Biodiversity and antimicrobial activity of endophytes associated with Egyptian medicinal plants

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One-hundred and thirty-two endophytic strains were isolated from 18 medicinal plants from Saint Katherine Protectorate, Egypt. Some of the endophytes were identified to genus or species level using traditional morphological methods, but most were classified as sterile mycelia. The relative frequency, isolation rate, and colonization rates of endophytes were used to express the diversity of endophytes. Most endophyte isolates were obtained from *Euphorbia sanctae-catharinae* (15 isolates). *Galium sinaicum* yielded the greatest endophytic diversity with eight taxa while *Thymus decussates* yielded only one taxon. Mycelia sterilia and *Acremonium* species were the dominant fungal endophytes, and some of these endophytes exhibited host specificity. Fifty-five of 99 tested endophytes showed a broad spectrum of inhibitory activity against different pathogenic bacteria and yeasts.

Key words – *Acromonium* sp. – antibacterial activity – anticandidal activity – biodiversity – endophytes – Mycelia sterilia – Saint Katherine – medicinal plants

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Introduction

Plants may serve as a reservoir of large numbers of microorganisms known as endophytes (Bacon & White 2000). Endophytes are microorganisms (mostly fungi and bacteria) that inhabit plant hosts for all or part of their life cycle. They colonize the internal plant tissues beneath the epidermal cell layers without causing any apparent harm or symptomatic infection to their host, living within the intercellular spaces of the tissues and its seems that they may penetrate the living cells (Strobel 2003). Endophytic fungi are an ecological, polyphyletic group of highly diverse fungi, mostly belonging to ascomycetes and anamor-phic fungi (Huang et al. 2001, Arnold 2007).

Individual plants may be host to one or more endophytes, and many endophytes may colonize certain hosts, suggesting that there may be many undiscovered endophyte species (Petrini 1991**,** Strobel & Daisy 2003, Huang et al. 2007). Most of endophytes produce a plethora of bioactive metabolites that may be involved in the host-endophyte relationship (Strobel 2003). These metabolites may serve as sources of novel natural products for exploitation in medicine, agriculture, and industry (Bacon & White 2000, Strobel & Daisy 2003). The described populations of endophytic strains are few, which means there are good opportunities to find new endophytes that colonize plants in different niches and ecosystems.

Endophytic fungi represent an important and quantified component of fungal biodiversity, and are known to affect on plant community diversity and structure (Sanders 2004, Gonthier et al. 2006, Krings et al. 2007). A variety of relationships can exist between endophytes and

their host plants, ranging from mutualism or symbiosis to antagonism or slight pathogenesis (Schulz & Boyle 2005, Arnold 2007).

Host-endophytes relationships can be described in terms of host-specificity, host-recurrence, host-selectivity, or host preference (Zhou & Hyde 2001, Cohen 2006). Host-specificity is a relationship in which a microorganism is restricted to a single host or a group of related species, and such specificity implies that complex biochemical interactions are occurring between the host and its associated endophytes (Holliday 1998, Strobel 2003, Strobel & Daisy 2003). Host-recurrence refers to the frequent or predominant occurrence of endophytes in a particular host or a range of plant hosts, although the endophytes may also be found infrequently in other host plants in the same habitat (Zhou & Hyde 2001). A single endophytic species may form relationships with two or many related host plants, but where there is a preference for one particular host the phenomenon is defined as host selectivity (Cohen 2006). The term host-preference is most frequently used to indicate a common occurrence or uniqueness of occurrence of endophytes in a particular host, and is also used to indicate the difference in endophytic community composition and relation frequencies from different host plants (Suryanarayanan & Kumaresan 2000). Endophytes are also able to colonize multiple host species of the same plant family within the same habitat, and the distribution of some endophytes can be similar in closely related plant species (Huang et al. 2008). The differences in endophytes in their metabolic profile, and hence in their biological activity, even if between isolates of a species, might be related to the chemical difference of host plants (Paulus et al. 2006); this raises the importance of studying host-endophytes relationships, and the effect of host plants on endophytic metabolite production. More attention is now being given to study endophytic biodiversity, the chemistry and bioactivity of endophytic metabolites, and the relation between endophytes and their host plants (Tan & Zou 2001, Schulz et al. 2002).

The Sinai Peninsula, which is a part of the Sahara-Arabian deserts, covers approximately 6% of the total land area of Egypt and is characterized by an arid to extremely arid climate with Mediterranean influences, i.e., aridity, winter precipitation and moderate temperature (Danin 1978). The Saint Katherine Protectorate covers 4350 km^2 of mountainous region (average 1500-2000 m above mean sea level) of Southern Sinai and is a protected area due to its immense biological and ecological interest. Saint Katherine Protectorate contains 472 plant species including 19 Egyptian endemic species, 115 of medicinal interest, and about 170 species used in folk medicine. Medicinal plants have been recognized as a repository of endophytes with novel metabolites of pharmaceutical and agriculture importance (Strobel et al. 2004). Due to the richness of medicinal plants in Saint Katherine Protectorate and its unique environmental characters, this area is receiving increased attention for biological and ecological studies, and is considered an interesting area for studying endophytic community (Strobel & Daisy 2003). The biodiversity of microbial community in Sinai is poorly known and thus, we focus this study on determining the structure, host specificity, and diversity of endophytic mycobiota of some medicinal plants in Saint Katherine Protectorate, and to study the effect of host plants on activity and metabolic profile of endophytes. Antimicrobial activity was used as pointer to compare between endophytic activities of different strains in this study.

Methods

Study area

The Saint Katherine Protectorate has an extremely arid climate with long, hot and rainless summers and cool winters. The mean annual precipitation in Saint Katherine area over 25 years is 45 mm per year, although the high mountains receive more precipitation (100 mm/year) as rain and snow. In some parts of this area, floods resulting from continual rain may occur during winter and spring. The mean maximum air temperature ranged from 15.1°C to 32.7°C and the mean minimum temperature ranges from 1.9°C to 20.2°C, with the lowest temperature in December and January and the highest temperature in July and August (Mosallam 2007).

Collection of host plants

Leaves and stems of 18 host medicinal plants from seven families (Adiantaceae, Asteraceae, Ephedraceae, Euphorbiaceae, Hypericaceae, Lamiaceae, and Rubiaceae) were collected from Saint Katherine Protectorate, in

September 2010. The plants were selected according to their ethnobotanical uses in traditional medicines, and only apparently healthy and disease free plants were selected in order to minimize the presence of plant pathogenic and saprobic species, and to prevent the isolation of localized pathogenic endophytic microorganisms. The collected plant materials were stored in separate plastic bags at 4°C in an ice box until isolation could commence (Strobel & Daisy 2003).

Isolation and identification of endophytic fungi and storage

Following Arnold et al. (2000) the plant material was washed in running tap water, leaves of each plant were cut into 2 mm^2, and branches were cut randomly to small pieces (5 mm long). The pieces were surface sterilization by sequentially dipping into 0.5% sodium hypochlorite (2 min) and 70% ethanol (2 min), then rinsed with sterile water and allowed to surface-dry under sterile conditions. Tissue pieces were placed on both PDA (potato-dextrose-agar) medium and Czapeks agar medium. Both media were supplemented with chloramphenicol (50 mg/L) to suppress bacterial growth, and incubated at 30°C. They were checked every day for 3 weeks and individual fungal colonies were transferred to fresh PDA plates for purification and identification purposes. Taxonomic identification of the fungi was based on their morphological characters and the mechanism of spore production (Moubasher 1993). Isolates that failed to produce reproductive structures after 3-4 months of incubation were referred to as mycelia sterilia, and divided into morphospecies according to their culture characteristics; this group of fungi is prevalent in endophyte studies (Lacap et al. 2003). Stock fungal cultures were deposited at Chemistry of Natural and Microbial Product Department, National Research Center, Egypt.

Cultivation and extraction of the fermentation broth

Pure strains of endophytes were inoculated into 100 ml PD (potato-dextrose) broth in Erlenmeyer flasks and incubated at 30°C, statically. After 21 days of incubation, the fungal mats were separated out by filtration. Dried fungal biomass was extracted by soaking in ethyl acetate (1:50, w/v) overnight. Supernatant was then extracted twice with equal volume of ethyl acetate (1:1, v/v), and the upper organic phase of supernatant were add to mycelia extract. The organic phase was concentrated to dryness under reduced pressure using a rotary evaporator below 55°C to obtain the crude fungal extract. In case of endophytic bacteria and yeasts the supernatant was centrifuged first at 3600 rev/min for 10 min before extraction with ethyl acetate.

Screening the relations between endophytes and their host plants

Colonization rate (CR) was calculated as the total number of plant tissue pieces colonized by endophytes divided by the total number of pieces incubated for that plant sample, and expressed as a percentage. Isolation rate (IR) is a measure of endophyte richness in a given sample of plant tissue, i.e., the incidence of multiple colonization of endophytes per piece, and was calculated as the number of isolates obtained divided by the total number of tissue pieces, but not expressed as a percentage. Relative frequency (RF) of isolation, used to represent fungal density, was calculated as the number of isolates of a species divided by the total number of isolates, and expressed also as a percentage (Photita et al. 2001; Huang et al. 2008).

Biological evaluation of endophytes using disc diffusion assay for antibacterial, and anticandidal activity

The antibacterial and anticandical activity of the endophytic extracts were tested against three Gram positive bacteria (*Staphylococcus aureus* ATCC 29213, *Bacillus subtilis* NRRL-B-4219, *Micrococcus luteus* B-287), four Gram negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27953, *Klebsiella pneumoniae* ATCC 10131, *Alcaligenes faecalis* B-170), and four yeasts (*Candida albicans* ATCC 10231, *C. tropicalis* ATCC 750, *C. parapsilosis* ATCC 22019, *Saccharomyces cerevisiae* ATCC 2180-1A). The assay was preformed according to Jorgensen & Turnidge (2007) with some modification. Nutrient agar (NA) media was used for test bacteria, and PDA media was used for test yeasts. The test organisms were inoculated over the surface of the sterilized media, with 1.5 $x10^8$ CFU/mL (colony-forming units) for bacteria and 4 x 10^7 CFU/mL for yeasts. The crude extracts were

dissolved in methanol, and sterile disks of Wattman No. 3 (6.0 mm in diameter) were loaded with 250 µg of different endophytic extracts, dried, and then each disk placed on agar surface of freshly inoculated medium with the test microorganisms. A control test for the solvent was also preformed. The Petri dishes were kept in a refrigerator for one hour to permit homogenous diffusion of the antimicrobial agent before growth of the test microorganisms, and the plates were then incubated at 37°C for 24 hours. The appearance of a clear inhibition zone around the disk in the inoculated Petri dishes is an indication of antimicrobial activity, and the diameters of the clear zones surrounding the discs were measured.

Result and discussion

Biodiversity of endophytic fungi associated with Saint Katherine medicinal plants

From 18 Saint Katherine medicinal plants 132 endophytic isolates were identified morphologically into 29 taxa (Table 1). Most isolates were obtained from *Euphorbia sanctae-catharinae* (15 isolates), followed by *Ephedra alata* and *Galium sinaicum* (12 isolates from each). Bacterial endophytes were obtained from only *Ephedra alata* and *Thymus decussates*, in the presence of antibiotic in the isolation media, this may reveal that these endophytic bacteria have special features in resistance to antibiotic. *E. alata* showed the presence of both endophytic bacteria and fungi, but only bacteria were obtained from *T. decussates*. Unusually, *Hypericum sinaicum* yielded an endophytic yeast.

Using traditional morphological techniques, only some fungal isolates could be identified to species level, and a few identified to genus level, but some could not be identified as they were sterile, a common problem associated with identification of fungal endophytes (Gamboa & Bayman 2001, Promputtha et al. 2005 a). Sterile fungi are prevalent in endophyte studies (lacap et al. 2003). The mycelia sterilia were divided into two morphospecies "Dark sterile mycelia and White sterile mycelia" according to their culture characters.

Mycelia sterilia were dominant endophytes, 19.69% for dark sterile mycelia and 18.93% for white sterile mycelia and these percentages agree with many endophytic studies (Fröhlich et al. 2000, Promputtha et al. 2005b, Huang et al. 2008). *Acremonium* species are frequently identified as endophytes (Wicklow et al. 2005, Wicklow & Poling 2009, De Almeida et al. 2011) and this genus was the second most frequent fungus with relative frequency of 12.87 %, followed by *Aspergillus*, *Penicillium*, *Pleospora tarda*, and *Ulocladium* spp., with relative frequencies of 10.6%, 9.09%, 4.5%, and 4.5%, respectively (Fig. 1).

Host recurrence and host specificity of endophytes

All 18 medicinal plants were found to be host to one or more endophytes. The colonization rate and the isolation rate of endophytes from these plants ranged from 33.3% to 100%, and 0.05 to 0.75, respectively. Different endophytic taxa showed different relative frequencies in different plants (Fig. 2). Among the 18 medicinal plants, *Galium sinaicum* had the highest endophytic diversity with eight taxa, followed by *Euphorbia sanctae-catbarinae*, *Hypericum sinaicum*, and *Teucrium polium* with six taxa. *Teucrium leucocladum* and *Thymus decussatus* had the lowest endophytic diversity, with only an endophytic bacterium for *Thymus decussatus*, and two taxa of endophytic fungi for *Teucrium leucocladum.* Most endophytic isolates (15) were obtained from leaves of *Euphor-bia sanctaecatbarinae* (6 taxa), Among these taxa, *Acromonium strictum* and white sterile mycelia were the most frequent with 26.66% relative frequency followed by dark sterile mycelia, *Aspergillus sydowii*, *Penicillium coleurophylium*, and *Phoma leveilleia*, with relative frequency of 20%, 13.33%, 6.66%, and 6.66%, respectively.

Endophytes with the wide distribution and highest isolate abundance were mycelia sterilia, found in most host plants, except *Adiantum capillus-veneris*, *Launea spino-sa*, *Thymus decussates*, and *Galium sinaicum.* Fourteen plants were colonized by sterile mycelia with high relative frequency (38.6%) for all plants, and sterile mycelia had high relative occurrence in nine plants with relative frequency above 40%, especially in *Teucrium leucocladum* with 100% relative frequency of sterile mycelia. *Aspergillus* and *Pencillium* were the second most abundant genera, found in 9 host plants, with major occurrence in *S. aegyptiaca* (relative frequency 42.8% for *Aspergillus*) and in *Adiantum capillusveneris* (relative frequency

Table 1 Number of endophyte colonies isolated from 18 Saint Katherine medicinal plants

Plant host /Fungal Isolates	Ab	Ac. sp.	Ac. st	Al	As. fla	As. nig	As. sp.	As. sy	B	Ch glo	Chs pr	Co	Em	Eu	Fu. ox	Fu. Sp.	D. M.S.	W. M. S.	Mu	Ni	Pn. ch	Pn. co	Pn. sp.	Ph	Pl	Sc	Ul. at	Ul. ch	Y	Total Endo-phyte	C.R. (%)	I.R.
Adiantaceae																																
Adiantum capillus-veneris				1						2													1						4	33.3	0.44
Asteraceae																																
Achillea fragrantissima			3														2	2				1	1						9	90	0.45
Artemisia herba alba					2												1	2		2									7	75	0.35
Chiliadenus montanus			1														1			1									3	50	.15
Launea spinosa		1	4			1															1								7	100	0.35
Pulicaria undulate																	3	1					1				1	2		8	90	0.4
Tanacetum sinaicum					1			1										3			1		1							7	45	0.35
Ephedraceae																																
Ephedra alata			3						1								5	3											12	100	0.6
Ephedra aphylla						1							1				2								1				5	60	0.25
Euphorbiaceae																																
Euphorbia sanctae-catbarinae			4					2									3	4				1		1					15	85	0.75
Hypericaceae																																
Hypericum sinaicum										1							1	2							1			1	8	95	0.4
Lamiaceae																																
Lavandula coronopifolia			1														3					1				1			6	40	0.3
Phlomis aurea					1						2						2	1					1						7	50	0.35
Stachys aegyptiaca					3													2	1						1				7	50	0.35
Teucrium leucocladum																	3	3											6	55	0.6
Teucrium polium				2		1												2		1	1	1							8	50	0.4
Thymus decussatus									1																				1	100	0.05
Rubiaceae																																
Galium sinaicum	1						1					2		1	2	1									3			1		12	100	0.6
Total no.	1	1	16	3	7	3	1	3	2	3	2	2	1	1	2	1	26	25	1	4	3	4	5	1	6	1	1	5	1	132	------	------

Morphologically identified taxa: Ab: *Absidia corymbifora*; Ac. sp.: *Acremonium* sp.; Ac. st: *Acremonium strictum*; Al: *Alternaria alternata*; As. fla: *Aspergillus flavus*; As. nig: *A. niger*; As. sp.: *Aspergillus* sp.; As. sy: *A. sydowii*; B: Bacteria; Ch. glo: *Chaetomium globosum*; Ch. spr: *C. spirale*; Co: *Cochliobolus lunatus*; Em: *Emericella versicolor*; Eu: *Eurotium* sp.; Fu. ox: *Fusarium oxysporum*; Fu. sp.: *Fusarium* sp.; D.M.S.: Dark Mycelia Sterilia; W.M.S.: White Mycelia Sterilia; Mu: *Mucor fuscus*; Ni: *Nigrospora sphaerica*; Pn. ch: *Penicillium chrysogenoum*; Pn. co: *Penicillium corylophilum*; Pn. sp.: *Penicillium* sp.; Ph: *Phoma leveillei*; Pl: *Pleospora tarda*; Sc: *Scopulariopsis* sp.; Ul. at: *Ulocladium atrum*; Ul. ch: *U. chartarum*; Y: Yeast; C.R.: olonization Rate; I.R.: Isolation Rate

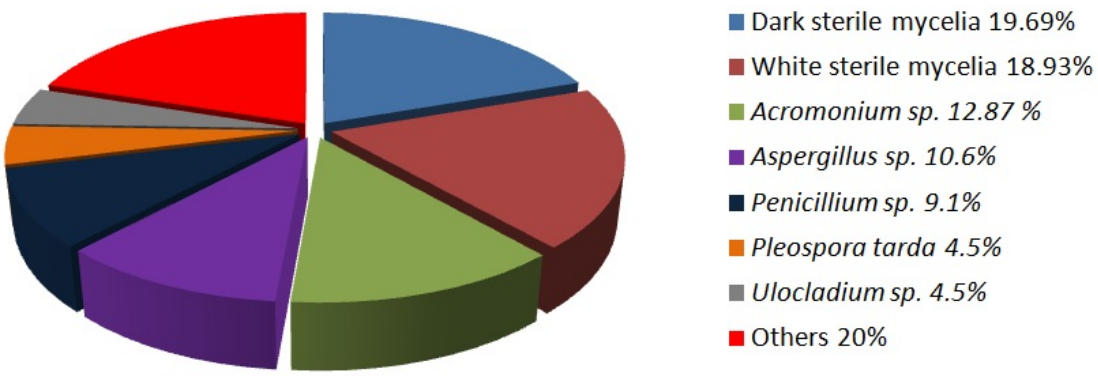

Fig 1 Relative frequencies of different endophytic taxa isolated from 18 Saint Katherine medicinal plants. "Others" include rare and infrequent endophytic fungal species.

25% for *Penicillum*). *Acremonium strictum* was obtained from 6 host plants, with major occurrence in *Launea spinosa* (relative frequency 57.1%).

Rare and infrequent endophytic fungal species were *Absidia corymbifora*, *Chaetomium globosum*, *C. spirale*, *Cochliobolus lunatus*, *Emericella versicolor*, *Eurotium* sp., *Fusarium* spp., *Mucor fuscus*, *Nigrospora sphaerica*, *Phoma leveillei*, and *Scopulariopsis* sp., all belonging to ascomycetes or anamor-phic fungi, except for the zygomycetes, *Absedia corymbifora* and *Mucor fuscus*. Previous studies have found that most endophytic fungi belong to the ascomycetes and anamorphic fungi (Huang et al. 2001).

Alternaria, *Chaetomium*, *Fusarium*, and *Phoma* are common endophytic genus that have been reported in many previous studies, but only one isolate of *Phoma leveilleia* was detected from host plant *Euphorbia sanctae-catharinae*, three *Alternaria* isolates were obtained from *Adiantum capillusveneris* and *Teucrium polium*, for *Chaetomium globosium*, also three isolates were obtained from *Adiantum capillus-veneris*, and *Hypericum sinaicum*, and three isolates of *Fusarium* were obtained from one host, *Galium sinaicum.*

Endophytic bacteria were obtained from *Thymus decussatus* and *Ephedra alata* (relative frequency 100% and 8.3%, respectively). However, the isolation medium contained an antibiotic to inhibit bacterial growth. Endophytic yeast was obtained from *Hypericum sinaicum* (relative frequency 12.5%). The four isolates of *Nigrospora spherica*, the six isolates of *Pelosora tarda*, and six isolates of *Ulocladium* came from three, four, and three different host plants, respectively. Some endophytes, were restricted to one specific host plant suggesting host specificity. For example, *Absedia corymbifora*, *Cochiobolus lunatus*, and *Eurotium* were found in only *Alium sinaicum*. *Chaetomiun spirali* was obtained from *Phlomis aurea*, *Emericella vercicolour* from *Ephedra aphyla*, *Mucor fuscus* from *Stachys aegyptiaca*, and *Scopulariopsis* from *Lavandula coronopifolia.*

Antibacterial and anticandial activities

Ethyl acetate crude extract of 132 endophytic isolates from the Saint Katharine medicinal plants were screened for their antibacterial and anticandidal activities, and the most active extracts are listed (Table 2). The remaining extracts showed a narrow spectrum of activity against only one or two pathogenic test organisms, or showed no activity. Fiftyfive isolates (55.5%) of 99 screened strains, exhibited significant inhibitory activity against a wide range of pathogenic test microorganisms, with diameters of inhibition zones ranging from 9 to 27 mm for the test bacteria, and from 8 to 31 mm for test *Candida* on disc diffusion assay.

It is clear from the results that most of 55 fungal extracts demonstrated broad spectrum activity against the tested bacteria, *Candida albicans* and *C. tropicalis*, but few showed antiyeast activity against *C. prabsiosis* or *Saccharomyces cerevisiae*. Many active isolates exhibited antibacterial activity compared with the isolates exhibiting antiyeast, this may due to the similarities in eukaryotic characteristics between the endophytic fungi and the test organ isms (Hugo 1998). Approximately 49% of dominant

Table 2 Antibacterial and Anticandidal activities of 55 endophytic isolates of 99 morphospecies endophytes

Host Plant	Endophytes/ Test organism	C. alb	C. t	C. p	Sac.	Staph	Bs.	M.	E. coli	Ps.	K.	A. f
Stachys aegyptiaca	*Mucor fuscus*	23	19	9	----	20	21	23	20	21	20	25
Stachys aegyptiaca	*Aspergillus flavus*	16	30	----	----	20	16	25	20	20	21	27
Galium sinaicum	*Pleospora tarda*	15	25	----	----	16	12	20	16	12	12	14
Galium sinaicum	*Aspergillus* sp.	18	19	----	----	23	20	23	24	22	20	24
Galium sinaicum	*Fusarium* sp.	12	18	----	----	10	12	14	10	12	12	12
Tanacetum sinaicum	white sterile mycelia	16	16	14	----	18	18	18	16	18	16	18
Tanacetum sinaicum	*Penicillium* sp.	14	21	----	12	11	15	12	----	----	11	9
Launea spinosa	*Acremonium strictum*	22	22	9	16	19	25	26	26	25	22	22
Launea spinosa	*Penicillium chrysogenoum*	16	23	----	9	15	20	20	15	15	19	20
Launea spinosa	*Aspergillus niger*	15	9	8	----	18	18	22	23	20	20	26
Achillea fragrantissima	Dark sterile mycelia	23	21	8	----	20	22	23	21	20	20	19
Achillea fragrantissima	Dark sterile mycelia	15	21	9	----	17	24	12	23	20	19	20
Achillea fragrantissima	white sterile mycelia	19	17	8	----	20	21	22	21	20	20	19
Achillea fragrantissima	white sterile mycelia	17	15	----	----	20	25	20	20	22	21	23
Achillea fragrantissima	*Penicillium corylophilum*	16	10	----	----	22	23	25	16	19	20	16
Ephedra aphyla	Dark sterile mycelia	19	22	----	-----	20	20	22	20	21	20	25
Ephedra alata	white sterile mycelia	16	16	12	16	18	19	22	20	20	21	23
Ephedra alata	white sterile mycelia	15	9	----	----	10	16	18	14	16	16	16
Ephedra alata	Dark sterile mycelia	16	24	----	-----	12	22	24	16	20	20	20
Ephedra alata	white sterile mycelia	15	15	----	----	14	16	17	13	17	16	19
Euphorbia sancte catherine	Dark sterile mycelia	16	17	----	----	20	20	21	19	19	20	24
Euphorbia sancte catherine	white sterile mycelia	12	12	----	13	15	13	15	15	14	13	17
Euphorbia sancte catherine	*Phoma leveillei*	18	16	----	----	19	19	21	19	19	20	20
Euphorbia sancte catherine	Dark sterile mycelia	16	20	----	----	17	16	20	15	16	16	20
Euphorbia sancte catherine	*Acremonium strictum*	13	31	11	8	14	11	15	15	12	13	12
Lavandula coronopifolia	Dark sterile mycelia	19	20	-----	-----	20	23	26	20	22	22	26
Chiliadenus montanus	Dark sterile mycelia	15	15	----	----	14	13	19	13	14	14	19
Chiliadenus montanus	*Nigrospora sphaerica*	16	17	----	----	16	20	22	17	20	20	22
Teucrium leucocladum	white sterile mycelia	15	20	10	----	17	16	18	17	17	16	18
Teucrium leucocladum	white sterile mycelia	16	18	15	12	23	18	22	20	18	20	24
Teucrium leucocladum	Dark sterile mycelia	----	20	----	-----	19	15	16	14	14	14	14
Teucrium polium	*Alternaria alternata*	18	17	----	----	18	19	17	17	18	18	22
Teucrium polium	*Nigrospora sphaerica*	15	19	----	9	15	15	20	15	16	15	20
Teucrium polium	white sterile mycelia	12	20	----	----	11	13	16	13	11	12	17
Teucrium polium	*Penicillium corylophilum*	18	31	----	----	18	18	16	24	18	19	24
Teucrium polium	*Penicillium chrysogenoum*	16	30	----	----	13	16	20	20	18	18	18
Teucrium polium	*Aspergillus niger*	19	30	----	----	15	16	20	12	16	17	23
Hypericum sinaicum	*Ulocladium chartarum*	15	22	11	----	13	15	20	13	14	14	20
Hypericum sinaicum	*Pleospora tarda*	18	21	----	----	20	20	25	21	21	20	26
Hypericum sinaicum	*Chaetomium globosum*	22	22	----	----	20	20	23	20	20	20	24
Hypericum sinaicum	Yeast	15	30	----	----	19	16	20	13	15	17	20
Hypericum sinaicum	white sterile mycelia	12	19	----	----	9	14	18	16	14	16	20
Artimisia herba alba	white sterile mycelia	14	20	-----	----	12	15	19	15	15	15	20
Artimisia herba alba	Dark sterile mycelia	17	13	----	-----	15	18	20	16	16	18	21
Artimisia herba alba	white sterile mycelia	20	18	----	15	11	18	17	16	16	18	16
Artimisia herba alba	*Aspergillus flavus*	21	20	9	----	17	19	21	----	----	19	22
Pulicaria undulate	Dark sterile mycelia	15	22	-----	16	14	20	20	14	20	20	20
Pulicaria undulate	*Ulocladium chartarum*	19	22	----	17	17	23	22	14	16	16	23
Pulicaria undulate	*Penicillium* sp.	14	21	-----	9	10	18	19	15	14	18	19
Pulicaria undulate	Dark sterile mycelia	13	20	----	----	15	18	22	14	18	18	22
Phlomis aurea	white sterile mycelia	14	8	----	-----	20	19	20	15	14	16	21
Phlomis aurea	Dark sterile mycelia	8	21	----	----	17	14	17	17	15	15	15
Phlomis aurea	*Chaetomium spirale*	8	21	----	----	12	10	14	13	13	13	14
Phlomis aurea	*Penicillium* sp.	15	20	----	----	16	22	21	18	17	20	18
Phlomis aurea	*Aspergillus flavus*	14	30	----	----	16	20	25	19	20	21	25

Code of pathogenic test organism: C. alb: *Candida albicans*; C. t: *Candida tropicalis*; C. p: *Candida prarapsilosis*; Sac.: *Saccharomyces cerevisiae*; Staph: *Staphylococcus aureus*; Bs.: *Bacillus subtilis*; M.: *Microcouss luteus*; E. coli: *Escherichia coli*; Ps.: *Pseudomonas aeuroginosa*; K.: *Klebsiella pneumonia*; A. f: *Alcaligenes faecalis.*

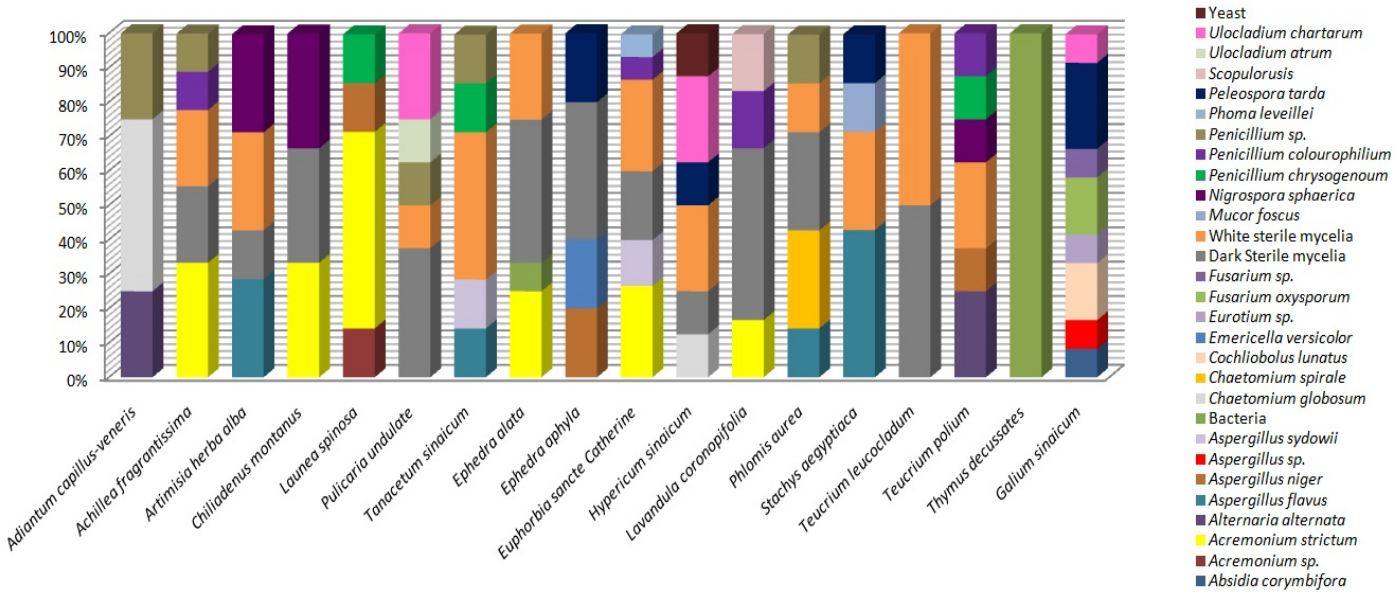

Fig 2 Relative frequances of different endophytic taxa isolated from 18 Saint Katherine medicinal plant

endophytic isolates showing antimicrobial activates were mycelia sterilia Four isolates, *Acremonium strictum Ulocladium chartarum* and two white sterile mycelia isolated from *Launea spinosa, Pulicaria undulata, Ephedra alata,* and *Teucrium leucocladum*, respectively, demonstrated most significant antimicrobial activity on the test organisms. They exihibited broad-spectrum antibacterial activity on both gram positive and negative bacteria, and broad anticandidal spectrum against all tested yeast. Three other isolates, *Ulocladium chartarum*, *Aspergillus niger*, and *Acremonium strictum* from *Hypericum sinaicum*, *Launea spinosa*, and *Euphorbia sanctae-catharinae*, respectively, showed moderate activity against all tested organisms. These endophytic isolates are potential candidates for further investigations into bioactive antimicrobial agents.

Isolates of *Acremonium strictum* from different hosts showed significant difference in biological activity, with a strong antimicrobial effect for those isolates from *Launea spinosa*, moderate antimicrobial activity when isolated from *Euphorbia sancte catherine*, and weak activity for other morphospecies from *Launea spinosa*. *Chaetomium globosum* isolated from *Hypericum sinaicum* showed strong antimicrobial activity with a concentration of 250 µg per disk and did not show any antioxidant activity (unpublished data), but isolates from *Adiantum capillus-veneris* did not show this strong antimicrobial activity, showed weak to moderate antimicrobial activity against pathogenic microorganisms at high concentration 500 µg per disk, but this isolate showed promising antioxidant activity (unpublished data). The same effect was noticed with isolates of *Ulocladium chartarum* from *P. undulata*, and from *Hypericum sinaicum*.

Only 55 isolates of 99 tested showed biological activity. For example, of two isolates of *Fusarium* from *Galium sinaicum* only one showed high activity against pathogenic test organisms. This has been noted in previous studies (Li et al. 1996). Many factors changing in the host, e.g., related to season, age, environment, and location, may influence the biology of the endophytes. This observation suggests the importance of the host plant as well as the ecosystem in influencing the general metabolites of endophytic microbes. Production of active metabolites by endophytes may be related to characters of the host plants and to a genetic recombination of the endophyte with the host that might have occurred in evolutionary time. It is possible to imagine that some endophytes developed a genetic system that allowed transferring of information between themselves and host plants (Tan & Zou 2001, Firáková et al. 2007). Endophytes are a poorly investigated group of microorganisms that represent an abundant source of bioactive agents with potential for exploitation in a wide variety of applications.

The great biodiversity of endophytes of Saint Katherine medicinal plants, and the presence of many of endophytic isolates exhibiting significant inhibitory activity against pathogenic microorganisms, indicate the ecological importance of studying the relationships between endophytes and their hosts. Furthermore, investigations on other biological activities of endophytes, beside isolation of pure antimicrobial agents, are crucial as an approach to search for novel natural products. On other hand, the use of endophytes as producers of bioactive agents will help in conservation of medicinal plants and maintenance of environmental biodiversity.

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