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# Environmental Aspergillus fumigatus Derived Antibiotics: Structure and Efficacy against Enteric Bacteria

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**KEYWORDS**

Bioactive, Strain, Salmonella, Typhi, Infections

**ABSTRACT:**

Antibiotic resistance poses significant clinical and public health challenges, leading to treatment failures, prolonged hospitalizations, and increased mortality. Enteric bacterial diseases are a major concern. The need for novel antibiotics from natural sources is pressing. This study explores the potential of antibiotics extracted from Aspergillus fumigatus mycelia against enteric bacterial pathogens. One hundred soil samples from hospital dumping sites were screened, yielding three Aspergillus fumigatus strains: DTO402 (AFO402), F7 (AFF7), and KMM4631 (AFK4631). The fungal isolates were grown in submerged culture, and their antibiotic production was evaluated. Structural elucidation using GC-MS revealed various bioactive compounds in each strain. AFK4631 produced oleic acid, hexacosanoic acid, erucic acid, and 10-methyl-E-11-tridece-1-ol-acetate, which showed significant ($p \leq 0.05$) inhibitory activity against Salmonella enterica subspecies enterica serovar Typhi strains. Similar compounds were identified in AFD402 and AFF7, including oleic acid and hexacosanoic acid. The study demonstrates the potential of these Aspergillus fumigatus strains as sources of alternative antibiotics for treating enteric bacterial infections. The findings suggest that these natural compounds could provide a promising solution to the growing problem of antibiotic resistance. Further research is warranted to explore the therapeutic applications of these bioactive compounds.

## INTRODUCTION

*Salmonella* Typhi is the etiological agent of enteric fever also known as typhoid fever, while *Salmonella* serovar. Paratyphi A, B and C causes paratyphoid fever [1]. Since the clinical symptoms of paratyphoid fever are indistinguishable from typhoid fever, the term 'enteric fever' is used collectively for both fevers, and both *Salmonella* serovar Typhi and *Salmonella s*erovar Paratyphi are referred as typhoid *Salmonella* [2]. For the two strains of typhoid *Salmonella,* humans are known to be the sole reservoir. The micro-organisms are passed through the eating of infected food or water with the waste of infected individuals. Prodromal symptoms of Enteric fever, such as headache, abdominal pain and diarrhea (or constipation) is characterized by an incubation period of one week or more, followed by the onset of fever [2]. Diarrhea is more normally observed in

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children, whereas patients with immunosuppression are more possible to display constipation [2].

The appearance of antimicrobial resistance in *Salmonella* strains is a severe health condition world [2]. In early 1960s, the first incidence of *Salmonella* resistance to a single antibiotic namely chloramphemcol, was reported [2]. Since then, the occurrence of separation of *Salmonella* strains with resistance towards one or more antimicrobial agents has improved in many countries, including the USA, the UK and Sandi Arabia [2]. Antimicrobial agents such as amplicillin, chlorampherical and trimethoprimsulfamethoxazole are used as the traditional first line treatments for *Salmonella* infection [1]. *Salmonella* spp resistant towards these agents are referred to as multi-drug resistant (MDR), with the emergence of resistance towards traditional antibiotics. Fluoroquinolones and extended-spectrum cephalosporins have been introduce as the antimicrobial agents of chance in treating MDR *Salmonella* serovar Typhi

From the ancient period, humankind had always been faced with a problem of spreading of bacterial infections [3]. The kingdom fungi is well-known as a valuable source of diverse bioactive compounds of therapeutic importance since the discovery of penicillin in 1928 by Alexander Fleming from *Penicillium notatum*. The treatment of bacterial infections is increasingly complicated by the ability of bacteria to develop resistance to antibacterial agents. Therefore, an urgent need to exploit new classes of antibacterial agents with a novel mechanism of action is required. The increasing need for new antibacterial agents able to control emerging diseases or resistant strains of bacteria inspired a number of research groups to explore diverse sources of producing potent alternative antibiotics [3].

Filamentous fungi represent an important group of microorganisms known to synthesize a huge diversity of bioactive molecules that are known as secondary metabolites or natural antibacterial agents (antibiotics). Secondary metabolites are biologically active organic compounds that are not required for normal cell growth and metabolism but enable the organism to minimize competition. Secondary metabolites are used as medicines, flavourings, pigments, and recreational drugs [1].

Research had revealed that a mold known as *Aspergillus fumigatus* has an ability to produce secondary metabolites otherwise known as antibiotics or antibacterial agents which exert a bactericidal effect on human pathogens such as sorbitol negative *Escherichia coli* that infects the gastrointestinal tract. The genus *Aspergillus* are highly ubiquitous saprophytic molds that thrive in diverse environment. Certain *Aspergillus* such as *Aspergillus fumigatus* are also pathogenic and infect human lungs especially the immune-compromised individuals.

It had been reported that about 10,000-15,000 liters of air that a typical person inhales each day are estimated to contain a few hundred *A. fumigatus* conidia. The conidia are 2-3 µm in diameter and can therefore penetrate deep into the lungs, reaching the alveoli. *A. fumigatus* is also heat tolerant, resistant to oxidative stress, has a high growth rate, and can survive on various nutrients [4].

Several studies have shown that the production of inhibitory substances/antibiotics from *Aspergillus fumigatus* against different bacterial isolates but little or no study has been done on inhibitory substances/antibiotics produced *Aspergillus fumigatus* from hospital dumping sites, and its activities against enteric bacteria majorly *Salmonella enterica* subspecies *enterica* serovar Typhi, which is the causative agent of enteric fever

## MATERIALS AND METHODS

**Collection of samples:** A total of 300 soil samples from hospital waste dumping site were randomly collected from different sites in Ihiala L.G.A, Anambra State. This was carried out using the method described in the study published by [5], [6] and [7]. The litter from the soil surfaces was carefully scrapped out using sterile stainless spoon. The soil auger was derived to a plough depth of 15 cm in the farm land, and soil sample was drawn up to 10 samples from each sampling unit into a sterile tray. The samples were thorough mixed and foreign materials such as roots, stones, pebbles and gravels were carefully removed. The soil sample was then reduced to half by quartering the sample. Quartering was carried out by dividing the soil sample into four equal parts and the two opposite quarters were discarded and the remaining two quarters were mixed. The process was repeated for the rest of soil samples used for this study. The samples were

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carefully labeled and then kept in a disinfected cooler, to maintain its temperature and stability of the number of the isolates. The samples were transported to the laboratory for analysis.

**Isolation of the Fungal Isolates:** The media used for this isolation was Sabouraud dextrose agar (SDA/BIOTECH). One gram of the soil sample was weighed into boiling test tube; 5 mL of normal saline was added and shake thoroughly and then make up to 10 mL using the normal saline ($10^{-1}$dilution). One milliliter of the suspension was added to four milliliter (4 mL) of normal saline (0.85% NaCl), which was give $5^{-1}$ dilution. From $5^{-1}$ dilution test tube, a five-fold serial dilution was carried out to obtain $5^{-5}$ dilution. One milliliter aliquot from $10^{-1}$, $5^{-1}$ and $5^{-5}$ test tubes were collected and aseptically plated onto solidified sabouraud dextrose agar plate (90 mm x 15 mm) which was prepared according to the manufacturers instruction and the procedures described in [8] supplemented with chloramphenicol (0.05 %) and spread using a spreading rod. The SDA was incubated in an inverted position for 5-7 days at 30±2$^0$C.

**Identification of Fungal Isolates:** The fungal isolates were identified to the genus/species level based on macroscopic, microscopic and molecular characteristics of the isolates obtained from pure cultures as described in the study published by [9], [10] and [11].

**Isolation and Characterization of Enteric Bacterial Organisms**

**Culture and Isolation of Enteric Bacteria:** This was carried out using the modified method of [8].The samples used for this study were drawn from the rivers and boreholes. The river water samples were also diluted to 1:10. One milliliter of the prepared soil sample, river sample and borehole sample were plated on Petri dishes (60 mm OD × 55 mm ID × 13mm high) containing Deoxycholate citrate agar medium (DCA/Biotech). All the plates in triplicates were incubated inverted at 37±2˚C for 24-48 h [8].

**Characterization and Identification of the Bacterial Isolates**

The isolates were sub cultured on nutrient agar (Biotech), incubated in inverted position at 37˚C for 24 h. The isolates were characterized and identified using their colonial and morphological descriptions [8], biochemical reactions [8] and molecular characterization [12,13,14,15,16]. The colonial description was carried out to determine the colours of the isolates on agar media plates, their sizes, edges, consistencies and optical properties of the isolates

**Screening the fungal isolates for antibiotic production:** For antibiotic production, Mueller Hinton Agar (MHA) medium was prepared according to the manufacturer's direction. This was allowed to cool and then poured in Petri dishes and kept in incubator at 37$^0$C for 24 h to check its sterility. Then the test organisms; *Salmonella species* were grown on broth culture at 37$^0$C for 24 h. After incubation, sterilized swab stick was dipped into the broth cultures and swabbed on MHA plates and allowed for 1 h. Then wells were made on the MHA plates using sterile cork-borer. Then the broth culture of the fungal isolates was carefully centrifuged at 6000 rpm for 10 minutes and their supernatants were poured in the wells and incubated at 37$^0$C for 48 h. zones of inhibition was observed after incubation [17].

**Extraction of Antibiotics:** The characterized fungal isolates were grown in a Brain heart Infusion broth: 10g/L, peptone 5g/L, dextrose 5g/L, NaCl 5g/L, Na2HPO4, 2.5g/L, (NH4)2SO4 1g/L, CaCl2 0.02g/L, KH2PO4, 15g/L, yeast extract 5g/L, starch 1g/L, cysteine HCl and 1g/L, MgSO4 0.2g/L. This was incubated at room temperature (30±2°C) for 7 days with intermittent manual shaking [17].

**Extraction and Elution of Antibiotic**: The culture medium was centrifuged at 8000 rpm for 15 min. This was filtered using Whatman No 1 filter paper (110 mm × 110 mm). The supernatant was eluted using column chromatographic technique using ethyl acetate/hexane/methanol/dichloromethane at ration of 2:2:1:1 [17].

**Purification and Elucidation of the Antibiotic**: The eluate that inhibited the growth of the tested bacteria were subjected to Thin Layer Chromatographic technique using chloroform/methanol (24:1 v/v), chloroform/methanol/water (1:1:1 v/v/v), benzene/acetic acid/water (4:1:5 v/v/v) and acetonitrile/water (92.5/7.5 v/v). The successive bands seen on the plates were crapped off carefully, dissolved in methanol, and centrifuged at 10,000 rpm for 10 min to remove the

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silica. The supernatant was subjected to structural elucidation using gas chromatography coupled with mass-spectrophotometer [18].

***In vitro* Antibacterial Activities of the Eluate using Agar Well Diffusion Method:** This was carried out by the modified method of [14], [19] and [20]. Each labeled plate was uniformly inoculated with the test organism (*Salmonella* species) using spread plate method. A sterile cork-borer of 5 mm diameter was used to make the wells on the medium. One tenth milliliter of the eluate was dropped into each labeled wells and then incubated at 35±2ºC for 24 h. Antibacterial activity was determined by measuring the diameter of the zones of inhibition (mm) produced after incubation.

**Data Analysis:** The data obtained in this study were presented in Tables and figures. Their percentages were also calculated. Significance of the study was carried out using one way Analysis of Variance (ANOVA) at 95% confidence level. Pair wise comparison was carried out using student "t" test [14,9,21,22,23,24].

**RESULTS**

The macroscopic and microscopic characterization of the fungal isolates are presented in Tables 1 and 2. The results showed that the fungal isolates initially appeared white on SDA within 2-3 days while gray-green with white edges was observed later within 5 days. The reverse colour of the isolates was pale and light yellow, and the growth rate was also rapid. The texture of the colony appeared cottony and woolly while the colour of the mycelium appeared gray-green. Similarly, the microscopic features of the fungal isolates showed septate hyphae and gray-green conidia. The shape of the conidia appeared ellipsoidal and the vesicle showed globose appearance. The molecular characteristics of the fungal isolates are presented in Table 3. The features showed that the fungus was *Aspergillus fumi01gatus* of different strains as showed in Table 3

The test bacterial isolates (code D, X and Z) exhibited similar appearances on Deoxycholate citrate agar (DCA) and Nutrient Agar (NA) plates as they showed colourless and dark centeredand greyish white colours respectively (Table 4). They exhibited circular and entire colonies on DCA and NA with smooth surfaces. They varied in colony sizes and elevations. Isolate D showed low convex elevation whereas isolates X and Z showed convex elevation. The isolates were Gram negative rods, arranged singly/pairs with peritichous flagella. They were non-spore formers and non-capsulated.

The bacterial test isolates were catalase, positive, oxidase, citrate, indole and urease as shown in Table 5. The isolates were able to provide hydrogen sulphide ($H_2S$) in triple sugar iron (TSI) agar. The isolates were methyl red positive as they were able to utilize the glucose in the medium to produce stable acid. The isolate was able to convert acetyl methyl carbinol produced from fermentation of glucose into diacetyl in the presence of α – naphthol, strong alkali (40% KOH) and atmospheric oxygen (VP Positive). The isolates reduced nitrates to nitrite, decarboxylated lysine but were not able to produce gelatinase (gelatine negative). The bacterial isolates utilized glucose and maltose, and were not able to utilize lactose, sucrose, arabitol and malonate as shown in Table 6. Isolate X and Z showed slight utilization of mucate, dulcitol, inositol and arabinose respectively. Also, Isolate D showed slight utilization of salicin. The isolates showed variation in the utilization of xylose, trehalose and mannitol. The isolates showed complete utilization of xylose whereas isolate X showed slight utilization. Isolate X showed complete utilization of trehalose whereas isolates D and Z showed slight utilization. Isolates X and Z showed complete utilization of mannitol whereas Isolates D and Z showed slight utilization.

The nucleic acid extracted from the bacterial isolates revealed that the nucleic acids were all DNA (1.80 - 1.90) as shown in Table 7. The sequence analysis of the bacterial isolates showed 100% identifies for all the three isolates and the identified isolates were: *Salmonella enterica* subspecies *entrica* serovar Typhi strain CMST (STCM), *Salmonella enterica* subspecies *entrica* serovar Typhi strain WG-S1146 (STWG), and *Salmonella enterica* subspecies *entrica* serovar Typhi strain ERL12960 (STER12) as shown in Table 8.

The inhibitory compounds eluted from the samples were Oleic acid, Hexacosanoic acid, Tetramethyl silicate, 1-Octadecene, 10-Methyl-E-11-tridece-1-ol acetate, 6-Octadecenoic acid, (Z)- and Erucic acid as shown in Tables 9, 10 and 11, and Figures 1(a-d), 2 (a-e) and 3 (a-c)

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The result of the diameter zones of inhibition of the fraction of the eluates from AFK4631 against the test isolates is presented in Table 12. The result showed that K1 showed highest zones of inhibition against the test isolates while K4 recorded the lowest zones of inhibition against the test isolates. Similarly, in Table 13, B1 recorded the highest zones of inhibition against the test isolates while B4 showed the lowest zones of inhibition against the test isolates. In Table 14, F3 revealed the highest zones of inhibition against the test isolates while the lowest was F2. Generally, all the eluates recorded highest zones of inhibition against STCM, followed by STWG while the lowest zones of inhibition were recorded against STER12.

Table 1: Macroscopic characteristics of the fungal isolates

| Parameter | Isolate V | Isolate U | Isolate W |
|---|---|---|---|
| Initial Appearance on SDA(2-3 days) | White | White | White |
| Later Appearance on SDA(5 days) | Gray-green with white edges | Gray-green with white edges | Gray-green |
| Reverse Colour | Light Yellow | Pale | Pale yellow |
| Growth Rate | Rapid | Rapid | Rapid |
| Colony Texture | Cottony | Wooly | Wooly |
| Colour of Mycelium | Gray-green | Gray-green | Gray-green |
| Fungus | Aspergillus species | Aspergillus species | Aspergillus species |

Table 2: Microscopic characteristics of the isolates

| Parameter | Isolate V | Isolate U | Isolate W |
|---|---|---|---|
| Nature of hyphae | Septate | Septate | Septate |
| Colour of Conidia | Gray-green | Gray-green | Gray-green |
| Conidia head | Columnare | Columnar | Columnar |
| Shape of Conidia | Ellipsoidal | Ellipsoidal | Ellipsoidal |
| Shape of Vesicle | Globose | Globose | Globose |
| Colour of Conidiophore | Hyaline | Hyaline | Hyaline |
| Texture of Conidiophore | Smooth | Smooth | Smooth |
| Length of Conidiophore | Short | Short | Short |
| Seriation (Sterigmata) | Uniseriate | Uniseriate | Uniseriate |
| Fungus | Aspergillus fumigatus | Aspergillus fumigatus | Aspergillus fumigatus |

Table 3: Molecular characteristics of the fungal isolates

| Parameter | Isolate V | Isolate U | Isolate W |
|---|---|---|---|
| Max Score | 1644 | 2442 | 2929 |
| Total Score | 1644 | 2442 | 2929 |
| Query Cover (%) | 100 | 100 | 100 |
| E-Value | 0.0 | 0.0 | 0.0 |
| Identity (%) | 100 | 100 | 100 |
| Accession Number | MT316338 | KR023997 | OR578448 |
| Description | *Aspergillus fumigatus* strain DTO402(AFD402) | Aspergillus fumigatus strain F7(AFF7) | *Aspergillus fumigatus* strain KMM4631(AFK4631) |

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Table 4: Cultural and morphological characteristics of the bacterial isolates

| Parameter | Isolate D | Isolate X | Isolate Z |
|---|---|---|---|
| Appearance on DCA | Colourless and dark centered | Colourless and dark centered | Colourless and dark centered |
| Appearance on NA | Greyish White | Greyish White | Greyish White |
| Size (mm) | 2.40 | 2.60 | 2.20 |
| Surface | Smooth | Smooth | Smooth |
| Colony Shape | Circular | Circular | Circular |
| Elevation | Low Convex | Convex | Convex |
| Edge | Entire | Entire | Entire |
| Gram reaction | ___ | ___ | ___ |
| Shape of Cell | Rod | Rod | Rod |
| Cell Arrangement | Singly/Pairs | Singly/Pairs | Singly/Pairs |
| Motility | + | + | + |
| Nature of Flagella | Peritrichous | Peritrichous | Peritrichous |
| Spore Test | ___ | ___ | ___ |
| Capsule Test | ___ | ___ | ___ |

+ = Positive , -- = Negative.

Table 5: Biochemical characteristics of the isolates

| Parameter | Isolate D | Isolate X | Isolate Z |
|---|---|---|---|
| Catalase | + | + | + |
| Oxidase | __ | __ | __ |
| Citrate | __ | __ | __ |
| Indole | __ | __ | __ |
| Urease | __ | __ | __ |
| $H_2S$ | + | + | + |
| MR | + | + | + |
| VP | __ | __ | __ |
| Nitrate Reduction | +/-- | + | + |
| Gelatin Hydrolysis | __ | __ | __ |

+ = Positive, - = Negative

Table 6: Sugar utilization potential of the isolates

| Sugar | Isolate 15 | Isolate 16 | Isolate 17 |
|---|---|---|---|
| Glucose | + | + | + |
| Maltose | + | + | + |
| Arabinose | __ | __ | __ |
| Lactose | __ | __ | __ |
| Sucrose | __ | __ | __ |
| Xylose | + | + | + |
| Trehalose | +/-- | + | +/-- |
| Mannitol | +/-- | + | + |
| Inositol | + | + | + |
| Dulcitol | __ | __ | __ |
| Arabitol | __ | __ | __ |
| Surbitol | + | + | + |

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| | | | |
|---|---|---|---|
| Salicin | +/-- | __ | __ |

Table 7: Purity of Nucleic Acids of the Bacterial Isolates

| Isolate | Conc. (ng/µl) | $ABS_{260}$ | $ABS_{280}$ | $ABS_{280}/ABS_{260}$ |
|---|---|---|---|---|
| D | 101.50 | 3.0142 | 1.6382 | 1.84 |
| X | 110.50 | 3.2146 | 1.7283 | 1.86 |
| Z | 104.20 | 3.0640 | 1.6743 | 1.83 |

Table 8: Characteristics and identities of the isolates

| Isolate Code | Max Score | Total Score | Query Cover (%) | E-value | Percent Identity | Accession Number | Description of the Isolates |
|---|---|---|---|---|---|---|---|
| D | 3620 | 3620 | 100 | 0.0 | 100 | CP053702.1 | *Salmonella enterica* subspecies *entrica* serovar Typhi strain CMST (STCM) |
| X | 3620 | 3620 | 100 | 0.0 | 100 | CP040575.1 | *Salmonella enterica* subspecies *entrica* serovar Typhi strain WG-S1146 (STWG) |
| Z | 3620 | 3620 | 100 | 0.0 | 100 | LT904894.1 | *Salmonella enterica* subspecies enterica serovar Typhi strain ERL12960 (STER12) |

Table 9: GC/MC products from AFK4631 eluates

| Fraction | Product | Molecular Formula |
|---|---|---|
| K1 | Oleic Acid | $C_{18}H_{34}O_2$ |
| K2 | Hexacosanoic acid | $C_{26}H_{52}O_2$ |
| K3 | Erucic acid | $C_{22}H_{42}O_2$ |
| K4 | 10-Methyl-E-11-tridece-1-ol acetate | $C_{16}H_{30}O_2$ |

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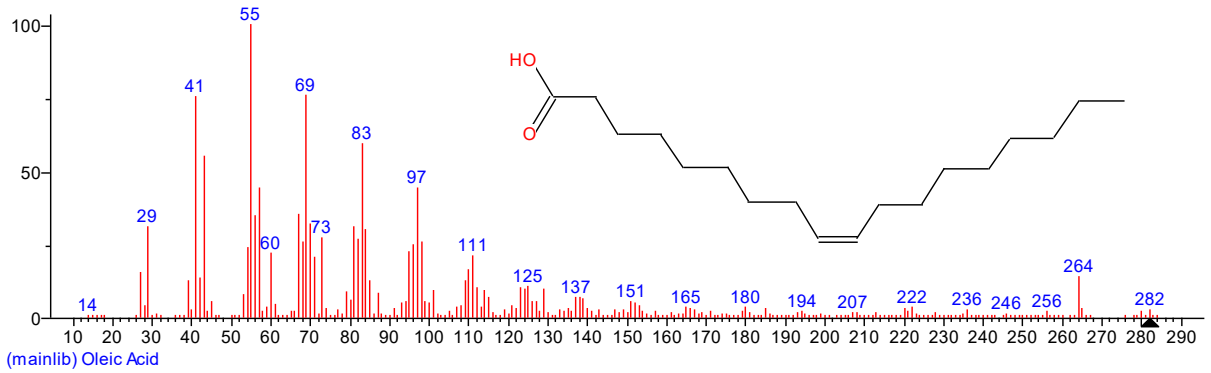
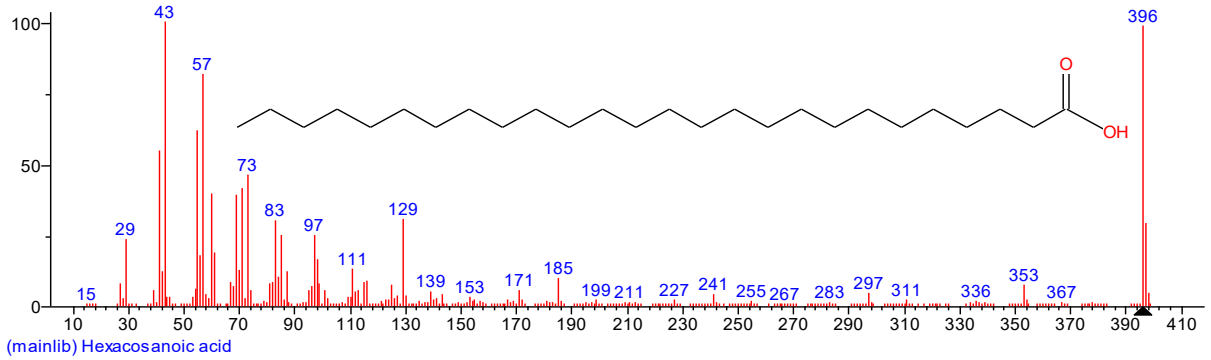
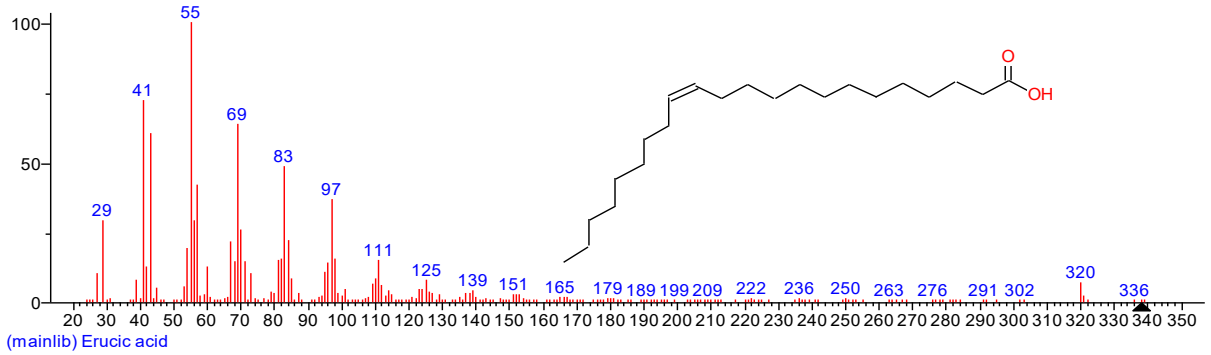



(a)

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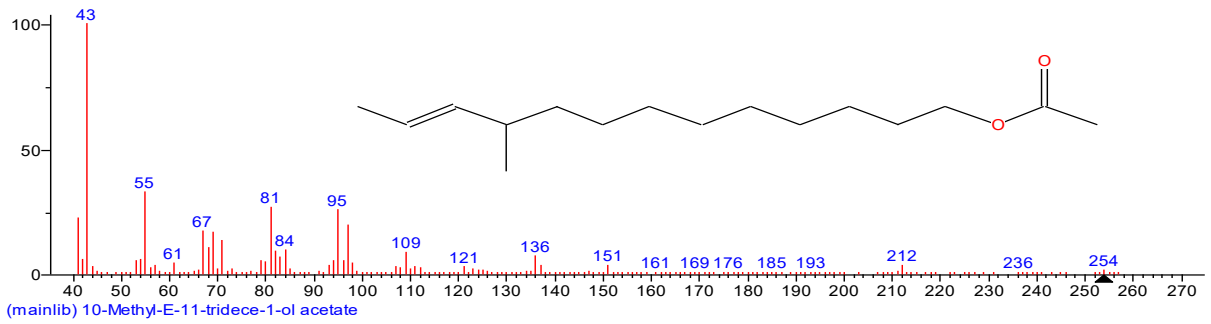


(c )

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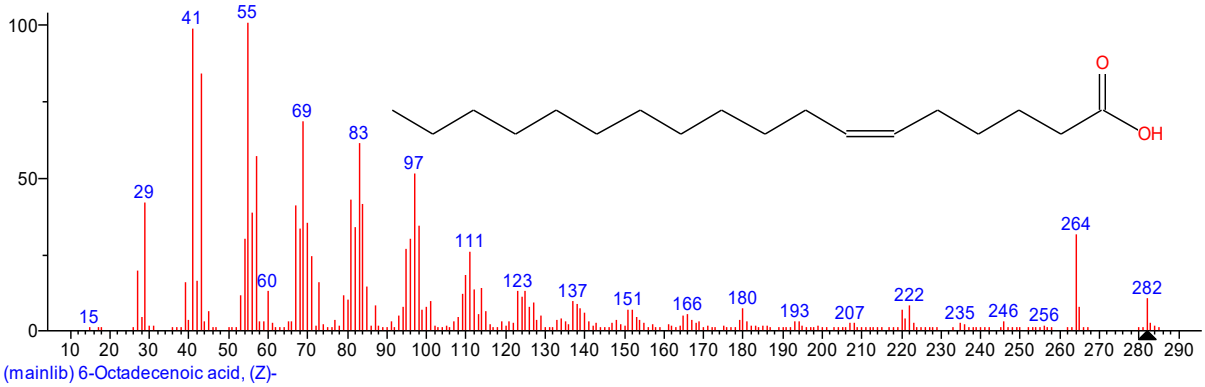
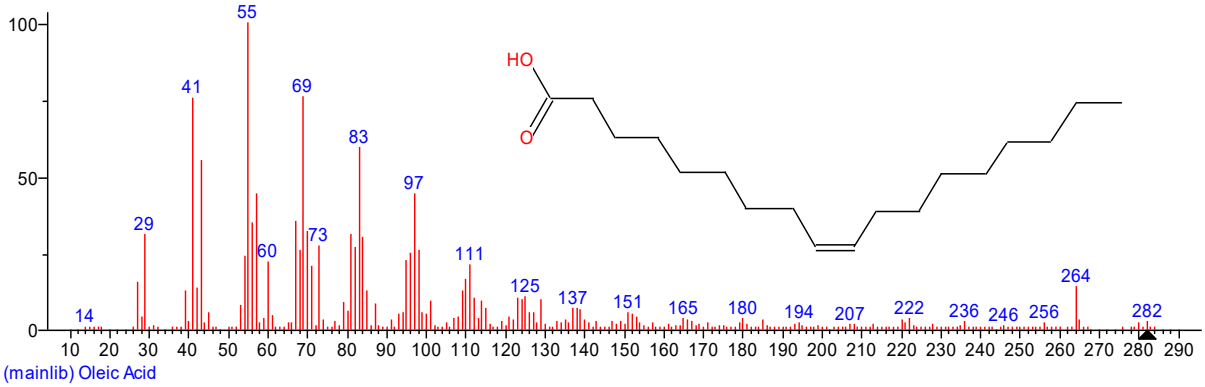



(d)

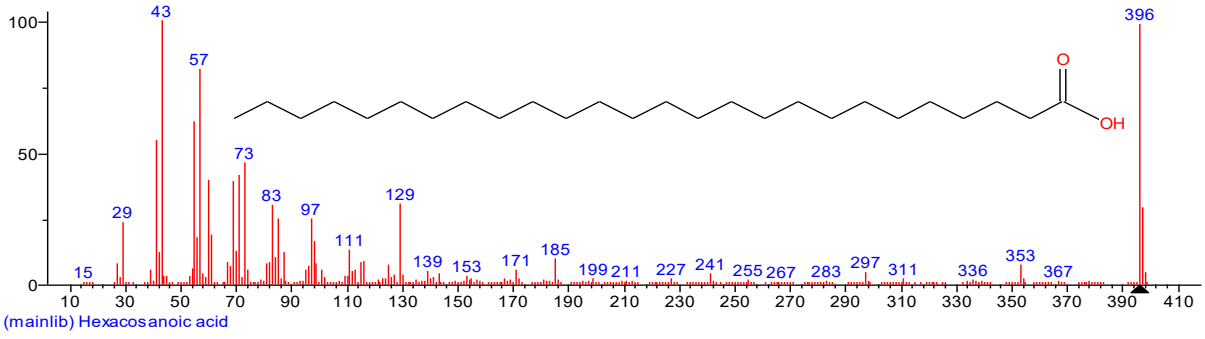
Figure 1 (a-d): Structural elucidation of the antibiotics

Table 10: GC/MC products from AFD402 eluates

| Fraction | Product | Molecular Formula |
|---|---|---|
| B1 | Oleic Acid | $C_{18}H_{34}O_2$ |
| B2 | Hexacosanoic acid | $C_{26}H_{52}O_2$ |
| B3 | 1-Octadecene | $C_{18}H_{36}$ |
| B4 | 10-Methyl-E-11-tridece-1-ol acetate | $C_{16}H_{30}O_2$ |
| B5 | 6-Octadecenoic acid, (Z)- | $C_{18}H_{34}O_2$ |




(a)



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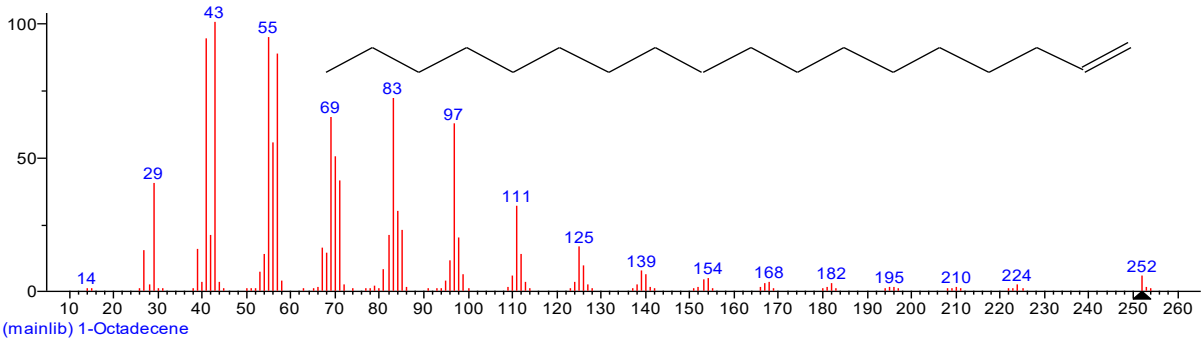


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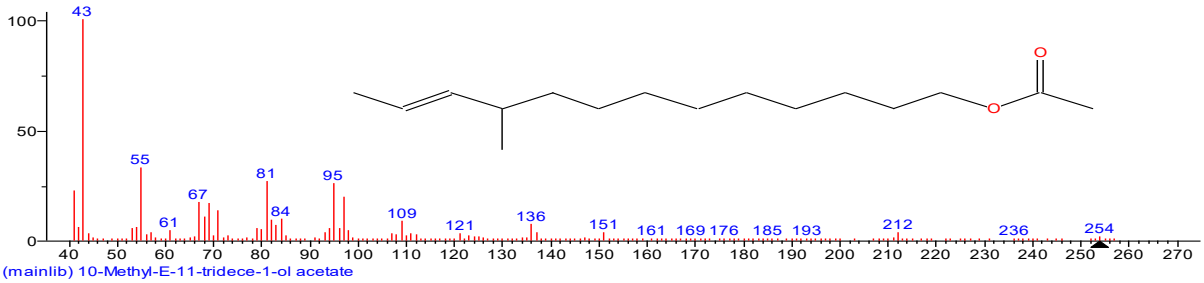
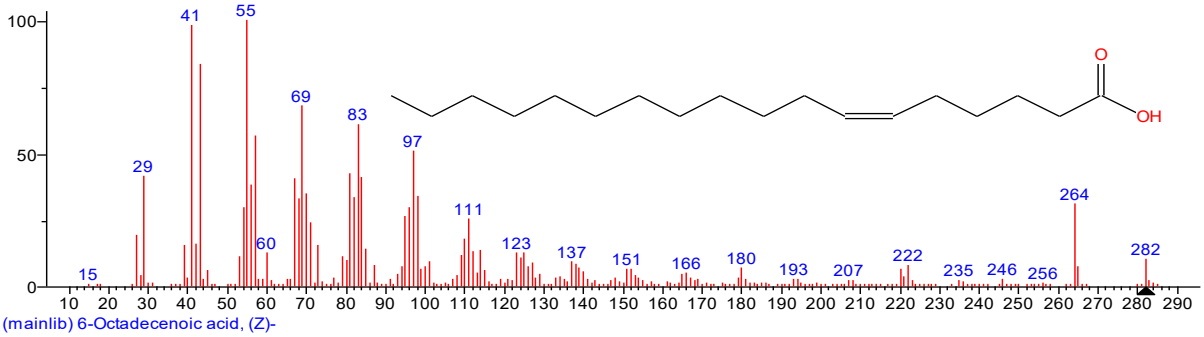


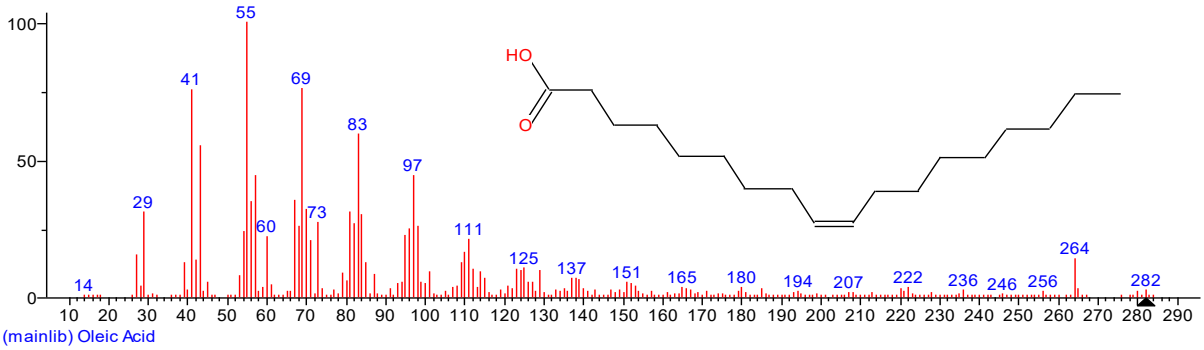




(c )

Figure 2 (a-c): Structural elucidation of the antibiotics

Table 11: GC/MC products from AFF7 eluates

| Fraction | Product | Molecular Formula |
|---|---|---|
| F1 | Oleic Acid | $C_{18}H_{34}O_2$ |
| F2 | Hexacosanoic acid | $C_{26}H_{52}O2$ |
| F3 | Tetramethyl silicate | $C_4H_{12}O_4Si$ |




(a)

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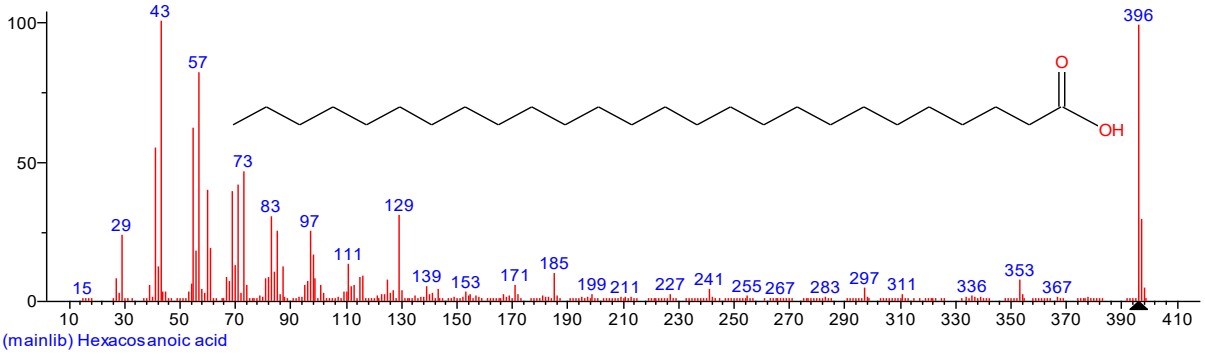
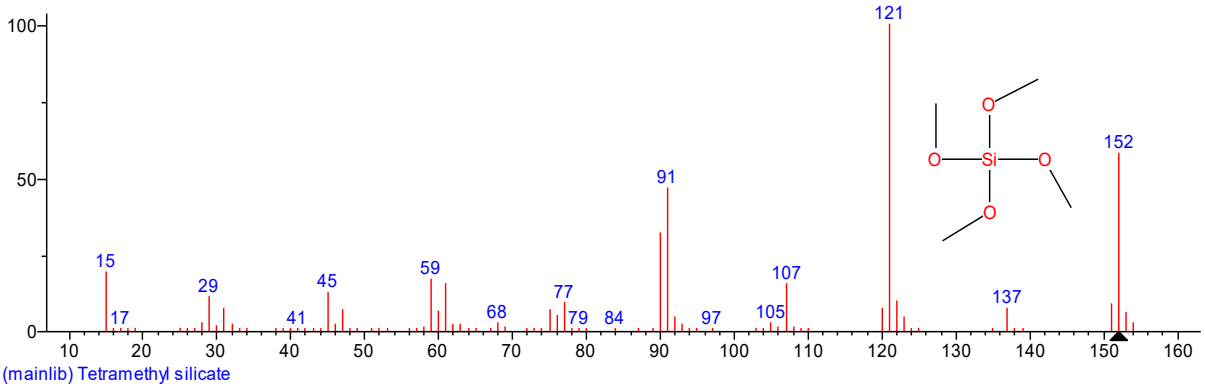



(b)




(c)

Figure 3 (a-c): Structural elucidation of the antibiotics

Table 12: Diameter zones of inhibition of the fraction of the eluates from AFK4631

| Fraction | STWG (mm) | STCM (mm) | STER12(mm) |
|---|---|---|---|
| K1 | 20.61 | 23.06 | 19.81 |
| K2 | 18.37 | 19.72 | 22.18 |
| K3 | 17.64 | 19.66 | 22.31 |
| K4 | 14.02 | 13.22 | 14.11 |

Diameter of Cork borer = 5 mm

Table 13: Diameter zones of inhibition of the fraction of the eluates from AFD402 against the test isolates

| Fraction | STWG (mm) | STCM (mm) | STER12 (mm) |
|---|---|---|---|
| B1 | 20.62 | 23.01 | 19.64 |
| B2 | 18.22 | 19.66 | 22.08 |
| B3 | 12.06 | 13.88 | 11.22 |
| B4 | 10.76 | 10.92 | 10.12 |
| B5 | 14.67 | 15.02 | 14.36 |

Table 14: Diameter zones of inhibition of the fraction of the eluates from AFK4631

| Fraction | STWG (mm) | STCM (mm) | STER12 (mm) |
|---|---|---|---|
| F1 | 20.64 | 23.14 | 19.76 |
| F2 | 18.36 | 19.76 | 22.14 |
| F3 | 21.54 | 24.61 | 21.06 |

Diameter of Cork borer = 5 mm

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## DISCUSSION

*Aspergillus* species isolated from various soils have been found to produce antibacterial, antifungal and anti tumour metabolites. Species of *Aspergillus* are known to produce mycotoxin, organic acids and antibiotics. *A. fumigatus* is an especially prolific producer of secondary metabolites such as fumiginin [25].

The characteristic features of *Aspergillus fumigatus* strain DTO402 (AFD402), *Aspergillus fumigatus* strain F7 (AFF7) and *Aspergillus fumigatus* strain KMM4631 (AFK4631) isolated from garden soil in the present study supported the reports of [26] and [27] who studied various soil samples for an antibiotic-producing *Aspergillus* species. The cultural features and morphology of the *Aspergillus* species isolated in their studies confirmed the observation made in this study concerning *Aspergillus* species. However, the previous researchers isolated other species of Aspergillus such as *niger, flavus*, and *fumigatus*, though their strains were not fully elucidated as was carried out in the present study.

The production of antibiotics from different strains of *Aspergillus fumigatus,* and the inhibitory activities of the extracted, eluted, and purified fractions against enteric bacterial pathogens; *Salmonella enterica* subspecies *entrica* serovar Typhi strain CMST (STCM), *Salmonella enterica* subspecies *entrica* serovar Typhi strain WG-S1146 (STWG), and *Salmonella enterica* subspecies *entrica* serovar Typhi strain ERL12960 (STER12) agrees with the reports of [28], [29] and [30]. These researchers observed that *Aspergillus* species are capable of synthesizing potent secondary metabolites in the stationary phase of their growth which they attributed to depletion of the required nutrients for growth. The researchers also stated that the secondary metabolites were able to inhibit the growth of certain bacterial pathogens.

The occurrences of *Salmonella enterica* subsp.*enterica* serovar Typhi strain CMCST (STCM), *Salmonella enterica* subsp.*enterica* serovar Typhi strain WGS1146 (STWG) and *Salmonella enterica* subsp.*enterica* serovar Typhi strain ERL12960 (STER12) in river, borehole and hospital dumping site samples supported the findings of many researchers [31,32,33,9]. Tracogna *et al.* [34] stated that the presence of *Salmonella* species in these samples reaffirms the need for monitoring in order to minimize the risks of infection to exposed persons. The researchers also stated that the presence of *Salmonella* species in the sampled water bodies is the major source of enteric fever that is commonly associated with intake of contaminated water from different sources, especially in rural areas where adequate water treatment is rarely achieved. They also stated that *Salmonella* species found in the soil are capable of contaminating water and food, thereby causing enteric fever to the infected patients.

The elution and purification of oleic acid [35,29,36], 10-methyl-E-11-tridece-1-ol-acetate [37], hexacosanoic acid, 1-octadecene [28] and tetramethyl silicate [30] agrees with the present study since these fractions were also extracted, eluted and purified in the study. The ability of the eluates to inhibit the growth of the enteric bacterial pathogens could be attributed to antibacterial potential of the antibiotics. This observation agrees with the findings of several researchers who investigated the efficacy of natural antibiotics produced by certain fungal species in the environment [38,39,40].

### Conclusion

This study demonstrates the potential of *Aspergillus fumigatus* strains (AFK4631, AFD402, and AFF7) isolated from garden soil as sources of antibiotics against enteric bacterial pathogens, particularly *Salmonella* Typhi strains. The significant production of inhibitory substances by these fungal isolates suggests their potential as alternative antibiotics for treating enteric bacterial infections, offering a promising avenue for further research and development

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### Conflict of interests

The authors declare that they have no conflict of interests.

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**Ethical approval**

Not applicable

**Authors Contributions**

All contributed towards the study design, experiment execution, data analysis, and manuscript drafting.

**Availability of Data and Materials**

All datasets analyzed and described during the present study are available from the corresponding author upon reasonable request.

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