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RESEARCH ARTICLE

Identification, GC-MS analysis and antibacterial activity of endophytic fungi isolated from *Trigonella foenum-graecum* leaf

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Abstract

The present study was designed to identify the endophytic fungi isolated from leaves of *Trigonella foenum-graecum* (local name: Fenugreek or Methi) from Bangladesh, followed by a GC-MS analysis of fungal culture filtrates to explore major components present in the ethyl acetate extract and finally to assess their putative antibacterial activity. Two pure fungal extracts, entitled TFLE-1 and TFLE-2 were isolated from the leaf of *T. foenum-graecum* followed by extraction with ethyl acetate. The fungal strains, TFLE-1 and TFLE-2 were identified as *Fusarium lichenicola* and *Trichoderma euskadiense* respectively as per microscopic and molecular identification. GC-MS analysis revealed the presence of a total of 47 bioactive compounds among which 2-((4-methylpentan-2-yloxy) carbonyl) benzoic acid (15.33%), pyrrolo[1,2-a]pyrazine-1,4-dione,hexahydro-3-(2-methylpropyl) (12.42%), benzeneethanamine, 4-benzyloxy-2-fluoro-beta-hydroxy-5-methoxy (3.45%) for TFLE-1 and pentatriacontane (8.61%), diethyl trisulfide (2.90%) and benzene, 3-heptynyl (2.78%) for TFLE-2 were the major ones. On antibacterial activity, by disc-diffusion method, the fungal strain *T. euskadiense* (code: TFLE-2) showed prominent antibacterial activity against both gram (+) and gram (-) multiple-resistant bacteria while compared to the standard drug kanamycin; values were found statistically significant ($p \leq 0.05$). The findings of the study indicated presence of potentially bioactive endophytic fungal extracts obtained from the leaves of *T. foenum-graecum* cultivated in Bangladesh.

Keywords

antibacterial activity; endophytic fungi; GC-MS analysis; *Trigonella foenum-graecum*

Introduction

Trigonella foenum-graecum L. (local name: Fenugreek or Methi) is an annual herbaceous plant found in various regions of Asia, Africa and Europe (1). The plant is a member of the Fabaceae family. Though originated in Northern Africa and Indian subcontinent, the plant is also extensively grown in Australia, Argentina, South Asia, Europe and Northern Africa. In many countries, Fenugreek leaves and seeds are utilized as a spice and component for food preparation. It is employed in nutraceuticals and physiological applications as well. In recent years, Fenugreek has gained popularity as a food stabilizer and emulsifier due to its high fibre protein content. Being one of the earliest recognized therapeutic herbs in the world, Fenugreek seeds and leaves are used to treat a variety of illnesses (2). *T. foenum-graecum* leaves are widely used to produce extracts and powder

for medicinal applications including hypoglycaemia, hypolipidemia and hypocholesterolemia. Additionally, *T. foenum-graecum* has been linked to anti-cancer, anti-parasitic and antimicrobial activities (3). These findings suggest that the Fenugreek plant parts contain a wide range of bioactive substances.

Using various techniques, researchers have identified and extracted numerous bioactive components present in Fenugreek. Chromatography coupled with mass spectrophotometry is one of the most popular techniques for isolation and characterization. Isoorientin, vitexin, trigonelline, isovitexin and orientin are just a few examples of the bioactive compounds that have been identified using the high performance liquid chromatography (HPLC) technology (4). Presence of polyphenols was identified from Fenugreek extract by HPLC in conjunction with negative ion electrospray ionization mass spectrometry (5). In a separate investigation, the chemical contents of Fenugreek seed oil were determined using FT-IR and gas chromatography coupled to mass spectrometry (GC-MS) investigations. The most prevalent substances were 4-pentyl-1-(4-propylcyclohexyl)-1-cyclohexene, methyl linoleate, palmitic acid and linoleic acid (6). However, profiling of the secondary metabolites isolated from endophytic fungi inhabited in Fenugreek leaf, especially planted in Bangladesh, has not yet investigated in a comprehensive way. We know well that difference in geographic conditions, nature of soil and climate could make a difference in composition (7).

Fungal endophytes are natural source of novel bioactive compounds (8, 9). The potential of endophytic fungi in the generation of bioactive metabolites, including the multibillion-dollar anticancer drug taxol, pestaloside, torreyanic acid (10) and a significant number of naturally occurring bioactive compounds (11, 12) has encouraged recent researchers to find out more and more promising lead compounds from this natural source (13, 14). The main objectives of the present research work were to isolate, purify and identify the endophytic fungal strains from the leaf of *T. foenum-graecum*, to perform a metabolite profiling of the fungal extracts by GC-MS analysis and to assess the putative antimicrobial activity of the fungal endophytes.

None of the isolated fungal endophytes, *Fusarium lichenicola* (15–17) and *Trichoderma euskadiense* (18) was novel one; however, these fungi isolated from the plant part of Fenugreek is for the first time ever, as far as we reviewed. Different endophytic fungal strains of *Fusarium* and *Trichoderma* isolated from different plants have been studied earlier leaving the isolated fungal endophytes of present study with minor importance.

Materials and Methods

Collection and identification of plant

Trigonella foenum-graecum leaves were collected from the BCSIR grounds in Dhaka and identified by a taxonomist at the Bangladesh National Herbarium, where a voucher specimen (DACB-55763) has been deposited for future reference.

Endophytic fungi isolation and purification

Endophytic fungal strains were isolated from leaves of *T. foenum-graecum* as per procedure, with slight modification (19). Fresh leaves were first washed with running tap water for 10 min, followed by treatments with 2% sodium hypochlorite for 3–4 min, a wash with 70% ethanol for 1 min and 3 rinses with sterile distilled water. On sterile filter paper, extra moisture was wiped. The surface-sterilized segments of length 1 cm × 1 cm were cut and put in petri dishes containing water agar (WA) medium supplemented with streptomycin (100 mg/L). Leaves were subsequently placed onto petri dishes containing WA media supplemented with streptomycin at a concentration of 200 g/mL. The culture process involved placing the petri dishes in a refrigerated incubator (Froilabo BRE 120, France) and maintaining a temperature of 28°C for duration of 21 days. Following visualization, hyphal tips were placed onto potato dextrose agar (PDA) media, and pure fungal colonies were obtained using a serial dilution procedure.

Morphological identification

Slides from mature fungal cultures were stained with lactophenol cotton blue. The specimens were observed using a microscope (Leica, Version 4.12.0, Switzerland), equipped with a 40x objective lens and a 0.65 numerical aperture, under a bright-field and phase contrast illumination, in order to identify the genus of endophytic fungal isolates. As the fungi grew to full maturity, various morphological traits, including growth pattern, hyphae, colony and medium color, surface texture, margin characteristics, sporulation, aerial mycelium and size plus color of the conidia etc. were examined sporadically and compared to the common taxonomic key (20).

Molecular identification

Fungal endophytes isolated from leaves were subjected to molecular identification and characterization as per standard (21). The internal transcribed spacer or ITS and 5.8s region sequences were amplified and sequenced. Here, ITS4 (5-TCCGTAGGTGAACCTGCGG-3 and ITS5 (5-TCCTCCGCTTATTGATATGC-3), the forward and reverse primers, both from Invitrogen-USA were used. The DNeasy Plant Minikit was used to isolate the DNA of the various fungi complying with the guidelines provided by the manufacturer (QIAgen, USA). The extracted DNA was then amplified using the HotStarTaq Master Mix Kit from QIAgen (USA) in a polymerase chain reaction (PCR). 5–10 ng of DNA from the genome, 1 M of each ITS4 and ITS5 primer and 1 U of Hot StarTaq Polymerase all were included in each PCR reaction mixture. These components were combined with 50 L total of reaction mixture using the HotStarTaq Master Mix Kit and DNA template. The prepared combination was then run through the scheduled PCR cycle as follows on the thermal cycler (BioRad, USA): 1 min of denaturation at 95°C, 1 min of annealing at 56°C, 1 min of extension at 72°C and 10 min of final extension at 72°C. Electrophoresis of 2% agarose gel at 75 V for 60 min in 1x TBE buffer was performed. An appropriate size, 550 bp stained fragment was retrieved

after the agarose gel was stained with 1% ethidium bromide. The perfect PrepGel Cleanup Kit (Eppendorf, USA) was used to purify the PCR product in line with the manufacturer's recommendations. The amplified pure fungal DNA (PCR product) was electrophoretically sequenced using an ABI370X1 DNA analyzer (Applied Biosystems, USA) and the Big Dye Terminator v3.1 cycle sequencing kit. The base sequences were compared with Genbank, a publicly accessible database, using the BLAST sequence match techniques. On the basis of 5.8S-rRNA-ITS sequences, evolutionary distances were calculated using the 'Maximum Composite Likelihood' approach. Phylogenetic trees for each sequence were generated using the 'Neighbor-Joining' (NJ) method with bootstrap support based on 1000 iterations.

Extraction of endophytic fungi

All of the isolated pure fungal strains were grown on PDA using roughly 500 mL of media. After the completion of 28 days incubation period at 28ºC, the culture media (PDA) along with the fungal mycelia was soaked into ethyl acetate at room temperature. Fungal mass was filtered three times for a 5-day interval. Through a rotary evaporator, the fungal extracts were concentrated into solid residue. The extracts were put into a weighted vial after evaporation.

GC-MS analysis

Gas chromatography-mass spectrometry analysis was carried out with Clarus®690 gas chromatograph (PerkinElmer, CA, USA) using a column (Elite-35, 30 m length, 0.25 mm diameter, 0.25 µm thickness of film) equipped with Clarus® SQ 8C mass spectrophotometer (PerkinElmer, CA, USA). Sample (1 µL) was injected (splitless mode) and pure helium (99.999 %) was used as a carrier gas at a constant flow rate (1 mL/min) of 35-min run time. The sample was analysed in EI (electron ionization) mode at high energy of 70eV. Column oven temperature was set at 60ºC (for 0 min), raised at 5ºC per minute to 240ºC and maintained for 4 min (22) while the inlet temperature remained constant at 280°C. The sample compounds were identified comparing to the National Institute of Standards and Technology (NIST) database.

Antibacterial activity assessment

The disc diffusion method, with a minor modification, was used to test the antibacterial activity (23). *Bacillus megaterium* (ATCC 25918) and *Staphylococcus aureus* (ATCC 25923), 2 gram-positive pathogenic bacterial strains as well as *Escherichia coli* (ATCC 28739) and *Salmonella typhi* (ATCC 19430), 2 gram-negative pathogenic bacterial strains were used as test organisms in the antibacterial study. The sample-treated discs were inoculated with bacteria in nutrient agar (NA) media. At 37°C, the plates were incubated for 24 h. The degree to which various microbial species were sensitive to fungal extracts (100 µg/disc) was determined by measuring the diameter of the inhibition zone (mm) around the sample-treated disc and comparing it to the standard drug kanamycin (30 µg/disc).

Statistical analysis

Each test was run 3 times in total. IBM SPSS version 29.0.1.0 was used to compute mean±SD and SE for the data, which was determined to be the average of 3 experiments (n=3). Statistical significance ($p \leq 0.05$) was also determined as per one-way ANOVA test.

Results and Discussion

Morphological and molecular identification

Following separation and purification, the isolates of fungi were identified to the genus level utilizing microscopic examination and morphological arrangement. Two endophytes named TFLE-1 and TFLE-2 were isolated from the leaf of *Trigonella foenum-graecum*. The morphological properties of the media or hyphal morphology of the fungal culture were used to identify the strains (Fig. 1). On the basis of morphological analysis, endophytic fungi were assigned to the genera *Fusarium* and *Trichoderma* respectively for TFLE-1 and TFLE-2.

TFLE-1, on PDA media, expressed the colony's diameter in between 3 to 4.5 cm after 3 days of incubation. The undersides of the colonies on PDA were violet with some faint orange, while an abundance of aerial mycelia was observed. Mycelia were heavily floccose to fluffy, displaying a range of colors from pink to light violet. On medium, the colony morphology was first white and cottony; however, after a few days it evolved into an orange or reddish centre with a lighter periphery. During the microscopic examination, septate hyphae were discovered; i.e., cellular compartments were found separated by wall. On the basis of all observable morphological features the strain TFLE-1 was identified as

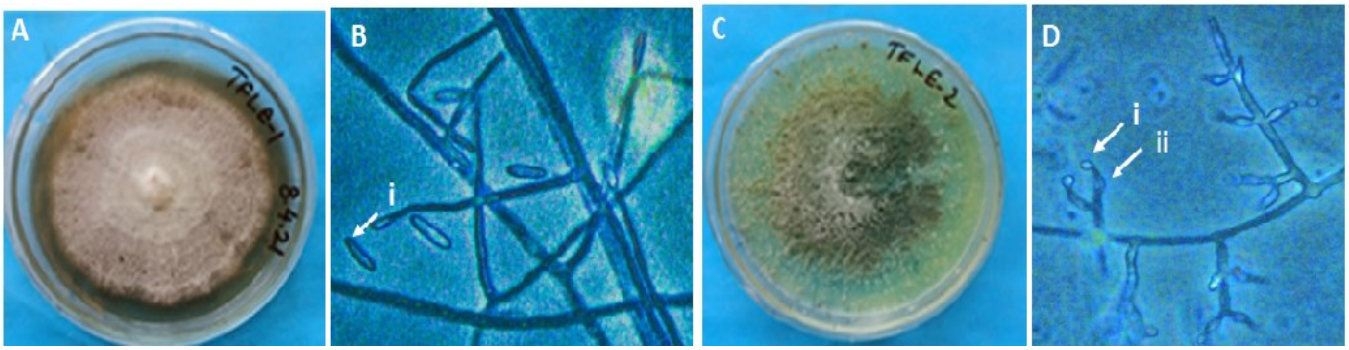

Fig. 1. Isolated fungal endophytes from leaf of *T. foenum-graecum*: A, B Microscopic view of pure fungal mycelia under 40x of strain TFLF-1; C, D Microscopic view of pure fungal mycelia under 40x of strain TFLF-2. **B-i** arrow shows elongated conidia of *Fusarium* sp. while **D-i** and **D-ii** show respectively round-shaped conidia and ampuliform phialides of *Trichoderma* sp.

the genus of *Fusarium*. Later, on molecular identification the strain was confirmed as *Furarium lichenicola* (Fig. 2-A).

TFLE-2 was found to be very rapidly growing, with mostly bright green conidia and a repetitively branched conidiophore structure; totally complies with the characteristics of *Trichodema* as mentioned in previous published data (24). Within a week full-plate growth of the fungal strain was observed on PDA media. On molecular identification the strain was confirmed as *Trichoderma euskadiense* obtained on nucleotide blast of sequence followed by phylogenetic tree construction (Fig. 2-B).

GC-MS analysis

After the extraction of identified fungal strains, a GC-MS analysis was performed to explore chemical constitutes present in fungal endophytic extracts (Table 1 and Table 2). Twenty compounds were identified from TFLE-1. The distinct GC MS chromatogram for TFLE-1 is shown in Fig.3. Table 1 shows the retention time (RT), molecular formula, molecular weight, and peak area (%) for each of the bioactive compounds found in TFLE-1. The major compounds found were 2-((4-methylpentan-2-yloxy) carbonyl) benzoic acid (15.33%), pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl) (12.42%), benzeneethanamine and 4-benzyloxy-2-fluoro-eta-hydroxy-5-methoxy (3.45%).

A total of 27 compounds were identified from TFLE-2. Fig. 4 represents the distinct GC-MS chromatogram and Table 2 shows the bioactive compounds identified from TFLE-2 along with their molecular formula, peak area (%), retention time (RT) and molecular weight. The major of the

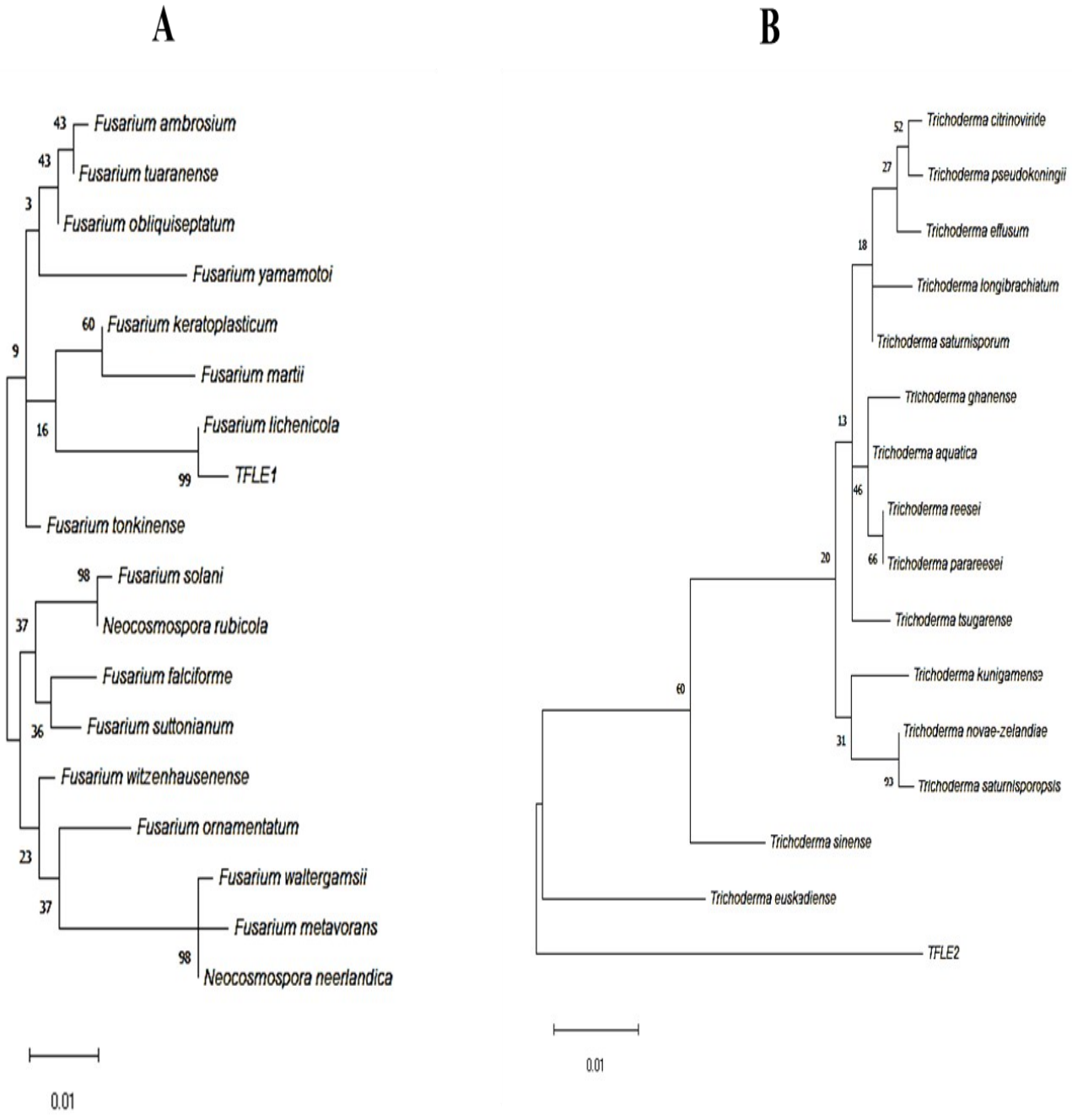

Fig. 2. The ITS regions of fungal endophytes used to construct the phylogenetic tree of fungal isolates: **A**. for TFLE-1 and **B**. for TFLE-2 confirming fungal isolates as *Fusarium lichenicola* and *Trichoderma euskadiense* respectively. The neighbour-joining method was used to infer phylogeny using MEGA version 12.

Table 1. Metabolite profiling of TFLE-1 by GC-MS analysis.

Serial no.	Retention time (RT)	Name of the compounds	Molecular weight	Molecular formula	Peak area (%)
1	3.70	Ethyl benzene	106	$C_6H_5CH_2CH_3$	0.88
2	4.67	Nonane, 2,2,4,4,6,8,8-heptamethyl 226	226	$C_{16}H_{34}$	1.55
3	5.46	Hentriacontane	436	$C_{31}H_{64}$	0.89
4	9.12	4-Butyl-2-methyltetrahydro-2H-thiopyran 1,1-dioxide	204	$C_{10}H_{20}O_2S$	0.64
5	10.65	2,6,10,14-tetra methyl-octadecane	310	$C_{22}H_{46}$	0.32
6	14.18	Trans-2-methyl-4-N-pentyl thiane, S,S-dioxide	218	$C_{11}H_{22}O_2S$	0.74
7	15.29	Octacosane, 1-iodo-	520	$C_{28}H_{57}I$	1.90
8	15.91	Heptadecane, 2,6,10,15-tetramethyl	296	$C_{21}H_{44}$	2.55
9	18.47	Benzaldehyde, 3-hydroxy-	122	$C_7H_6O_2$	2.65
10	19.02	Tetradecyl chloroacetate	290	$C_{16}H_{31}O_2Cl$	0.90
11	19.91	2,4-ditert-butylphenyl 5-hydroxypentanoate	306	$C_{19}H_{30}O_3$	0.76
12	20.78	2-Methylhentriacontane	450	$C_{32}H_{66}$	2.16
13	23.38	1-Hexacosanol	382	$C_{26}H_{54}O$	0.86
14	25.11	Docosane, 1-iodo-	436	$C_{22}H_{45}I$	2.65
15	27.37	Heptacosanoate, 25- methyl-methyl ester	438	$C_{29}H_{58}O_2$	1.11
16	29.35	2-((4-methylpentan-2-yloxy)carbonyl)benzoic acid	250	$C_{14}H_{18}O_4$	15.33
17	31.15	Trans, cis-1, 8-dimethylspiro (4.5) decane	166	$C_{12}H_{22}$	0.96
18	32.16	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	210	$C_{11}H_{18}N_2O_2$	12.42
19	32.61	Benzeneethanamine, 4-benzyloxy-2-fluoro-beta-hydroxy-5-methoxy-	291	$C_{16}H_{18}O_3NF$	3.45
20	34.16	Benzaldehyde, 4,5-dibenzyloxy-2-chloro-	352	$C_{21}H_{17}O_3Cl$	0.88

Table 2. Metabolite profiling of TFLE-2 by GC-MS analysis.

Serial no.	Retention time (RT)	Name of the compounds	Molecular weight	Molecular formula	Peak area (%)
1	3.66	Benzene, 1,3-dimethyl-	106	C_8H_{10}	2.01
2	4.64	Heptane. 2,2,3,3,5,6,6-heptamethyl-	198	$C_{14}H_{30}$	1.50
3	5.43	Hentriacontane	436	$C_{31}H_{64}$	2.06
4	9.10	3-N-hexylthiane, S,S-dioxide	218	$C_{11}H_{22}O_2S$	1.27
5	10.32	Oxalic acid, 6-ethyloct-3-yl isobutyl ester	286	$C_{16}H_{30}O_4$	1.04
6	10.63	Hexadecane	226	$C_{16}H_{34}$	0.93
7	11.61	Glutaric acid, dodec-2-en-1-yl 2-methylbutyl ester	368	$C_{22}H_{40}O_4$	0.13
8	13.07	*n*-Benzyl-2-phenethylamine	211	$C_{15}H_{17}N$	0.089
9	14.19	1-Decanethiol	174	$C_{10}H_{22}S$	0.61
10	15.16	2-Coumaranone	134	$C_8H_6O_2$	1.80
11	15.91	Triacontane	422	$C_{30}H_{62}$	1.48
12	19.01	1-Tetracosene	336	$C_{24}H_{48}$	2.14
13	19.91	Pentanedioic acid, (2,4-di-t-butylphenyl) mono-ester	320	$C_{19}H_{28}O_4$	1.27
14	20.79	Carbonic acid, octadecyl vinyl ester	340	$C_{21}H_{40}O_3$	1.53
15	21.86	D-Fructose,1,3,6-trideoxy-3,6-epithio-	162	$C_6H_{10}O_3S$	0.53
16	23.40	Octacosanol	410	$C_{28}H_{58}O$	1.59
17	25.14	Galactopyranoside, 1-heptylthio-1-deoxy-	294	$C_{13}H_{26}O_5S$	1.26
18	27.39	Hexacosyl acetate	424	$C_{28}H_{56}O_2$	1.44
19	30.27	L-Proline, N-valeryl-, hexadecyl ester	423	$C_{26}H_{49}NO_3$	1.34
20	31.05	2,6,10,14-tetramethyl-7-(3-methylpent-4-enylidene) pentadecane	348	$C_{25}H_{48}$	0.58
21	31.32	Propyl 9,12-octadecadienoate	322	$C_{21}H_{38}O_2$	1.40
22	32.17	Diethyl trisulfide	154	$C_4H_{10}S_3$	2.90
23	32.42	Phenol, 2-(1,1-dimethylethyl)-4-(1-methyl-1-phenylethyl)-	268	$C_{19}H_{24}O$	1.43
24	32.64	Benzene, 3-heptynyl-	172	$C_{13}H_{16}$	2.78
25	33.08	Pentatriacontane	492	$C_{35}H_{72}$	8.61
26	34.20	Fencamfamin propionyl	271	$C_{18}H_{25}NO$	1.05
27	34.39	Dichloro(l-menthyl) phosphine	240	$C_{10}H_{19}Cl_2P$	0.37

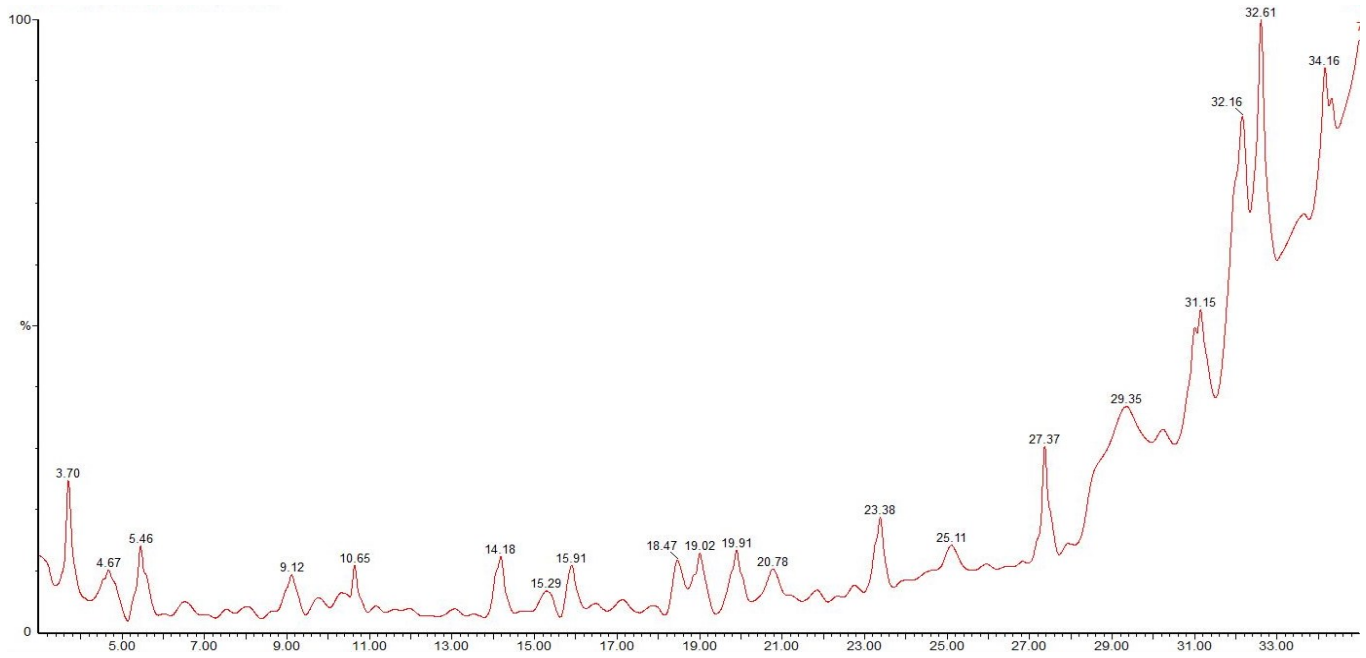

Fig. 3. GC-MS chromatogram of the ethyl acetate fungal extract, TFLE-1 of *T. foenum-graecum* leaf.

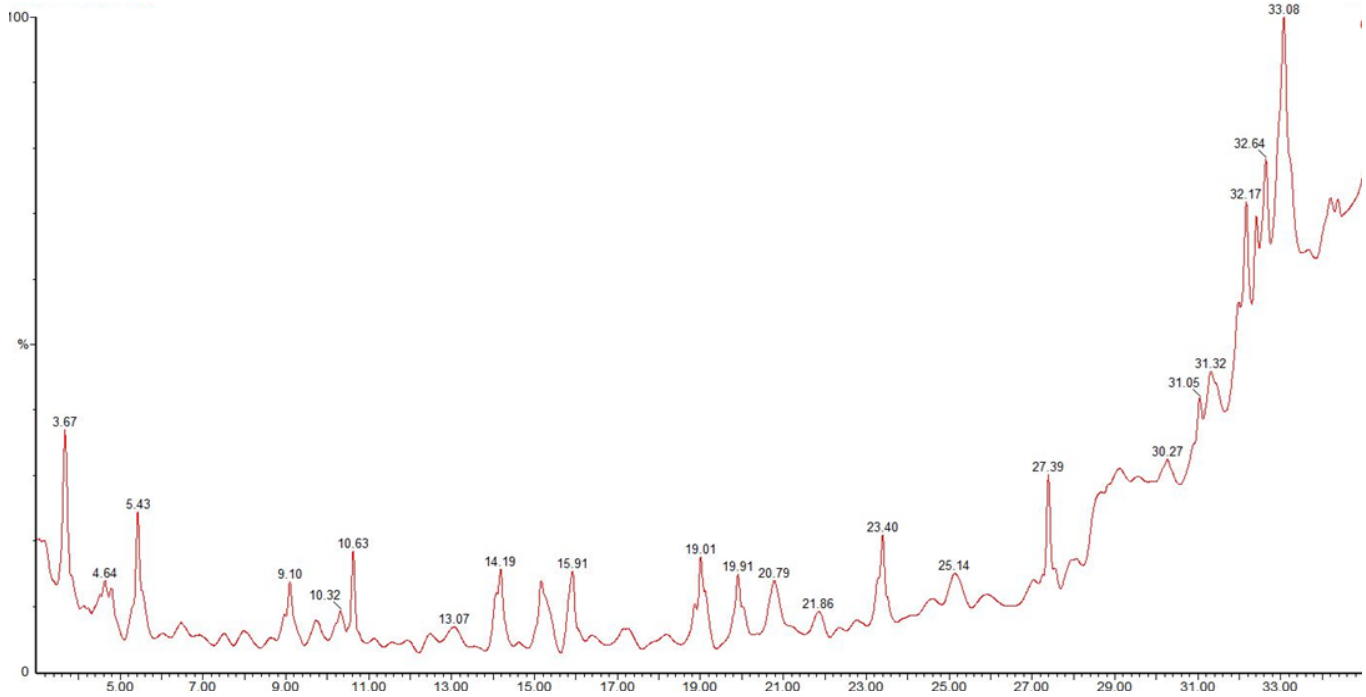

Fig. 4. GC-MS chromatogram of the ethyl acetate fungal extract, TFLE-2 of *T. foenum-graecum* leaf.

27 identified compounds are pentatriacontane (8.61%), diethyl trisulfide (2.90%) and benzene, 3-heptynyl (2.78%).

The secondary metabolites like flavonoids (25), saponins (26), polyphenols (27), cardiac glycosides (28), coumarins (29), tannins (30), alkaloids (28, 31, 32) and triterpenes (33) have been reported previously to have considerable antimicrobial activities. Different plant parts of medicinal plants as well as endophytic microbes contain the aforementioned secondary metabolites which has well documented in the review (34). Another prominent issue importantly required for having antimicrobial activity of a compound is the presence of various functionalities, e.g., hydroxyl and carboxylic groups (35). If we carefully have a look at a glance, a significant number of the compounds in Table 1 and Table 2 contain hydroxyl and carboxylic functional groups in their structure. As a whole, characteristics of the compounds prompted us to go for a preliminary antibacterial study of the fungal isolates.

Antibacterial activity assessment

On a preliminary antibacterial study as per protocol of standard disc diffusion method, the zone of inhibition was utilized to figure out how effective the fungal extracts were at retarding growth of pathogenic bacteria in contrast to standard kanamycin. *Bacillus megaterium* (ATCC 25918) and *Staphylococcus aureus* (ATCC 25923), 2 gram-positive pathogenic bacterial strains, as well as *Escherichia coli* (ATCC 28739) and *Salmonella typhi* (ATCC 19430), 2 gram-negative pathogenic bacterial strains were used as test organisms in the antibacterial study. Since these bacterial strains are well-defined multiple-resistant strains, they were chosen for the present study.

We conducted a one-way ANOVA test to exploit the

data of the zones of inhibition (mm) obtained from all endophytic fungal extracts and standard kanamycin; One-way ANOVA test was utilized and the zone of inhibition was found statistically significant (*p≤0.05; Fig. 5). However, *Kirby-Bauer* (KB) disc diffusion susceptibility testing protocol describes the extent of the zone of inhibition in a more specific way; we therefore, also tried to explain our finding on the basis of the KB protocol. The bacterium is regarded as being sensitive to the antibiotic/drug/extract if the observed zone of inhibition is higher than or equal to the standard size of the zone of inhibition. On the other hand, the microorganism is regarded as resistant if the detected zone of inhibition is less than that of the conventional size (36). As per the KB protocol, for a drug if the zone of inhibition ≥18 mm (in respect of standard kanamycin) against *Staphylococcus* species, the species would be considered as susceptible; while if a zone of inhibition is ≤14, *Staphylococcus* species would be resistant. As our study result shows, *S. aureus* is susceptible to TFLE-2 while resistant to TFLE-1. If we consider susceptibility against *E. coli* the outcome is similar; *E. coli* is susceptible to TFLE-2 while resistant to TFLE-1. As a whole, all the bacterial strains show susceptibility against TFLE-2 (Fig. 5b) while almost resistance against TFLE-1 at a dose of 100 µg/mL. It is possible that if the doses of TFLE-1 are increased gradually we would find susceptibility of the fungal extract against tested bacterial strains. Researchers of this study also suggest for further extensive studies like MIC studies, time-kill tests etc. in order to quantify the degree of susceptibility.

Crude endophytic fungal extracts from different *Fusarium* species earlier reported a broad antimicrobial spectrum against various pathogenic microorganisms (37–42). These findings are consistent with the general hypothesis that the endophytic fungi have the ability to

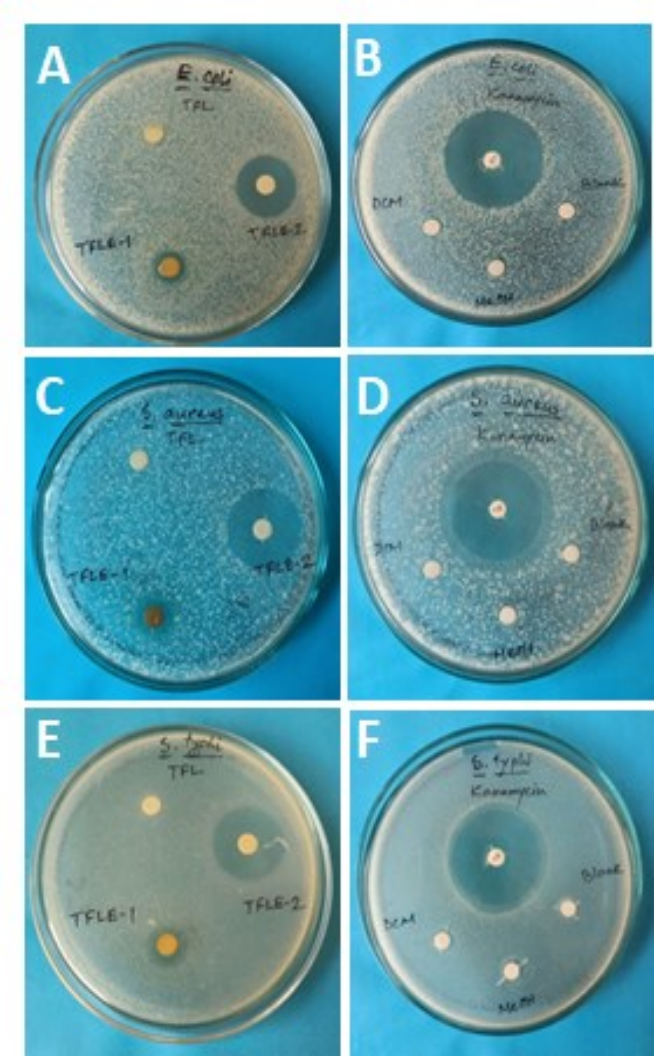

Fig. 5b. Zone of inhibition of visible for TFL (Leaf plant extract of *T. foenum-graecum*), fungal extracts TFLE-1 and TFLE-2 (A, C, E) in respect to standard antibacterial kanamycin (B, D, F) against *E. coli, S. aureus* and *S. typhi* demonstrating significant level of antibacterial effect of TFLE-2.

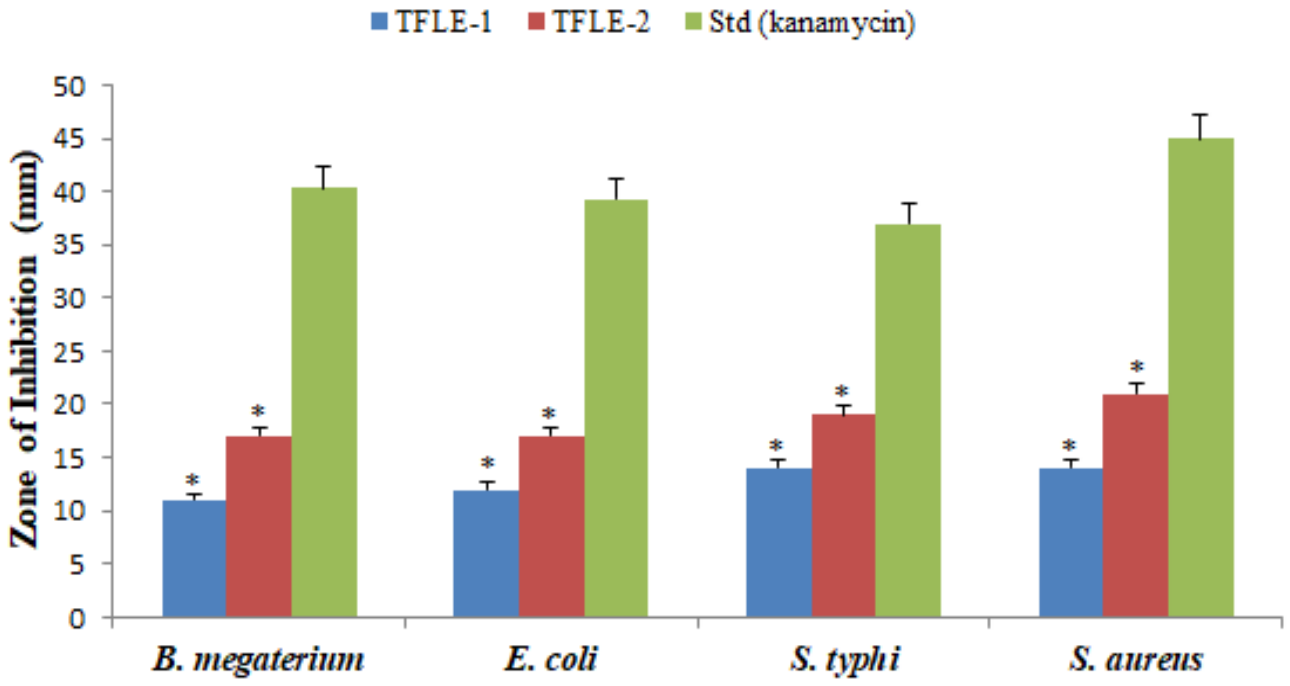

Fig. 5a. Average zone of inhibition (mm) of the endophytic fungal extracts, TFLE-1 and TFLE-2, isolated from the leaf of *T. foenum-graecum* showing the antibacterial effect in contrast to standard kanamycin. One-way ANOVA test (IBM SPSS version 29.0.1.0) was utilized and the zone of inhibition was found statistically significant (*p≤0.05). Data were means of 3 replicates (n=3).

generate a diverse array of compounds possessing antimicrobial properties. Endophytic fungi isolated from *Fusarium* species also obtained a number of compounds having potential antimicrobial effect (43). Significant antibacterial activity of the compounds isolated from *Fusarium* species included fusolanone-B (44), aurofusarin and bikaverin from *F. solani* (45), antiviral activity by coculnol (46), the compounds isolated from *F. napiforme*, namely, 6-hydroxy-astropaquinone B and astropaquinone D (47), karimunones-B isolated from sponge-associated *Fusarium* sp (48), fusariumin C and fusariumin D from *F. oxyoporum* (49), fusariumester B from merine-derived *Fusarium* sp.(50), amoenamide C from *F. sambucinum* TE-6L (51), fusaribenzamide A from *Fusarium* sp. showing antifungal activity (52), fusarithioamide A from *F. chlamydosporium* (53), fusariumin A obtained from *Fusarium* sp. (54), sambacide from *F. sambucinum* B10.2 (55) and fusartricin from *F. tricinctum* (56). All these compounds isolated from endophytic *Fusarium* species showed moderate to strong activities against bacteria, fungi and virus in respective instances.

Trichoderma species of endophytic fungi, which is in general a rich source of bioactive compounds (57) were also previously assessed for their antimicrobial activity. Assessment was conducted on both isolated *Trichoderma* endophytes fungal strains as well as the secondary metabolites isolated from respective fungal variant(s). Few antimicrobial compounds isolated from *Trichoderma* species include viridin from *Trichoderma viride* (58), Koninginin C and Koninginin E from *T. koningii* (59, 60), viridiofungins from *T. viride* (61), Trichokonins from *T. koningii* (62), Harzianolide from *T. harzianum* (63, 64) and so on. Furthermore, pure fungal extracts of a number of endophytic *Trichoderma* species were also evaluated for antibacterial activity; namely: *T. koningiopsis*, *T. longibrachiatum*, *T. brevicompactum*, *T. longibrachiatum*, *T. brevicompactum*, *T. longibrachiatum* and *T. asperellum*. All showed significant antibacterial activities against different bacterial strains, including notorious *S. aureus* and *E. coli* showing significant minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) (65). Earlier, other fungal species like *T. atroviride* and *T. koningii* were also reported to demonstrate significant antibacterial and antifungal activities against model phytopathogenic bacteria including *Pseudomonas syringae*, *Erwinia amylovora* and *Bacillus* sp. (66). *T. harzianum* was also found to show antimicrobial activities against bacteria and fungi (67). In general, most *Trichoderma* species as well as the compounds isolated from *Trichoderma* species were found having prominent antimicrobial activities.

While we decided in our study to go for bioactivity assessment of the isolated fungal species, *F. lichenicola* and *T. euskadiense,* we considered and reviewed all these outcomes; knowing that, interestingly, these 2 particular fungal strains are not yet being studied for their bioactivity. Positive outcome in terms of preliminary antibacterial activity test in the present study against different bacterial strains would encourage us to isolate and characterize pure bioactive compounds from these fungal strains using column chromatography and preparative thin layer chromatography followed by structure elucidation using NMR technique as the continual future plan. Putative positive outcome from bioassay of compounds in the later phase of the study will hopefully encourage the researchers for planning animal trial.

Conclusion

Endophytic fungi isolated from *Trigonella foenum-graecum* leaf, especially planted in the soil of Bangladesh, have not been studied so far. Our objective, therefore, was to isolate, identify and characterize the extracted pure fungal endophytes from the leaf of *T. foenum-graecum*. After the isolation and identification, we conducted GC-MS analysis and revealed major phytochemical components of the isolated fungal extracts. Applying disc-diffusion method, we also evaluated the bioactivity of the extracts and revealed that one of the fungal strains *Trichoderma euskadiense*, TFLE-2, possess promising antibacterial property against tested pathogenic bacteria while compared to standard kanamycin. This finding could be a basis to find out lead antibacterial compound(s) from these endophytic fungal variants in future endeavour.

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Authors' contributions

GMMM conducted the experiments and drafted the manuscript. MHS conceived of the study and participated in its design and coordination. MMM revised the manuscript. MAM supervised on designing study protocols. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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