Original article

Antimicrobial and cytotoxic potential of an endophytic fungus *Alternaria tenuissima* AUMC14342 isolated from *Artemisia judaica* L. growing in Saudi Arabia

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ABSTRACT

In this study, 19 endophytic fungal isolates associated with *Artemisia judaica* L. were isolated from Al-Shihiyah, Ha᾽il, Saudi Arabia. Those isolates represented five genera, including: *Alternaria* sp., (predominantly), *Aspergillus* sp., *Chaetomium* sp., *Cladosporium* sp., and *Fusarium* sp. All isolates (6 species) were grown on a solid rice medium and their ethyl acetate crude extracts, beside *A. judaica* L. extract, were tested for their antimicrobial activity. One endophytic fungal isolate demonstrated the highest activity and was chosen for further investigation. Based on its phenotypic, ITS ribosomal gene sequences, and phylogenetic characterization, this isolate was identified as *Alternaria tenuissima* AUMC14342 with accession number (MT468650). Ethyl acetate (EtOAc) extract from the isolated strain, AUMC14342, was fractioned using VLC. The active fraction of the EtOAc exhibited good antimicrobial effect against *P. aeruginosa*, *S. aureus*, *F. solani*, and *A. niger* at conc. 30 μ/mL using disc diffusion assay. A moderate cytotoxic effect was reported against a Hepatocellular carcinoma cell line (HepG2), with the lowest inhibitory concentration (IC_{50}) of 0.52 μM. The active fraction was characterized by the presence of different chemically terpene, ester, volatile, and other organic compounds, determined by GC–MS. Conclusively, the endophytic fungus, *A. tenuissima,* derived from *A .judaica* L., is an ideal candidate for the potential industrial production of antimicrobial agents with a variety of applications.

1. Introduction

The genus *Artemisia* L. belonging to family Asteraceae, includes a variable number of species, with over 500 species found throughout the northern hemisphere of the world (Hayat et al., 2009). *Artemisia* is a good source of various secondary metabolites that has a high value in medicine and bio-pesticides (Cosoveanu and Cabrera, 2018). *A. judaica* L. is a perennial, fragrant little shrub that grows broadly in the southern desert of Algeria, the Sinai desert of Egypt, and in the Middle East across Jordan and Saudi Arabia (Wyk and Wink, 2004; Abu-Darwish et al., 2016). Many plants have the potential to produce bioactive compounds that help in preventing or relieving illnesses and providing a special environment for endophytic fungi (Rebecca et al., 2011). Endophytes are prevalent livening microorganisms in the internal of plant tissues without affecting obvious signs of infection. Most of these endophytic fungi that have a mutualistic association with their host, protecting them from microbial pathogen (Strobel and Daisy, 2003; Kharwar

et al., 2008). The majority of endophytes are well known to have biosynthetic abilities more prominent than their host plant, because of their long co-evolution combined with genetic recombination (Fernandes et al., 2009).

Endophytic fungi are pervasive to plants and are mainly members of Ascomycota or their mitosporic fungi; some are members of Zygomycota, Oomycota, and Basidiomycota (Zheng and Jiang, 1995). Various endophytic fungi isolated from different plant groups have been identified using polyphasic approaches, including phenotypic and genotypic methods (Govindarajulu et al., 2013). About 80% endophytic fungi are responsible for production of bioactive products that have antimicrobial, and herbicidal effects (Schulz et al., 2002). Numerous antimycotic products have been created using endophytic fungi, belonging to different structural groups in particular alkaloids, phenols, flavonoids, terpenoids, and peptides (Kumar et al., 2008; Fernandes et al., 2009).

The genus *Alternaria* Nees (Dematiaceae), in the fungi Imperfecti, is extensive if the species found worldwide are counted, in association with an enormous assortment of substrates comprising plants, animals, soil, agricultural products, and the atmosphere (Lou et al., 2013; Eramet al., 2018). They live as plant pathogens, saprophytes, weak facultative parasites, and endophytes (Thomma, 2003). Simmons (2007) summarized the *Alternaria* taxonomy based on morphological characteristics, particularly 275 fungal species were identified as *Alternaria* spp. Metabolites from *Alternaria* showed a variety of bioactivities, including antimicrobial, phytotoxic, and cytotoxic activities (Bräse et al., 2009; Tsuge et al., 2013). *A. tenuissima*, as an endophytic fungus isolated from *Tylophora indica*, was found through genotypic and phenotypic examination to be more active against *S. sclerotiorum* and *F. oxysporum*, using the dual culture technique (Kumar et al., 2011). The objective of the current investigation has been to isolate endophytic fungi from *A. judaica* collected in Saudi Arabia, and further screen them for their antibacterial, antimycotic, and cytotoxic activity. In addition, this study has aimed to explore the bioactive metabolites profile using GC–MS.

2. Materials and methods

2.1. Collection of plant samples

Artemisia judaica L. was randomly selected from Al-Shihiyah, (27.8942°N, 42.6832°E), Haʾil, Saudi Arabia, during October–December 2018. Healthy plant parts were collected for further isolation of endophytic fungi; plant samples were transported to the lab under aseptic conditions.

2.2. Isolation of fungal endophytes

The collected plant materials were surface sterilized for the isolation processes described by Kumar et al. (2011) with some modifications. The isolation of endophyic fungi was carried out on Petri dishes that contained a potatoes dextrose agar medium (PDA). All plates were incubated at 28 ± 2 °C for up to two weeks. The fungal mycelium that were growing from the plant fragments were then isolated, purified, and maintained on PDA slants at 4 °C, for further investigations. Endophytic fungal mycelia were grown in PDA media, in order to assess their characterization and morphological identification.

2.3. Fermentation and extraction of fungal metabolites

Fermentation of pure fungal isolates was carried out in a solid rice medium (100 g rice and 110 mL H_2O in 1 L Erlenmeyer flasks), autoclaved for 20 min at 121 °C. After cooling down, 2 mL spore suspension (scraped from a freshly cultivated culture) were inoculated into flasks, and incubated at 28 °C under static conditions for 30 days. After that, 500 mL of EtOAc was added to the fermented rice medium, and EtOAc extract was filtered and dried up with a vacuum rotary evaporator (BÜCHI R-114, Switzerland) at 40 °C until dry (Pretsch et al., 2014).

2.4. Preparation of plant extract

Dried *A. judaica* was ground into a coarse powder, and 100 g was soaked in EtOAc at room temperature for 48 h, then the filtered solution was concentrated by rotary evaporator to a small volume at 45 °C, in order to yield the crude extract for further antimicrobial activities (Pandey, 2007).

2.5. Antimicrobial activity using fungal and plant crude extracts

2.5.1. Test microorganisms

In our study microorganisms were obtained from the Bacteriology and Mycology Labs of the Botany and Microbiology Department, Faculty of Science at Al-Azhar University (Assiut), Egypt. The bacterial strains obtained for testing were *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC6538P, *Escherichia coli* ATCC 8739, *Klebsiella pneumonia* ATCC43816, and *Pseudomonas aeruginosa* ATCC9027, and pathogenic fungi *Aspergillus flavus* (Hassane et al., 2018), *A. niger* ATCC 16404, *Alternaria alternata* MLBM09, *A. solani* MLBM 23, *Fusarium oxysporum* MLBM212, and *F. solani* MLBM227). The bacteria were grown in Mueller Hinton broth (MHB) at 37 °C for 24 h, and the fungi were grown in PDA medium, incubated for 5 days at 28 °C.

2.5.2. Antimicrobial activity of extracts

The antimicrobial assay of the fungal and plant crude extracts underwent preliminary evaluation using the well diffusion method (Jahangirian et al., 2013), on MHA and PDA medium. Wells (diameter ~ 6 mm) made in the inoculated plates were filled with 50 μL of a 10% (*w/v*) dissolved extract of dimethyl sulfoxide (DMSO). All plates were incubated for 24 h at 37 °C for bacteria and 3–5 days at 28 °C for fungi. The diameter of zone of inhibition around the well was measured in millimeters (mm). Chloramphenicol (1 mg/mL) was used as a positive control for the bacteria, and nystatin (100000 Iu/mL) for fungi, while DMSO was used as a negative control. All tests were performed in triplicate.

2.6. Identification of fungal culture

The *A. tenuissima* fungal isolate was identified based on colonial morphological features, microscopic observation, and well-established molecular biological protocols for the amplification and sequencing of DNA in the Internal Transcribed Spacer (ITS) region of the rRNA gene. DNA was extracted using a Patho-gene-spin DNA/RNA extraction kit, provided by the Intron Biotechnology Company (Seongnam, South Korea). The fungal DNA was then sent to SolGent Co. Ltd (Daejeon, South Korea) for polymerase chain reaction (PCR) and rRNA gene sequencing. The 18S rRNA encoding gene was amplified by PCR from purified genomic DNA using the fungal-specific primers, ITS1 (5′ - TCC GTA GGT GAA CCT GCG G − 3′), and ITS4 (5′- TCC TCC GCT TAT TGA TAT GC −3′). The purified PCR product (amplicon) was sequenced with the same primers, with the incorporation of ddNTPs, in the reaction mixture (White et al., 1990). The fungal ITS sequence fragment that was obtained was then aligned with closely-related species sequences, obtained from the NCBI nucleotide database using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The phylogenetic analyses of sequences were constructed using MegAlign (DNA Star) software, version 5.05.

2.7. Fractionation of the most active extract

Fungal and plant crude extracts were obtained through extraction by (VLC) vacuum liquid chromatography (10 × 15 cm) on a sintered funnel filled with silica gel (60–200 mesh size, Fisher, UK). Column chromatography (50 × 3 cm) was eluted with various solvent systems—methanol:water (80:20, v/v), n-hexane 100%, ethyl acetate 100%, n-hexane:ethylacetate (50:50, v/v), dichloromethane 100%, dichloromethane:methanol (50:50, v/v), and butanol—and successive fractions were collected. (TLC) Thin layer chromatography of the fractions was carried out on sheets (Silica gel 60 F$_{254}$, Merck, Germany). Fractions that had the same spots on the TLC with similar Rf values were pooled, then antimicrobial activity was checked, and the most active fraction was used for further analysis.

2.8. Antimicrobial activity of AUMC14342 fractions

The antimicrobial activities of different fungal fractions were carried by agar disc diffusion method (Parekh et al., 2005). Presterilized filter paper discs (Whatman no. 3, with 5 mm in diameter) were soaked in all fractions at a 30 μg/mL concentration, along with positive and negative controls, and dried in a laminar flow biological safety cabinet. The soaked discs were placed on the surface of the inoculated solidified plates, at equal distances, and then plates were incubated at 37 °C for 24 h for bacteria, and at 30 °C for 72 h for fungi. The plates were observed for the presence of inhibition of microbial growth and measured in mm.

2.9. Cytotoxicity activity

A hepatocellular carcinoma cell line (HepG2) was obtained from Nawah Scientific Inc. (Al-Mokattam, Cairo, Egypt). The cells were maintained in DMEM media, supplemented with 100 mg/mL streptomycin, 100 units/mL penicillin, and 10% heat-inactivated fetal bovine serum, in a humidified, 5% (v/v) CO$_2$ atmosphere at 37 °C. The percentage of cell viability was calculated with the following:

$$Percentage of cell viability = \frac{OD of treated cells}{OD control} \times 100\%$$

Cell viability was assessed by WST-1 assay using Abcam® kit ab155902 WST-1 Cell Proliferation Reagent, as previously reported by Alaufi et al. (2017).

2.10. Gas chromatography-mass spectroscopy (GC–MS) analysis

The active fraction of the *A. tenuissima* strain that was obtained, along with plant ethyl acetate extract, were individually dissolved in 100% methanol and dehydrated with anhydrous Na$_2$SO$_4$, then filtered through a syringe filter (0.45 μm pore size) prior to injection. A Trace GC Ultra-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA), was used for the chromatographic analysis, and the compounds were separated according to Salem et al. (2016). The extract components were determined and identified using the mass spectra and retention time database that is found in the Wiley 09 and NIST 11 library.

2.11. Statistical analysis:

All the experiments were conducted in triplicate and analysis of variance was made using SPSS, program version 16. The mean of the data was calculated by analysis of variance (ANOVA), and statistical analysis was observed (at $P < 0.05$) to establish significant differences.

3. Results

3.1. Isolation of endophytic fungi

In this study, 19 fungal isolates were obtained from the 150 fragments analyzed (75 leaves and 75 stems fragments), which were obtained from healthy leaves and stem parts of *A. judaica* L. Samples of the leaves and stem parts differed in their endophytic fungal colonization, with 15 isolates (78.9%) obtained from leaves, and 4 isolates (21.1%) obtained from stem parts. The isolates belonged to Ascomycota and Hyphomycetes. *Alternaria* sp. isolated from the leaves was found to be predominant, with 63.1% (*Alternaria alternata* (Fries) Keissler (26.3%), and *A.tenuissima* (Kunze ex Pers.) Wiltshire (36.8%); followed by *Aspergillus niger* Van Tieghem from the leaves, with 15.7%; and from stem, *Chaetomium* sp. Kunze ex Fresen., with 5.2%; *Cladosporium cladosporioides* (Fresen.) G. A. de vries, with 5.2%; and *Fusarium oxysporu*m Schlechtendal, with 10.5%.

3.2. Preliminary antimicrobial screening of isolated fungi and host plant extracts

The EtOAc crude extracts of the isolated fungi (6 species), obtained from the fermented medium and *A. Judaic*a extract, were evaluated for antimicrobial activities by agar well diffusion method. The data obtained (Supplementary material Table S1, and Fig. 1) showed that the antibacterial activities of the fungal and plant extracts presented different degrees of inhibition, and a significant difference was observed as compared to controls. The results recorded high antibacterial activity when compared with the bacterial strains that were tested (*B. subtilis*, *S. aureus*, *E. coli*, *K. pneumonia,* and *P. aeruginosa),* with inhibition zones that ranged between 14.66 ± 1.45 to 38.66 ± 0.88 mm. Two isolates, *A. tenuissima* and *Aspergillus niger* crude extracts, exhibited no significant antibacterial activity when compared with all the bacteria that were tested, as compared to chloramphenicol. Meanwhile, the *A. judaica* plant crude extract showed varied significance with an inhibition zone that ranged between 20.33 ± 0.33 to 26.33 ± 0. 88 mm. Other crude extracts of endophytic fungi showed significant inhibition when compared with the control. *A. alternata*and *C. cladosporioides* did not affect *E. coli*, while *Chaetomium* sp. showed no activity on *K. pneumonia*.

The antifungal activities of these extracts were also evaluated against toxigenic and plant pathogenic fungi (*A. flavus*, *A. niger*, *A. alternata*, *A. solani*, *F. oxysporum,* and *F. solani*); inhibition zones ranged from 15.00 ± 0.57 to 33.00 ± 2.51 mm. The results demonstrated that the *A. tenuissima*, *A. niger,* and *A. judaica* plant crude extracts exhibited a significant antimycotic activity, when compared with nystatin. On the other hand, *C. cladosporioides* showed no antifungal activity when compared against all of the fungi that were tested. Meanwhile, the *A. alternata* and *F. oxysporu*m crude extracts only showed inhibition against pathogenic *A. alternata*and *A. solani* (Supplementary material Table S2, and Fig. 2). The results above have demonstrated that bacteria were more susceptible to ethyl acetate extracts than fungi. The *A. tenuissima* crude extract presented high broad-spectrum activity among other extracts; therefore, it was selected for further study.

3.3. 18S rDNA sequencing and phylogenetic analysis of the most active culture

The molecular identity and phylogenetic characterization of the promising isolate, AUMC14342, were determined using 18S rDNA gene sequencing. The partial 18S rDNA gene sequence was detected using gel electrophoresis as a clear band at a region
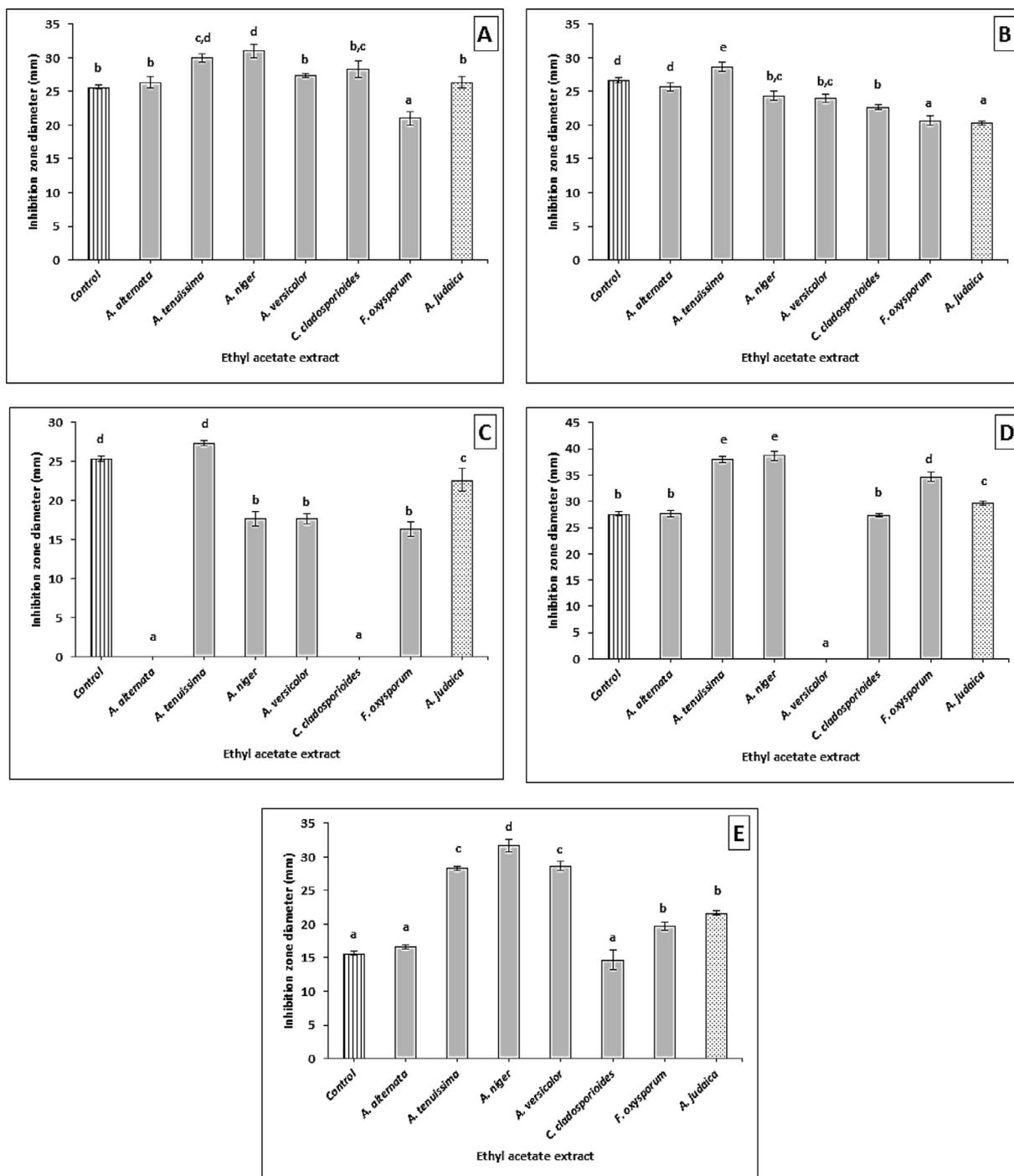
Fig. 1. Antibacterial activity of the crude extracts against (A) *B. subtilis*, (B) *S. aureus*, (C) *E. coli*, (D) *K. pneumoni,* and (E) *P. aeruginosa*. Error bars represent standard error of the means (*n* = 3).

of ~500 bps. Sequence homology analysis of the AUMC14342, with previously published reference sequences found using the BLASTn tool on the GenBank Database, indicated that the AUMC14342 isolate belongs to the genus *Alternaria,* with the greatest homology (100%) to *A. tenuissima* published sequences (Fig. 3). Therefore, the isolated strain was designated as A. *tenuissima* AUMC14342 with the registered accession number MT468650.

3.4. Fractionation of the AUMC14342 crude extract and its antimicrobial activity

The EtOAc crude extract (3.4 g) of AUMC14342 was separated by silica gel for VLC, followed by partial purification via TLC plates, affording 7 different fractions (Fr.1-Fr.7). All of these fractions were evaluated for antimicrobial activities using the disc diffusion method (30 µg/mL). Results found that the fraction no. 3 was the
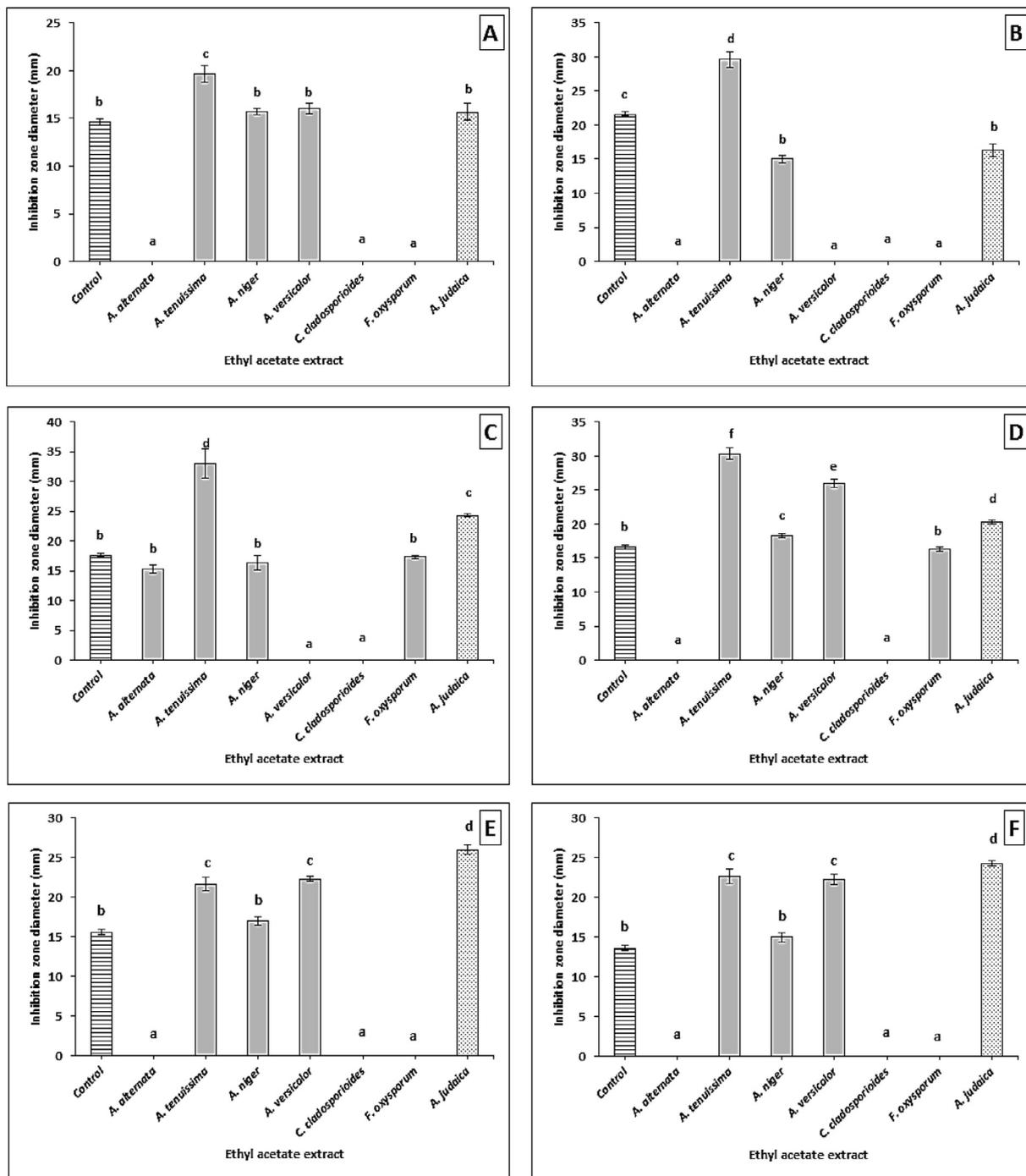
Fig. 2. Antifungal activity of crude extracts against (A) *A. flavus*, (B) *A. niger*, (C) *A. alternata*, (D) *A. solani*, (E) *F. oxysporum*, and (F) *F. solani*. Error bars represent standard error of the means (*n* = 3).

most potent; it showed significantly higher activity than even the positive control used in this experiment, against different pathogenic bacteria that were tested with inhibition zones from 13.00 ± 0.57 to 21.33 ± 0.88 mm. Most of the bacteria that were tested were sensitive to the applied fractions with different degrees, and a few strains were resistant to certain fractions (Supplementary material Table S2, and Fig. 4). In addition, the antifungal activity of *A. tenuissima* fractions showed that fr. 3 was also the most promising active fraction, with zones of inhibition that ranged from 7.00 ± 0.57 to 16.00 ± 0.57 mm. Our data demonstrated that fungi were more resistant to most of the tested fractions,

showing significant sensitivity in some fractions in comparison to control (Supplementary material Table S2, and Fig. 5). Fraction no. 3 was selected as the most potent antimicrobial extract, for further study to determine its chemical components using GC/MS analysis as well as the crude extract of the *A. Judaica* plant.

3.5. WST-1 cytotoxicity activity

The results obtained from WST-1 cytotoxicity assay were rated based on cell viability compared with a control (diluted DMSO). The different degrees of cell viability were categorized as strongly
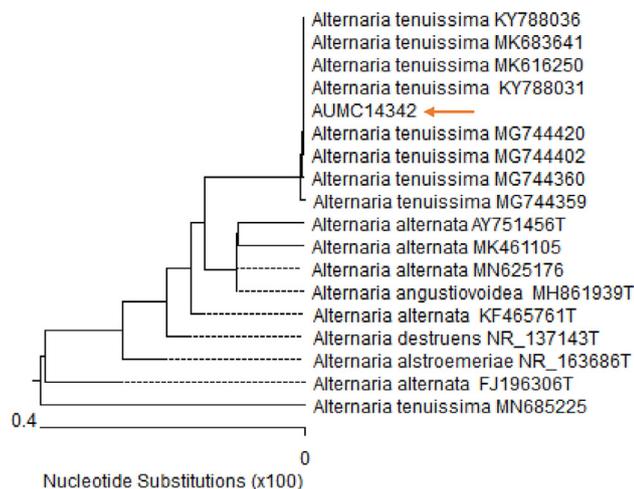
Fig. 3. The neighbour-joining (NJ) phylogenetic tree based on ITS gene sequences of AUMC14342, with closely related strains accessed from the GenBank, (arrowed) showed a 100% identity with several strains of *A. tenuissima*.

cytotoxic (>30%), moderately cytotoxic (30–59%), slightly cytotoxic (60–90%), and non-cytotoxic (>90% cell viability). Cytotoxicity results showed moderate cytotoxic activity of the *A. tenuissima* ethyl acetate active fraction (no. 3) against the HepG2 Hepatocellular carcinoma cell line. The lowest IC_{50} 0.52 μM of the *A. tenuissima* fractions was recorded at a viability of 43.33%, compared with the negative control which did not exhibit any activity.

3.6. GC–MS based identification of bioactive compounds

The chemical composition of the ethyl acetate extract of *A. judaica* leaves was analyzed using GC–MS. Eighteen chemical compounds were identified, supported by a NIST database comparison in which specific peaks bore a resemblance with the nearest compound. The most abundant constituents were; camphene, artemisia ketone, camphor, (+)-dihydrocarvone, farnesene epoxide, α-bisabolol, humulol, nerolidol-epoxyacetate, and limonene dioxide (Table 1, and Fig. 6). Furthermore, GC–MS analysis of the most potent ethyl acetate fraction of *A. tenuissima* (no. 3) identified seventeen chemical components. The dominant compounds were hexadecanoic acid, methyl ester; 1-hexadecanol; 9-octadecenoic acid (Z)-, methyl ester; 1-hexadecanol, 2-methyl-; methyl octadeca-9,12-dienoate; and 1,2-benzenedicarboxylic acid. The peak area of each compound was directly proportional to its quantity in the extract.

4. Discussion

Endophytic fungi are well known to be valuable sources of a wide range of bioactive metabolites, with antimicrobial, cytotoxic, anticancer, antioxidant, antimalarial, and antiviral activities (Aly et al., 2011). There is an urgent necessity to research new medications and pharmaceuticals because of the rise in drug-resistant pathogens and emergence of new infectious diseases (Espinel et al., 2001). In the present study, *A. alternata, A. tenuissima, A. niger, Chaetomium* sp., *C. cladosporioides* and *F. oxysporum,* were isolated from *A. judaica.* Qian et al. (2014) identified the *A. alternata, A. tenuissima, C. globosum, C. cladosporioides, F. nematophilum,* and *P. radicum* strains of fungal endophytes, deriving these from *Artemisia*

argyi via ITS rDNA sequence data. While, Zhang et al. (2012) identified 11 endophytic strains isolated from the stems of *Artemisia annua* and shown to have four genera (*Aspergillus, Fusarium, Cephalosporium,* and *Mucor*). Cosoveanu et al. (2018) indicated *A. alternata, A. flavus,* and *Cladosporium* sp. endophytes, from *Artemisia thuscula,* in a study based on morphology and phylogenetic analysis.

Our results have shown that the *A. tenuissima* and *A. niger* ethyl acetate crude extracts exhibit a good antibacterial activity against the bacteria they are tested upon, while the *A. judaica* plant crude extract shows bioactivity of varied significance. In this respect, *Artemisia thuscula* fungal endophytes have exhibited antibacterial activity against *E. coli, S. aureus* (Zhang et al., 2012), and ethyl acetate extracts of *A. argyi* fungal endophytes at 200 μg/mL have demonstrated varied antibacterial activity against *Clavibacter michiganensis* and *Pseudomonas solanacearum* (Qian et al., 2014). Al-Wahaibi et al. (2020), meanwhile, proved the antibacterial susceptibility of *B. subtilis, S. pneumonia,* and *E. coli* to *A. judaica* essential oil, while Guetat et al. (2017) reported antibacterial activities of taxa belonging to the genus *Artemisia* against the common human pathogens *E. coli, S. typhimurium, S. agalactiae, E. feacium,* and *S. aureus.*

In the current study, the impacts of the ethyl acetate extracts that were tested upon pathogenic fungi showed that *A. tenuissima* and *A. niger,* as well as *A. judaica,* plant crude extracts all exhibited significant antimycotic activity. Qian et al. (2014) cleared that a 1 mg/mL EtOAc extract of fungal endophytes, from *A. argyi,* has an antifungal effect upon the phytopathogenic fungi *F. graminearum, R. solani* and *P. capsici. A. judaica* essential oil has also been proven to have an antifungal effect upon *A. fumigatus, S. racemosum, G. candidum,* and *C. albicans* (Al-Wahaibi et al., 2020). Moreover, Abu-Darwish et al. (2016) reported that the essential oil of *A. judaica* is active against *A. flavus* and *A. fumigatus,* as well as against *A. niger.* Badawy and Abdelgaleil (2014) noted that the oil of *A. judaica* caused the highest spore germination inhibition of *F. oxysporum,* and that the *A. tenuissima* ethyl acetate fraction recorded the most potent antibacterial and antimycotic efficiency among seven derived fractions of the crude extracts. This fraction also showed moderate cytotoxic activity against a HepG2. An endophytic fungus *A. tenuissima* fermentation broth has too exhibited moderate anticancer activity (Wu et al., 2014). However, Qian et al. (2014) reported no inhibition at 20 μg/mL of *A. tenuissima* ethyl acetate extract against human tumor cell lines [breast (MCF-7), colon (COLO205), and leukemia (HL-60)].

With regards to the GC–MS analysis of the *A. judaica* ethyl acetate extract in this study, camphene, artemisia ketone, camphor, (+)-dihydrocarvone, α-bisabolol, humulol, and limonene dioxide were identified. In similar results to ours, Sallam et al. (2011) isolated camphor and piperitone as major compounds in *A. judaica* from Egypt; and Abu-Darwish et al. (2016) detected camphene, camphor, and artemisia ketone in *A. judaica* from Jordan; while Al-Wahaibi et al. (2020) reported carvone, camphor, camphene, artemisia ketone, α-humulene, α-bisabolol, farnesol, and farnesyl acetate in essential oils of *A. judaica* from Saudi Arabia.

In this study, GC–MS analysis of the most potent ethyl acetate fraction of *A. tenuissima* identified seventeen chemical components, in which the dominant compounds were 9-octadecenoic acid (Z)-, methyl ester; hexadecanoic acid, methyl ester; and methyl octadeca-9,12-dienoate. *Alternaria* species have been recognized among the literature as a rich fungal origin of new pharmacologically active metabolites, such as pyrones, steroids, terpenoids, phenolics, quinones, and nitrogen-containing compounds, which present diverse biological activities and include
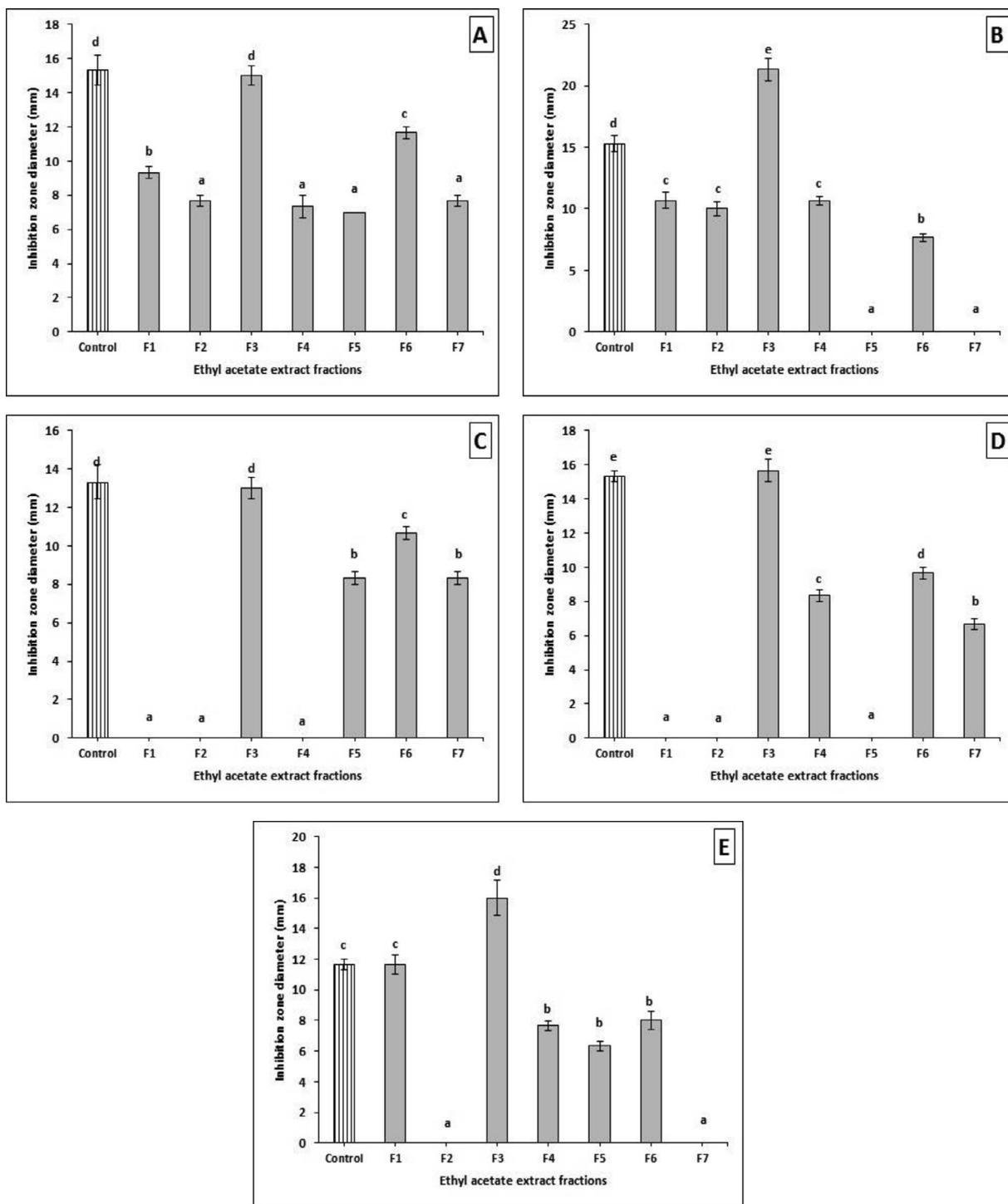
Fig. 4. Antibacterial activity of *A. tenuissima* crude extract fractions against (A) *B. subtilis*, (B) *S. aureus*, (C) *E. coli*, (D) *K. pneumonia*, and (E) *P. aeruginosa*. Error bars represent standard error of the means (*n* = 3).

among them phytotoxic, cytotoxic, and antimicrobial properties (Lou et al., 2013). Pyrone derivatives from the endophytic fungus *A. tenuissima* SP-07, were evaluated for their antibacterial activities against six bacteria (*B. megaterium*, *B. subtilis*, *C. perfringens*, *E. coli*, *M. tetragenus,* and MRSA) (Wang et al., 2014). Elgorban et al. (2019) used GC–MS to identify bis(2-ethylhexyl) phthalate; 1,2- dibutyl phthalate; phenol, 2,4-di-t-butyl-6-nitro; and 1-octadecene; 1-tetradecene;these demonstrate antimicrobial and anticancer activity in the ethyl acetate extract from the culture filtrate of *Alternaria* sp. (A8), from *Salvadora persica* from Saudi Arabia.
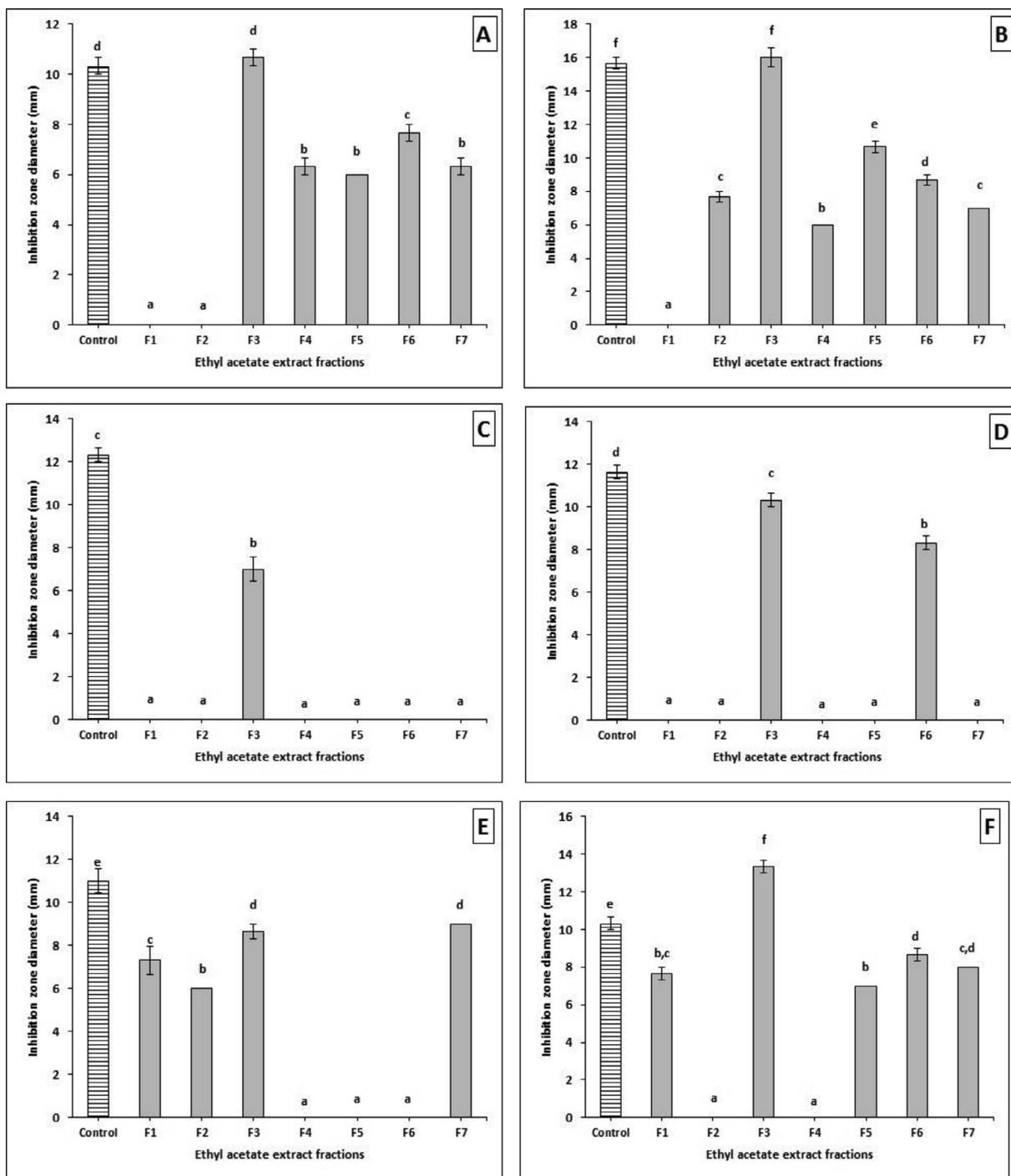
Fig. 5. Antifungal activity of *A. tenuissima* crude extract fractions against (A) *A. flavus*, (B) *A. niger*, (C) *A. alternata*, (D) *A. solani*, (E) *F. oxysporum,* and (F) *F. solani*. Error bars represent standard error of the means (*n* = 3).

5. Conclusion

Endophytic fungi show high bioactive versatility, as well as their host *A. judaica*. Both extracts of *A. tenuissima* (AUMC14342) and *A. judaica* have been shown to contain a variety of bioactive compounds, including antibacterial and antifungal agents, in addition to bioactivity against the HepG2 Hepatocellular carcinoma cell line.

This study has used GC–MS for the bioactivity-guided active fraction of the ethyl acetate organic solvent of the isolated strain and host plant extract, leading to the determination of various chemical compounds that possess broad bio-active properties. This study concludes that *A. tenuissima* can be considered to be an ideal candidate for use in the industrial production of antimicrobial agents, and in pharmaceutical development.

Table 1

GC–MS analysis of ethyl acetate extract of *A. judaica* (a), and fraction (no. 3) of *A. tenuissima* (b).

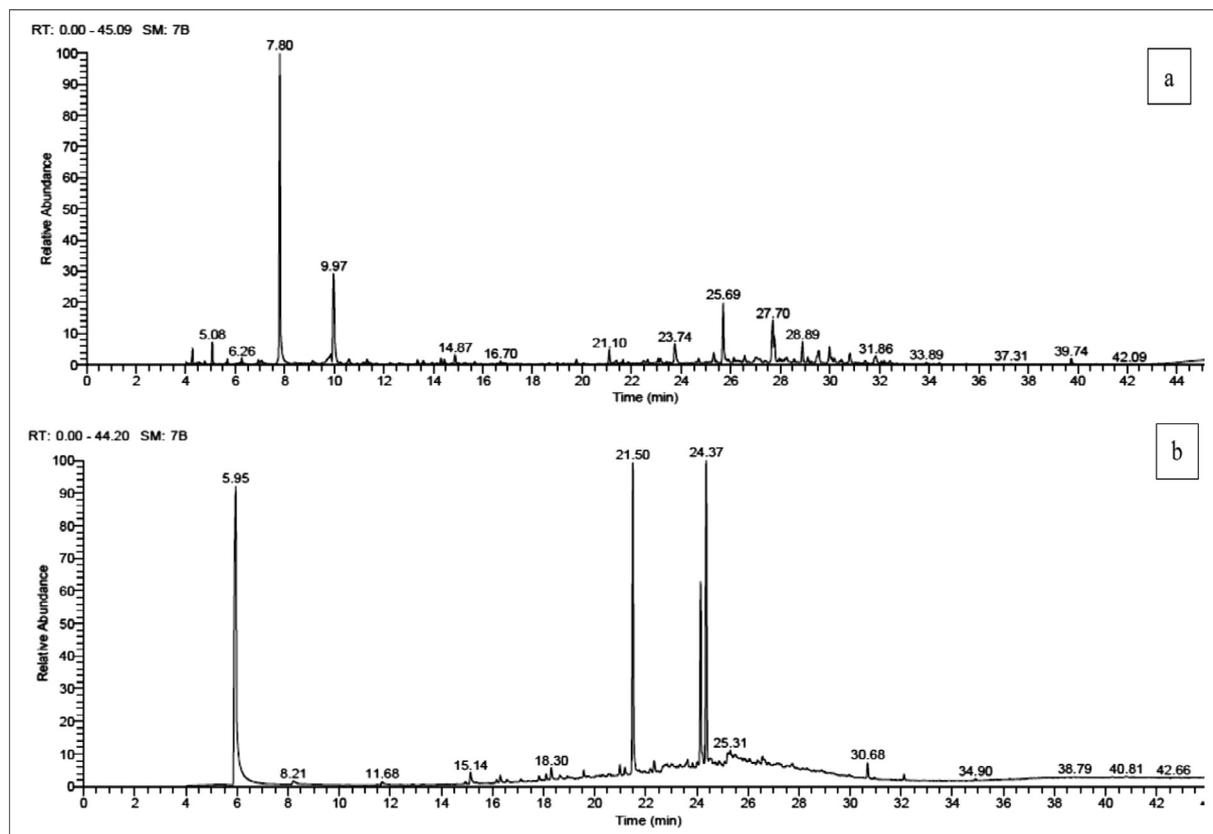| **a**	GC–MS analysis of ethyl acetate extract of *A. Judaica*					
No.	Compounds	Chemical formula	Molecular weight	RT (min.)	Match Factor	Area (%)
1	Camphene	$C_{10}H_{16}$	136	5.08	969	2.20
2	Artemisia ketone	$C_{10}H_{16}O$	152	7.80	937	37.03
3	Camphor	$C_{10}H_{16}O$	152	9.97	964	16.62
4	(+)-dihydrocarvone	$C_{10}H_{18}O$	154	14.87	782	1.31
5	Farnesene epoxide	$C_{15}H_{24}O$	220	21.10	786	1.83
6	α-bisabolol	$C_{15}H_{26}O$	222	23.74	953	3.72
7	Humulol	$C15H26O$	222	25.69	743	8.53
8	Nerolidol-epoxyacetate	$C_{17}H_{28}O_4$	296	27.70	792	7.79
9	Limonene dioxide	$C_{10}H_{16}O_2$	168	28.89	758	3.13
b	GC–MS analysis of ethyl acetate fraction (no. 3) of *A. tenuissima*					
1	1-Hexadecanol	$C_{16}H_{34}O$	242	15.14	910	1.02
2	1-Eicosanol	$C_{20}H_{42}O$	298	18.30	891	0.92
3	Tert-Hexadecanethiol	$C_{16}H_{34}S$	258	19.57	790	0.65
4	Tetradecane, 2,6,10-trimethyl-	$C_{17}H_{36}$	240	20.99	812	0.84
5	*cis*-11-Eicosenoic acid	$C_{20}H_{38}O_2$	310	21.18	764	0.63
6	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	21.50	941	21.15
7	1-Hexadecanol, 2-methyl-	$C_{17}H_{36}O$	256	22.33	746	1.08
8	Isochiapin B	$C_{19}H_{26}O_6$	350	23.63	762	0.82
9	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	24.14	913	12.64
10	Methyl octadeca-9,12-dienoate	$C_{19}H_{34}O_2$	294	24.37	918	20.95
11	Erucic acid	$C_{22}H_{42}O_2$	338	25.31	816	0.92
12	1,2-Benzenedicarboxylic acid	$C_{24}H_{38}O_4$	390	30.68	909	1.22
13	Squalene	$C_{30}H_{50}$	410	32.11	823	0.46

Fig. 6. GC–MS chromatogram of (a) ethyl acetate extract of *A. Judaica*, and (b) ethyl acetate fraction (no. 3) of *A. tenuissima*.

Declaration of Competing Interest

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2021.101462.

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