Secondary metabolites from two phenotypic variants of *Colletotrichum gloeosporioides* show antibacterial and antioxidant activities

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**Abstract:** Crude polar and nonpolar extracts of the secondaray metabolites of the sabouraud broth pure cultures of two genetically identical but phenotypically distinct strains of *Colletotrichum gloeosporioides* were tested for antimicrobial and antioxidants activities *in vitro*. Extracts from both *Colletotrichum gloeosporioides* (Black sp.) and *Colletotrichum gloeosporioides* (White sp.) showed the presence of unique solvent-dependent combinations of multiple phytochemicals that included terpenoids, flavonoids, anthraquinones, tannis and saponins. For both *Colletotrichum gloeosporioides* (Black sp.) and *Colletotrichum gloeosporioides* (White sp.) strains, crude polar fungal extracts recorded higher antibacterial activities against the panel of 9 tested bacterial pathogens than did the nonpolar extract in the agar diffusion assay (relatively larger zone diameters of inhibition) and in the broth dilution assay (relatively lower MICs). Polar extracts of both fungal metabolites were also stronger antioxidants than the nonpolar extracts and displayed in both cases IC50s in DPPH radical scavenging assays that were at least 3-fold difference lower than that of their corresponding nonpolar extracts. But the DPPH radical scavenging antioxidant activities of both fungal extracts were meagre compared to that of the Ascorbic acid control as both extracts showed IC50s that were at least 6-fold difference higher than that of Ascorbic acid. Future studies can assess the potential of single purified secondary metabolites from either *Colletotrichum gloeosporioides* (White sp.) or *Colletotrichum gloeosporioides* (Black sp.) or from both fungi to act as leads for the development of novel antibacterial agents.

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**Capsule Summary:** Crude polar (Ethylacetate) and non-polar (petroleum ether) extracts of the secondary metabolite of pure cultured *Colletotrichum gloeosporioides* (Black sp.) and *Colletotrichum gloeosporioides* (White sp.) demonstrate phenotypic variant-specific antimicrobial and antioxidant activities *in vitro*.


**INTRODUCTION**

Although secondary metabolites biosynthesized by pure cultured fungi have, historically, been utilized as leads for the production of antibiotics including tetracycline, screening
Fungal isolates for potential novel antibiotics has ebbed. Fungal secondary metabolites can still be sourced as leads for the development of novel antimicrobial agents with effective activity against multi-drug resistant pathogenic microbes. Additionally, fungal secondary metabolites can be utilized as leads for the production of much needed novel antioxidants to replenish the stagnant stock of antioxidant molecules.

A long-term goal of screening fungal extracts from the repository of endogenous soil-derived and endophytic plant-derived fungi cultured in various media has been initiated (Mensah et al. 2015; Mensah et al. 2016; Mensah et al. 2017). In this continuing effort, crude extracts of the secondary metabolites derived from two genetically identical but phenotypically distinct strains of fungi: Colletotrichum gloeosporioides (White sp.) and Colletotrichum gloeosporioides (Black sp.) were assessed for antibacterial activities and for antioxidant activities in vitro. Phenotypically, Colletotrichum gloeosporioides (Black sp.) differs from Colletotrichum gloeosporioides (White sp.) by the presence of dark-walled stromatic structures in the culture of the fungi (Weir et al., 2012).

Filamentous fungi of the Colletotrichum gloeosporioides genera are endophytic fungal species known for its contamination of agricultural products including maize, vegetables and citrus (Mills et al., 1992). As a diverse group, Colletotrichum gloeosporioides species are highly variable and exists in phenotypic forms that often vary with its host as well as with its geographical location (Mills et al., 1992).

Individual Colletotrichum fungi are endophytic (Weir et al., 2012) and together with closely related genotypes, the species exhibit a range of phenotypic differences that often vary with geographical location or with its source matrix [(Cannon et al., 2012); (Mills et al., 1992); (Lima et al., 2012); (Gonzaga et al. 2015); (Deshmukh et al., 2015)]. Due to intra-species genetic relatedness, mycologists have often supplemented morphological methods with molecular approaches as diagnostic and confirmatory tools for characterization [(Mills et al., 1992); (Alvim et al., 2009)]. For Colletotrichum species that show clear phenotypic differences, utilization of morphological distinctiveness as the differentiation tools suffice (Alvim et al., 2009). Few secondary metabolites biosynthesized by distinct Colletotrichum species have been isolated and structurally characterized [(Sharma et al., 2017); (Zou et al., 2000); (Strobel et al., 2004); (Liu et al. 2017; García-Pajón and Colrado 2003)]. In few cases, isolated bioactive fungal metabolites have demonstrated substantial fungicidal activities in vitro [(Chapla et al., 2014); (Rabha et al., 2014)].

Both Colletotrichum gloeosporioides (White sp.) and Colletotrichum gloeosporioides (Black sp.) have stimulated broad interest not just for their distinct phenotypic appearance but also for their potential utilization as sources for bioactive lead compounds. No prior study has either examined their secondary metabolites for bioactivity against bacterial pathogens or for their antioxidant activities. Although genetically identical, the possibility that Colletotrichum gloeosporioides (White sp.) and Colletotrichum gloeosporioides (Black sp.) might utilize distinct secondary metabolic pathways for the biosynthesis of chemically diverse bioactive compounds, on a phenotype-specific basis, was intriguing and offered the basis for screening both White sp. and Black sp. phenotypic variants of Colletotrichum gloeosporioides on the same bioassay platforms. The total pool of secondary metabolites available from the two species was reasoned to provide access to a substantial library of chemically diverse secondary metabolites that may include potentially potent antibacterial and/or antioxidant compounds.

This study reports that crude polar fungal extracts for both Colletotrichum gloeosporioides (White sp.) and Colletotrichum gloeosporioides (Black sp.) recorded relatively higher antibacterial activities against a larger pool of bacterial pathogens than the nonpolar extracts. Although both polar and nonpolar extracts displayed meagre DPPH radical scavenging activities in vitro, relative to Ascorbic acid, the polar extracts of both fungi were stronger antioxidants than the nonpolar extracts. Future studies can explore the potential utilization of singly isolated purified secondary metabolite(s) as lead for the development of novel antibacterial agents.

MATERIAL AND METHODS

Isolation, culture, identification of fungi and inoculation of media with fungal isolate

Fungal species were isolated from soil as previously reported (Mensah et al., 2015; Mensah et al., 2016; Mensah et al., 2017). Isolated species were identified using routine methods that relied on carefully documented and highly reported morphologic and phenotypic properties of each fungi (Mensah et al., 2015; Mensah at al., 2016; Mensah et al., 2017). Microscopic observation that were utilized in species characterization is based on the culture colour, culture pigmentation, the general appearance of aerial mycelium, the conidia length and breadth, and the number of conidia produced.

Fungal isolates were maintained as Petri-dish pure cultures and inoculated as pure culture into sabouraud broth media are previously described (Mensah et al., 2015; Mensah et al., 2016; Mensah et al., 2017). Previously reported quality control measures involving re-culturing and re-identification of aliquots of each of the sabouraud media liquid culture of fungal specimen was also performed (Mensah et al., 2015; Mensah et al., 2016; Mensah et al., 2017).

Extraction of secondary metabolites from culture supernatant

Culture supernatant was filtered and the secondary metabolites extracted with ethylacetate and with petroleum ether as previously described (Mensah et al., 2015; Mensah et al., 2016; Mensah et al., 2017). The extract was evaporated at room temperature to dryness and the dried fungal extracts were stored at -20 ºC until needed.
Phytochemical analyses of extracted secondary metabolites

Standard tests were utilized to assess the phytochemical composition of the media extracts (Mensah et al., 2015; Mensah et al., 2016; Mensah et al., 2017). Examined phytochemicals included tannins, flavonoids, alkaloids, saponins, coumarins, anthraquinones, glycosides, terpenoids and steroids (Mensah et al., 2015; Mensah et al., 2016; Mensah et al., 2017).
Thin Layer Chromatography (TLC)

TLC analyses were performed on 20 cm x 20 cm silica gel 60 (Darmstadt, Germany) that were prepared in-house. Prior to use, TLC plates were activated at 60 °C for 30 min and allowed to cool in a desiccator. Spotting of fungal extract samples, running of spotted plates in chloroform as eluent, visualization of resolved bands and calculation of Rf values.
were performed as previously described (Mensah et al., 2015; Mensah et al., 2016; Mensah et al., 2017).

**Culture and maintenance of pathogenic bacteria**

The panel of bacteria pathogens used for the assessment of antimicrobial activity of the fungal extracts were acquired from ATCC (USA). The bacteria pathogens included *Escherichia coli* (ATCC 25922), *Salmonella typhi* (ATCC 19430), *Bacillus subtilis* (ATCC 6633), *Enterococcus faecalis* (ATCC 29212), *Streptococcus pneumoniae* (ATCC 49619), *Klebsiella pneumoniae* (ATCC 10031), and *Neisseria gonorrhoea* (ATCC 49226).

**Table 7: MIC values (mg/mL) of extracts against test bacteria**

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>White sp. Ethyl acetate extract</th>
<th>Petroleum ether extract</th>
<th>Black sp. Ethyl acetate extract</th>
<th>Petroleum ether extract</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. Coli</em></td>
<td>0.2734</td>
<td>8.750</td>
<td><em>E. Coli</em></td>
<td>2.25</td>
</tr>
<tr>
<td><em>S. typhi</em></td>
<td>0.2734</td>
<td>1.0938</td>
<td><em>S. aureus</em></td>
<td>2.25</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>0.2734</td>
<td>0.5469</td>
<td><em>S. pneumoniae</em></td>
<td>2.25</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>0.2734</td>
<td>0.5469</td>
<td><em>E. faecalis</em></td>
<td>4.50</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>0.2734</td>
<td>0.5469</td>
<td><em>N. gonorrhoea</em></td>
<td>4.50</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>0.2734</td>
<td>0.5469</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 8: Broth dilution for standard drug (Ciprofloxacin)**

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Concentration (mg/mL)</th>
<th>0.01</th>
<th>0.005</th>
<th>0.0025</th>
<th>0.00125</th>
<th>0.000625</th>
<th>0.0003125</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. Coli</em></td>
<td>Ciprofloxacin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>S. typhi</em></td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>S. pneumoniae</em></td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>N. gonorrhoea</em></td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

**Table 9: DPPH radical scavenging activities of ethyl acetate and petroleum ether extracts of the White sp, Black sp and ascorbic acid expressed as IC$_{50}$**

<table>
<thead>
<tr>
<th>Sample extract</th>
<th>IC$_{50}$ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>White sp.</em></td>
<td></td>
</tr>
<tr>
<td>Ethyl acetate extract</td>
<td>378.12</td>
</tr>
<tr>
<td>Petroleum ether extract</td>
<td>1127.47</td>
</tr>
<tr>
<td><em>Black sp.</em></td>
<td></td>
</tr>
<tr>
<td>Ethyl acetate extract</td>
<td>298.539</td>
</tr>
<tr>
<td>Petroleum ether extract</td>
<td>811.97</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>49.02</td>
</tr>
</tbody>
</table>

*www.bosaljournals/csp*
(ATCC 29212), *Klebsiella pneumoniae* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923), *Streptococcus pneumoniae* (ATCC 49619) and *Neisseria gonorrhoeae* (ATCC 43069). Storage, culture and maintenance of the pathogenic bacteria were performed as previously reported (Mensah et al., 2015; Mensah et al., 2016; Mensah et al., 2017).

### Agar Diffusion Assay

Details of the agar diffusion assay including the adjustment of the turbidity of the pathogenic bacteria, the spreading of the pathogenic bacteria suspension over the Mueller-Hinton agar plates containing the five 7 mm diameter wells and the application of fungal extracts of varying concentrations to the wells have been reported elsewhere (Mensah et al., 2015; Mensah et al., 2016; Mensah et al., 2017). The overall experimental setup and the data interpretation were guided by previously reported literature (Schwarz et al., 2010). Fungal extract concentrations utilized for the assay are listed in Tables 2 and 3. Methanol was used as the negative control while different concentrations of Ciprofloxacin (Table 4) was used as positive control. Incubation of the petri-dish and the reading of the zone diameters of inhibition was performed as previously described (Mensah et al., 2015; Mensah et al., 2016; Mensah et al., 2017). Inhibition zone diameters were measured to the nearest whole millimeter from the center to the point where there was no visible growth (clear zones) after 24 h incubation.

### Broth dilution assay

The experimental protocol utilized for the broth dilution assay is previously reported (Mensah et al., 2015; Mensah et al., 2016; Mensah et al., 2017). The concentration of fungal extracts and the list of pathogenic bacteria are listed in Tables 5, 6 and 7. Ciprofloxacin (at concentrations presented in Table 8) were used as positive controls. MICs (expressed in mg/mL in Table 7) were taken as the lowest extract concentration that showed complete growth inhibition as demonstrated by a lack of visible violet coloration following the addition of 0.1 mL of MTT. The experimental setup and the data interpretation was performed by following the guidelines described earlier (Schwarz et al., 2010).

### 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity

Prior published report has provided details of the experimental protocol used for the assessment of the antioxidant activities via the DPPH free radical scavenging assay (Mensah et al., 2016; Mensah et al., 2017). Briefly, fungal extracts of different concentrations were separately treated with 0.1 mM DPPH solution in methanol as previously described (Mensah et al., 2016; Mensah et al., 2017). Control samples were prepared without extracts and Ascorbic acid was used as standard free radical scavenger. Radical scavenging activity was expressed as the inhibition percentage after absorbance determination at 517 nm (Mensah et al., 2016; Mensah et al., 2017).

### RESULTS AND DISCUSSION

#### Thin Layer Chromatography (TLC)

All samples of extracts applied on TLC plates showed single individual band for each extract (Table 1). Besides the ethylacetate extract of Black sp. that had a measurable Rf of 0.60, spotted aliquots of the other three extracts (ethylacetate and petroleum ether extracts of White sp.; petroleum ether extract of Black sp.) did not move beyond the point of application (Table 1). It is unclear whether individual extracts contain a single chemical constituent or whether the combination of mobile phase and the silica gel stationary phase utilized in the TLC study could not resolve any of fungal extracts into constituent fractions.

#### Phytochemical composition of fungal extracts

The ethylacetate extract of both White sp. and Black sp. were relatively more phytochemical-rich as both displayed more phytochemical presence (terpenoids, flavonoids, anthraquinones and tannins for White sp.) and (terpenoids, flavonoids, saponins and tannins for Black sp.) than the Petroleum ether extracts (Table 1). Petroleum ether extract of White sp. showed the least number of phytochemicals: terpenoids and tannins while the petroleum ether extract of Black sp. profiled flavonoids, anthraquinones and saponins (Table 1).

### Agar well diffusion assay

Agar well diffusion assay was used to assess the antimicrobial effects of the extracts on a panel of bacterial strains. Zone diameters of growth inhibition (estimated in millimeters) at different extract concentrations were utilized as correlates of extract antibacterial efficacy. Both Tables 2 and 3 depict the utilized range of extract concentrations and the corresponding zone diameters of growth inhibition extracts triggered against the panel of tested bacteria strains in the agar well diffusion assay. Zone diameters of inhibition varied by bacteria strains and were generally low and fell within 10 – 36 mm range.

#### Pattern of bacteria growth inhibition by the extracts of the black sp

The ethylacetate extract of Black sp. showed only marginal antibacterial activity against *Streptococcus pneumoniae* and *Neisseria gonorrhoeae* at all applied extract concentrations (zones of inhibition were lowest in the series for both bacteria strains in Table 2). *Escherichia coli* inhibition occurred only at the two highest applied doses but the quantitative pattern in the zone diameters of inhibition was erratic and was devoid of a dose-dependence effect (17.0 mm zone diameter of inhibition in each case). Against *Staphylococcus aureus* and *Enterococcus faecalis*, the ethylacetate extract showed dose-dependent inhibitory
activities with higher effectiveness against *Enterococcus faecalis* (zone diameter of inhibition at 18 mg/mL was the highest among the series at 16.5 mm) than against *Staphylococcus aureus* (zone diameter of inhibition at 18 mg/mL is 13.0 mm).

The petroleum ether extract of Black sp. showed its strongest activity against *Enterococcus faecalis* displaying a concentration-dependent gradation in activity demonstrated with a relatively higher zone diameter of inhibition per each applied extract dose (zone diameter of inhibition at 18 mg/mL was the second highest among the series at 14.5 mm as recorded in Table 2). *Escherichia coli* was also subjected to a dose-dependent inhibitory activities by the petroleum ether extract with zone diameters of inhibition per applied extract dose that was comparable to that observed for *Enterococcus faecalis* (zone diameter of inhibition at 18 mg/mL was again at 14.5 mm). The inhibitory activity of the petroleum ether extract against *Staphylococcus aureus* was only manifested at the highest applied dose (zone diameter of inhibition at 18 mg/mL was at 12.5 mm) while inhibitory activity against *Streptococcus pneumoniae* and *Neisseria gonorrhoeae* was modest but failed to show any gradation with increasing concentration of applied extracts (zone diameter of inhibition was at 10.0 mm at all applied concentration). The ethylacetate extract of Black sp. produced slightly larger zones of inhibition per applied extract concentration compared to that of the petroleum ether extract (Tables 2) (mean difference, 0.4 mm [*P* < 0.001; ANOVA]).

**Pattern of bacteria growth inhibition by the extracts of the white sp**

The ethylacetate extract of White sp. showed dose-dependent inhibition of *Salmonella typhi*, *Bacillus subtilis*, *Enterococcus faecalis* and *Klebsiella pneumoniae* in a pattern of inhibition that differed in intensity for each bacterial species in Table 3. The strongest activity in the series was displayed against *Enterococcus faecalis* (largest zone diameters of inhibition per applied dose) while comparable activities were displayed by the three other strains *Salmonella typhi*, *Bacillus subtilis* and *Klebsiella pneumoniae* (marginal differences in their respective zone diameters of inhibition). Extract concentrations above 0.7 mg/mL were dose-dependent but effective inhibitors of *Escherichia coli* growth (zone diameter of inhibition at 18 mg/mL was the third highest within the series at 31.0 mm). Ethylacetate extract of White sp. was ineffective in inhibiting the growth of *Pseudomonas aeruginosa* at all applied concentrations (lowest observable zones diameter of inhibition among the series of bacterial pathogens).

The petroleum ether extract of White sp. displayed maximum inhibitory activity against *Enterococcus faecalis* where applied concentrations yielded the second largest dose-dependent zones diameters of inhibition per bacteria species in the series of extracts (Table 3). Individual inhibitory activities afforded by the petroleum ether extract against *Salmonella typhi*, *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumoniae* were appreciable but not dose-dependent. Similar to the ethylacetate extract of White sp., the petroleum ether extract of white sp. was inactive against *Pseudomonas aeruginosa* at all applied concentrations except at the highest utilized dose where inhibitory activity was barely marginal.

In a pattern of inhibition synonymous with that of the Black sp. extract, the ethylacetate extract of White sp. yielded slightly larger zones of inhibition per applied dose per bacteria strains than that delivered by the petroleum ether extract (Table 3), with a mean zone size difference of 0.5 mm (P < 0.001; ANOVA).

Zones of inhibition triggered by the positive control drug (Ciprofloxacin) per bacterial strain were by comparison relatively large per applied dose (zone diameter of inhibition at the low 0.35 mg/mL concentration of Ciprofloxacin was the highest recorded per each bacteria strain) (Table 4). Recorded zones diameters of inhibition show that the ethylacetate fungal extract of each fungal species was comparably a more potent inhibitor of bacteria growth than the corresponding petroleum ether extract. By far, the ethylacetate extract of White sp. was the most potent bacterial growth inhibitor of the series of extracts accounting for the largest observable zone diameters of inhibition per applied extract concentration (Tables 2 and 3).

**Broth dilution assay**

Relative bacteria susceptibilities to the bactericidal activities of the extract was additionally assessed through broth dilution assays that utilized the minimum inhibitory concentration (MIC) as relative estimates of bactericidal efficacies. MIC was taken as the lowest concentration of the assayed extract that triggered complete and visible growth inhibition of the specific bacterium under investigation. MIC determinations for each of the 4 extracts was made against the 9 pathogenic bacteria. Tables 5 and 6 lists the MIC values for all the four extracts (ethylacetate and petroleum ether for White sp.; ethylacetate and petroleum ether for Black sp.) against the panel of 9 bacteria pathogens.

**Pattern of bacteria growth inhibition by the extracts of the black sp**

Bacteria strains *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus pneumoniae* displayed higher susceptibilities to the ethylacetate extract of Black sp. recording in each case a relatively low MIC of 2.25 mg/mL (Table 5). By comparison, bacterial growth inhibition of the ethylacetate extract for *Enterococcus faecalis* and *Neisseria gonorrhoeae* was observed at a two-fold higher MICs of 4.50 mg/mL (Table 5). A similar qualitative pattern of bacteria susceptibilities was demonstrated by the petroleum ether extract of Black sp. with recorded quantitative MICs that varied for two distinct groups of bacterial strains (Table 6). With the petroleum ether extract, *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus pneumoniae* recorded lower MIC of 2.25 mg/mL while *Enterococcus faecalis* and *Neisseria gonorrhoeae* presented with MIC at a higher 9.0 mg/mL concentration (Table 6).
Pattern of bacteria growth inhibition by the extracts of the white sp
For the ethylacetate extract the range of MICs did not vary by bacteria strain (Table 5). All bacteria strains recorded a relatively low MIC at an extract concentration of 0.55 mg/mL. Higher MICs were recorded for the petroleum ether extracts than for the ethylacetate extract as bacterial susceptibilities to its growth inhibitory effects varied by strain (Table 5). The range of quantitative values for the MIC of the petroleum ether extract start from 17.5 mg/mL for Escherichia coli, goes through 2.19 mg/mL for Salmonella typhi and end at 1.09 mg/mL for Bacillus subtilis, Enterococcus faecalis, Klebsiella pneumoniae and Pseudomonas aeruginosa (Table 6).

Table 7 lists the recorded MICs for both polar and nonpolar extracts of both fungi. For comparison, the positive control drug Ciprofloxacin MIC values (Table 8) were lower than 0.6 µg/mL. The ethylacetate extract of White sp. exhibited the strongest relative bactericidal activities as demonstrated by the consistently larger zone diameters of inhibition in agar diffusion assays and by the relatively lower MICs in broth dilution assays for all bacteria strains. The ethylacetate extract of White sp. again displayed much broader spectrum of bactericidal action than all the other extracts, inhibiting the growth of all bacteria species in the panel in the broth dilution assay.

DPPH radical scavenging assay
All samples showed a dose-dependent scavenging of DPPH radicals, with a much more prevalence at the initial low extract concentrations (Figure 1). The graph for the Ascorbic acid control showed a slight plateau at high sample concentrations (Figure 1). Fungal extracts demonstrated comparable dose-dependent DPPH scavenging activities to that of Ascorbic acid control at the initial lowest concentrations and the differences in scavenging activities between extracts and the Ascorbic acid control became relatively large at higher extract concentrations (Figure 1). Antioxidant activities varied with extract type as demonstrated by the recorded IC50 in Table 9 and as shown in the graph in Figure 1. The IC50 records from the DPPH radical scavenging assay show higher levels of antioxidative activity for Ascorbic acid control relative to that of all fungal extracts (Table 9). Scavenging of DPPH radicals for all fungal extracts was dose-dependent in a concentration range between 10-500 mg/mL while that for Ascorbic acid showed gradation only between 10-120 mg/mL concentration range.

For both fungal species, the ethylacetate extracts showed stronger antioxidant activities than the PE extracts recording in each case a 3-fold lower IC50 than that of the petroleum ether extracts. Relative to Ascorbic acid, ethylacetate extracts demonstrated moderate antioxidant potential with IC50s that were 6-fold (Black sp) and 8-fold (White sp) higher. Antioxidant activities of the petroleum ether extract were significantly lower than that of the ethylacetate extracts by a 3-fold difference lower IC50s. The
ethyelacetate extract for the Black sp. displayed the highest antioxidant activity among all four fungal extracts. Individual fungal extracts from the Black sp. (both ethyelacetate and petroleum ether) were correspondingly stronger antioxidants than the individual extracts (both ethyelacetate and petroleum ether) from White sp.

To explain the trend in antioxidant potential between the extracts, the negative linear correlation between IC50 and antioxidant activities was invoked. All fungal extracts had lower antioxidant potentials than Ascorbic acid, and consequently displayed IC50 values that were higher in quantitative values than that for Ascorbic acid (Table 9). Ascorbic acid was, at least, six times more active antioxidant than any of the fungal extracts. For both fungal species, the polar media extracts were the more active antioxidants with the lowest relative recorded IC50s. The IC50-based decreasing order of antioxidant potential for all the examined DPPH radical scavengers is: Ascorbic acid < ethylacetate media extract of Black sp.<ethylacetate media extract of White sp.< Petroleum ether extract of Black sp.<Petroleum ether extract of White sp. The difference in antioxidant potential for the fungal extracts is apparently determined by the differences in phytochemical compositions.

The biosynthesis of novel chemotypes of secondary metabolites by cultured fungi is a well-known method for production of successful leads for the discovery of new drugs to treat pathogenic infections. The two Colletotrichum gloeosporioides strains (White sp. and Black sp.) utilized as source organisms for this study are phylogenetically identical but phenotypically distinct fungi responsible for the contamination of agricultural produce such as maize, vegetables and citrus (Mills et al., 1992). These two strains of Colletotrichum gloeosporioides fungi have hitherto been unexamined for bioactive secondary metabolites that are either bactericidal or antioxidants.

Although the TLC failed to conclusively identify the presence of multiple compounds in each of the crude extracts, the high number of phytochemicals, particularly in the polar extracts, are indicative of the possible presence of multiple chemical entities. The array of phytochemicals present in both extracts of both fungal phenotypes are diverse and the solvent-dependence of its composition are in line with the repertoire of phytochemical composition observed from precedents for fungal extracts (Mensah et al., 2015; Mensah et al., 2016 and Mensah et al., 2017). The differences in phytochemical composition between the two phenotypes suggests genotype-specific biosynthesis of secondary metabolites by each strain of Colletotrichum gloeosporioides fungi.

Bioactive fungal secondary metabolites that are bactericidal have wide and varied phytochemical compositions and, thus, it is difficult to predict which of the phytochemicals are primarily responsible for the observed bactericidal activities of its extracts. It is, however, noteworthy that phytochemicals such as tannins are well known to confer antioxidant properties to compounds. Unsurprisingly the crude polar extracts that showed the presence of tannins exerted moderate antioxidant activities that was distinct from its bactericidal activities.

Although the cumulative antibacterial data shows qualitative agreement between the MICs of the broth dilution method and the zone diameters of inhibition in the agar diffusion assay, an apparent discrepancy existed in the higher extract concentrations needed for microbial growth inhibition in the agar diffusion and in the lack of activity for some extracts on some bacterial pathogens in the agar diffusion assay. These inconsistencies is partly attributable to the slow rate of diffusion of the extract from its site of application through the agar matrix to the microbes (Mensah et al., 2017).

The display of antibacterial activities by the polar extracts of both fungi in agar diffusion and in broth dilution assays at concentrations that are indicative of moderate bioactivity support the assertion that individual constituents of the polar extracts when isolated and structurally characterized, may become lead candidates for future antibacterial pharmaceutical drug development. Anti-oxidant EC50 values suggests that the polar extracts from both fungi represent better sources of anti-oxidant due primarily to its higher radical scavenging activities. Although anti-oxidant bioactivity was minimal with EC50s above the range of clinically useful concentrations, the possibility that one or more synthetic analogs of the constituent compounds might exhibit potent antioxidant activity should spur further research designed to derive useful anti-oxidants from the extracts of these cultured fungi.

Since higher antibacterial and antioxidant bioactivities resided in the polar extracts of both fungal cultures, future studies can delineate the chemical structures of the bioactive compounds responsible for the antimicrobial and antioxidant activities of the extracts using activity-guided fractionation with agar diffusion and DPPH assays as primary screens. Synthesis of analogs of the characterized promising active constituents may afford useful antibacterial and antioxidant drug leads. This study ultimately hoped to uncover novel chemical entities that could be lead candidates with novel mechanism of action and with moderate potency against bacterial pathogens. Such an effort will partly mitigate the proliferation of multi-drug resistant bacterial pathogens.

CONCLUSIONS

Examined Colletotrichum gloeosporioides fungi are unique sources of phytochemicals such as terpenoids, flavonoids, anthraquinones, tannis and saponins, that were common to both species. Colletotrichum gloeosporioides (Black sp.) had an additional phytochemical content that was distinct from that of Colletotrichum gloeosporioides (White sp.). Crude polar and nonpolar extracts of these two genetically similar but phenotypically distinct Colletotrichum gloeosporioides fungi show varying antibacterial potency and differing antioxidant activities. Agar diffusion and broth dilution assays show crude polar extracts, from both Colletotrichum gloeosporioides species, demonstrated higher antibacterial activity against the panel of 9 tested pathogenic bacteria. Polar extract of Colletotrichum gloeosporioides (Black sp.) and Colletotrichum gloeosporioides (White sp.) were also stronger...
antioxidants as demonstrated by their relatively lower IC50 values in the DPPH radical scavenging assay. The IC50 value for the ethylacetate extract of Black sp. is only about 0.8-fold lower than that of the White sp. but is significantly higher, by about 6-fold difference, when compared with that of the IC50 of Ascorbic acid. The observation of moderate bactericidal and substantial antioxidant activities of the polar extracts of examined Colletotrichum gloeosporioides (Black sp) and Colletotrichum gloeosporioides (White sp.) species should be a critical starting point for future studies focussed on the isolation and the structural characterization of bioactive antimicrobial and antioxidant fungal metabolites.

REFERENCES


