Introduction

Throughout evolution, the dependence of human beings on nature has been everlasting. Natural products have among the important sources of medicine for millennia to alleviate and treat various diseases. However, despite the rise of combinatorial chemistry as an integral part of lead discovery process, natural products still play a major role in providing novel and interesting chemical scaffolds for drug discovery with an outstanding development in the areas of separation science (Liu et al. 2019). Although plants are considered as the biofactories of many valuable bioactive compounds, they possess the disadvantage of slow growth rate, while harvesting rare and endangered species also poses a risk (Jia et al. 2016). Therefore, it is necessary to find alternative approaches to produce medicinal plant-derived bioactive metabolites. In recent decades, endophytes have been recognized as sources of several bioactive compounds and are studied as potential sources of novel natural products for medical and commercial exploitation (Selvakumar and Panneerselvam 2018).

The term “endophyte” originally introduced by de Bary (1866) to distinguish fungi – living inside host tissues – from epiphytes, is derived from the Greek word “endon”, meaning inside or within and “phyton”, meaning plant. The meaning of the word has evolved to include any microorganism that inhabits plants during a period of its life cycle, especially within leaves, branches, and stems, without causing significant damage to its host (Wilson 1995). Endophytes possess a complex relationship with their hosts. They are symbiotic in nature, which may be mutualism, commensalism or saprophytism (Clay and Schardl 2002; Strobel and Daisy 2003). Endophytes can increase the competitive abilities and fitness of plants by increasing their nutrient uptake, resistance to drought and water stress, tolerance to heavy metal stress and high salinity, or their growth rate through biochemical pathways by producing plant growth hormones. For example, researchers proved that most of the endophytic fungi produce indole-3-acetic acid (Tan and Zou 2001; Turbat et al. 2020). It was also suggested that these endophytes initiate the biological degradation of the dead or dying host tissues (Tan and Zou 2001). Although almost all higher plants contain at least one endophytic micro-
organism, the relationship between microbes and their plant hosts remains one of the least studied biochemical systems, because it is difficult to find the exact physical relationship in the interaction. Given this fact, this long-held association might have created a specific genetic system in endophytes regarding their relationship with plants or vice versa (Strobel 2003).

Endophytic fungi are highly diverse, and more than 1 million species of this fungal group is estimated to be undiscovered (Sun and Guo 2012). They represent an important component of fungal biodiversity and it has also been observed that almost every plant examined to date harbors at least one species of endophytic fungi, while many plants, particularly woody plants, contain hundreds of endophytic species (Petrini 1986; Sahoo et al. 2017). However, various factors affect the distribution of the endophytic fungal community, such as environmental factors (temperature, humidity), and the type and age of the colonized host tissue (Sanchez-Azofeifa et al. 2012). Most of the studies reported that Dothideomycetes and Sordariomycetes are the dominant classes found in medicinal plants, but the diversity of the endophytic fungi also differs according to the geographical regions (Kharwar et al. 2008; Dhayanithy et al. 2019).

Endophytic fungi of medicinal plants are potential sources of novel bioactive compounds and some have also been proved to produce plant-associated therapeutic metabolites (Huang et al. 2007; Vigneshwari et al. 2019). The endophytic fungi such as Acremonium, Alternaria, Aspergillus, Cephalosporium, Chaetomium, Chloridium, Choanephora, Colletotrichum, Fusarium, Gliocladium, Hypoxylon, Paecilomyces, Penicillium, Pestalotiopsis, Talaromyces, and Trichoderma from different medicinal plants have been reported as sources of several bioactive compounds (Rana et al. 2019). A diverse array of endophytic metabolites exhibited antimicrobial activity against various pathogenic microorganisms, and these can be used in pharmaceuticals, medicine, and agriculture (Gunatilaka et al. 2006; Yo et al. 2017; Sim et al. 2020).

The common juniper (Juniperus communis L.) is a bush or small evergreen tree which has been commonly referred to as herbal medicine in ancient times. It contains various chemical constituents including flavonoids, volatile oils, coumarins and therapeutically important anticancer lignans, podophyllotoxin and deoxypodophyllotoxin (Hartwell et al. 1953). Previous studies reported the isolation and characterization of endophytic fungi harboured in Juniperus plants sampled from the natural populations in Dortmund and Haltern (Germany), and Jammu and Kashmir (India). This resulted in the discovery of a deoxypodophyllotoxin-producing endophytic fungus harboured in J. communis (Kusari et al. 2009). The endophytic fungi isolated from Juniperus trees such as Penicillium and Aspergillus were also found to exhibit antimicrobial activities (Gherbawy and Elhariry 2016).

In our study, isolation and identification of endophytic fungi from J. communis were undertaken and their biodiversity parameters evaluated. Furthermore, the antimicrobial activities of metabolites extracted with different organic solvents from both the ferment broth and mycelia of isolated endophytic fungi were also determined.

Materials and Methods

Collection of J. communis samples

Fresh, healthy parts of the J. communis plants were collected during the late autumn of 2015, 2016 and 2017 (Table 1). GPS coordinates of the sampling sites were recorded. All plant specimens have been identified and authenticated by experts. Collected specimens were placed into sealed plastic bags, labelled with the number and date of collection and stored at 4 °C until processing.

Isolation of endophytes

Isolation of endophytic fungi from plant parts was performed according to the method described by Garyali (2013) with minor modifications. The plant materials were rinsed in running tap water to remove dust and debris, and the specimens were cut into small segments of about 0.5 to 1 cm in length using a sterile blade. The leaf, twig, root, and cone parts were separated, and these parts were examined for their fungal endophyte content.

The plant segments were surface sterilized to kill the epiphytic microorganisms by sequentially immersing the plant material in 70% ethanol for 60 sec, washing with sterile distilled water and then steeping in 0.01% mercuric chloride (VWR International, Hungary) for 30 sec. Finally, the specimens were washed again with sterile distilled water 2-3 times and then allowed to dry on a sterile blotting paper. Each segment was placed onto the surface of PDA medium (VWR International, Hungary) supplemented with ampicillin (50 µg/mL, Merck, Hungary) in Petri dishes. They were incubated at 25 °C for 5-10 days and checked daily for the growth of fungal colonies. Pure isolates were obtained by picking individual colonies from the plates and transferring them onto fresh PDA medium where they were incubated at 25 °C for 10 days. Each fungal culture was checked again for purity, transferred separately to PDA slants, maintained at 4 °C and deposited in the Szeged Microbiological Collection (SZMC, Hungary; http://www.wfcc.info/ccinfo/collection/by_id/987).

Molecular identification of isolates

Genomic DNA was isolated from fungal mycelia grown in PDB medium (VWR International, Hungary) at 25 °C.
for 7 days. Isolation and purification of genomic DNA was performed using the EZNA Fungal DNA Mini Kit (Omega Bio-tek, Norcross, USA) according to the manufacturer’s instructions.

DNA fragments containing the ITS region of the ribosomal DNA gene cluster were amplified using the primers ITSS1 and ITSS4 (White et al. 1990). Polymerase chain reaction (PCR) was performed in a total volume of 25 µL consisting of 2.5 µL of PCR reaction buffer (10×Standard Reaction Buffer), 2 µL of 2mM dNTP, 0.5 µL of 10 µM ITSS1 forward primer (5'-TCCGTAGGTGAACCTGCGG-3'), 0.5 µL of 10 µM ITSS4 reverse primer (5'-TCCTCGCTTATTGATATGC-3'), 2 µL of DNA template and 0.125 µL of Pfu DNA-polymerase (Fermentas). The PCR amplification profile consisted of an initial denaturation at 94 °C for 5 min, followed by 35 amplification cycles (30 sec at 94 °C, 40 sec at 48 °C, and 1 min 72 °C) and a final extension at 72 °C for 3 min. PCR products were separated using horizontal gel electrophoresis in 1% agarose gel supplemented with ethidium bromide at 0.1 µg/mL final concentration. Electrophoresis was performed for 30 min at 100 V. Separated DNA fragments were visualized using a UV transilluminator (UVP-BioDoc-ItTM imaging Systems, Analytik Jena, Jena, Germany). 1000 bp marker (Fermentas) was used to determine the size of the products.

Amplified DNA fragments were sequenced (BaseClear, The Netherlands) and used for BLAST similarity search at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST). Genus level identification was carried out from the lowest expect value of the BLAST output.

**Screening of bioactive metabolite-producing endophytic fungi**

**Secondary metabolite extraction**

The isolated endophytic fungi were cultured for 7 days at 25 °C in 50 mL PDB medium. Then the mycelia were separated from the broth by filtration through a cheese cloth and dried overnight in an oven until constant weight. Then 25 mL distilled water was added to the dry material, which was then sonicated for 20 min after the addition of an aliquot of liquid nitrogen to maintain the chilled condition. After that the extraction of the aqueous samples was done with a 25 mL mixture of chloroform and methanol (4:1, V/V) and extraction was repeated 3 times. The ferment broths were extracted 3 times sequentially with 50-50 mL of hexane, ethyl acetate and chloroform, respectively, and both extract series were pooled. The organic solvents were removed by a rotary evaporator (IKA HB10 basic, VWR International, Hungary) in vacuum at 30 °C from each pooled extract including ethyl acetate, chloroform, as well as chloroform and methanol (4:1, V/V) fractions. The resulted 4 dry samples per each isolate were stored at -20 °C and resuspended in 1 mL of HPLC grade methanol (VWR International, Hungary) prior to use.

**Activity assays against bacteria and yeast**

For testing the antibacterial potential of the crude extracts, 400 µL of the methanolic extracts were transferred into new Eppendorf tubes and dissolved in 1 mL 10% methanol after evaporation. These extracts were tested against two Gram-negative (Escherichia coli SZMC 6271 and Pseudomonas aeruginosa SZMC 23290), two Gram-positive bacteria (Staphylococcus aureus SZMC 14611 and Bacillus subtilis SZMC 0209) and two yeasts (Candida albicans SZMC 1533 and C. krusei SZMC 1352). For the assay, the suspensions of the microbes were prepared from overnight cultures, which were cultivated in Luria-Bertani broth (10 g tryptone, 5 g yeast extract and 5 g NaCl in 1 L distilled water) and yeast extract peptone dextrose broth (20 g peptone, 10 g yeast extract and 20 g glucose in 1 L distilled water) for the bacteria and yeasts, respectively, at 37 °C. Their concentrations were set to 4 × 10^5 cells/mL with sterile media. Then, 96-well plates were prepared by dispensing 100 µL suspension containing the bacterial or yeast cells, 100 µL of the extract dissolved in 10% of methanol was added into the wells, which were then incubated for 24 h at 37 °C. The mixture of 100 µL broth and 100 µL 10% methanol was used as the blank sample for background correction, while 100 µL of the microbial suspension supplemented with 100 µL 10% methanol was applied as the negative control. The positive control contained ampicillin (100 µg/mL, Merck, Hungary) for bacteria and nystatin (10 µg/mL, Merck, Hungary) for fungi. The inhibitory effects of each derivative were spectrophotometrically (SPECTROstar Nano, BMG Labtech, Ordenberg, Germany) determined at 620 nm after incubation, and the inhibition rate was calculated as the percentage of the positive control after blank correction.

**Activity assay against filamentous fungi**

To determine the potential antifungal activity of the fungal extracts against filamentous fungi, agar well diffusion assay was carried out. Evaporated samples of crude extracts (400 µL) were dissolved in 1 mL 10% methanol. Four holes with a diameter of 8 mm were bored into PDA plates, at the 2.5 cm distances around the centre of the plate. Then precultured (25 °C, 7 days) Fusarium culmorum SZMC 11039 and Rhizoctonia solani SZMC 21048 strains were placed in the centre of plates with agar plugs. After that, 100 µL of samples was applied into each hole. As solvent control, 10% methanol was used. Mycelial plug inoculated without any extracts was used as a control.
Antifungal activity of the samples was determined by the size of the inhibition zone.

**Biodiversity mapping of endophytic fungi of *J. communis***

**Calculating isolation rate and diversity index**

The isolation rate of endophytic fungi was calculated as the total number of tissue segments infected by fungi divided by the total number of tissue segments incubated (Kumar and Hyde, 2004).

The diversity of endophytic fungi isolated from 3 plant parts were evaluated using the Shannon-Weiner Index (H'), Simpson's diversity index (1-D), evenness Index (J) and Margalef richness index (D mg) (Hoffman et al. 2008; Suryanarayanan and Kumaresan 2000; Kusari et al. 2012). All the diversity indices were calculated plantwise and also tissuewise to analyse the host and tissue specificity of endophytic fungi.

**Statistical analysis**

Statistical analyses for biodiversity calculations were carried out in R 3.5.2 (R core Team 2019). The diversity indices were calculated using the Vegan package from R 3.5.2 (Oksanen et al. 2018). One-way analysis of variance (ANOVA) was carried out to test the effect of plant species or tissue type (stem and root) on the isolation rate and genus richness of endophytic fungi. Post hoc Tukey’s Honest Significant Difference tests were performed to observe the significant differences among the plant species or tissue types at P < 0.05 level.

**Results**

**Investigation of endophytic fungi isolated from *J. communis***

The *J. communis* plant parts were collected from Southern Hungarian areas and their endophytes were isolated and purified, which was followed by sequence-based molecular identification (Table 1).

Altogether, 240 parts were tested involving 60 cuttings of leaf, stem, root and cone from 12 different plant samples of *J. communis*. A total of 75 endophytic fungi distributed into 3 main classes and 7 main orders were isolated from *J. communis* (Fig. 1). The isolation rates were 0.51, 0.3, 0.35 and 0.18 for stem, leaf, root and cone, respectively. The predominant class was found to be Sordariomycetes, similarly to previous studies on *Juniperus* endophytes (Kusari et al. 2009). Most of the isolates belonged to Hypocreales, while the rest of them were members of the taxa Pleosporales and Eurotiales (Table 1).

To characterize the biodiversity of *J. communis* EF, the Shannon diversity index (H') Simpson's diversity index (1-D), and Margalef's richness (D mg) have been calculated. The Shannon-index revealed higher certainty of endo-
| Collection code | GPS coordinates                   | Plant part | Species        | Genbank ID of ITS |
|----------------|----------------------------------|------------|----------------|------------------|
| SZMC 27149     | N 46°53.338' / E 019°24.483'     | Stem       | Alternaria sp. | MT940776         |
| SZMC 27150     | N 46°53.340' / E 019°24.528'     | Stem       | Alternaria sp. | MT940777         |
| SZMC 27151     | N 46°53.340' / E 019°24.528'     | Stem       | Alternaria sp. | MT940778         |
| SZMC 27152     | N 46°53.338' / E 019°24.483'     | Stem       | Alternaria sp. | MT940779         |
| SZMC 27153     | N 46°53.345' / E 019°24.501'     | Stem       | Alternaria sp. | MT940780         |
| SZMC 27154     | N 46°53.345' / E 019°24.501'     | Stem       | Alternaria sp. | MT940781         |
| SZMC 27155     | N 46°53.345' / E 019°24.501'     | Leaf       | Alternaria sp. | MT940782         |
| SZMC 27156     | N 46°53.345' / E 019°24.501'     | Stem       | Alternaria sp. | MT940783         |
| SZMC 27157     | N 46°53.345' / E 019°24.501'     | Stem       | Alternaria sp. | MT940784         |
| SZMC 27158     | N 46°53.338' / E 019°24.483'     | Leaf       | Alternaria sp. | MT940785         |
| SZMC 27159     | N 46°53.345' / E 019°24.501'     | Stem       | Alternaria sp. | MT940786         |
| SZMC 27160     | N 46°53.345' / E 019°24.501'     | Stem       | Alternaria sp. | MT940787         |
| SZMC 27161     | N 46°53.345' / E 019°24.501'     | Stem       | Alternaria sp. | MT940788         |
| SZMC 27162     | N 46°53.345' / E 019°24.501'     | Stem       | Alternaria sp. | MT940789         |
| SZMC 27163     | N 46°53.338' / E 019°24.483'     | Stem       | Alternaria sp. | MT940790         |
| SZMC 27164     | N 46°53.345' / E 019°24.501'     | Stem       | Aspergillus sp. MT993364 |
| SZMC 27165     | N 46°53.345' / E 019°24.501'     | Stem       | Aspergillus sp. MT993365 |
| SZMC 27166     | N 46°53.338' / E 019°24.483'     | Stem       | Aspergillus sp. MT993366 |
| SZMC 27167     | N 46°53.345' / E 019°24.501'     | Stem       | Aspergillus sp. MT993367 |
| SZMC 27168     | N 46°53.345' / E 019°24.501'     | Stem       | Aspergillus sp. MT993368 |
| SZMC 27169     | N 46°53.330' / E 019°24.478'     | Stem       | Cladosporium sp. MT993369 |
| SZMC 27170     | N 46°53.330' / E 019°24.478'     | Stem       | Cladosporium sp. MT993370 |
| SZMC 27171     | N 46°53.330' / E 019°24.478'     | Stem       | Cladosporium sp. MT993371 |
| SZMC 27172     | N 46°53.338' / E 019°24.483'     | Stem       | Cladosporium sp. MT993372 |
| SZMC 27173     | N 46°53.330' / E 019°24.478'     | Root       | Cladosporium sp. MT994503 |
| SZMC 27174     | N 46°53.330' / E 019°24.478'     | Stem       | Colletotrichum sp. MT994504 |
| SZMC 27175     | N 46°53.330' / E 019°24.478'     | Leaf       | Colletotrichum sp. MT994505 |
| SZMC 27176     | N 46°53.330' / E 019°24.478'     | Stem       | Colletotrichum sp. MT994506 |
| SZMC 27177     | N 46°53.342' / E 019°24.474'     | Stem       | Curvularia sp. MT994507 |
| SZMC 27178     | N 46°53.330' / E 019°24.478'     | Stem       | Curvularia sp. MT994508 |
| SZMC 27179     | N 46°53.330' / E 019°24.478'     | Stem       | Didymella sp. MT994509 |
| SZMC 27180     | N 46°53.338' / E 019°24.483'     | Stem       | Fusarium sp. MT994510 |
| SZMC 27181     | N 46°53.342' / E 019°24.474'     | Stem       | Fusarium sp. MT994511 |
| SZMC 27182     | N 46°53.340' / E 019°24.528'     | Stem       | Fusarium sp. MT994512 |
| SZMC 27183     | N 46°53.338' / E 019°24.483'     | Leaf       | Fusarium sp. MT994513 |
| SZMC 27184     | N 46°53.342' / E 019°24.474'     | Root       | Fusarium sp. MT982177 |
| SZMC 27185     | N 46°53.340' / E 019°24.528'     | Root       | Fusarium sp. MT982178 |
| SZMC 27186     | N 46°53.338' / E 019°24.483'     | Root       | Fusarium sp. MT982179 |
| SZMC 27187     | N 46°53.340' / E 019°24.528'     | Root       | Fusarium sp. MT982180 |
| SZMC 27188     | N 46°53.340' / E 019°24.528'     | Root       | Fusarium sp. MT982181 |
| SZMC 27189     | N 46°53.340' / E 019°24.528'     | Root       | Fusarium sp. MT982182 |
| SZMC 27190     | N 46°53.338' / E 019°24.483'     | Root       | Fusarium sp. MT982183 |
| SZMC 27191     | N 46°53.340' / E 019°24.528'     | Root       | Fusarium sp. MT982184 |
| SZMC 27192     | N 46°53.340' / E 019°24.528'     | Root       | Fusarium sp. MT982185 |
| SZMC 27193     | N 46°53.340' / E 019°24.528'     | Root       | Fusarium sp. MT982186 |
| SZMC 27194     | N 46°53.342' / E 019°24.474'     | Root       | Fusarium sp. MT982187 |
| SZMC 27195     | N 46°53.340' / E 019°24.528'     | Root       | Fusarium sp. MT982188 |
| SZMC 27196     | N 46°53.340' / E 019°24.528'     | Root       | Fusarium sp. MT982189 |
| SZMC 27197     | N 46°53.338' / E 019°24.483'     | Root       | Penicillum sp. MT982190 |
| SZMC 27198     | N 46°53.340' / E 019°24.528'     | Leaf       | Penicillum sp. MT982191 |
phytic fungal genus consistency in the stem compared to that of the other parts of *J. communis*. Moreover, the Simpson’s-index clearly showed that the stem harboured highly diverse fungal endophytes compared to those harboured by other plant parts. Finally, based on Margalef’s-index the stems had high taxonomic richness, while the cone had the lowest compared to the other tissues of *J. communis* (Table 2.).

The stems of *J. communis* harboured 11 unique fungi, whereas 4 and 2 were found in leaf and cone samples (Fig. 2). Interestingly, the roots of *J. communis* did not harbour any unique fungi. This shows that some genus seems to be tissue specific. *Xylaria* sp. were found only in the cone, while *Pestalotiopsis* and *Bipolaris* were found only in the leaf and stem respectively, whereas *Curvularia*, *Aspergillus*, *Didymella* and *Purpureocillium* sp. were specifically found in stems (Fig. 3). *Fusarium* strains were more abundant in roots than in other tissues.

### Antimicrobial effects of fungal extracts of *J. communis* endophytes

Gram-positive bacteria were found to be more susceptible to the extracted endophytic metabolites than Gram-negative ones due to the higher number of highly active (>90%) extracts (Fig. 4). For *B. subtilis*, the highest number of highly active extracts was recorded in the case of the

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**Table 2.** Biodiversity parameters of endophytic fungi isolated from *J. communis*.

| Diversity index     | Stem  | Root  | Leaf  | Cone  | Total |
|---------------------|-------|-------|-------|-------|-------|
| Simpson’s Dominance (D) | 0.912 | 0.775 | 0.788 | 0.666 | 0.92  |
| Shannon (H’)         | 2.582 | 1.630 | 1.950 | 1.214 | 2.85  |
| Pielou’s evenness (j) | 0.931 | 0.910 | 0.847 | 0.48  | 0.89  |
| Margalef richness    | 4.218 | 1.894 | 3.176 | 1.365 | 5.32  |
ethyl acetate extracts of ferment broth (55), while the lowest amount of effective extracts (19) was obtained from the hexane-based solvent partitions. The mycelial extracts proved to be the most effective against *E. coli* and *P. aeruginosa*, as the numbers of the active extracts were 25 and 27, respectively. In the case of *S. aureus*, the highest number of effective extracts was obtained for the ethyl acetate extracts (43), followed by chloroform-partitioned ferment broth samples (37). It is important to highlight that strain SZMC 27155 was highly active against all bacteria, but it was not active against the tested yeasts and plant pathogenic fungi. The ethyl acetate and chloroform extracts of strains SZMC 27164 and SZMC 27031 showed remarkable inhibitory effects to all tested bacteria and the mycelial extracts of these isolates were also active against plant pathogenic fungi and yeasts. The *Trichoderma* isolates of this plant showed activity at least against one test microbe. The extracts of strain SZMC 27205 showed significant inhibitory activity to both Gram-positive and

| Collection code | *F. culmorum* | *R. solani* |
|-----------------|--------------|-------------|
|                 | HEX | CLF | EtOAc | C:M | HEX | CLF | EtOAc | C:M |
| SZMC 27198      | -   | -   | -     | -   | -   | -   | -     | +   |
| SZMC 27206      | -   | -   | -     | +   | -   | -   | ++    | -   |
| SZMC 27209      | -   | -   | -     | +   | -   | -   | +     | -   |
| SZMC 27210      | -   | -   | -     | -   | -   | -   | -     | +   |
| SZMC 27211      | -   | -   | -     | -   | -   | -   | +     | -   |
| SZMC 27212      | -   | -   | -     | +   | -   | -   | +     | +   |
| SZMC 27213      | -   | -   | -     | -   | -   | -   | +     | +   |
| SZMC 27214      | -   | -   | -     | -   | -   | +   | -     | -   |
| SZMC 27215      | -   | -   | -     | -   | -   | +   | -     | ++  |
| SZMC 27216      | -   | -   | -     | -   | -   | +   | -     | +   |
| SZMC 27218      | -   | -   | -     | -   | -   | -   | +     | +++|

**Table 3.** List of the endophytic fungi extracts showing inhibitory activities to plant pathogenic fungi (HEX – hexane; CLF – chloroform; C:M - chloroform:methanol (4:1) extract of mycelia; EtOAc – Ethyl acetate).

![Figure 3. Distribution of endophytic fungi of *J. communis* at the genus level.](image)

![Figure 4. Summary of the antibacterial effects of endophytic extracts isolated from *J. communis* (C:M - chloroform:methanol (4:1, V/V) extract of mycelia).](image)

![Figure 5. Summary of the antifungal effects of endophytic extracts isolated from *J. communis* (C:M - chloroform:methanol (4:1, V/V) extract of mycelia).](image)
Gram-negative bacteria. With respect to taxa, Fusarium, Pestalotiopsis, Trichoderma, Aspergillus and Purpureocillium strains showed high bioactivities and will be suitable for further investigations.

Altogether, 27 extracts showed inhibitory effects to yeasts, which is over 90% (Fig. 5). Interestingly, both the chloroform extract of the ferment broth and the mycelial extracts inhibited C. albicans, while C. krusei was mainly susceptible to the ethyl acetate extract of the ferment broth and to the mycelial extracts.

Previous works showed that the endophytic fungi of J. communis were excellent sources of antimicrobial compounds (Gherbawy and Elhariry 2016). In our study at least one solvent partition of 58 isolates was active against B. subtilis, S. aureus and C. albicans. However, only a few extracts, particularly the mycelial extracts were found to be active against the tested filamentous fungi (Table 3). Specifically, mycelial extracts of Trichoderma and Purpureocillium strains were active against both of the tested fungi. F. culmorum was found to be more resistant than R. solani.

**Discussion**

Endophytic fungi are highly diverse, and their investigation is very important from different plants to understand the biodiversity and structure of the endophytic fungal community, which mostly depends on the plant physiology, biogeographical factors and their interplay with other pathogenic microorganisms associated with their host plant (Arnold et al. 2007). Only a few studies have been carried out to study the endophytic fungal communities in Hungary (Knapp et al. 2012). Therefore, in the present work, 75 endophytic strains were isolated from J. communis from the Southern part of Hungary and the antimicrobial activities of their metabolites evaluated.

In our study, the culture-dependent method was followed for the molecular identification of fungal isolates. Based on ITS sequence analysis, the isolates were characterized into 7 orders of 3 classes. All the isolated fungi belonged to Ascomycota, which includes the classes Dothideomycetes, Sordariomycetes and Eurotiomycetes. Sordariomycetes was the dominant class (52%) followed by Dothideomycetes (32%) and Eurotiomycetes (16%). Such dominance of Sordariomycetes as endophytes has been reported from several plants, e.g., Phragmites (Sim et al. 2018) and lichens (U’ren et al. 2016) indicating that Sordariomycetes are ubiquitous among the plant kingdom. Totally, 14 genera (Alternaria, Aspergillus, Bipolaris, Cladosporium, Colletotrichum, Curvularia, Didymella, Fusarium, Penicillium, Pestalotiopsis, Phomopsis, Purpureocillium, Trichoderma and Xylaria) were identified (Table 1), where the relative abundances of Fusarium, Alternaria and Trichoderma were the highest (Fig. 3). The isolation rates of endophytic fungi were found to be the highest from stems followed by leaf, similarly to the results of previous studies on J. communis endophytes (Gherbawy and Elhariry 2016), whereas similar studies in other plants showed higher isolation rate values from the leaf compared to stems (Alurappa and Chowdappa 2018).

One of the most important properties of endophytic fungi is that they produce a wide variety of compounds that protect the plants from plant pathogens (Tan and Zou 2001; Strobel and Daisy 2003). In our cases, a total of 58 isolates (77%) showed antibacterial activity against at least one test microorganism. Altogether, 16% of the isolates had antibacterial effects with wide spectrum. Five strains showed remarkably high inhibitory values (>90%) to all the tested strains. Furthermore, regarding the antifungal activity, 43 and 31 extracts were active against C. albicans, and C. krusei, respectively, while 3 and 11 showed inhibitory effects against F. culmorum and R. solani.

Future examinations could reveal the chemical nature of the active metabolites and their potential for practical (e.g., pharmaceutical, agricultural) applications.

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