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Antimicrobial and antimycotoxigenic activities of (3s)-3, 6, 7-trihydroxy-α-tetralone isolated from endophytic *Phoma moricola*

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Abstract
Endophytic fungi are known to biosynthesis a plethora of bioactive secondary metabolites with promising antimicrobial activities. The present investigation evaluated the antimicrobial and antimycotoxigenic activities of the bioactive compound (3S)-3, 6, 7-trihydroxy-α-tetra lone (TT) isolated from *Phoma moricola* an endophytic fungus of *Withania somnifera*. The compound TT showed concentration-dependent broad-spectrum antimicrobial and anti-mycotoxin activities. The biosynthesis of aflatoxin B_1 (AFB_1) from *Asprgilllus flavus* and fumonisin B_1 (FB_1) from *Fusarium verticillioides* were strongly inhibited by TT at 200 µg/mL. The compound TT significantly interferes *A. flavus* and *E. coli* growth by inhibiting membrane-specific cellular ergosterol biosynthesis and release of potassium ions, respectively. The docking study confirms that TT strongly active against the enzyme polyketide synthase, with a docking score of -6.77 kcal/mol units. The obtained results confirm the antimicrobial and antimycotoxin activities of TT; hence this compound could be explored as a green eco-friendly strategy for managing pathogenic microbial and mycotoxins contamination in food- and feedstuffs.

Keywords: *Phoma moricola*, *Withania somnifera*, (3S)-3, 6, 7-trihydroxy-α-tetralone, antimicrobial activity, mycotoxins, mode of action, docking studies

Introduction
Endophytic fungi are a diverse and versatile group of poorly investigated eukaryotic microorganisms. They have been mutually residing in all types of plant species, including algae, bryophytes, pteridophytes, gymnosperms, and angiosperms (Stone and Polishook 2004; Gouda *et al.* 2016) [36, 10]. As per the survey of Royal Botanic Gardens (United Kingdom), there are about 391,000 species of vascular plants currently known to science (Jin and Qian 2019) [16], and each species hosted an active community of endophytic fungi (Kumar *et al.* 2014) [20]. In the association, endophytic fungi produce an array of bioactive secondary metabolites and protect its host plant against microbial invasions and pest attacks (O'Hanlon *et al.*, 2012) [25]. The bioactive compounds isolated from fungal endophytes belong to the group's terpenoids, steroids, quinones, isocoumarin derivatives, phenols, phenolic acids, coumarins, alkaloids, flavonoids, and peptides. They showed anticancer, antimicrobial, immunomodulatory, antioxidant, and insecticidal properties (Huang *et al.* 2008; Suryanarayanan *et al.* 2009; Wang *et al.* 2012; Kaul *et al.* 2012; Palem *et al.* 2015; Ayob and Simarani 2016; Sudharshana *et al.* 2019) [14, 38, 40, 17, 26, 3, 37]. Many researchers have proven that endophyte is a new and potential source of novel natural products for exploitation in modern medicine, agriculture, and industry (Yu *et al.* 2010) [42]. Kumar *et al.* (2014) [20] reported that more than 100 compounds isolated from endophytic fungi showed anticancer activity. Even though endophytes are a promising way to overcome the increasing threat of drug-resistant microbial strains, antimicrobial compounds from endophytes are promising. Still, only about 7% of the estimated 1.5 million fungi species have been screened for drug production (Hawksworth *et al.* 2004; Suryanarayanan *et al.* 2009; Chowdhary *et al.* 2012; Bhagat *et al.* 2019) [12, 38, 7, 5]. The present investigation was undertaken to evaluate the antimicrobial and antimycotoxigenic properties of an endophytic fungus *Phoma* sp. isolated from the leaves of *W. somnifera*, a well-known medicinal plant, belongs to the family Solanaceae. It is known as Indian Ginseng for its wide range of therapeutic uses in ayurvedic and other traditional medicine systems, and more than 91 pharmaceutical products were obtained from this plant (Agarwal *et al.* 1999; Scartezzini *et al.* 2000; Rai *et al.* 2001; Rasool *et al.* 2006; Yang *et al.* 2007) [2, 32, 27, 28, 41].

Material and Methods

Isolation and identification of endophytic fungi from *W. somnifera*

The endophytic fungi associated with the leaves of *W. somnifera* were isolated following Ezra *et al.* (2004) [8]. The plant *W. somnifera* was authenticated by Dr. Y.N. Seetharam, Professor, Department of Botany, Tumkur University, Tumakuru (India), and the established voucher specimens were deposited in the Herbarium laboratory, Bangalore University, Bangaluru with appropriate voucher numbers BUB/MB-BT/DCM/2017/26. The leaf samples were collected and subjected to endophytic fungal isolation, as described in our previous paper (Sudharshana *et al.* 2019) [37]. The isolate DCM-EF-S9 showed promising antimicrobial activity was selected for identification using 5.8S rDNA sequence analysis following the procedure of Mohana *et al.* (2016) [22]. Briefly, the genomic DNA was isolated from five days old culture and amplified using a PCR machine (Q cycler, CM 6050, Quanta Biotech, England). The fungal-specific internal transcribed spacer (ITS) regions of 5'TGATCCTTCYGCAGGTTCAC3' and 5'ACCTGGTTGATCCTGCCAG3' were used as forward and backward primers to amplify a fragment within the gene coding for 5.8 S rRNA. The PCR product was sequenced with Eurofins Genomics Pvt. Ltd., Bengaluru (India). The obtained base sequences were searched against GenBank using the NCBI-BLAST search tool, and a phylogenetic tree was constructed. The base sequence of the identified fungal isolate of DCM-EF-S9 was deposited in NCBI-GenBank.

Solvent extraction and bioactive compound isolation from *P. moricola*

The culture filtrate of *P. moricola* was subjected to solvent extraction following the procedure of Srivastava and Anandrao (2015) [35]. Briefly, 500 ml culture filtrate of 15 days grown *P. moricola* was collected and extracted with the same amount of ethyl-acetate using a separatory funnel and concentrated to dryness using a vacuum evaporator (Lyoquest–85, Telstar Technologies, SL Terrassa, Spain). The collected ethyl-acetate extract was fractionated using column chromatography following the procedure of Mohana *et al.* (2010) [21] with some modification. The silica gel column (mesh size 60–120, SRL, India) loaded with ethyl acetate extract was eluted sequentially with the mixtures of $CHCl_3$ and MeOH (10:0, 7.5:2.5, 1:1, 2.5:7.5, 0:10, v/v). The fraction which showed the highest activity was collected and further purified by TLC using $CHCl_3$: MeOH (9:1) as a mobile phase. The separated chromatograms on the TLC plate were scraped out separately, dissolved with MeOH, filtered, and allowed to dry. The crystals obtained from the 4th band, which showed activity was collected and subjected to LC-MS, FT-IR and NMR analysis. The active compound was identified by comparing the spectral data with the published literature and spectral libraries.

Evaluation of antimicrobial activities of TT

Seven bacterial species, *viz*., *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Streptococcus faecalis*; two yeast species, *viz*., *Candida albicans* and *Cryptococcus neoformans*; and fifteen species of fungi, *viz*., *Alternaria brassicicola*, *A. geophila*, *Aspergillus flavus* (aflatoxin producing strain), *A. fumigatus*, *A. ochraceus*, *A. tamarii*, *A. terreus*, *Curvularia tetramera*, *Fusarium oxysporum*, *F. lateritium*, *F. equiseti*, *F. udum*, *F. verticillioides* (fumonisin producing strain), *Penicillium citrinum*, and *P. expansum*. The collection centers and sources of these isolates have been described in our earlier reports (Abhishek *et al.* 2015) [1]. The 24 h, 48 h, and seven days old culture of bacteria, yeast, and fungi were selected as test microorganisms for evaluation, respectively.

The TTs antimicrobial activity against the tested microorganisms was determined using the disc diffusion method (Hajji *et al.* 2010). Briefly, six mm discs impregnated with TTs (100 µg/disc) were placed on the Mueller-Hinton agar (MHA) plate pre-inoculated with 10^8 CFU/mL of bacteria and 10^6 CFU/mL of yeast and incubated at 37 °C for 24 h for bacteria and 48h for yeast. The disc without TT served as a negative control. The zone of inhibition (ZOI) around the discs was measured after spraying iodo-nitro-tetrazolium chloride (INT 2 mg/mL) on the cultured plate.

TT's antifungal activity against the tested fungi was determined using a poisoned food technique (Mohana *et al.* 2010) [21]. Briefly, mycelial discs (5 mm in diameter) of the test fungi were placed on Sabouraud Dextrose Agar medium impregnated with TT (100 µg/mL) and incubated at 28±2 °C for seven days. The medium without TT served as a negative control. The mycelial growth inhibition (%MI) was determined using the formula.

$$\%MI = (C–T/C) \times 100$$

C is the diameter of mycelial growth in the control plate, and T is the diameter of mycelial growth in a treated plate.

The MICs of TT against tested microbes was determined using broth dilution techniques (Hajji *et al.* 2010). Briefly, 15 µL of bacteria, yeast, and fungi were independently inoculated into a microtiter plate containing 200 µL of MHB/SDB impregnated with TT (3.125 µg/mL to 800 µg/ml, and incubated at a specific period and temperature as explained above. After incubation, 50 µL of INT was added to each well and incubated for another 30 min. The pale yellow-colored INT was reduced to pink, indicating viable cells, and no change in color demonstrated the inhibition of microbial growth. The color intensity was measured using an ELISA reader. The lowest concentration at which the color remained unchanged was considered as MIC. Erythromycin (15 mcg), itraconazole (10 mcg), and zinc ethylene bithiocarbamate (Indofil Z–78) (2mg/mL) served as a positive control for bacteria, yeast, and fungi, respectively.

Evaluation of antimycotoxigenic activities of TT

The anti-aflatoxin B_1 and anti-fumonisin B_1 activity of TT was determined following the procedure of Abhishek *et al.* (2015) [1]. Briefly, 100 µL of spore suspension *A. flavus* and *F. verticillioides* was inoculated into the SMKYB and SDA contains the requisite amount of TT (25, 50, 100, 200 µg/mL), and incubated at 28 °C for 15 days. The medium devoid of TT served as control. After incubation, AFB_1 was extracted from the culture filtrate of *A. flavus* by adding an equal volume of $CHCl_3$ (Shukla *et al.* 2008) [33], and FB_1 was extracted from a culture of *F. verticillioides* by adding an equal amount of acetonitrile-water mixture (1:1, v/v) (Bailly *et al.* 2005) [4].

The extracted AFB_1 was separated using TLC plate adjacent to the AFB_1 standard (Sigma, Germany) and eluted using $CHCl_3$-acetone (96:4, v/v) as a mobile phase, and then observed under ultraviolet light at 365 nm (UV-cabinet, Labline, India). AFB_1 was estimated quantitatively by measuring the light absorbance of the samples using a spectrophotometer at 600 nm wavelengths (UV–1800, Shimadzu, Japan). The amount of AFB_1 content was

calculated by the formula given below.

AFB_1 (µg/L) = [D × M/E × L] 1000

Where *D* is the absorbance, *M* is the molecular weight of AFB_1 (312), *E* is the molar extinction coefficient of AFB_1 (21,800) and *L* is the path length (1 cm).

The extracted FB_1 was spotted on the TLC plate adjacent to the standard FB_1 (Sigma, Germany) and eluted using butanol-acetic acid-water (20:10:10, v/v/v) a mobile phase. The solution of *p*-anisaldehyde in methanol-acetic acid-H_2SO_4 (85:10:0.5, v/v/v) (0.5%) was sprayed and incubated at 110°C for 10 min. The amount of FB_1 was measured by comparing the band's intensity with standard spots using a Spectro photo-densitometer at 600 nm wavelengths (Bio Rad, Universal Hood II 720BR/02170, USA).

Efficacy of TT on inhibition of AFB_1 and FB_1 production in viable maize seed samples determined following the procedure of Bailly *et al.* (2005) [4]. Briefly, 100 µL spore suspension of toxigenic strains of *A. flavus* and *F. verticillioides* (10^4 CFU/mL) was inoculated separately on viable maize samples treated with different amount of TT (25, 50, 100 and 200 µg/g). The maize seeds (water activity (a_w) 0.95) impregnated with TT without fungal inoculums served as control. Both treated and control maize samples were stored in plastic containers (200 g/pack) separately up to 15 days at 25 ±2 °C. After 15 days, all samples were powdered separately, *A. flavus* treated samples were used for AFB_1 extraction followed by quantification, whereas *F. verticillioides* treated maize samples were used for FB_1 extraction followed by quantification using the same procedure as explained above *in vitro* studies.

Effect of TT on the ergosterol content of *A. flavus*

TT's effect on the synthesis of cell membrane ergosterol from *A. flavus* was determined following Abhishek *et al.* (2015) [1]. Briefly, fifty µl spore suspension of *A. flavus* (10^4 cfu/ml) was inoculated into SDB containing desired concentrations of TT and incubated at 28±2 °C for 4 d. A control set was kept parallel to the treatment sets without TT Sterols were extracted from each sample by adding a mixture of 2 ml of distilled water and 5 ml of *n*-heptane. The mixture was then sufficiently mixed by vortex for 2 min, allowing the layers to separate for 1 h at room temperature, and the *n*-heptane layer was analyzed by scanning spectrophotometry (Shimadzu, Japan) between 230 and 300 nm. The ergosterol content was calculated as a percentage of the wet weight of the cell by the following formula:

% ergosterol + %24(28)-dehydroergosterol = (A_{282}/290)/pellet weight,

%24(28)-dehydroergosterol = (A_{230}/518)/pellet weight,

% ergosterol = (% ergosterol + % 24(28)-dehydroergosterol) − %24(28) dehydroergosterol

Where 290 and 518 are the *E* values (in percentages per cm) of crystalline ergosterol and 24(28)-dehydroergosterol, respectively, and pellet weight is the net wet weight (g).

Effect of TT on the release of potassium ion from *E. coli*

The potassium ion released from *E. coli* cells was measured by using a flame photometer. The bacterial cells (10^8 CFU/mL) were incubated in test tubes containing 1mL of deionized water and test compound of TT (50µg/mL) and incubated at 37°C for 20, 40, 60, 80, 100, and 120 min. After incubation, the cell suspension was centrifuged 10,000rpm for 15min, and the supernatant was diluted to 100-fold. The amounts of releasing potassium ion were measured using a flame photometer. The control was kept without treatment triplicate readings that were made for each supernatant (George *et al.* 2019) [9].

In-silico targeting studies of aflatoxin biosynthetic pathways of *A. flavus* by using TT.

The key enzyme polyketide synthase responsible for aflatoxin biosynthesis in *A. flavus* was used as a target enzyme for in-silico targeting the aflatoxin biosynthetic pathway of *A. flavus* following the procedure of (Biswal *et al.* 2019) [6]. The protein of the enzyme polyketide synthase was retrieved from the Protein Data Bank database and used for the docking study. The bioactive compounds TT was docked against polyketide synthase using the comprehensive bioinformatics tool Auto Dock 4.2.6 software. The method of incorporation was done using X-ray diffraction. The Auto Dock 4.2.6 relies on the principle of the Lamarckian genetic algorithm and is the most reliable automated tool used by the researchers to understand the protein-ligand interactions and protein-protein interactions. The software provides us with the necessary information about small and large molecules' interactions taking part in the interaction. The two-dimensional and three-dimensional interaction images displayed in this study were developed through this software. The chemical structure reported research papers, and the compounds were retrieved from the Pub Chem database (https://pubchem.ncbi.nlm.nih.gov/).

Results and Discussions

The isolate DCM-EF-S9 commonly associated with all the samples were isolated and identified using 5.8S rDNA sequence analysis. The ITS region of 5.8S rDNA nucleotide sequence of the isolate DCM-EF-S9 showed 98.0% similarity with *Phoma* spp. The nucleotide sequence analysis, followed by the microscopic image analysis, the isolate DCM-EF-S9was identified as *P. moricola*. The complete 5.8S rDNA nucleotide sequence of *P. moricola* was submitted to the NCBI GenBank (India), and the collected accession number was MG207679. Among the 24 column chromatography fractionation of *P. moricola* (25 mL per fraction), the activity guided 9th fraction was collected and purified by TLC. In the TLC chromatogram, five bands with the R_f values of 0.1, 0.36, 0.45, 0.54, 0.63, and 0.81 were eluted, among which, the 4th band with R_f value 0.54 showed antimicrobial activity was collected and subjected to LC-MS FT-IR and NMR analysis for identification of the bioactive compound. In the positive mode LC-MS followed by ESI-MS analysis, the isolated bioactive compound showed a molecular weight of 195 $[M+H]^+$ (Figure 1a). In FT-IR spectroscopic analysis, absorptions bands appeared at 3338.32 cm^{-1} is a characteristic of O- H stretching, 2917.21cm^{-1} (CH_2) and 2849.47cm^{-1} is indicating aliphatic or C- H stretching or (CH_3), 1613.16 and 1573cm^{-1} (C=C absorption peak), 1429.41cm^{-1} due to double (C=C) stretching, 1088.07 cm^{-1} due to (C-O). Other absorption frequencies include 1158.69 and 1245.72 cm^{-1} is a bending frequency for cyclic (CH2) n. The absorption frequency at 795.55 cm^{-1} signifies cycloalkane (Figure 1b). The ^1H NMR spectra (400MHz) showed a set of proton signals at 2.447 (dd, 1H, J = 16.8, 7.6 Hz); 2.856 (dd, 1H, J = 16.8, 4.0 Hz); 4.2 (dddd, 1H, J = 7.6, 4.0, 3.6, 3.2 Hz); 2.776 (dd, 1H, J = 16.0, 3.6 Hz), Hax-4; 3.102 (dd, 1H, J = 16.0, 3.2 Hz); 6.256 (s, 1H); 6.145 (s, 1H). The ^{13}C NMR spectrum showed a signals at 202.5; 47.4; 66.9; 39.2, 109.4; 166.6; 166.5; 101.7; 117.0; 146.0. Based on a comparison of

LC-MS, ESI-MS, FT-IR, and NMR reported values in the literature (Wang *et al.*, 2012; Nong *et al.* 2013; Mousa *et al.* 2015; Santra and Banerjee 2020) [40,], the bioactive compound was predicted to be (3S)-3,6,7-trihydroxy-α-tetralone ($C_{10}H_{10}O_4$) (TT) with a molecular weight of 194 Da (Figure 1c).

The ZOIs and MICs values of TT against tested bacteria were ranged between 16.2–24.2 mm at 100 µg/disc, and 6.25–100 µg/mL, respectively (Table 1). The TT also showed significant antifungal activities against 15 different seed-borne phytopathogenic fungi with % MI and MICs values ranging from 34.4–78.3% at 100 µg/mL, and 12.5–200 µg/mL, respectively (Table 2). The TT showed more activity against Gram-positive bacteria and field fungi than Gram-negative bacteria and storage fungi. On a comparative evaluation, TT's antimicrobial activity was comparable with erythromycin, itraconazole, and indofil Z–78. The present study confirms that TT shows broad-spectrum antimicrobial activity against bacteria and fungi.

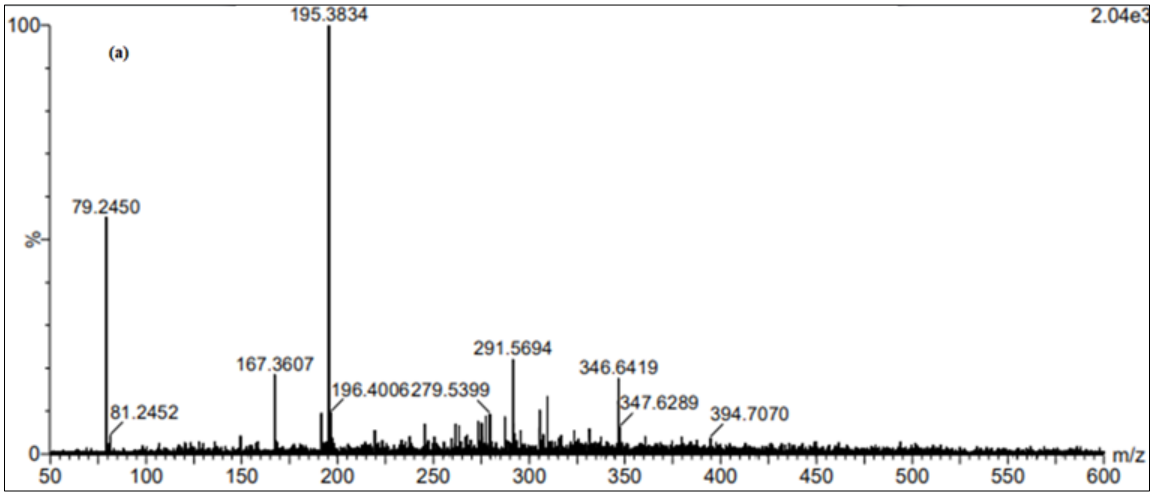
The AFB_1 production from *A. flavus* and FB_1 production from *F. verticillioides* was significantly inhibited by TT (Table 3). The control sets showed the highest amount of AFB_1 and FB_1, but TT completely inhibited these toxin's production at 200 µg/mL. In the viable maize model assay, TT significantly protected maize seeds from AFB_1 and FB_1 accumulation by preventing fungal growth at 200 µg/g. In the *in vivo* studies, the viable maize model conditions were similar to the conditions prevalent during the storage (Soares *et al.* 2013; Mohana *et al.* 2016) [34, 22]. The obtained results confirm that TT effectively suppressed a vast number of seed-borne fungi and mycotoxin biosynthesis.

Kelly *et al.* (1995) [18] and Thippeswamy *et al.* (2014) [39] reported that the antifungal drug azole inhibits fungal growth by inhibiting ergosterol biosynthesis. The depletion of ergosterol synthesis leads to disruption of the plasma membrane and makes it more vulnerable to further damage by altering membrane-bounded enzymes' activity. Keeping this point in view, TT's antifungal mode of action was assessed by measuring the ergosterol content of *A. flavus* with increasing TT Comparison concentrations with control, the percent inhibition of ergosterol content was 58.6% and 79.8% at 100 and 200µg/mL (Figure 3). The impairment of the biosynthesis of ergosterol is correlated with the inhibition of aflatoxin biosynthesis in *A. flavus*. In the mechanism of membrane action of TT on *E. coli*, the amount of releasing potassium ions from treated cells was compared with the untreated cells (Figure 4). After 60 min of treatment, around a 3-fold increase of K+ leakage was observed in treated cells compared to control. The increased amount of released K+ from *E. coli* after treatment showed that the TT probably acted on the plasma membrane by increasing permeabilization, causing the leakage of K+ ions from the cell.

The molecular docking revealed the structure-based drug designing by knowing ligand and drug target protein (Kortemme *et al.* 2003) [19]. The docking study confirms that TT significantly active against polyketide synthase with a docking score of -6.77, and its interaction score was Arg1500, Lys1493, and Lys1489. The binding of TT inhibits the activity of enzyme resulting in the inhibition of aflatoxin biosynthesis.

The perusal of literature reveals that endophytic species of *Phoma* symbiotically associated with a wide range of medicinal plants, and it produces many biologically active secondary metabolites. The bioactive compounds (3S)-3,6,7-trihydroxy-a-tetralone, cercosporamide, b-sitosterol, and trichodermin isolated from endophytic *Phoma* sp. of *Arisaema erubescens* showed antimicrobial activity against *Fusarium oxysporum*, *Rhizoctonia solani*, *Colletotrichum gloeosporioides*, *Magnaporthe oryzae*, *Xanthomonas campestris* and *Xanthomonas oryzae* (Wang *et al.* 2012) [40]. Similarly, Phomodione isolated from endophytic *Phoma* sp. of *Saurauia scaberrinae* was effective against *Staphylococcus aureus*, and the MIC was 1.6 µg/ml (Hoffman *et al.* 2010) [13]. Santiago *et al.* (2012) [30], investigated that the bioactive compound 5-hydroxyramulosin isolated from endophytic *Phoma* sp. of *Cinnamomum mollissimum* showed antifungal activity against *Aspergillus niger*. Phomoxanthone A and B, Phomoenamide, and Phomonitroester isolated from the endophytic *Phomopsis* sp. of *Garcinia* sp. showed significant antibacterial activity against *Mycobacterium tuberculosis* (Isaka *et al.* 2001; Rukachaisirikul *et al.* 2008) [15, 29]. Even though some reports are available on the bioactivity of (3S)-3,6,7- trihydroxy-a-tetralone isolated from species of *Phoma* in the earlier studies, as per our knowledge, we are the first to report the broad-spectrum antimicrobial activity, antimycotoxigenic, in-silico targeting studies of aflatoxin (AFB_1) biosynthetic pathway, and their mode of action.

a)



b)

c)

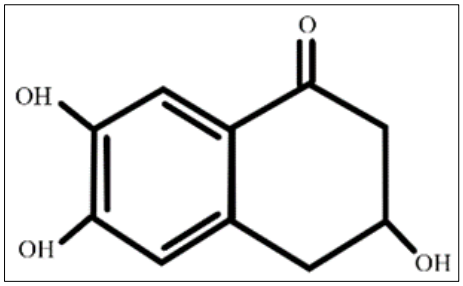


Fig 1: a) LC-MS spectra, b) FT-IR spectra, and c) Chemical structure of (3S)-3, 6, 7-trihydroxy-α-tetralone (TT)

a) b)

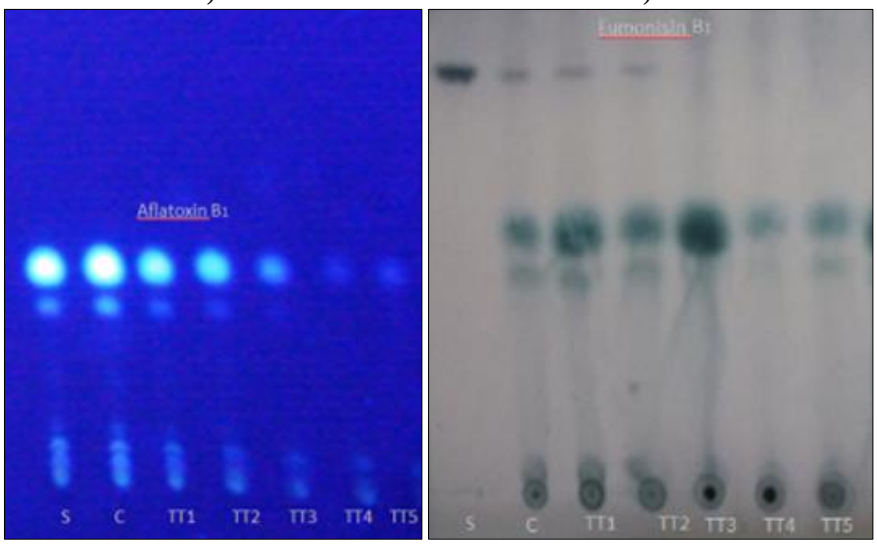


Fig 2: TLC chromatogram of a) Aflatoxin B_1 and b) Fumonisin B_1

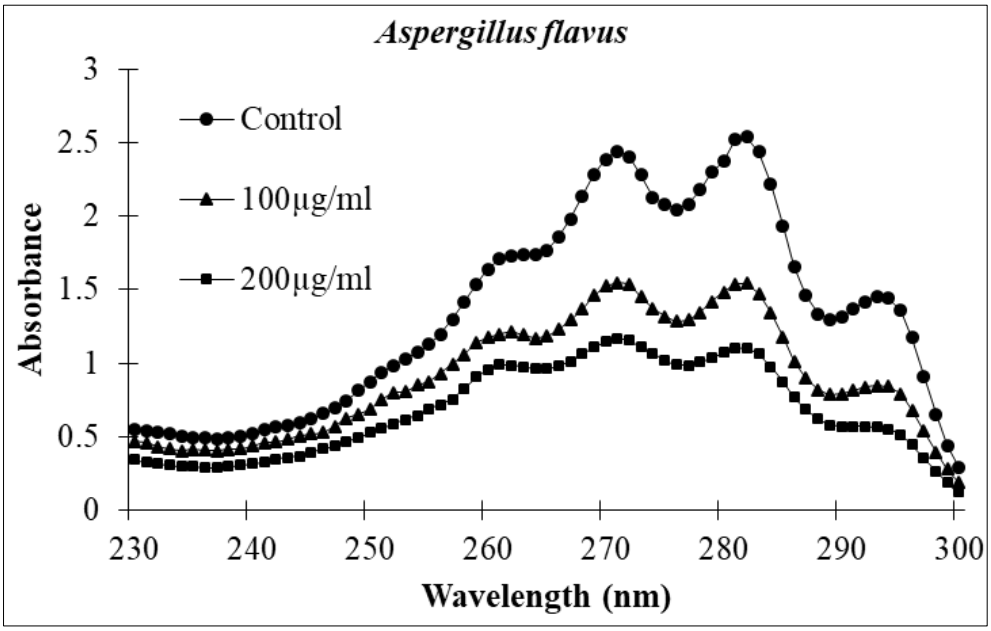


Fig 3: Efficacy of (3S)-3, 6, 7-trihydroxy-α-tetralone on inhibition of ergosterol biosynthesis from *A. flavus*.

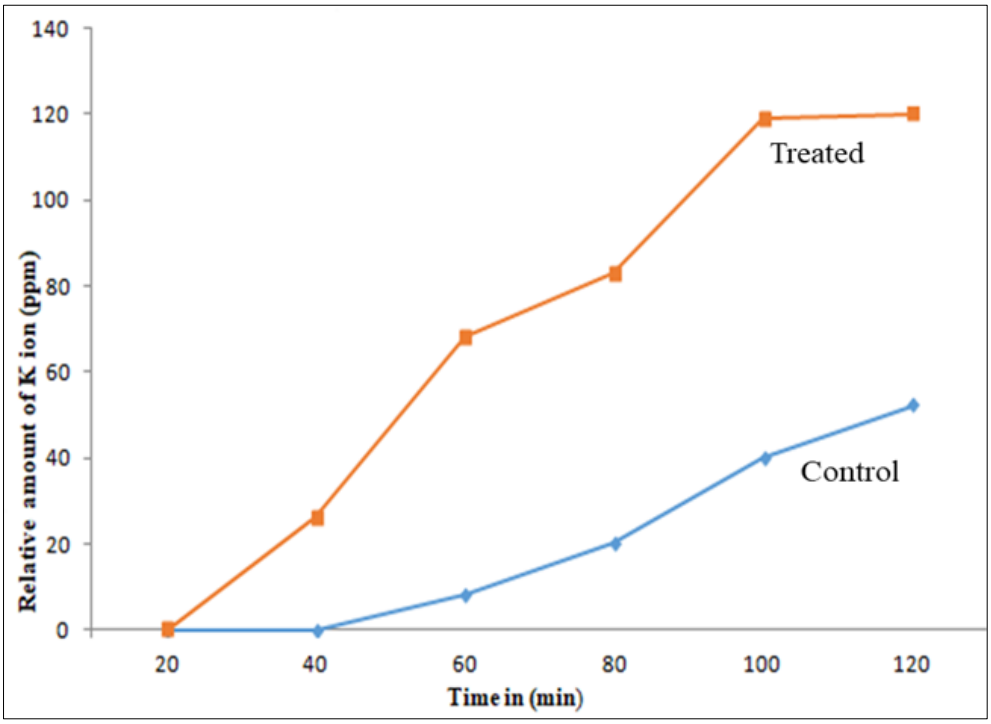


Fig 4: Efficacy of (3S)-3, 6, 7-trihydroxy-α-tetralone on leakage of potassium ion in *E.coli*

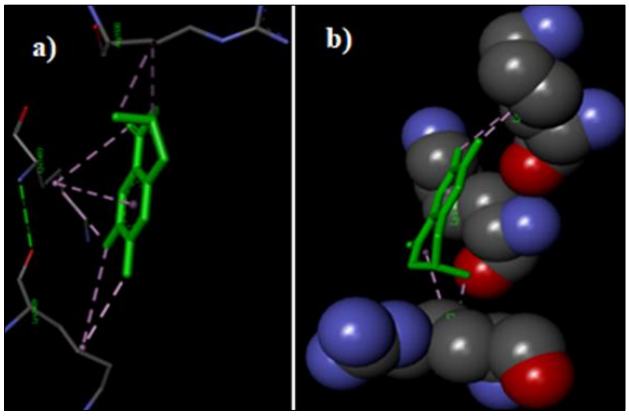


Fig 5: Molecular interaction between (3S)-3, 6, 7-trihydroxy-α-tetralone and polyketide synthase in Docking studies

Table 1: Antimicrobial activity of (3S)-3, 6, 7-trihydroxy-α-tetralone (TT) against some human pathogenic bacteria and yeast

Test organisms	TT		MICs values of Erythromycin / Itriconozole (µg/mL)
	ZOI at 100 µg/disc	MIC (µg/mL)	
E. coli	16.2±0.5	50	50
K. pneumoniae	23.5±0.4	12.5	25
P. vulgaris	21.3±0.9	25	25
P. aeruginosa	19.3±0.8	100	50
S. typhi	20.7± 0.4	25	62.5
S. aureus	23.8±0.5	25	12.5
S. faecalis	24.2±1.6	6.25	6.25
C. albicans	21.8±0.3	25	12.5
C. neoformans	19.2±0.8	50	50

Erythromycin and Itriconozole served as a positive control for bacteria and yeast, respectively. Data given are the mean of three replicates ± standard error ($p \leq 0.05$).

Table 2: Antifungal activity of (3S)-3, 6, 7-trihydroxy-α-tetralone (TT) and Indofil Z-78 against different field and storage fungi

Test fungi	TT		MIC of indofil Z-78 (µg/mL)
	%MI at 100 µg/mL	MIC (µg/mL)	
A. brassicicola	72.78±1.5	12.5	50
A. geophila	65.76±1.1	25	50
A. flavus†	38.34±0.9	100	100
A. fumigatus	51.56±0.8	50	100
A. ochraceous	59.78±0.5	25	25
A. tamarii	40.34±0.8	100	200
A. terreus	54.65±1.4	25	50
C. tetramera	78.34±1.7	12.5	12.5
F. equiseti	70.41±1.9	25	25
F. lateritium	68.76±0.9	50	50
F. oxysporum	54.23±1.1	100	50
F. udum	67.89±0.5	12.5	12.5
F. verticillioides‡	62.58±0.5	50	50
P. citrinum	43.78±0.4	100	100
P. expansum	34.56±0.7	200	200

Data given are the mean values of three replicates ± standard error ($p \leq 0.05$).
†Aflatoxin B_1 producing strain. ‡Fumonisin B_1 producing strain

Table 3: *In-vitro* and *in-vivo* efficacy of (3S)-3, 6, 7-trihydroxy-α-tetralone (TT) on mycelial inhibition, AFB_1 and FB_1 production from *A. flavus* and *F. verticillioides*

TT*	*A. flavus*			*F. verticillioides*		
	AFB_1 (*In vitro*) (µg/L)	AFB_1 (*In vivo*) (µg/kg)	%MI	FB_1 (*In vitro*) (mg/L)	FB_1 (*In vivo*) (mg/kg)	%MI
Control	1395.4±4.2	1467.6±5.8	0.0±0.0	82.6±3.2	51.6±3.6	0.0±0.0
25	725.6±4.5	1120.5±3.1	15.6±0.8	38.4±2.6	39.4±3.0	29.6±0.4
50	377.4±3.1	811.5±3.2	29.6±0.8	12.6±0.8	25.7±0.8	48.7±0.2
100	88.2±0.3	415.2±2.0	37.8±1.2	0.0±0.0	18.6±2.2	62.6±1.6
200	0.0±0.0	175.4±0.8	46.8±1.4	0.0±0.0	9.8±0.8	74.8±2.5

*TT concentration: *In vitro* evaluation (µg/mL) and *in vivo* evaluation (µg/g)
Data given are the mean values of triplicates ± standard error ($P \leq 0.05$).

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