

Article

# Antimycotoxigenic Activity of Beetroot Extracts against *Alternaria alternata* Mycotoxins on Potato Crop

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**Abstract:** *Alternaria* species, mainly air-borne fungi, affect potato plants, causing black spots symptoms. Morphological identification, pathogenicity assessment, and internal transcribed spacer (ITS) molecular identification confirmed that all isolates were *Alternaria alternata*. The annotated sequences were deposited in GenBank under accession numbers MN592771–MN592777. HPLC analysis revealed that the fungal isolates KH3 (133,200 ng/g) and NO3 (212,000 ng/g) produced higher levels of tenuazonic acid (TeA) and alternariol monomethyl ether (AME), respectively. Beet ethanol extract (BEE) and beet methanol extract (BME) at different concentrations were used as antimycotoxins. BME decreased the production of mycotoxins by 66.99–99.79%. The highest TeA reduction rate (99.39%) was reported in the KH3 isolate with 150 µg/mL BME treatment. In comparison, the most effective AME reduction rate (99.79%) was shown in the NO3 isolate with 150 µg/mL BME treatment. In the same way, BEE application resulted in 95.60–99.91% mycotoxin reduction. The highest TeA reduction rate (99.91%) was reported in the KH3 isolate with 150 µg/mL BEE treatment, while the greatest AME reduction rate (99.68%) was shown in the Alam1 isolate with 75 µg/mL BEE treatment. GC-MS analysis showed that the main constituent in BME was the antioxidant compound 1-dodecanamine, n,n-dimethyl with a peak area of 43.75%. In contrast, oxirane, methyl- (23.22%); hexadecanoic acid, methyl ester (10.72%); and n-hexadecanoic acid (7.32%) were the main components in BEE found by GC-MS. They are probably antimicrobial molecules and have an effect on the mycotoxin in general. To our knowledge, this is the first study describing the antimycotoxigenic activity of beet extracts against *A. alternata* mycotoxins-contaminated potato crops in Egypt, aimed to manage and save the environment.

**Keywords:** *Alternaria*; potato; beetroot extract; mycotoxin; TeA; AME; ITS

## 1. Introduction

Potato (*Solanum tuberosum*, L.) is one of the most important vegetable crops worldwide [1]. In Egypt, the amount of land allocated to potato production represents about

20% of the cultivated area [2]. Phytopathogenic fungi cause significant agricultural problems and reduce crop production, resulting in substantial economic losses for growers [3]. *Alternaria* is one of the most common plant fungal genera found ubiquitously and comprises species that may be saprophytic, endophytic, or pathogenic [4].

*Alternaria* species produce more than 70 phytotoxins—which can be designated into several groups of mycotoxins—including alternariol (AOH), alternariol monomethyl ether (AME), altenuene (ALT), altertoxins (ATX-I, -II and -III), and tenuazonic acid (TeA) [3,5]. AOH, AME, TeA, and ATX toxins were reported to cause adverse teratogenic effects in mammals [6]. AOH and AME toxins, extracted from grains infected by *Alternaria*, were responsible for genotoxicity and throat cancer in China [7].

TeA is a beta-diketone with metal-chelating properties and contains a secondary amine moiety [8]. It exhibited acute oral toxicity in many mammalian species (LD<sub>50</sub> 37.5 and 225 mg/kg b.w. for chicks and mice, respectively) and inhibited protein synthesis [9]. TeA was reported in infected plants and also in the processed products, including gapes, beer and cereal grains [10–13]. Furthermore, some evidence has been reported that AME and AOH are genotoxic and cytotoxic in human organs [14].

Currently, *Alternaria* mycotoxins are detected in different geographic regions worldwide, including Egypt. Beetroot (*Beta vulgaris* L. subsp. *vulgaris*) is a member of the *Amaranthaceae* family, *Chenopodiaceae* sub-family (Angiosperm polygamy group), which contains high levels of betalains, polyphenols, and other phytochemical compounds that are associated with antioxidant and antimicrobial activities [15,16].

Our study aimed to (i) isolate, morphologically identify, and genetically characterize (by ITS) the *Alternaria* isolates; (ii) examine the pathogenicity of the isolates; (iii) investigate the occurrence of *Alternaria* toxins naturally contaminating potatoes and their geographic distribution in Egypt; (iv) evaluate the effect of beetroot methanol and ethanol extracts against the produced mycotoxins; and (v) identify the active compounds in beetroot extracts by GC-MS.

## 2. Materials and Methods

### 2.1. Collection, Isolation, and Morphological Identification of *Alternaria* Species

Infected potato leaves with brown spots were collected from different governorates in Egypt, as indicated in Table 1. All fungal isolates were obtained by cutting a portion of the infected leaf tissue, putting it on potato dextrose agar (PDA), and incubating for 3–4 days at 28 ± 2°C until the fungal mycelium appeared [17]. The pure hyphae were then picked and transferred onto a new PDA plate. The examined *Alternaria* isolates were identified by their morphological traits, including conidial length and width (µm), and the L/W ratio was determined using an ocular and stage micrometer or, later, by molecular methods [18].

**Table 1.** Screening of mycotoxins produced by *Alternaria* isolates isolated from infected potato plants from different areas.

Collection Sites	Geographical Data	Fungal Code	Mycotoxin Detection	
			Tenuazonic Acid (TeA) ng/g	Alternariol Monomethyl Ether (AME) ng/g
Beban, Kom Hamada, El Beheira Governorate	30°46'00.4" N 30°39'46.8" E	KH3	133,200 a	138,320 c
Demeito, Kom Hamada, El Beheira Governorate	30°47'19.5" N 30°44'07.0" E	KH1	22,560 b	94,340 g
An Nubariyah, Gharb El Noubareya, El Beheira Governorate	30°40'13.5" N 30°02'53.0" E	NO1	24,000 b	114,000 f
Al Natron Valley, El Beheira Governorate	30°33'12.0" N 30°18'04.9" E	W2	27,768 b	118,800 e

Quora Kheriguine, Gharb El Noubareya	30°42'36.7" N	NO3	49,270 ab	212,000 a
Quora Kheriguine, El Beheira Governorate	29°54'43.2" E			
Al Hamam Desert, Matrouh Governorate	30°44'56.2" N 29°36'50.6" E	Alam1	38,160 b	147,278 b
Al Natron Valley, El Beheira Governorate	30°34'40.6" N 29°58'43.1" E	W3	43,680 b	131,560 d
L.S.D.0.05			40.2411	68.04136

Data with similar letters are not significant.

## 2.2. ITS rDNA

DNA was extracted from the *Alternaria* isolates using the DNA isolation kit (EZ<sup>TM</sup> Genomic DNA Prep Kit, enzymomics, Korea). The quality and concentration of DNA was assessed by Nanodrop ND1000 spectrophotometer. ITS-specific primers, ITS1 (5'-TCCG-TAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'), were used to amplify the ITS region (500–600 bp in length) from the selected fungal isolates. The PCR, under standard conditions, were performed in 0.2 mL tubes containing a mixture of 10 µL Master mix (2x TOPsimple<sup>TM</sup> DyeMIX-*n*Taq, enzymomics, Korea), 1 µL template DNA (50ng/µL), an equal amount (1 µL) of the forward and reverse primers (5 pmol/µL), and sterile water up to 20 µL. The PCR cycle consisted of initial denaturation at 95 °C for 2 min, 35 cycles (30 s for each denaturation at 95 °C, annealing at 56 °C, and extension at 72 °C), and a final extension at 72 °C for 7 min. The PCR products were run in a 1% agarose gel stained with redsafe (iNtRon, Seoul, Korea) and visualized under ultraviolet light. The purified fragments were sequenced directly at Macrogen Inc., Seoul, Korea [19]. The assembled sequences were compared to those on the GenBank website (<http://www.ncbi.nlm.nih.gov/>) using the BLAST<sup>n</sup> tool. An ITS full-length alignment revealed relationships to published *Alternaria* sequences. The obtained *Alternaria* sequences were submitted to GenBank to generate accession numbers.

## 2.3. Mycotoxin Production and Extraction In vitro

Mycotoxin production was examined for each isolate using a modified Czapek-Dox medium (MCD), according to Brzonkalik et al. [20]. Plugs from fungal isolates were cultured in 100 mL of broth medium and incubated for a week at 28 ± 2 °C. After that, the fungal mats were removed, filtered, and further analyzed. Mycotoxins were extracted from the filtered medium by decreasing the acidity to pH 2 with 6 N hydrochloric acid, then 10 mL of acidified medium were mixed with 20 mL of CH<sub>2</sub>Cl<sub>2</sub> in a separating funnel shaker for 1 min, repeated three times, the CH<sub>2</sub>Cl<sub>2</sub> phase was collected in a flask and let to evaporate in a rotary evaporator at 35 °C. The dried material was dissolved in 1 mL of 1:1 water-methanol before the HPLC analysis [21].

## 2.4. Pathogenicity Test

The pathogenicity test of the seven isolates was performed on the potato cultivar “Diamond”. Potato tubers were bleached in NaOCl, washed with sterilized water, air-dried, and then cultivated separately in 25 × 25 pots and grown at 28 ± 3 °C and relative humidity of 40–60%. The optimal inoculum concentration was approximately calculated (2 × 10<sup>6</sup> spores/mL) and sprinkled on the potato leaves. Plants were inspected for infection, and the disease severity was evaluated one week after the inoculation. Areas of black necrotic lesions on the leaves (10–100%) were recorded and compared to the control. The disease severity was assessed as slightly pathogenic (10–30%), moderately pathogenic (30–60%), or highly pathogenic (60–100%).

## 2.5. Preparation of Beetroot Extracts

The beetroots (*Beta vulgaris*, L.) from the “Early flat-round” variety were purchased from a local market. The taproots were washed, cut, frozen at −80°C, freeze-dried, and

milled into a fine powder using a laboratory blender. The dried powder (60 g) was extracted with 200 mL of 95% methanol or ethanol for 48 h at 25 °C in the dark. The extracts were filtered, concentrated using DAIHAN—Premium Rotary Evaporator (RVE-05, Singapore) at 45 °C, lyophilized, and kept at −20 °C until use. The beetroot methanol extract (BME) and beetroot ethanol extract (BEE) were dissolved in dimethyl sulfoxide (DMSO) at 100 mg/mL and kept at −20 °C [22].

#### 2.6. Total Phenolic Content Determination

Total phenolic content (TPC) was determined by the Folin–Ciocalteu method using 10 mg of crude extract dissolved in 10 mL of the solvent. A volume of 100 µL was then mixed with 750 µL of the Folin–Ciocalteu reagent (1:9 parts of deionized water) and left standing for 10 min at 25 °C. Then, 750 µL of 10% Na<sub>2</sub>CO<sub>3</sub> was added to the mixture and it was left in a dark place for 60 min. Its absorbance was measured by a spectrophotometer SP-300 (OPTIMA Inc., Tokyo, Japan) at 725 nm. Gallic acid was used to make the calibration curve (ranging from 0.01 to 0.05 mg/mL) as a standard reference. TPC was measured as gallic acid equivalents (GAE mg/g of the extract).

#### 2.7. Effect of Beetroot Extracts on TeA and AME Production

To evaluate the TeA and AME production by *Alternaria* spp. in the presence of BME or BEE, 50 µL of an *Alternaria* inoculum (10<sup>3</sup> conidia/mL) was inoculated in 50 mL of MCD medium supplemented with: (1) DMSO (control); (2) 75, 150, and 300 µg/mL BME; and (3) 75, 150, and 300 µg/mL BEE. The inoculated flasks were incubated at 28 °C with agitation at 200 rpm for up to a week. The mycotoxin extraction procedure was performed as mentioned before, in Section 2.3.

#### 2.8. HPLC Analysis

Chromatographic detection and quantification of TeA and AME was performed using an HPLC system (Agilent 1260, Waldbronn, Germany), quaternary pump, auto sampler, and diode array detector. The column used for separation was a Phenomenex C18 column (100 × 4.6 mm) with 2.6 µm particle size. Mobile phases consisted of 0.02% formic acid–water (solvent A) and methanol (solvent B). Samples were injected at a volume of 10 µL and eluted with a binary gradient starting at 40% mobile phase A and reaching 100% B in 12 min with a flow rate of 0.6 mL/min. Presence and quantity of *Alternaria* mycotoxins were determined at the wavelengths of 256 nm (AME) and 278.7 nm (TeA). Standards of TeA and AME were prepared by serial dilutions of stock solutions with methanol.

#### 2.9. GC-MS Analysis of BME and BEE

The BME and BEE were analyzed for their components using a Trace GC Ultra-ISQ mass spectrometer (Thermo Scientific, TX, USA) with a direct capillary column TG-5MS (30 m × 0.25 mm × 0.25 µm film thickness). Program conditions and sampling were performed as published previously [23]. The constituents were identified based on the Wiley and NIST MS library databases and the comparison between mass spectra literature data and their retention times [24,25].

#### 2.10. Statistics Analysis

The experiments were performed in triplicate in a completely randomized design. Data were analyzed by one-way ANOVA and the results were compared by the least significant difference (LSD) according to Duncan's Multiple Range test using Costat statistical package (CoHort Software, CA, USA) [26].

### 3. Results and Discussion

#### 3.1. Morphological Identification

Seven isolates of *Alternaria* spp., selected based on their high capability to produce mycotoxins, were collected from different locations in Egypt. The initial morphological identification based on conidial length or width, as mentioned in the literature [18], revealed that the isolates were *A. alternata*. However, the isolates showed different cultural and morphological characteristics, which could have been due to the environment and the area temperature profile in the temperate regions of Egypt where the isolates were collected from [27,28]. The isolates exhibited significant diversity in phenotypic characteristics and mycotoxin productions (Tables 1 and 2).

**Table 2.** Morphological characteristics of *Alternaria alternata* isolates, their pathogenicity and GenBank accession numbers.

Isolate	Conidial Length ( $\mu\text{m}$ )	Conidial Width ( $\mu\text{m}$ )	L/W Ratio	Pathogenicity	Accession Number
KH1	27.32	5.30	5.15	High	MN592776
KH3	34.45	4.68	7.36	Moderate	MN592775
NO1	30.58	5.34	5.73	Moderate	MN592772
NO3	35.48	6.10	5.82	High	MN592771
W2	29.6	7.20	4.11	Low	MN592773
W3	32.77	6.89	4.76	High	MN592774
Alam1	26.75	4.83	5.54	Low	MN592777

#### 3.2. ITS Sequence Analysis

ITS rDNA sequencing was used as a valuable marker for fungal species identification and classification to confirm the morphological characteristics [29]. The ITS-specific primers amplified one fragment (500–600 bp long) from the DNA of all examined isolates. The purified amplicons were sequenced. The obtained nucleotide sequences were aligned using the BLAST tool at NCBI site and revealed that all the isolates were *A. alternata*, with homology ranging from 99% to 100%. The annotated sequences were accessioned with numbers MN592771–MN592777 (Table 1).

#### 3.3. Mycotoxin Production Ability

The obtained isolates were tested to investigate their abilities to produce mycotoxins. Most of the tested isolates could produce two mycotoxins, as illustrated in Table 1. Our results were in agreement with the findings of Meena et al. [30], who reported that *Alternaria* spp. could produce several toxins (TeA, AOH, and AME). Our results showed that *Alternaria* spp. isolates could produce two mycotoxins: alternariol monomethyl ether (AME) and tenuazonic acid (TeA). The isolates KH3, NO3, and Alam1 showed the highest production of the two mycotoxins [3]. All isolates were kept at 4 °C for further tests.

#### 3.4. Pathogenicity Test

All isolates were tested for their pathogenicity on the “Diamond” potato cultivar. A highly pathogenic isolate was defined as the one that caused severe disease symptoms on the potato leaves. We recorded a variation in the disease incidence in case of all the isolates. They were designated into three categories—(i) highly pathogenic, (ii) moderately pathogenic, and (iii) slightly pathogenic—based on the symptom incidence (Table 2).

Our results (Tables 1 and 2) showed that the pathogenicity was depended on the plant susceptibility to the fungus isolate and on the type and concentration of the mycotoxin. Our findings also demonstrated that the produced mycotoxins were related to fungal pathogenicity, indicating that these mycotoxins were phytotoxic. These results were in accordance with those of other authors [3,21,22], who reported that AME, TeA, and AOH produced by *Alternaria* species were considered phytotoxins that played a crucial role in plant pathogenesis.

### 3.5. Total Phenolic Content of Beetroot

The total phenolic content (TPC) was determined in BME and BEE. Our results showed that the TPC of BME (2.52 mg GAE/g) was approximately 2-fold of the TPC of BEE (1.34 mg GAE/g). Our findings were in accordance with previous studies [15,31,32], which reported that the methanol extract had higher concentration than the beetroot ethanol extract. The TPC of *Beta vulgaris* roots was 20-fold of the TPC of the other parts.

### 3.6. Effect of Beetroot Extracts on *Alternaria* Mycotoxins Production

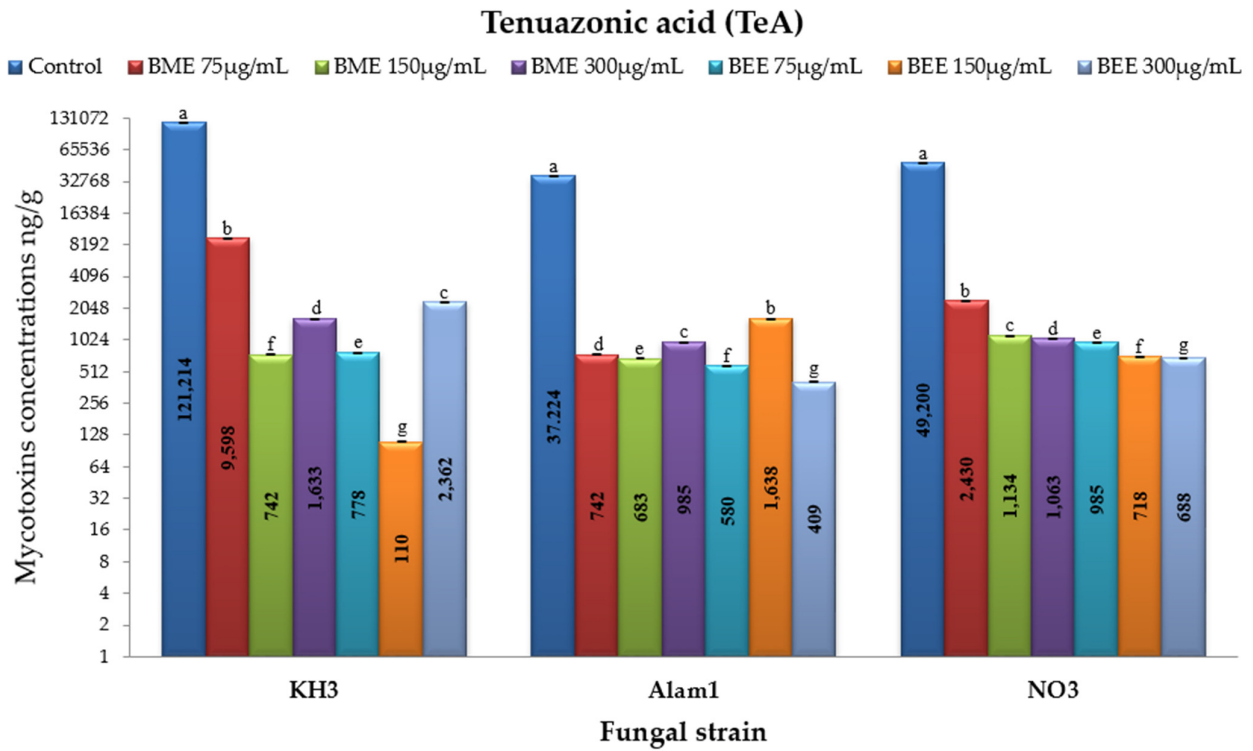
The KH3, NO3, and Alam1 isolates were used in this experiment according to their observed pathogenicity and their different abilities to produce mycotoxins (Table 1). The results showed that BEE was more effective in reducing the TeA and AME mycotoxins (Figures 1 and 2) than the methanol extract. That might have been due to the presence of the antioxidants betalains, which are more stable in ethanol extracts than in methanol extracts. To our knowledge, no data are available in the literature about the effect of the two extracts on *A. alternata* mycotoxin production. However, several studies have examined the antifungal activity of different extracts on the *Alternaria* spp. [33–39].

The ability of plant extracts to reduce fungal growth did not mean that they could stop or decrease the mycotoxin synthesis. Consequently, if the fungus can still grow in the presence of a plant extract, the toxin biosynthesis may be accelerated. Inhibitory actions on growing fungi are also not always correlated with declines in mycotoxin concentrations [34]. While *Alternaria* is a common food product contaminant, its toxins are little documented, possibly because a control of its presence in food is unavailable. This issue is particularly important in the context of potato products. In developing countries, fruits with high fungal contamination are frequently used for processed food products, resulting in higher mycotoxin accumulation.

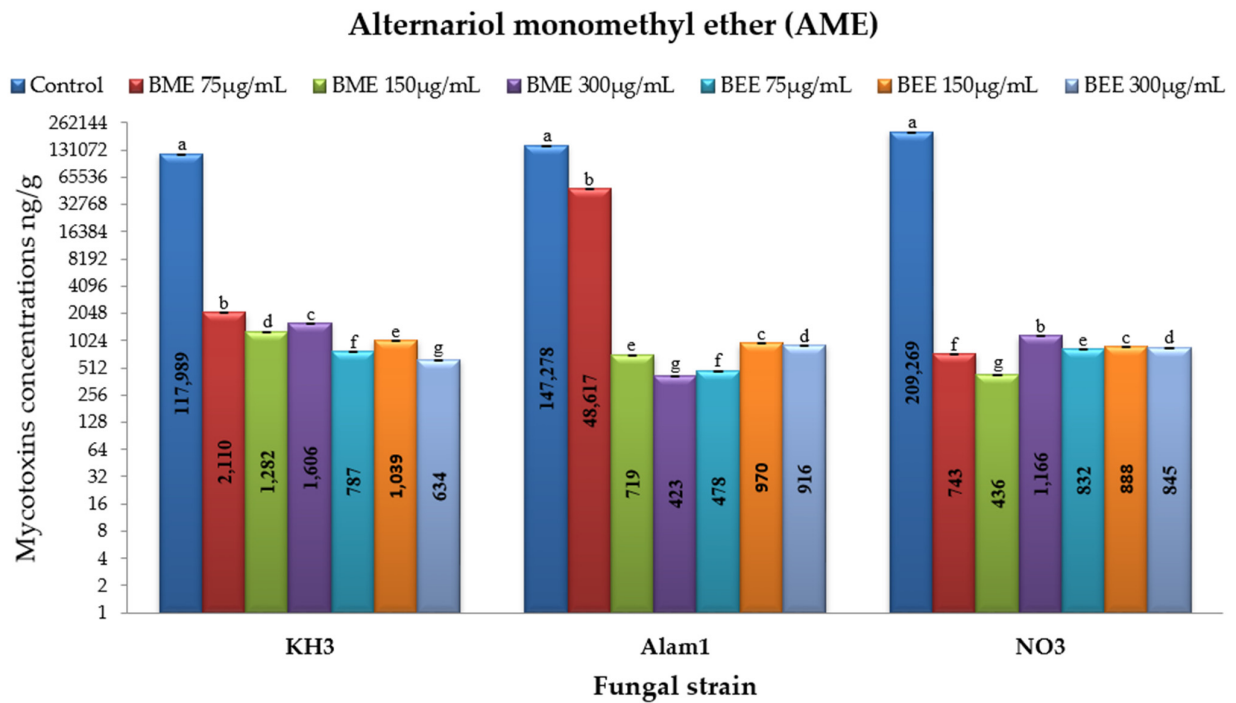
The amount of synthesized mycotoxin varied with the toxin, the extract concentration, and the isolate studied. BEE and BME showed high inhibition of production of these metabolites by *A. alternata* at most doses. Consequently, these fractions could have promising applications in treatments of foods where the two mycotoxins can simultaneously accumulate. It is worth mentioning that certain extracts increased the synthesis of some of these metabolites in comparison with corresponding control values [40]. Moreover, some of them resulted in an inhibition at a particular concentration but enhanced toxin production at another. BEE at a 150 µg/mL concentration was the most effective in reducing TeA produced by the KH3 isolate.

In comparison, BEE at 300 µg/mL was very promising in reducing TeA produced by Alam1 and NO3. BME decreased the mycotoxin production by 66.99% to 99.79%. The highest TeA reduction rate (99.39%) was observed in the KH3 isolate with 150 µg/mL BME treatment, while the most significant AME reduction rate (99.79%) was shown in the NO3 isolate with 150 µg/mL BME treatment.

In the same way, BEE ranged from 95.60% to 99.91% mycotoxin reduction. The highest TeA reduction rate (99.91%) was reported in the KH3 isolate with 150 µg/mL BME treatment. In comparison, the most surprising AME reduction rate (99.68%) was shown in Alam1 isolate with 75 µg/mL BEE treatment. Our results were in accordance with the findings of Youssef [41]. It is interesting to remark that in different isolates of *A. alternata*, the TeA and AME productions were affected differently. When the BEE concentration was increased, at least the same reduction—or, in some cases, a lower one—was observed in the mycotoxin accumulation, compared with the control (Figures 1 and 2).



**Figure 1.** Effects of beetroot extracts on tenuazonic acid mycotoxin production in three isolates of *Alternaria alternata*. Data with the same letters are not significantly different at  $p \leq 0.05$ . BEE = beetroot ethanol extract, BME = beetroot methanol extract.



**Figure 2.** Effects of beetroot extracts on alternariol monomethyl ether mycotoxin production in three isolates of *Alternaria alternata*. Data with the same letters are not significantly different at  $p \leq 0.05$ . BEE = beetroot ethanol extract, BME = beetroot methanol extract.

BEE controlled the higher amounts of TeA when the extract concentration was increased in the isolates Alam1 and NO3. Other authors have reported similar behavior regarding aflatoxigenic isolates. The decrease or rise in the amount of aflatoxin produced by several *Aspergillus* or *Alternaria* spp. depended on the extract concentration combined with environmental conditions [40,42–44]. For this reason, the effect of vegetal extracts on mycotoxin biosynthesis must be evaluated to determine the adequate dose for the development of natural antifungals. Both AME and TeA have been considered as phytotoxins that played a crucial role in plant pathogenesis, according to Meena and Samal [3]. It is vital to avoid suboptimal doses that could reduce the fungal activity but increase the mycotoxin accumulation.

The data showed that BEE and BME had anti-mycotoxigenic properties that were not dependent on the TPC. This was in accordance with the results of others [45], who reported that beetroot was among the tested plants which extracts had the best antifungal properties. However, its TPC and total flavonoid content are lower than those of *Hibiscus sabdariffa*. The two mycotoxins produced by the three tested isolates did not have the same sensitivity against the tested treatments. The tolerability of each mycotoxin depended on the fungus isolate that produced it. The data were in accordance with those of Youssef [41], who found that citrinin produced by *Penicillium citrinum* did not have the same sensitivity as the same mycotoxin produced by *Penicillium verrocosum*, and the ochratoxin A was more tolerant than citrinin against chitosan.

### 3.7. GC-MS Analysis of Beetroot Extracts

The GC-MS analysis of the methanolic beetroot extract led to identification of the 17 compounds presented in Figure 3. The active principles of beetroot (taproot) are various components whose compound names, molecular weights, peak areas (%), molecular formulas, and retention times are presented in Table 3. It was found that the most effective constituents of BME were 1-dodecanamine, n,n-dimethyl (43.75%); hexadecanoic acid, methyl ester (synonym: palmitic acid, methyl ester) (3.02%); 9-octadecenoic acid (Z)-, methyl ester (2.47%); 9-octadecenoic acid (Z)-(2.32%); 9,12-octadecadienoic acid (Z,Z)-, methyl ester (1.89%); n-hexadecanoic acid (1.74%); 9-octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester (0.90%); and octadecanoic acid, methyl ester (synonym: methyl stearate) (0.53%) (Table 3). In the ethanolic extract (BEE), 25 chemical constituents were identified (Table 4 and Figure 3), and the most abundant of them were oxirane, methyl- (23.22%); 9-octadecenoic acid (z)-(1.03%); ethanol,2-(9-octadecenyloxy)-, (Z)- (1.81%); cyclopentane acetic acid, 3-oxo-2-pentyl-, methyl ester (2.13%); patchouli alcohol (1.43%); propanal (6.23%); 2H-pyran-3-ol,tetrahydro-2,2,6-trimethyl-6-(4-methyl-3-cyclohexen-1-yl),[3S-[3à, 6à (R\*)]]- (5.09%); hexadecanoic acid, methyl ester, (10.72%), n-hexadecanoic acid (9-octadecenoic acid (z)-) (7.32%); 11,14-octadecadienoic acid, methyl ester (3.76%); 9-octadecenoic acid (Z)-, methyl ester (5.99%); octadecanoic acid, methyl ester (2.99%); 9,12-octadecadienoyl chloride, (Z,Z)-(linoleoyl chloride) (5.35%); hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (1.49%); hexadecanoic acid, 2,3-dihydroxypropyl ester (1.14%); n-methyl-n-benzyltetradecanamine (2.05%); and 9-octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester (2.74%).



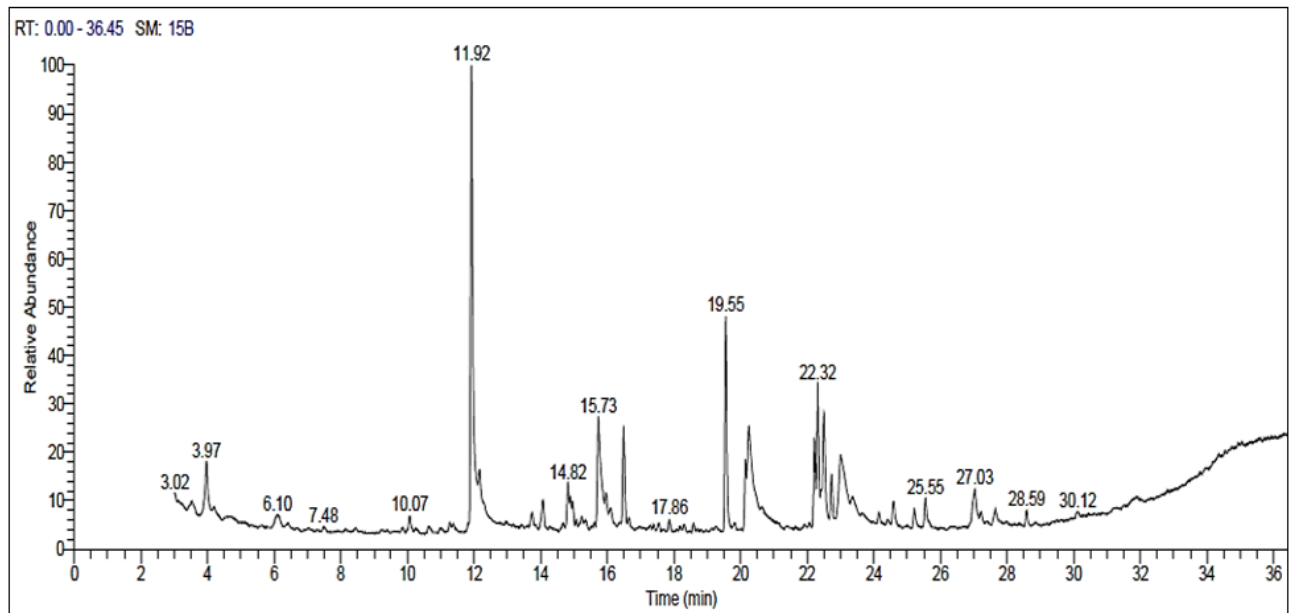
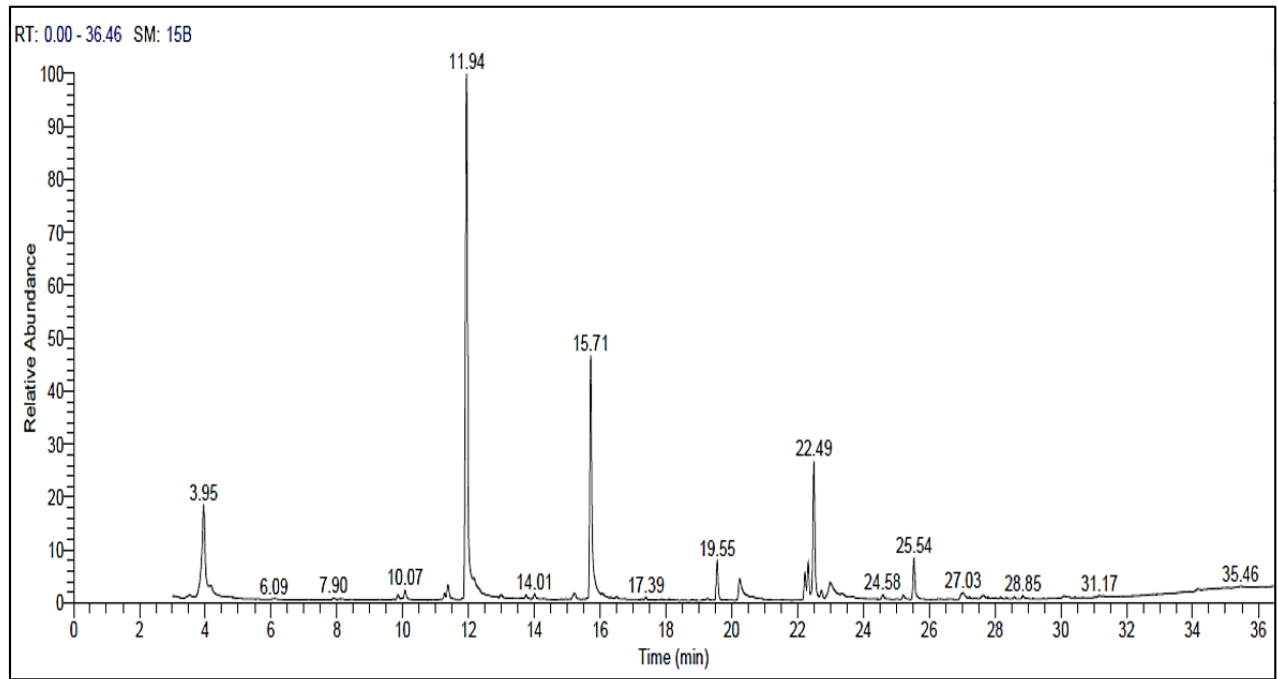
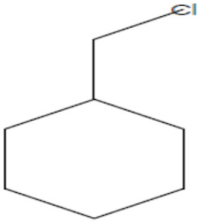
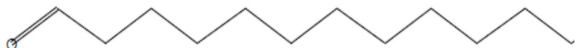


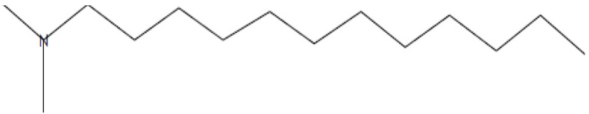


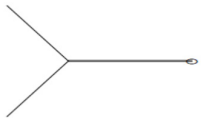
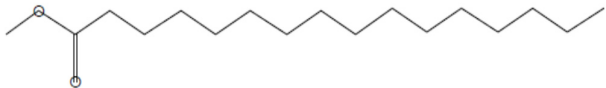
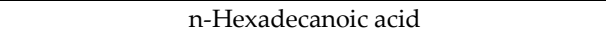
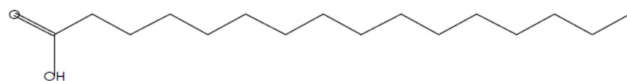


Figure 3. GC-MS chromatograms of beetroot methanolic (up) and ethanolic (bottom) extracts.

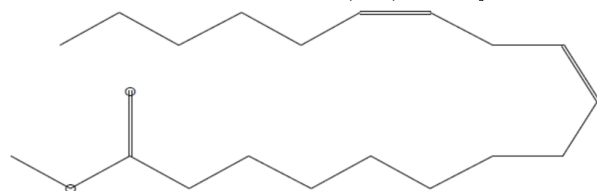
**Table 3.** Compounds identified in the methanolic beetroot extract by GC-MS.

Retention Time	Compound Name	Area (%)	Molecular Formula	Molecular Weight
3.95	Benzyl chloride 	7.49	C <sub>7</sub> H <sub>7</sub> Cl	126
10.07	Dodecanal (Tetradecanal) 	0.71	C <sub>12</sub> H <sub>24</sub> O	184
11.27	1-Chloroundecane 	0.42	C <sub>11</sub> H <sub>23</sub> Cl	190
11.37	1-Dodecanol 	1.17	C <sub>12</sub> H <sub>26</sub> O	186
11.95	1-Dodecanamine, n,n-dimethyl 	43.75	C <sub>14</sub> H <sub>31</sub> N	213
14.00	Tetradecanal 	0.49	C <sub>14</sub> H <sub>28</sub> O	212
15.22	1-Hexadecanol 	0.67	C <sub>16</sub> H <sub>34</sub> O	242
15.71	2-Propanone 	18.50	C <sub>3</sub> H <sub>6</sub> O	58
19.55	Hexadecanoic acid, methyl ester (Palmitic acid, methyl ester) 	3.02	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270
20.24	n-Hexadecanoic acid 	1.93	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256



9,12-Octadecadienoic acid (Z,Z)-, methyl ester

22.22



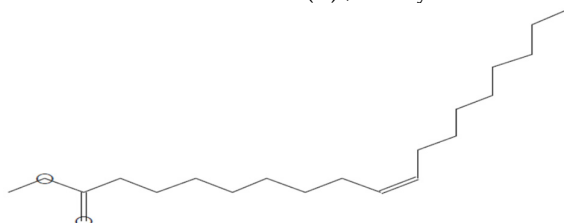
1.89

C<sub>19</sub>H<sub>34</sub>O<sub>2</sub>

294

9-Octadecenoic acid (Z)-, methyl ester

22.32



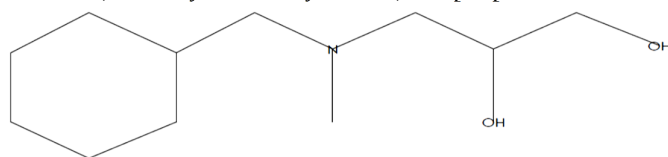
2.47

C<sub>19</sub>H<sub>36</sub>O<sub>2</sub>

296

3-(N-Benzyl-N-methylamino)-1,2-propanediol

22.49



10.52

C<sub>11</sub>H<sub>17</sub>NO<sub>2</sub>

195

Octadecanoic acid, methyl ester (Methyl stearate)

22.73



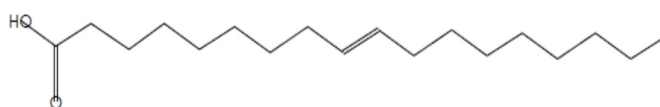
0.53

C<sub>19</sub>H<sub>38</sub>O<sub>2</sub>

298

9-Octadecenoic acid (z)-

22.99



2.32

C<sub>18</sub>H<sub>34</sub>O<sub>2</sub>

282

25.54

2-Methyleneborexane

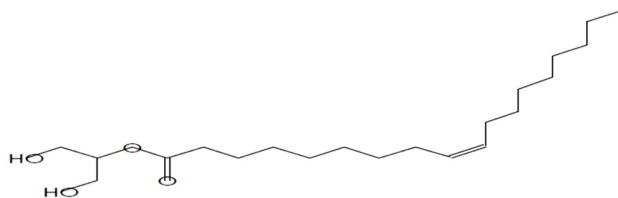
3.22

C<sub>10</sub>H<sub>14</sub>

134

9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester

27.03



0.90

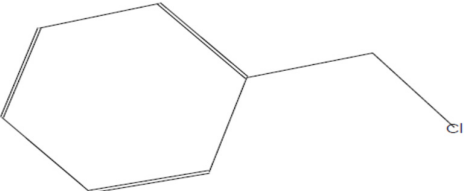
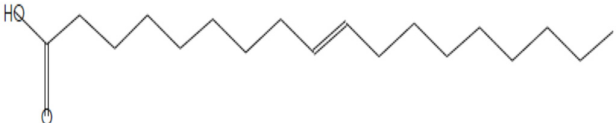
C<sub>21</sub>H<sub>40</sub>O<sub>4</sub>

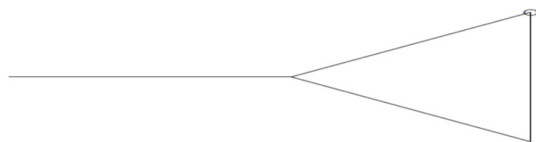
356

The composition we determined for the methanolic extract corresponded to 86.63% of the entire GC-MS chromatogram, while in case of BEE, it corresponded to 100% of the GC-MS chromatogram curves. Beetroot extracts were found to contain a variety of constituents that may be the cause of their therapeutic potential. 1-dodecanamine, *n,n*-dimethyl is an antioxidant which acts as a natural or synthetic food preservative against oxidative deterioration during storage and processing due to its high stability and low volatility. It is essential for animal and plant life since it is involved in complex metabolic and signaling mechanisms [46]. Antioxidants have essential roles in deactivation of mycotoxins and avoiding oxidative stress. Kaur and Kapoor [47] evaluated the antioxidant activities of 36 vegetable extracts, using a model system consisting of  $\beta$ -carotene and linoleic acid, and found that the ethanolic extract of beetroot had a high antioxidant activity (>70%). Gilby and Few [48] reported that 1-dodecanamine, *n,n*-dimethyl, used as a surfactant, could be more active in bacterial protoplast lysis than quaternary ammonium bromide and dodecyl sulfate. Hueck et al. [49] noticed that dodecanamine had antifungal and antibacterial effects on many gram-negative or positive bacteria. Consequently, we propose that dodecanamine and/or its derivatives may have antimycotoxigenic activity.

The GC-MS analysis of the crude extracts showed some significant compounds that contributed to the taproot antimicrobial activity. These included oleic acid; (*Z,Z*)-9,12-octadecadienoic acid; *n*-hexadecanoic acid, methyl ester; (*Z,Z*)-9,12-octadecadienoic acid; and (*E*)-9-octadecenoic acid, which are known to have antifungal potential [50,51]. Additionally, benzyl chloride, detected in BME, is used as a precursor of a wide range of quaternary ammonium derivatives, including benzalkonium chloride, which has a practical antimicrobial application [52]. Basaran [53] found that benzalkonium chloride could prevent the growth of *Aspergillus* spp., *Penicillium* spp., and *A. alternata* in inoculated fruits and prevented them from decaying in vitro. The secondary plant metabolites could absorb mycotoxin molecules to minimize their exposure and health side effects. There is a possibility of using the identified constituents as lead compounds in the development of new valuable drugs. The components of the extracts might have contributed to antimicrobial action of traditional medicines in treating various diseases [54–56]. There is an increased demand for using plant extracts and their compounds, which raises concerns about the safety, toxicity, and quality of these products [57–59]. Studies have reported mycotoxin contaminations and roles for medicinal properties of plant materials [60,61].

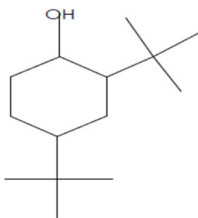
**Table 4.** Compounds identified in the ethanolic extract of beetroot in GC-MS.

Retention Time	Compound Name	Area (%)	Molecular Formula	Molecular Weight
3.96	Benzene, (chloromethyl)- 	3.01	C <sub>7</sub> H <sub>7</sub> Cl	126
10.07	9-Octadecenoic acid ( <i>z</i> )- 	1.03	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282
11.92	Oxirane, methyl-	23.22	C <sub>3</sub> H <sub>6</sub> O	85



2,4-Di-tert-butylphenol

12.17



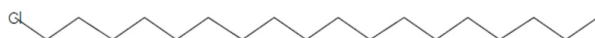
1.17

C<sub>14</sub>H<sub>22</sub>O

206

1-Chlorooctadecane

13.74



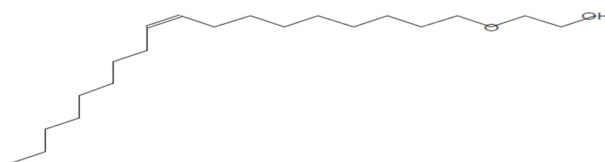
1.05

C<sub>18</sub>H<sub>37</sub>Cl

288

Ethanol,2-(9-octadecenyl)-,(Z)-

14.07



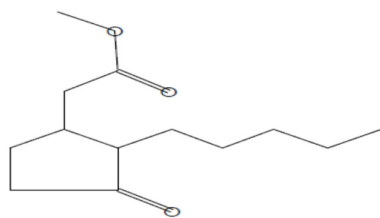
1.81

C<sub>20</sub>H<sub>40</sub>O<sub>2</sub>

312

Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester

14.82



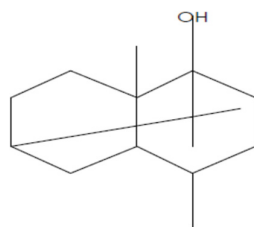
2.13

C<sub>13</sub>H<sub>22</sub>O<sub>3</sub>

226

Patchouli alcohol

14.89



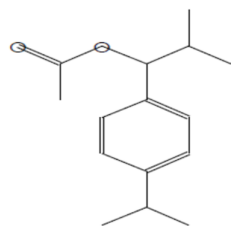
1.43

C<sub>15</sub>H<sub>26</sub>O

222

1-(4-isopropylphenyl)-2-methylpropyl acetate

14.97



1.09

C<sub>15</sub>H<sub>22</sub>O<sub>2</sub>

234

Propanal

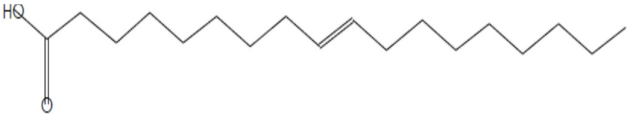
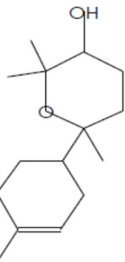
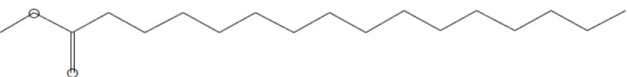
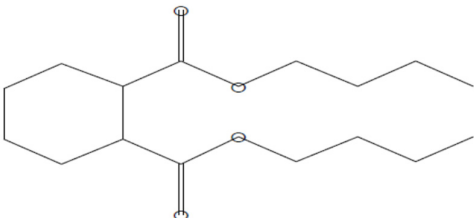
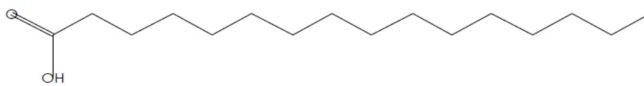
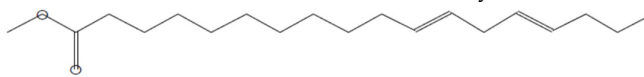
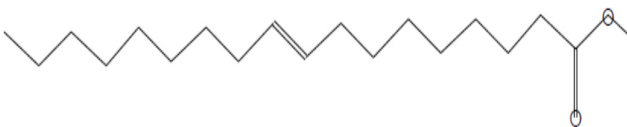
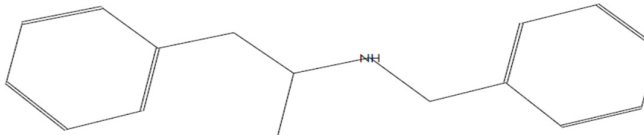
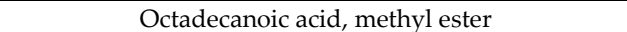
15.72



6.23

C<sub>3</sub>H<sub>6</sub>O

58

15.96	<p>9-Octadecenoic acid (z)-</p> 	0.66	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282
16.49	<p>2H-Pyran-3-ol, tetrahydro-2,2,6-trimethyl-6-(4-methyl-3-cyclohexen-1-yl), [3S-[3à,6à(R*)]]-</p> 	5.09	C <sub>15</sub> H <sub>26</sub> O <sub>2</sub>	238
19.55	<p>Hexadecanoic acid, methyl ester</p> 	10.72	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270
20.15	<p>Dibutyl phthalate</p> 	2.82	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	278
20.24	<p>n-Hexadecanoic acid (9-OCTADECENOIC ACID (Z)-)</p> 	7.32	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256
22.22	<p>11,14-Octadecadienoic acid, methyl ester</p> 	3.76	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294
22.32	<p>9-Octadecenoic acid (Z)-, methyl ester</p> 	5.99	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296
22.50	<p>Benzylamphetamine</p> 	5.61	C <sub>16</sub> H <sub>19</sub> N	225
22.73	<p>Octadecanoic acid, methyl ester</p> 	2.19	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	298

	9,12-Octadecadienoyl chloride, (Z,Z)-(Linoleoyl chloride)			
23.00		5.35	C <sub>18</sub> H <sub>31</sub> ClO	298
	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester			
24.58		1.49	C <sub>19</sub> H <sub>38</sub> O <sub>4</sub>	330
	Hexadecanoic acid, 2,3-dihydroxypropyl ester			
25.21		1.14	C <sub>19</sub> H <sub>38</sub> O <sub>4</sub>	330
	N-Methyl-N-benzyltetradecanamine			
25.55		2.05	C <sub>22</sub> H <sub>39</sub> N	317
	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester			
27.03		2.74	C <sub>21</sub> H <sub>40</sub> O <sub>4</sub>	356
	1,2-Benzenedicarboxylic acid			
28.58		0.93	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	390
	1,2-Benzenedicarboxylic acid			

Most of plant extracts constituents have been found to show interesting biological activities: against specific pathogens, antioxidant, hypocholesterolemic [62], and antibacterial [63]. Several activities have been reported for *n*-hexadecanoic acid, suggesting the

rationale for the traditional use of *Albizia adianthifolia* and *Pterocarpus angolensis* extracts. Moreover, *n*-hexadecanoic acid isolated from heartwood and stem bark of *A. adianthifolia* (*n*-hexane extract) was reported as anti-inflammatory [64], an antioxidant, hypocholesterolemic, a nematocide, a pesticide, anti-androgenic, hemolytic, a 5- $\alpha$  reductase inhibitor [62], and a potent mosquito larvicide [63]. Oleic acid could have antibacterial activity [65]. A chloroform extract of *A. adianthifolia* contains 9,12-octadecadienoic acid (*Z,Z*-), methyl ester and exhibited an anti-cancer activity [66]. Li et al. [67] indicated that volatile compounds, such as tetradecanal, dodecanol, and 2,4-dimethyl-6-tert-butylphenol, individually exhibited a strong antifungal effect against the fungal isolates of *Verticillium dahliae* and *Fusarium oxysporum*, although those compounds were found in low amounts in BEE and BME by GC-MS. Tetradecanoic acid showed larvicidal and repellent activities [68]. 2-Propanone, also called acetone or dimethyl ketone, seemed to be an effective compound in our study. Similarly, many authors previously found, by GC-MS, that the active compounds included alcohols, esters, ketones, and acids and they inhibited phytopathogenic fungi [69]. The fatty acid methyl ester could play a role in the decomposition of mycotoxins, based on the antibacterial and antifungal activity. Hexadecanoic acid, methyl ester and octadecanoic acid proved to be antimicrobial molecules [63,70]. Finally, there is a need for further studies on the most abundant components found by the GC-MS analysis to evaluate their antimycotoxigenic activities individually.

#### 4. Conclusions

The mycotoxins TeA and AME, produced by *A. alternata* isolates, did not have the same sensitivity to BEE or BME. The tolerability of each mycotoxin depended on the producing fungus isolate. BEE at 150  $\mu\text{g/mL}$  could be recommended for inhibiting TeA. BEE and BME mycotoxin reduction percentages were 95.60%–99.91% and 66.99%–99.79%, respectively. The GC-MS spectra of the ethanol and methanol extracts showed different constituents; fatty acid methyl esters were the most abundant compounds, which suggested more effective decomposition of the *Alternaria* toxins.

**Author Contributions:** Conceptualization, N.H.Y. and S.I.B.; methodology, N.H.Y.; software, A.A.; validation, A.A., A.A.H. and E.S.D.; formal analysis, A.A.H. and A.K.; resources, N.H.Y.; data curation, S.I.B.; writing—original draft preparation, N.H.Y., M.M.E., and S.I.B.; writing—review and editing, V.M., A.A., S.H.Q. and S.I.B.; visualization, A.A.H.; supervision, S.I.B.; project administration, E.S.D. and E.I.E.-H.; funding acquisition, E.S.D. and E.I.E.-H. All authors have read and agreed to the published version of the manuscript.

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