of fraction EC3-1 showed that it has moderate antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* and moderate antifungal activity against *Aspergillus niger* at concentration ranging from 0.5 to 1 mg/mL. *Staphyococcus aureus* and *Pseudomonas aeruginosa* were seen to be most susceptible. EC3-1 did not show any inhibition against *Escherichia coli*, *Salmonella typhi, Bacillus subtilis as* well as *C. albican* at the tested concentrations. The fraction EC3-3 displayed inhibition against *Staphylococcus aureus, Salmonella typhi and Aspergillus niger* at concentration ranging from 0.25 to 1 mg/mL. *Salmonella typhi* was seen to be the most susceptible. The fraction EC3- 1 showed MIC at 0.34 mg/mL against *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria strain and MIC at 0.40 mg/mL against *Aspergillus niger* while EC3-3 showed MIC at 0.19 mg/mL against *Aspergillus niger*. However, the fractions did not show any inhibitory activity against *Escherichia coli, Bacillus subtilis* and *Candida albicans* at the tested concentrations. The failure for the tested fractions to inhibit some of these microorganisms may be due to inactivation which is a mechanism by which microorganisms may circumvent the inhibitory action of antimicrobial agents. Similarly, it has been reported that the metabolites of endophytic fungus, *Collectotrichum spp*. had strong antimicrobial activity [37, 38]. The fraction, EC3-3 showed a very high antioxidant activity with IC_{50} value of 3.16µg/mL compared to ascorbic acid (50.72µg/ml). Compounds that can scavenge free radicals have great potentials in ameliorating disease processes causing oxidative damage of tissues and biomolecules. The antioxidant potential demonstrated by the fraction EC3-3 may be attributed to the presence of the secondary metabolites. Palitantin identified by HPLC-DAD and two other major compounds, which were not identified due to lack of library hits. These two unidentified major peaks are likely new bioactive metabolites and their isolation and structure elucidation is currently in process in our laboratory.

5. Conclusion

HPLC-DAD analysis of the metabolites of *Colletotrichum spp* (Anamorph) isolated from *Vernonia amygdalina* revealed the presence a wide array of compounds that could serve as leads in drug development. The Sephadex fractions EC3-1 and EC3-3 displayed inhibitory effect against *Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa* and *Candida albicans* indicating their broad-spectrum antimicrobial activity. EC3-3 displayed strong antioxidant activity and thus could have great potential as therapeutic agent in managing degenerative diseases. This study further highlights the vast potentials of endophytic fungi as reservoir of bioactive metabolites that might serve as alternative sources for the production of therapeutic agents.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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