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ARTICLE

Revealing of biodiversity and antimicrobial effects of *Artemisia asiatica* endophytes

Aruna Vigneshwari[1], Saruul Erdenebileg[1], Kata Fujkin[1], Dezső Csupor[3], Judit Hohmann[3], Tamás Papp[1,2], Csaba Vágvölgyi[1], András Szekeres[1]*

[1]Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary
[2]MTA-SZTE Fungal Pathogenicity Mechanisms Research Group, Hungarian Academy of Sciences - University of Szeged, Szeged, Hungary
[3]Department of Pharmacognosy, Faculty of Pharmacy, University of Szeged, Szeged, Hungary

ABSTRACT Endophytic fungi produce a plethora of secondary metabolites, which may open new avenues to study their applicability in pharmaceuticals. Therefore, the present study focuses on the fungal endophytic community of *Artemisia asiatica*. During our work, fungal endophytes were isolated from a medicinal plant, *A. asiatica*. The culturable endophytic fungi were identified using molecular techniques and biodiversity, richness and tissue specificity were examined. As these microorganisms have been generally identified as an abundant reservoir of novel antimicrobial compounds, the antimicrobial (i.e. antibacterial and antifungal) activities of the metabolites produced by the isolated fungi were studied. Numerous extracts containing the endophytic metabolites proved to be active against the applied test microorganisms including Gram-positive and Gram-negative bacteria, as well as yeasts and filamentous fungi, which can be examined in detail in the future and, based on the the chemical nature of these active metabolites, allow to discover novel bioactive metabolites. **Acta Biol Szeged 64(2):111-119 (2020)**

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***Corresponding author**
E-mail: andras.j.szekeres@gmail.com

Introduction

Endophytic fungi can be defined as an ecological group of fungi colonizing the inner tissues of plants without any recognizable features of their presence (Wani et al. 2016). The plant's endophytic fungi spend their lifecycle colonizing inter- and/or intra-cellularly the healthy tissues of the host plants, without causing any apparent indication of disease.

For agriculture, pharmaceutical and food industry, plant-associated endophytic fungi have been observed as important and novel sources of natural bioactive products (Zhao et al. 2010; Yo and Ting 2017). Until now tens of thousands of natural products have been identified in the world, but still a vast number of unknown compounds are waiting for discovery and to be utilized for the benefits of mankind (Sarker 2012). The comprehensive review by Newman and Cragg (2016), provides detailed information about the natural compounds discovered between 1981-2014. Of the 1562 new chemical entities discovered in this period, 73% belongs to natural products and their derivatives and only 27% of the drugs were of synthetic origin. Furthermore, the increasing prevalence of new diseases results in the continuous need of exploiting natural products for drugs (Cragg and Newman 2013). Especially, the emergence of multidrug-resistant microbes increases the urge to find novel therapeutic leads (Talebi et al. 2019). Demain and Sanchez (2009) estimate that currently more than 1 million natural compounds have been isolated, from which 50-60% have plant and 5% have microbial origins. In recent decades, endophytes have been recognized as a source of several bioactive compounds and are studied as potential sources of novel natural products for the health sector and for drug discovery (Jalgaonwala et al. 2011; Lam 2007; Strobel and Daisy 2003; Shukla et al. 2014; Vigneshwari et al. 2019). However, it could provide an important alternative to overcome the increasing levels of drugs resistance to various pathogenic microorganisms, only a few plant species have been investigated for their endophytic diversity and their bioactive secondary metabolites until now (Shukla et al. 2014).

The medicinal plants are potential sources of fungal endophytes producing novel bioactive compounds (Kaul et al. 2012), plant hormones (Khan et al. 2017; Turbat et al. 2020), and plant associated therapeutic metabolites (Huang et al. 2007; Vigneshwari et al. 2019). The plant genus *Artemisia* consists of around 400 species and these are one of the most important sources of medicinal compounds (Koul et al. 2018). Among the *Artemisia* species, *A.*

annua is the best known due to its artemisinin content, which is an important antimalarial drug (Weathers et al. 2011). Liu et al. (2001) identified 14 fungal endophytes, which produced antagonistic compounds against four phytopathogens, in *A. annua*. Another study showed the bioactive potential of endophytic fungi isolated from *A. annua*, including *Aspergillus* sp. and *Cephalosporium* sp., which showed the highest antibacterial activity (Zhang et al. 2014).

A. asiatica Nakai is also the member of the *Artemisia* (mugwort) genus. This is a perennial plant and abundantly found in the northern temperate regions of Asia, Europe and North America. This species is widely known for its medicinal properties and their essential oil is commonly used in medicine and food products (Oh et al. 2005; Ryu et al. 1998; Ahuja et al. 2018). Characteristic secondary metabolites of *A. asiatica* include flavonoids, coumarins, terpenes, sesquiterpene lactones, monoterpenes, guaianolidem secoguianolide, lignans, phenylpropanoids and steroids (Hajdú et al. 2014). Despite the broad-spectrum bioactivity of *A. asiatica,* its endophytic fungal community and their bioactive compounds has not been investigated. Therefore, in our study, isolation and identification of endophytic fungi from *A. asiatica* were carried out and their biodiversity parameters were evaluated. Furthermore, antimicrobial activities of metabolites extracted using different organic solvents from both the ferment broth and the mycelia of the isolated endophytic fungi were also determined.

Materials and Methods

Collection of A. asiatica samples and isolation of the endophytes

The *A. asiatica* Nakai plants were provided by the Department of Pharmacognosy, University of Szeged (Szeged, Hungary). The samples were collected in 2016 and the plant specimens have been identified and authenticated by experts. Collected specimen was placed in a sealed plastic bag and was labelled with the number and date of collection and stored at 4 °C until processing.

Isolation of endophytic fungi from plant parts was done based on the method described by Gariyali (2013) with minor modifications. The plant materials were rinsed in running tap water to remove contaminations and the specimens were cut via a sterile blade into small segments of about 0.5 to 1 cm in length. The leaf and stem parts were separated, and these parts were examined for their fungal endophyte content. The plant segments were firstly surface sterilized by sequentially immersing the plant material in 70% (60 sec) ethanol, washing with sterile distilled water and then, steeping in 0.01% (30 sec) mercuric chloride (VWR International, Hungary). Finally, the specimens were washed again 2-3 times with sterile distilled water and then dried on a sterile blotting paper. After the surface sterilisation, each segment was placed onto the surface of PDA medium (VWR International, Hungary) supplemented with 50 μg/mL ampicillin (Merck, Hungary) in a Petri dish. Then, the plates were incubated at 25 °C and the growth of fungal colonies were checked daily for 5-10 days. Pure isolates were collected by picking up individual colonies from the plates and transferring them onto a fresh PDA medium, which were incubated at 25 °C for 10 days. Each fungal culture was checked again for purity and transferred separately to PDA slants. These colonies were maintained at 4 °C and were deposited into the Szeged Microbiological Collection (SZMC, Hungary; http://www.wfcc.info/ccinfo/collection/by_id/987).

Molecular identification of isolates

For DNA isolation, fungal isolates were grown in PDB (VWR International, Hungary) at 25 °C for 5 days. Isolation of the genomic DNA from the mycelia was performed using the E.Z.N.A. Fungal DNA Mini Kit (Omega Bio-tek, Norcross, USA) based on the manufacturer's instructions. The internal transcribed spacer (ITS) region of the rDNA was amplified using the primers ITS1 and ITS4 as described previously (White et al. 1990). Sequencing was performed commercially by BaseClear B.V. (Leiden, The Netherlands). Sequences were first analyzed by BLAST similarity search at the website of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST) and the species were identified based on their identity values (>97%).

Screening of bioactive metabolite producing endophytic fungi

Secondary metabolite extraction

Endophytic isolates were cultivated in 50 mL PDB medium at 25 °C, 120 rpm for 7 days. At the end of the incubation period mycelia was filtered through a cotton wool. The ferment broths were extracted three times sequentially with 50-50 mL of hexane, ethyl acetate and chloroform, respectively, and the extracts containing same solvents were pooled. The mycelial samples were overnight dried in an oven until constant weight and 25 mL distilled water was added to each. Then, the mixtures were 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 three times with the mixture of 25 mL of chloroform and methanol (4:1, V/V). Both the ferment broth and the mycelial extracts were evaporated by a rotary evaporator (IKA HB10 basic, VWR International, Hungary) in vacuum at 30 °C. The

resulted four 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.

Microdilution based antimicrobial assay

To test the antibacterial effects of the metabolites, 400 µL of the methanolic solution of all extracts were transferred into Eppendorf tubes and after the evaporation were dissolved in 1 mL 10% methanol. These solutions were tested against two Gram-negative bacteria, *E. coli* (SZMC 6271) and *P. aeruoginosa* (SZMC 23290), two Gram-positive bacteria, *S. aureus* (SZMC 14611) and *B. subtilis* (SZMC 0209) and two yeasts, *C. albicans* (SZMC 1533) and *C. krusei* (SZMC 1352) according to the M07-A10 CLSI guideline (Weinstein 2018), all of which were obtained from the SZMC, Szeged, Hungary. For the test, 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 in1 L distilled water) for the bacteria and yeasts, respectively, at 37 °C. The concentrations of the suspensions were set to 4×10^5 cells/mL with sterile media and 100 µL of this suspension were transfered into the wells of 96-well plates. Then, 100 µL of the extract was added into each well, and the plates were then incubated for 24 h at 37 °C. The mixture of 100 µL of ferment broth and 100 µL of 10% methanol was used as the blank sample for background correction, while 100 µL of the microbial suspension supplemented with 100 µL of 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 yeasts. The inhibitory effects of the extracts 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.

Agar diffusion test

To determine the potential antifungal activity of the fungal extracts against plant pathogenic fungi 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 and 100 µL of the 10% methanolic extracts prepared for the microdilution assay were applied into the wells. As a solvent control, 10% methanol was used and a mycelial plug inoculated without any extracts was applied as a growing control. Antifungal activities of the samples were determined by the detection of the inhibition zone.

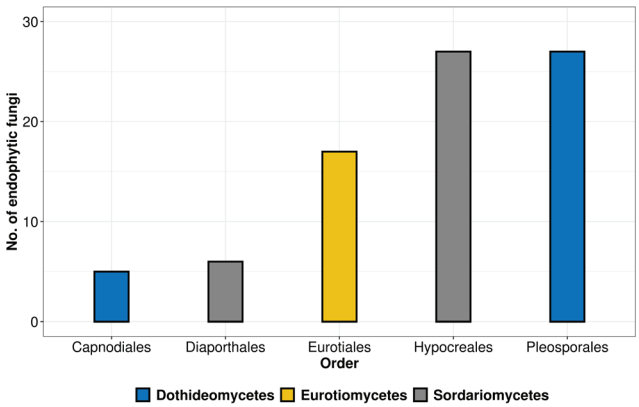

Figure 1. Distribution of endophytic fungi isolated from *A. asiatica* into classes and orders.

Biodiversity mapping of endophytic fungi of A. asiatica

Calculating isolation rate and diversity parameters

The isolation rate of the 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 were evaluated using the Shannon-Weiner Index (H′), Simpson's diversity index (1-D), evenness Index (J) and Margalef richness index (Hoffman et al. 2008; Suryanarayanand and Kumaresan 2000; Kusari et al. 2012). All the diversity indexes were calculated both plant wise and also tissue wise.

Statistical analysis

Statistical analyses for biodiversity calculations were carried out by R 3.5.2 (Team 2019). One-way analysis of variance (ANOVA) was carried out to test the effect of tissue type (stem and leaf) on the colonization rate and species richness of endophytic fungi. Post hoc Tukey's Honest Significant Difference tests were performed to observe the significant differences among the tissue types at $P < 0.05$ level.

Results

Investigation of endophytic fungi isolated from A. asiatica

In our study, 82 fungal endophytes were isolated from the *A. asiatica* plants collected from the southern Hungarian areas and the strains were identified using molecular taxonomical tools (Table 1). The endophytic fungi distributed into 3 classes and 5 orders, where the members

Table 1. Endophytic fungi isolated in this study.

Plant part	Species	Collection code	Genbank ID
Leaf	*Alternaria* sp.	SZMC 27067	MT879608
Leaf	*Alternaria* sp.	SZMC 27068	MT879609
Leaf	*Alternaria* sp.	SZMC 27069	MT879610
Leaf	*Alternaria* sp.	SZMC 27070	MT879611
Leaf	*Alternaria* sp.	SZMC 27071	MT879612
Leaf	*Alternaria* sp.	SZMC 27072	MT879613
Leaf	*Alternaria* sp.	SZMC 27073	MT879614
Leaf	*Alternaria* sp.	SZMC 27074	MT879615
Leaf	*Alternaria* sp.	SZMC 27075	MT879616
Stem	*Aspergillus* sp.	SZMC 27076	MT994591
Stem	*Aspergillus* sp.	SZMC 27077	MT994592
Leaf	*Aspergillus* sp.	SZMC 27078	MT994593
Stem	*Aspergillus* sp.	SZMC 27079	MT994594
Stem	*Aspergillus* sp.	SZMC 27080	MT994595
Stem	*Aspergillus* sp.	SZMC 27081	MT994596
Stem	*Aspergillus* sp.	SZMC 27082	MT994597
Stem	*Aspergillus* sp.	SZMC 27083	MT994598
Stem	*Clonostachys* sp.	SZMC 27084	MT883288
Stem	*Cladosporium* sp.	SZMC 27085	MT883289
Stem	*Cladosporium* sp.	SZMC 27086	MT883290
Stem	*Cladosporium* sp.	SZMC 27087	MT883291
Stem	*Cladosporium* sp.	SZMC 27088	MT883292
Stem	*Cladosporium* sp.	SZMC 27089	MT883293
Leaf	*Penicillium* sp.	SZMC 27090	MT994617
Stem	*Clonostachys* sp.	SZMC 27091	MT940229
Leaf	*Clonostachys* sp.	SZMC 27092	MT940230
Leaf	*Curvularia* sp.	SZMC 27093	MT994617
Leaf	*Curvularia* sp.	SZMC 27094	MT994617
Leaf	*Diaporthe* sp.	SZMC 27095	MT940231
Leaf	*Diaporthe* sp.	SZMC 27096	MT940232
Leaf	*Diaporthe* sp.	SZMC 27097	MT940233
Leaf	*Didymella* sp.	SZMC 27098	MT940234
Leaf	*Didymella* sp.	SZMC 27099	MT940235
Leaf	*Didymella* sp.	SZMC 27100	MT940236
Leaf	*Didymella* sp.	SZMC 27101	MT940237
Leaf	*Didymella* sp.	SZMC 27102	MT940238
Leaf	*Didymella* sp.	SZMC 27103	MT994617
Stem	*Alternaria* sp.	SZMC 27104	MT997192
Stem	*Alternaria* sp.	SZMC 27105	MT997193
Stem	*Fusarium* sp.	SZMC 27106	MT997194
Leaf	*Fusarium* sp.	SZMC 27108	MT997196
Leaf	*Fusarium* sp.	SZMC 27109	MT997197
Leaf	*Fusarium* sp.	SZMC 27110	MT997198
Stem	*Fusarium* sp.	SZMC 27111	MT881636
Stem	*Fusarium* sp.	SZMC 27112	MT881637
Leaf	*Fusarium* sp.	SZMC 27113	MT881638
Stem	*Fusarium* sp.	SZMC 27114	MT881639
Leaf	*Fusarium* sp.	SZMC 27115	MT997199
Leaf	*Penicillium* sp.	SZMC 27116	MT997200
Leaf	*Penicillium* sp.	SZMC 27117	MT997201

Table 1. Continued.

Plant part	Species	Collection code	Genbank ID
Leaf	*Penicillum* sp.	SZMC 27118	MT997202
Leaf	*Penicillum* sp.	SZMC 27119	MT994761
Leaf	*Penicillum* sp.	SZMC 27120	MT994762
Stem	*Penicillum* sp.	SZMC 27121	MT994763
Leaf	*Penicillum* sp.	SZMC 27122	MT994764
Leaf	*Penicillum* sp.	SZMC 27123	MT994765
Leaf	*Phomopsis* sp.	SZMC 27124	MT994766
Leaf	*Phoma* sp.	SZMC 27125	MT994650
Leaf	*Phoma* sp.	SZMC 27126	MT994651
Leaf	*Phomopsis* sp.	SZMC 27127	MT994652
Stem	*Phomopsis* sp.	SZMC 27128	MT994653
Leaf	*Microsphaeropsis* sp.	SZMC 27129	MT994654
Leaf	*Simplicillium* sp.	SZMC 27130	MT994655
Stem	*Stemphylium* sp.	SZMC 27131	MT994656
Stem	*Trichoderma* sp.	SZMC 27132	MT881591
Stem	*Trichoderma* sp.	SZMC 27133	MT881592
Stem	*Trichoderma* sp.	SZMC 27134	MT994657
Stem	*Trichoderma* sp.	SZMC 27135	MT881593
Stem	*Trichoderma* sp.	SZMC 27136	MT881594
Stem	*Trichoderma* sp.	SZMC 27137	MT881595
Stem	*Trichoderma* sp.	SZMC 27138	MT881596
Leaf	*Trichoderma* sp.	SZMC 27139	MT881597
Stem	*Trichoderma* sp.	SZMC 27140	MT881598
Stem	*Trichoderma* sp.	SZMC 27141	MT881599
Stem	*Trichoderma* sp.	SZMC 27142	MT881600
Stem	*Phoma* sp.	SZMC 27143	MT994658
Stem	*Phoma* sp.	SZMC 27144	MT994659
Stem	*Phoma* sp.	SZMC 27145	MT994660
Stem	*Microsphaeropsis* sp.	SZMC 27146	MT994661
Stem	*Fusarium* sp.	SZMC 27147	MT994662
Stem	*Fusarium* sp.	SZMC 27148	MT994661

of Sordariomycetes were the most abundant (Fig. 1).

The isolation rate was recorded as 0.66 for leaf and 0.63 for stem and isolated fungi belong to 15 different genera. To characterize the biodiversity of endophytic fungi in the *A. asiatica*, 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 endophytic fungal species consistency in the stem compared to the leaf. Moreover, the Simpson's-index clearly showed that the stem harbored slightly diverse fungal endophytes compared to the leaf. Finally, based on Margalef's-index the stems have higher taxonomic richness than the leaf in *A. asiatica* (Table 2). Although the number of fungi in the leaves was higher than those in the stems, the notable difference was comparatively low (Fig. 2).

Strains belonging to *Alternaria, Clonostachys, Didymella*, *Fusarium, Microsphaeropsis* and *Penicillium, Phoma* genera were isolated both from stem and leaves. However, the members of *Curvularia, Simplicillium* and *Phomopsis* genera were found only in leaves, while *Aspergillus*, *Stemphylium.* and *Trichoderma* were isolated only from the stem (Fig. 2, Table 1). These genera could be even tissue specific in *A. asiatica*, but to clarify this statement larger sample set would be favorable. It should also be considered that the host specificity of endophytic fungi can change the prevalence of their taxa in a particular plant and the divergence in the endophytic fungal community might be harbored in specific host tissues due to the histological difference and nutritional availability (Arnold et al. 2007).

Antimicrobial effects of fungal extracts of A. asiatica endophytes

Altogether, 328 extracts were tested against four bacte-

Table 2. Biodiversity parameters of endophytic fungi isolated from *A. asiatica*.

Diversity index	Stem	Leaves	Total
Simpson's (1-D)	0.1	0.13	0.07
Shannon (H')	2.62	2.36	2.96
Pielou's evenness (J)	0.89	0.87	0.9
Margeref richness	4.72	3.82	5.86

ria, two yeasts and two filamentous fungi (Fig. 3, Fig. 4, Table 3). Our results revealed that altogether, 54 hexane, 78 ethyl acetate, and 73 chloroform extracts of the ferment broth and 78 mycelial extracts were active against at least one test strain. Remarkably high number of extracts (53) were active against *B. subtilis*, and 50% of the extracts were active against *S. aureus* (Fig. 2). However, lower percentage of extracts were active against Gram-negative bacteria including *E. coli* (31%) and *P. aeruginosa* (28%). The extracts of *Didymella* sp. SZMC 27102 strain exhibited high activity against all of the tested bacteria and yeasts, while the extracts of *Phoma* sp. SZMC 27125 and SZMC 27126 showed a remarkable activity against Gram-positive bacteria and the mycelial extract of these endophytic fungi showed high activity against Gram-negative bacteria. However, their extracts did not show any activity against yeasts and filamentous fungi (Table 3). Most of the *Fusarium* extracts exhibited remarkable antimicrobial activities against yeasts, but none of them were active against the two tested filamentous fungal strains. The ethyl acetate extracts of two *Aspergillus* isolates (SZMC 27077, SZMC 27078) showed a significant inhibitory activity (>90%) against *B. subtilis, S. aureus, P. aeruginosa* and *C. albicans*.

During the yeast inhibition testing, *C. krusei* was found to be more resistant against the extracts than *C. albicans* (Fig. 3). In total, extracts of 46 strains were found to be possessing more than 90% inhibition against at least one test pathogen (Fig. 3). Taxa wise, *Aspergillus*, *Alternaria*, *Fusarium* and *Didymella* were found to have metabolites with effective yeast inhibition activity. Moreover, chloroform and mycelial extracts of *Trichoderma* isolates were mainly active against *R. solani* and *F. culmorum* (Table 3).

Discussion

Despite the biotechnological potential of endophytic fungi, the basic ecology about their relationship with the host plants is poorly understood. Furthermore, given the high biodiversity of plants in Hungary, examining their fungal endophytes could lead to the discovery of novel metabolites. Therefore, in our study the fungal endophytic community of *A. asiatica* was examined. The culturable endophytic fungi were identified using molecular techniques and their biodiversity, richness and tissue specificity were described. As these microorganisms have been generally identified as an abundant reservoir of novel antimicrobial compounds, the antimicrobial (i.e., antibacterial and antifungal) activities of the metabolites produced by the isolated fungi were also studied.

The fungi isolated from *A. asiatica* were characterized into 3 classes and 5 orders. All of the isolated fungi belonged to the phylum Ascomycota, which includes three classes, Dothideomycetes, Sordariomycetes and Eurotiomycetes. Our findings revealed that the leaves and stem parts of the *A. asiatica* are excellent reservoirs for endophytic fungi, where the most abundant genera were *Fusarium, Trichoderma, Penicillium* and *Alternaria*. Although *Alternaria* and *Fusarium* species are considered as plant pathogens, they might be latent when they are inside the living tissues until the environmental conditions are favourable and certain strains of them might have evolved to endophytic lifestyle due to loss of virulence (Freeman and Rodrigues 1993). These species are gaining a lot of attention recently for their bioactive

Table 3. Table 3. List of the endophytic fungi extracts showing inhibitory activities against plant pathogenic fungi.

Strain code	*F. culmorum*				*R. solani*			
	HEX	CLF	EtOAc	C:M	HEX	CLF	EtOAc	C:M
SZMC 27132	-	-	-	-	-	+	-	++
SZMC 27133	-	-	-	-	-	-	-	+
SZMC 27134	-	-	-	-	-	+	-	+
SZMC 27135	-	-	-	-	-	-	-	+
SZMC 27136	-	-	+	-	-	-	-	+
SZMC 27137	-	-	-	+	-	-	-	++
SZMC 27138	-	-	-	+	-	-	-	-
SZMC 27141	-	-	-	-	-	+	-	+++

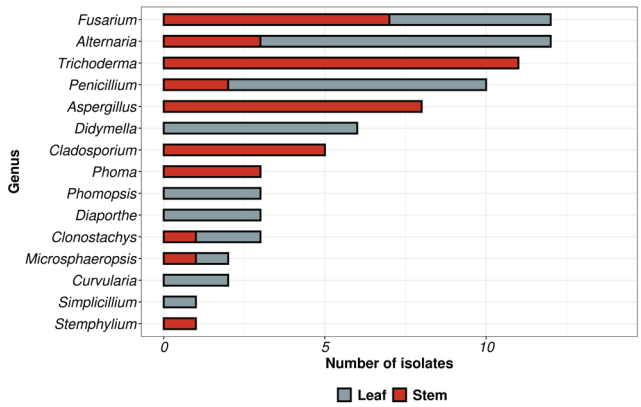

Figure 2. Distribution of endophytic fungi of *A. asiatica* at genus level.

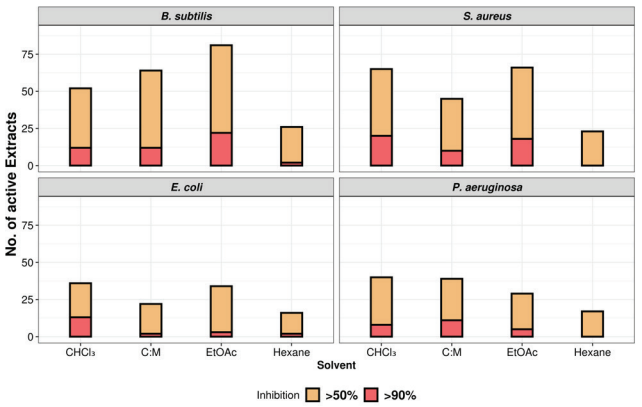

Figure 3. Summary of the antibacterial effects of endophytic extracts isolated from *A. asiatica*. C:M = chloroform:methanol (4:1 V/V) extract of mycelia.

compounds (Toghueo et al. 2019; Hellwig et al. 2002; Kaushik et al. 2020). Members of the *Trichoderma* genus were also found dominant in our study colonizing only the stems. In the literature, most of the studies reported the tissue specificity of *Trichoderma* species to roots and leaves (Rosemana et al. 2018). In addition, the colonization mechanism of *Trichoderma* sp. was also reported through systemic infection, which proved that the fungus could be re-isolated from stems rather than leaves and roots after the infection of roots (Rosemana et al. 2018).

In this work, five *Phoma* strains were isolated from stem and leaf. This genus is ubiquitous and inhabits a diverse range of hosts, from soil to air or from plants to animals (Aveskamp et al. 2010). Previously, extensive studies were carried out to clarify the significant generic boundaries in Didymellaceae, however, due to the lack of phylogenetic support of nearly 70 *Phoma* species belonging to Didymellaceae could not be assigned to definite genera (Aveskamp et al. 2010; Gruyter et al. 2013; Chen et al. 2017).

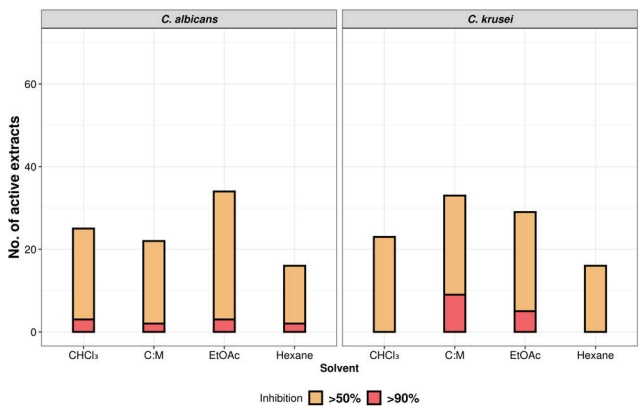

Fig. 4. Summary of the antifungal effects of endophytic extracts isolated from *A. asiatica*. C:M = chloroform:methanol (4:1 V/V) extract of mycelia.

In our cases, the isolation rate of the fungi were found to be the highest in stem, followed by leaf, whereas similar studies in other plants showed higher isolation rates in the leaf compared to stems (Alurappa and Chowdappa 2018). The tissue specificity are also affected by different environmental conditions. Previous studies showed that certain fungi, such as *Aspergillus* (El-Hawary et al. 2020) and *Penicillium* (Devi et al. 2012) species, did not exhibit tissue specificity. However, in our study, the *Aspergillus* strains were isolated only from stem.

Recently, an intensive research is required for effective antimicrobial drugs and the endophytic fungi are potential sources of these metabolites because they have been identified as an abundant reservoir of novel antimicrobial compounds (Strobel 2003). In the present work, 82 endophytic strains were isolated from the plant parts and the antimicrobial activity of their metabolites was evaluated. Altogether, 14.63% of the isolates had antibacterial effects with wide spectrum. Gram-positive bacteria were found to be more susceptible than Gram-negative bacteria and the observed antibacterial activity was higher than the antifungal activity. Altogether, 86.2% of the extracts were active against bacteria, whereas only 53% were active against yeasts and regarding Gram-positive bacteria, especially *B. subtilis* showed the highest sensitivity to the extracts. *Alternaria* and *Aspergillus* were the most predominant genera found to be exhibiting higher antibacterial activity than other fungi. Six out of 9 *Alternaria* strains had inhibitory activity against all the test organisms and at least one extract of all the *Aspergillus* isolates were active against all the test organisms. Furthermore, three and seven isolates showed inhibitory effects against the filamentous fungi *F. culmorum* and *R. solani*, respectively.

Acknowledgments

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