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Isolation, purification and characterization of Trichothecinol-A produced by endophytic fungus *Trichothecium* sp. and its antifungal, anticancer and antimetastatic activities

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Abstract

Background: A total of 30 endophytic fungi (AAP-PS 1–30) were isolated from the medicinal herb *Phyllanthus amarus* and screened for the production of Trichothecinol-A. Out of all the endophytic strains screened for Trichothecinol-A production, the culture filtrate of AAP-PS-1 extracted with ethyl acetate yielded Trichothecinol-A extracellularly in appreciable amounts. Trichothecinol-A was purified, quantified and completely characterized by different standard chromatographic and spectroscopic techniques including reverse phase HPLC, 1D and 2D NMR spectroscopy, etc. The compound was tested for antifungal activity against filamentous fungi and yeast, apoptotic activity against B16F10 cells, anticancer activity against MDA-MB-231, HeLa and B16F10 cells as well as antimetastatic activity against MDA-MB-231 cell line.

Results: The endophyte producing Trichothecinol-A was identified as *Trichothecium* sp. by morphological, cultural and molecular methods. RP-HPLC analyses performed on a Waters model using a C₁₈ symmetry pack column with a flow rate of 0.5 ml/min and the eluting compounds were detected by a dual mode wavelength detector set at 220 nm and 240 nm. The 1D (¹H, ¹³C) and 2D NMR (COSY, NOESY, TOCSY, DEPT, ¹³C–¹H HMBC, ¹³C–¹H HSQC), ESI-MS, HRMS, IR and UV–vis show conclusively that the isolated compound was Trichothecinol-A. One liter of *Trichothecium* sp. yielded 4.37 mg of Trichothecinol-A. Trichothecinol-A exhibited antifungal activity against *Cryptococcus albidus* (NCIM 3372) up to 20 µg/ml. Cytotoxicity studies indicate that Trichothecinol-A causes 50% cell death at 500nM concentration in HeLa and B16F10 cells and induces apoptosis in later. Inhibition of wound migration assay performed on MDA-MB-231 cells reveals that 500nM of Trichothecinol-A was able to inhibit wound migration by 50% indicating its remarkable antimetastatic property.

Conclusion: The compound Trichothecinol-A has previously been isolated from *Trichothecium roseum* and characterized by various standard techniques. Anti-cancer studies conducted on Trichothecinol-A showed that it significantly inhibits cancer cell migration and can thus be developed as a new class of anti-metastatic drug. Here, we for the first time report the anti-metastatic as well as anti-fungal activity exhibited by Trichothecinol-A isolated by us from the endophytic fungus *Trichothecium* sp. of medicinal plant *Phyllanthus amarus*. Trichothecinol-A also exhibited apoptotic activity.

Keywords: Trichothecinol-A, *Trichothecium* sp., Antimetastatic, Antiproliferative, Cancer cell migration, Antifungal

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Background

Endophytic fungi are microorganisms which spend the whole or part of their life-cycle residing symbiotically within the healthy tissues of host plants, inter-and/or intra-cellularly and produce bioactive natural products or drugs and derivatives; meanwhile causing no damage or disease to their hosts [1].

Owing to the huge developments in the fields of genetic engineering, microbial fermentation technology, etc. the past two decades have seen a major increase in the number of researchers working hard to explore the endophytic fungal diversity, and better understand the relationships between endophytic fungi and their host plants, in an attempt to employ endophytes to obtain valuable compounds of plant origin without exploiting plant parts and to improve the productivity of the ones already being derived by optimizing fermentation conditions to reap benefits of abundant renewable supply [2,3]. Thus, endophytes hold tremendous promise as an alternative eco-friendly source for efficiently producing valuable bioactive compounds in the future with varied applications in both the research and applied fields of medicine, food industry, agriculture, pest management, etc.

Trichothecenes are a vast group of structurally and chemically related mycotoxins which have a strong impact on the health and well-being of humans, plants and farm animals. These mycotoxins are complex sesquiterpene secondary metabolites produced by fungi belonging to various species of *Fusarium*, *Trichothecium*, *Trichoderma*, *Myrothecium*, *Cephalosporium*, *Stachybotrys*, etc. These mycotoxins are highly stable even during the milling, processing and cooking of food stuffs and thus it's very difficult to get rid of them [4,5]. It is these trichothecenes which are responsible for the toxicity and spoilage of grains, fruits, vegetables, tubers and other vegetative products worldwide.

Trichothecenes are minute, amphiphatic molecules which are extremely powerful inhibitors of protein synthesis as these have their specific site of action located on a very crucial site on the ribosomal RNA (rRNA), thus interfering with the normal polypeptide chain initiation, elongation and termination [6,7]. Altering the biological activity of trichothecenes has helped in inducing anti-bacterial, anti-viral, insecticidal, phytotoxic, cytotoxic, anti-biotic and anti-tumour properties in the resultant molecules [8]. Konishi *et al.* reported the trichothecins A–C, all showing remarkable potential at inhibiting EBV-EA activation induced by TPA (12-O-tetradecanoylphorbol-13-acetate) in Raji cells [9]. Out of these, the most active compound, Trichothecinol-A also suppressed TPA-induced tumour promotion on mouse skin initiated with DMBA (7,12-dimethylbenz[a]anthracene) in a two-stage carcinogenesis experiment.

As part of our ongoing research for plant based drugs using endophytic fungi, we started the isolation and screening of endophytic fungi from medicinal herb *Phyllanthus amarus* for the production of various beneficial bioactive secondary metabolites and identified a fungal strain *Trichothecium* sp. which during the present investigation produced only antifungal secondary metabolites against *Aspergillus niger*. The compound was identified as sesquiterpenoid trichothecene mycotoxin and completely characterized as Trichothecinol-A. *P. amarus* was used as it is a known astringent, deobstruent, stomachic, diuretic, febrifugal, antiseptic, employed in dropsy and diseases of urinogenital system; its leaves are expectorant, diaphoretic; seeds are carminative, laxative, astringent to the bowels, tonic to the liver, and used as a remedy for bronchitis, earache, griping, ophthalmia and ascites [10-13]. Fresh roots and leaves of this plant have also been reported to be potent remedy for jaundice. All these features made *P. amarus* a very worthy candidate for our study.

The present work focuses on the isolation, purification and complete characterization of Trichothecinol-A from the endophytic fungus *Trichothecium* sp. isolated from *P. amarus*. The anti-fungal, anti-cancer (anti-proliferative), anti-metastatic and apoptotic activities of trichothecinol-A revealed its class-apart potential to be developed as a drug for each mentioned field.

Results and discussion

Isolation, purification and identification of endophytic fungus producing Trichothecinol-A

Total thirty endophytic fungi were isolated from leaves and stems of *P. amarus* and only one culture (AAP-PS-1) isolated from leaves was found to produce Trichothecinol-A extracellularly. Trichothecinol-A producing fungal culture was identified using molecular and morphological approaches. Morphological observations revealed that conidiophores were long, slender, simple, bearing conidia singly, apically, sometimes held together in groups or chains; conidia were hyaline, 2-celled and ovoid to ellipsoid.

Amplification of fungal genomic DNA by primers ITS1 and ITS4 yielded 650 bps fragment (Accession No. JN603460). The analysis of sequence revealed 99.9% identity with that of *Trichothecium roseum*. Phylogenetic analysis of this strain AAP-PS-1 based on ITS sequences exhibited 98% nucleotide sequence similarity with *Trichothecium roseum*, the phylogenetically closest validated genus (Figure 1). The ITS sequence analysis and homology alignment of isolate AAP-PS-1 using BLAST and Clustal-W programme respectively revealed similarity with *T. roseum*. Also, morphological and cultural studies confirm that the isolate belongs to genus *Trichothecium* [14].

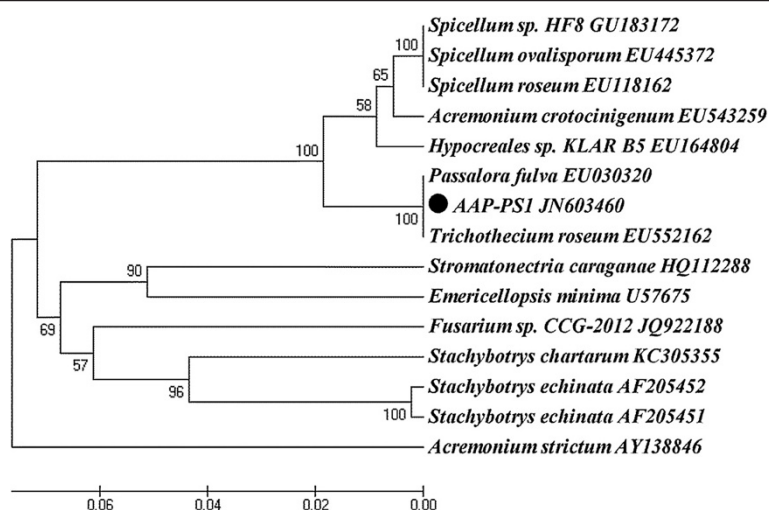


Figure 1 Phylogenetic tree highlighting the position of *Trichothecium* sp. within the closely related genus. GenBank accession numbers are indicated in parentheses. The scale bar represents a 5% nucleotide sequence divergence.

Purification, quantification and complete characterization of Trichothecinol-A

Purified Trichothecinol-A eluted at retention time of 34 minutes when loaded on analytical C_{18} symmetry pack column with a flow rate of 0.5 mL/min. EtOAc extract of one litre of filtrate of *Trichothecium* sp. fermented in modified S7 medium yielded 4.37 mg of Trichothecinol-A. Maximum UV absorption was recorded at 251 nm in ethyl acetate. The mass spectrum showed molecular ion at m/z 349.57 and at m/z 371.28 attributing to $M + H$ and $M + Na$ ions of Trichothecinol-A respectively. The HRMS spectrum showed molecular ion at 348.0683 attributing to ions of Trichothecinol-A ($C_{19}H_{24}O_6$). The FTIR spectrum showed characteristic bands of Trichothecinol-A at 1679, 1721 and 3452 cm^{-1} . (HPLC details, UV-visible spectroscopy measurements, FTIR spectroscopy measurements, ESI-MS details are included as supporting information (Additional file 1: Figure S1 – S4).

The ^1H NMR spectrum of the Trichothecinol-A showed the presence of 24 protons with the olefinic peaks in side chain ester linkage at $\sim 6.48\delta$, 5.89δ and the ring olefinic proton at $\sim 6.60\delta$. The methyl groups (Me 14, 15, 16 and 20) appeared at $\sim 0.76\delta$, $\sim 1.03\delta$, $\sim 1.83\delta$ and $\sim 2.17\delta$, respectively. The protons of epoxide methylene group (13a, 13b) appeared as doublets at $\sim 2.81\delta$ and $\sim 3.08\delta$. The other methylene group (7a, 7b) resonated at $\sim 2.33\delta$ and $\sim 2.96\delta$. The most noticeable feature of the ^1H spectrum is the presence of a broad peak at $\sim 3.48\delta$ which belongs to OH group proton. ^{13}C NMR spectrum showed the presence of 19 carbons which included two carbonyls (198.52δ , 168.05δ), four olefinic (148δ to 119δ) and thirteen sp^3 carbons in the region 85δ to 5δ . The assignments

of the ^1H and ^{13}C spectra are presented in Table 1 and Figure 2. The spectral details are included as Supporting information (Additional file 1: Figures S5-S12, Tables S1 and S2). The NMR spectral features are found to match very well with the one reported by Konishi *et al.* [9].

Antifungal activity of Trichothecinol-A

In contrary to earlier reports [9], we for the first time demonstrated antifungal activity of Trichothecinol-A against a range of filamentous fungi and yeast. Trichothecinol-A exhibited moderate activity against yeast *Cryptococcus albidus* var *diffluens* NCIM 3372 up to $20\text{ }\mu\text{g/mL}$. MIC and IC_{50} values were determined as described below (Table 2). Dose response curves of Trichothecinol-A are included as supporting information (Additional file 1: Figure S13).

Trichothecinol-A exhibit cytotoxicity at nanomolar concentrations against B16F10 and HeLa cells and effectively inhibit cell migration in MDA-MB-231 cells

Cytotoxicity studies indicated that B16F10 and HeLa cells show enhanced sensitivity to Trichothecinol-A compared to MDA-MB-231 cells. Trichothecinol-A induced about 50% cell death in HeLa and B16F10 cells compared to about 25% in MDA-MB-231 cells at 500 nM concentrations (Figure 3).

The effect of Trichothecinol-A on B16F10 cell death was performed by cell cycle analysis. Briefly, cells were treated with Trichothecinol-A in a dose of 0–5 μM for 12 and 24 h respectively and then stained with PI (propidium iodide). The DNA fluorescence histogram of PI-stained cells revealed a distinct quantifiable region beyond G1 peak (Figure 4).

Table 1 ^1H and ^{13}C NMR assignments* of Trichothecinol-A

Position	δ ^1H (J_{HH}) Hz	δ ^{13}C
2	3.80(4.95)	79.31
3	4.27(4.95,3.00)	78.93
	OH- 3.48;br	
4	4.93(3.00)	83.38
5		48.87
6		44.71
7a	2.33(15.18, 1.54)	42.06
7b	2.96, (15.18,1.10)	
8		198.52
9		137.52
10	6.60(5.80,1.54)	137.27
11	4.40(5.80)	71.02
12		64.54
13a	2.81,(3.90)	46.63
13b	3.08,(3.90)	
14(CH ₃)	0.76, s	6.00
15 (CH ₃)	1.03, s	18.46
16 (CH ₃)	1.83, s	15.43
17		168.05
18	5.89(11.50,1.80)	119.90
19	6.48(11.50,7.30)	147.26
20 (CH ₃)	2.17(1.80,7.30)	15.65

* Numbering of various atoms are given in Figure 2.

Significant increase in the sub G_0 population was observed in Trichothecinol-A treated cells in a time dependent manner. 500 nM of Trichothecinol-A had 12.7% sub G_0 population as compared to 3.88% in vehicle control in 12 h. However, cells treated with 500 nM of Trichothecinol-A for 24 h resulted in an

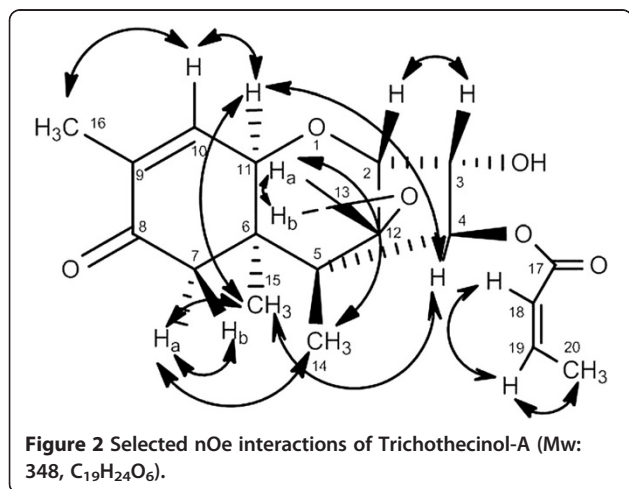


Table 2 MIC and IC₅₀ values of Trichothecinol-A

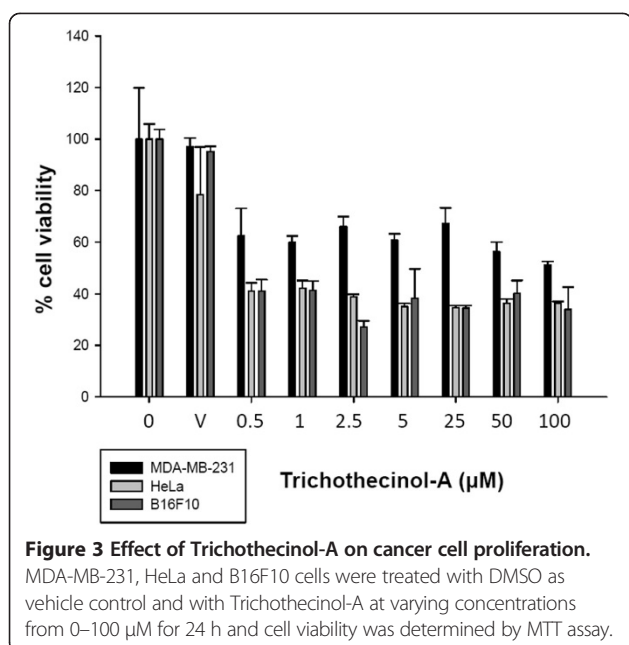
Sr.No.	Organism ⁵	IC ₅₀ (μg/mL)	MIC (μg/mL)
1	<i>Saccharomyces cerevisiae</i> NCIM 3045	17.16	36
2	<i>Cryptococcus albidus</i> var <i>diffluens</i> NCIM 3371	4.08	36
3	<i>Cryptococcus albidus</i> var <i>diffluens</i> NCIM 3372	2.5	20
4	<i>Fusarium oxysporum</i> NCIM 1043	7.16	>100
5	<i>Aspergillus flavus</i> NCIM 535	24.3	>100
6	<i>Trichoderma reesei</i> NCIM 992	15	90
7	<i>Penicillium expansum</i> NCIM 939	6.61	50
8	<i>Trichoderma viride</i> NCIM 1051	36.6	>100
9	<i>Paecilomyces varioti</i> NCIM 1217	8.69	70
10	<i>Aspergillus niger</i> NCIM 773	9.12	>100

⁵NCIM corresponds to National Collection of Industrial Microorganisms, Pune, India.

increase of apoptotic fraction to 52.96% versus 16.68% in vehicle control.

Enhanced migratory capacity is one of the hall marks of highly invasive tumor cells. Inhibition of cancer cell migration could be an effective means of preventing cancer metastasis, thereby enabling confinement of primary tumors in manageable form, making surgical options more viable [15]. During opening phase of the metastatic cascade, cancer cells penetrate into surrounding tissues and blood vessels and it has been shown to be the rate limiting step in an experimental model [16]. Therefore, inhibition of the cell migration represents a potential therapeutic approach for the treatment of tumor metastasis. Here, we studied the inhibitory effect of Trichothecinol-A in MDA-MB-231 cell migration at various concentrations. Percentage of wound closure was determined by the difference in area covered by migrated cells in control versus treated with Trichothecinol-A at 0 or 18 h. The results show that the 500 nM of Trichothecinol-A was able to inhibit wound closure by 50% where as 2.5 and 5 μM concentrations of Trichothecinol-A inhibited migration up to 75% as compared to vehicle control (Figure 5a & b).

Because, the common approach of chemotherapy is to decrease the growth rate (cell division) of the cancer cells, the side effects are seen in bodily systems that naturally have a rapid turnover of cells including skin, hair, gastrointestinal and bone marrow. These healthy, normal cells also end up damaged by the chemotherapy program. Hence, it's reasonable to look out for compounds with antimetastatic activity which could be further developed as lead against cancer metastasis. As, Trichothecinol-A is showing promising antimetastatic activity against human breast carcinoma (MDA-MBA-231) and antiproliferative activity against skin carcinoma (B16F10) at nanomolar concentration, therefore, it can be used as scaffold in drug



discovery programme to synthesize analogues with better specificity and favourable pharmacological properties. Further efforts are needed to modify the compound to remove sites of non specific toxicity and understand the mechanism by which this compound inhibits cancer cell growth and migration.

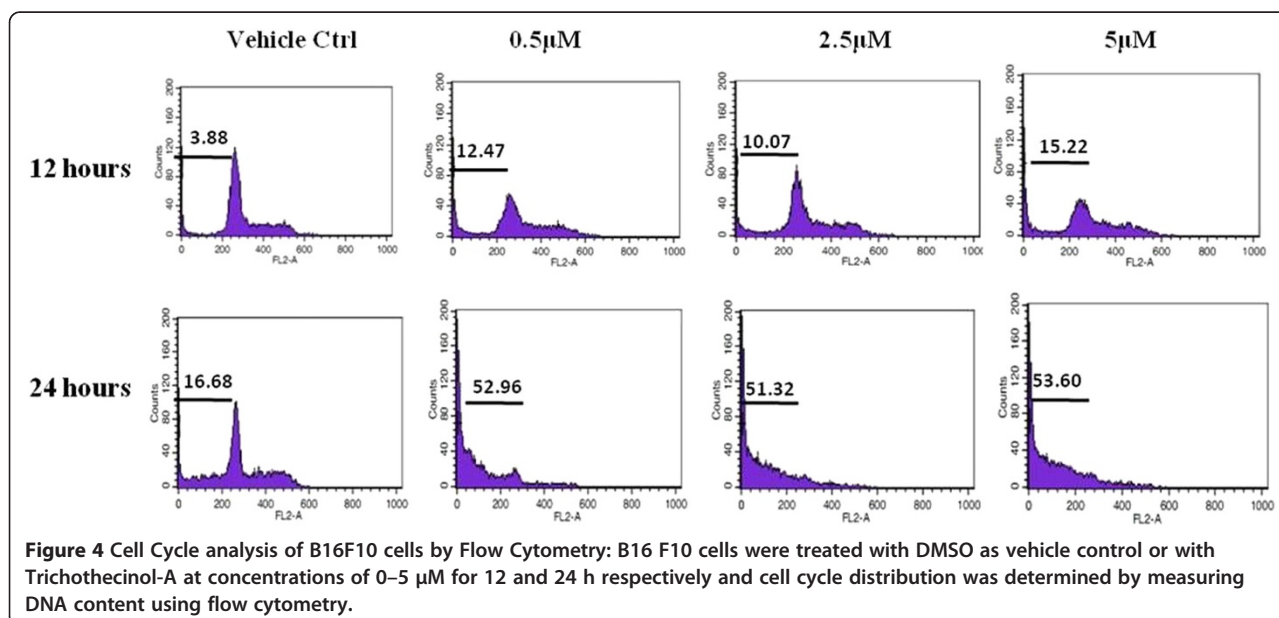
Materials and methods

Isolation, purification, identification and screening of endophytic fungi for the production of Trichothecinol – A
 A total of 30 endophytic fungi (AAP-PS 1–30) were isolated from medicinal herb *P. amarus* as described elsewhere

[17–19]. Each fungal culture was checked for purity and transferred to agar slants by hyphal tip as well as single spore isolation method [17,20]. Production of Trichothecinol-A by endophytic fungi was studied by a two stage fermentation procedure [21]. The flasks containing MGYP media (Malt extract 0.3%, Glucose 1%, Yeast extract 0.3%, Peptone 0.5%) were inoculated with agar plugs of mycelium from 7 day old slants. The inoculated flasks were incubated at 28°C on rotary shaker at 180 rpm for 6 days. These cultures were used as seed culture (first stage). For Trichothecinol –A production, 20 mL seed culture was transferred to 500 mL Erlenmeyer flask containing modified S7 medium [21]. The flasks were incubated at 28°C for 30 days as stationary culture (second stage). After incubation, the cultures were harvested and passed through sterile cotton to separate mycelial mat from culture filtrate. The culture filtrate was lyophilized and extracted thrice with equal volumes of ethyl acetate. The extracts were pooled and dried with anhydrous sodium sulphate and concentrated at 40°C under reduced pressure to yield crude extract. The antifungal activity and a method developed by Sorenson *et al.* was used to detect Trichothecinol-A in crude extract [22]. The culture (AAP-PS-1) producing Trichothecinol-A was used for further studies.

Identification of endophytic fungus producing Trichothecinol-A by morphological and molecular methods

Identity of endophytic fungal culture (AAP-PS-1) producing Trichothecinol-A was established by using morphological and molecular approaches. For studying the morphological features, the fungus was grown on potato dextrose agar (potato 25%, dextrose 2%, agar 2%).



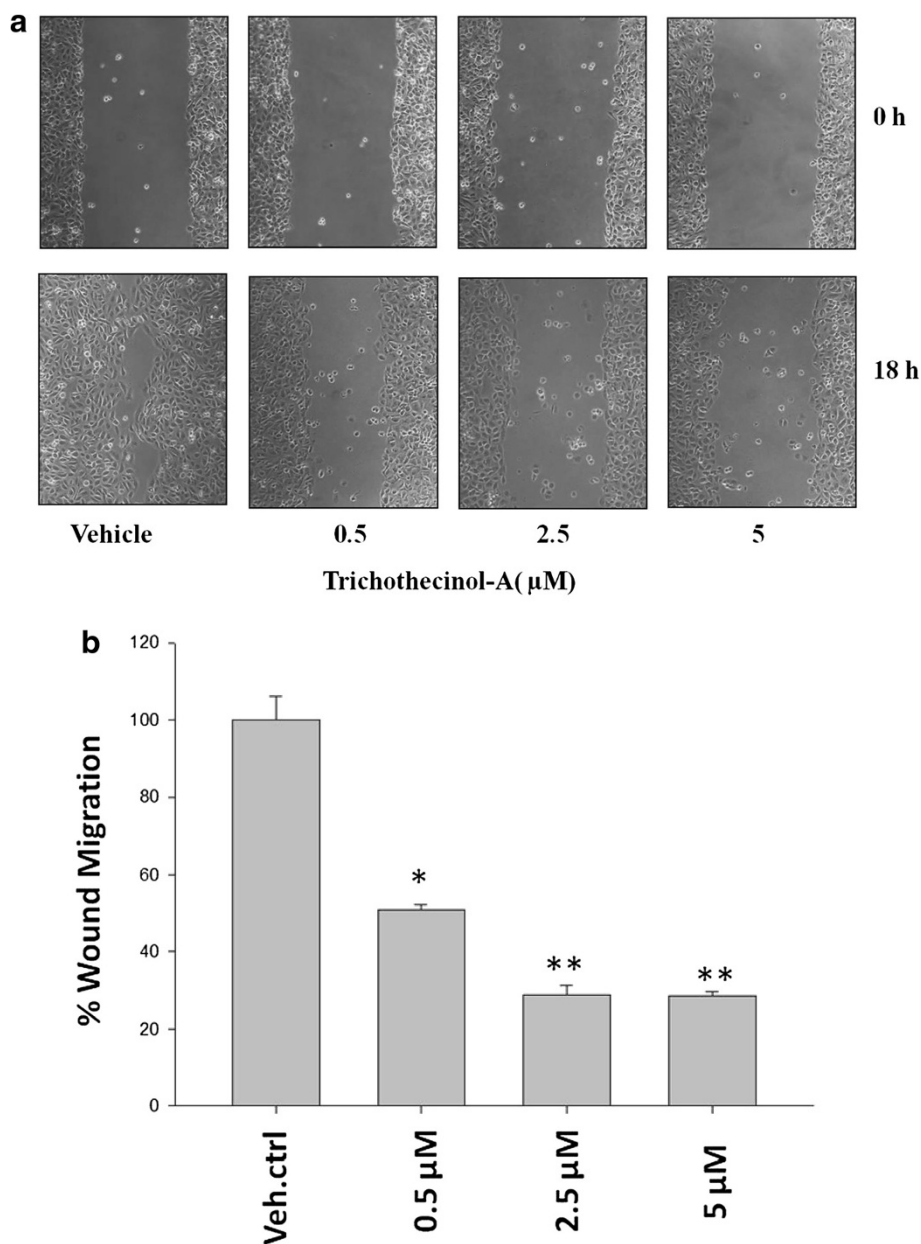


Figure 5 Inhibition of cell migration by Trichothecinol-A: **a.** MDA-MB-231 cells were treated with DMSO as vehicle and Trichothecinol-A at concentrations of 0–5 μM for 18 h and photographs were taken. **b.** The areas of migrated cells were quantified and represented as bar graph. * $p = .0013$, ** $p = .0003$ vs. vehicle control.

Morphological characteristics of the fungus like mycelia, conidiophores and conidia were microscopically studied (Carl Zeiss Axiovert 25 Inverted microscope and Nikon Eclipse E200). The genomic DNA was isolated by salting out method with little modification [23]. Sonication for a period of 5 min was done before lysozyme treatment. ITS region of genomic DNA was amplified using ITS1 TCCGTAGGTGAACCTGCGG (forward) and ITS4 TCCTCCGCTTATTGATATGC (reverse) primers. PCR products were eluted from agarose gel by protocol

described in Axygen™ gel elution kit, Biosciences USA. The purified PCR product was ligated with pGEM-T vector and transformed into competent *E.coli* XL-1 cells [24]. Plasmid containing the insert was isolated using alkaline lysis method [24]. The cloned fragments were sequenced using Sangers dideoxy method using ABI prism big dye terminator cycle sequencing kit from ABI system [25]. The sequence obtained was further analysed for its homology by using online tool nucleotide BLAST. The multiple sequence alignments were carried out using

CLUSTAL-W and phylogenetic inferences were obtained using the maximum-likelihood method within MEGA program. The bootstrap of consensus tree was inferred from 500 replicates. The evolutionary distances were computed using the Kimura2 parameter. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). *Acremonium strictum* were used as an out-group.

Purification and quantification of Trichothecinol-A

Trichothecinol-A was purified using preparative TLC and RP-HPLC. TLC plates coated with 0.5 mm thickness of silica were used for preparative TLC. Approximately 0.5 mL of crude sample dissolved in methanol was loaded on to plate and developed in hexane: ethyl acetate (2:1) solvent system. The developed plates were air dried and exposed to iodine vapours. Individual bands were scrapped, eluted with ethyl acetate, concentrated *in vacuo* and tested for antifungal activity against *Aspergillus niger* by agar well diffusion method. Out of eight bands, second and fifth band showed good antifungal activity. The second band was further processed for purification by analytical RP HPLC. HPLC analysis was performed on a Waters model using a C₁₈ symmetry pack column with the flow rate of 0.5 mL/min and eluted with a 40 minutes gradient of water: acetonitrile (95: 5). The Trichothecinol-A was detected by a dual mode wavelength detector set at 220 nm and 240 nm.

Quantification of Trichothecinol-A by HPLC

Stock solution of different concentrations (1 mg/ml, 3 mg/ml, 5 mg/ml and 10 mg/ml) of a purified Trichothecinol-A was prepared in HPLC grade acetonitrile. 10 µl of each stock solution was injected in HPLC to generate a standard graph of peak area v/s concentration. Then, the known volume of (10 µl) crude dissolved in HPLC grade acetonitrile containing unknown concentration of Trichothecinol-A was analysed by HPLC and the peak area of Trichothecinol-A containing peak was compared to standard graph to calculate its concentration.

Complete characterization of Trichothecinol-A

UV-visible spectroscopy measurements were carried out on Jasco dual beam spectrophotometer (model V- 570) operated at resolution of 1 nm. Molecular mass of the compound was determined by M/S Applied Biosystems API QSTAR pulsar (ESI-MS) mass spectrometer. HRMS analysis was carried out using mass spectrometry instrument (model – Autoconcept) by direct injection probe with resolving power of 6000. FTIR spectroscopy measurements of purified compound taken in KBr pellet were carried out using a Perkin-Elmer spectrum one instrument. Spectrometer was used in the diffuse reflectance

mode of resolution 2 cm⁻¹. All the NMR measurements were carried out on Bruker AV 500 spectrometer operating at 500 MHz and 125 MHz, respectively for ¹H and ¹³C. ~10 mg of the compound was dissolved in CDCl₃ in a 5 mm NMR tube and the ¹H, ¹³C, COSY, NOESY, TOCSY, ¹³C–¹H HSQC and ¹³C–¹H HMBC experiments were performed under standard conditions using a 5 mm BBFO probe at ambient temperature (~ 25°C). Chemical shifts in the ¹H spectra were referenced to residual CHCl₃ peak (7.26 ppm), while ¹³C spectra were referenced to CDCl₃ (77.0 ppm).

Antifungal assay

The purified compound was analyzed for antifungal activity against different plant pathogenic, saprophytic fungi and yeast. Antifungal assay was performed in microtiter plates as reported earlier [26,27]. Fungal spores were removed from a 4 days old culture, transferred to YM broth (Glucose 1%, Malt extract 0.3%, Yeast extract 0.3%) and adjusted to 2x10⁵ spores or cells/mL. The compound was diluted in DMSO to get desired concentrations. 50 µL aliquots of spores were mixed with different concentrations of compounds (10 to 100 µg/mL in 10 µL of DMSO). Final volume was adjusted by adding 140 µL YM media. Total assay volume was 200 µL. The plates were incubated at 28°C for 48 h.

OD 600 measurements

The OD 600 measurements were carried out on microtiter plate reader (Bio-Rad xMark). The MIC was determined as the lowest concentration of Trichothecinol-A that completely inhibits visible growth of the microorganism. The dose response curves were obtained by plotting concentration of compound v/s% growth inhibition. The IC₅₀ values were determined as the concentration of Trichothecinol-A that could show 50% of maximum inhibition.

Cell culture and reagents

Human breast adenocarcinoma (MDA-MB-231), human cervical cancer (HeLa) and murine melanoma (B16F10) cell lines were obtained from American Type Culture Collection and were grown in L-15, DMEM and RPMI media respectively, supplemented with 10% fetal bovine serum and 100 U/mL Penicillin and Streptomycin (Invitrogen). All cells were grown in humidified atmosphere with or without 5% CO₂ and 95% air at 37°C. Tetrazolium dye and Propidium Iodide was purchased from Sigma.

Cell viability assay

MDA-MB-231, HeLa and B16F10 cells (2x10⁴) were seeded in 96 well plates. Cells were treated with Trichothecinol-A for 24 h with indicated concentration.

Treatment was terminated by removing media and MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (0.5 mg/ml) was added. After 4 h, crystals were dissolved in isopropanol and A₅₇₀ was recorded.

Flow cytometry analysis

Flow cytometry experiments were performed as described earlier [28]. Murine melanoma B16F10 cells were treated with indicated concentrations of Trichothecinol-A in RPMI media supplemented with 10% fetal bovine sera for 12 and 24 h. DNA analysis was done and cell undergoing apoptosis were determined following propidium iodide staining (FACS Calibar, Becton Dickinson).

Wound assay

Wound assay was performed with post confluent MDA-MB-231 cells as described [29]. Briefly, uniform sized wounds were made. MDA-MB-231 cells were treated with 2.5 μM of Trichothecinol-A. DMSO was used as vehicle control. After 18 h, photographs were taken and the area of migrated cells was quantified using Image Pro Plus 6.0 software (Nikon).

Statistical analysis

The data reported in cytotoxicity and cell migration experiments are expressed as mean ± S.E. Statistical differences were determined by Student's *t* test. The *p* value < 0.05 was considered significant.

Conclusion

We for the first time have isolated a sesquiterpene viz. Trichothecinol-A produced by an endophytic fungus *Trichothecium* sp. isolated from medicinal herb *Phyllanthus amarus*. The compound was purified and characterized by using different chromatographic and spectroscopic techniques. Being non proteinaceous in nature, this compound holds the promise of potent antiproliferative and most importantly antimetastatic lead, if suitably modified by employing combinatorial chemistry approaches to enhance its drug like properties. Trichothecinol-A exhibits appreciable antimetastatic activity against human breast carcinoma (MDA-MBA-231) and antiproliferative activity against skin carcinoma (B16F10) at nanomolar concentrations. Also, Trichothecinol-A showed good antifungal activity against *Cryptococcus albidus* var *diffluens* (NCIM 3371 and 3372) and *Penicillium expansum* (NCIM 939). Therefore, the above compound could be used as scaffold in drug discovery programmes to synthesize effective and more specific analogues which can be further developed as drugs. Further studies are required to understand the mechanism by which this compound inhibit cancer cell growth and metastasis.

Additional file

Additional file 1: Figure S1. HPLC was carried out using waters separation module. **Figure S2:** UV-visible spectroscopy measurements were carried out on Perkin Elmer spectrophotometer (model lambda - 750) operated at resolution of 1nm. Maximum Uv absorption was observed at 251nm in ethyl acetate. **Figure S3:** FTIR spectroscopy measurements of purified compound taken in Kbr pellet were carried out using a Perkin-Elmer spectrum one instrument. **Figure S4:** Molecular mass of the compound was determined by M/S Applied Biosystems API QSTAR pulsar (ESI-MS) mass spectrometer. **Figure S5:** 500 MHz ¹H NMR spectrum of Trichothecinol -A in CDCl₃. **Figure S6:** 125 MHz ¹³C NMR spectrum of Trichothecinol -A in CDCl₃. **Figure S7:** DEPT 135 spectrum of Trichothecinol -A, where the CH₂ carbons appear with a negative phase. **Figure S8:** 500 MHz COSY spectrum of Trichothecinol -A in 99.8% deuterated CDCl₃. The cross peaks indicates scalar couplings. **Figure S9:** 500 MHz NOESY spectrum of the Trichothecinol-A in 99.8% deuterated CDCl₃. A mixing time of 1 second was employed for the measurement. **Figure S10:** 500 MHz TOCSY correlations of the Trichothecinol-A in CDCl₃. **Figure S11:** ¹H-¹³C HSQC spectrum of the Trichothecinol-A with the ¹H and ¹³C DEPT spectra shown as projections in the F₂ and F₁ dimensions, respectively. **Figure S12:** ¹H-¹³C HMBC correlations of the Trichothecinol-A. **Figure S13:** Dose response curves of Trichothecinol-A. **Table S1:** Homo nuclear correlations observed in Trichothecinol-A. **Table S2:** Major ¹H-¹³C correlations observed in Trichothecinol-A.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

RT isolated, purified and characterized the compound and helped in preparing the manuscript. PA assisted during antifungal assay. DP and PRR helped in evaluation of NMR measurements. RR, GS and GK carried out cytotoxicity experiments with cell lines. AA conceived the study, designed the experiments and wrote the manuscript. All authors have read and approved the final manuscript.

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