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Foliar Fungal Endophytes of Selected Medicinal Plants from the Province of Albay, Philippines

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ABSTRACT

Fungal endophytes were isolated from the leaves of the 10 most frequently used medicinal plants in the province of Albay, Philippines at three different locations: upland, lowland, and coastal areas. Their occurrence, frequency, and isolation rates were compared. A total of 120 isolates belonging to 17 species were identified. *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk and *Colletotrichum gloeosporioides* M.B. Dickman were the most frequent fungi occurring in 10 and nine plants, respectively. No significant difference in the total number of isolates, as well as the total number of unique species from among sampling sites, was detected. *Blumea balsamifera* (L.) DC harbored the most endophytes with 16 isolates, while banana leaves yielded the least with eight isolates. There were species of fungi that cut across all sampling sites, while a few occurred only in one site. The collection of additional samples from other sites within the province and the testing of the biological properties of the isolates are recommended.

Keywords: Albay, endophytes, *Glomerella cingulata*, medicinal plants, upland

INTRODUCTION

Traditionally, fungal endophytes are species of fungi residing within their hosts without causing apparent harm, emerging only during host-tissue senescence (Rodriguez et al. 2009) or when some physiological changes happen in the host tissue. Endophytism has long been regarded to be a form of mutualism. However, Saikkonen et al. (1998) strongly suggested that this forms a continuum of

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interaction, and that endophytism simply refers to where the fungus resides and not exactly how it interacts with its plant host. All plants, therefore, would host at least one fungal endophyte throughout its life cycle.

The continuous documentation of fungal endophytes of plants emerges from numerous objective endpoints. It can be towards the practical application of their natural products because of the superiority of natural selection over combinatorial chemistry in novel substance discovery (Schulz et al. 2002). Moreover, an ecological standpoint examines the changing roles the fungi play in relation to its plant host (Carroll 1988; Saikkonen et al. 1998; Rodriguez et al. 2009). Diversity, taxonomy, and phylogeny also are important drivers for endophyte research because of their ubiquity and geographical distribution (Arnold and Lutzoni 2007). More importantly, endophytes are significant in estimating overall fungal diversity (Arnold et al. 2000).

Regardless of the objective, it remains clear that endophyte documentation is a common denominator of nearly every, if not all, fungal endophyte research. Plant grouping is the basis for many processes, such as bioprospecting, ecological inferences, and diversity analysis. These groupings could be formal taxonomic grouping, or an informal grouping based on phenotypic, functional, or geographical similarities. The absence of species identification, or at least characterization, may be an obstacle and can substantially limit future prospects.

Medicinal plants form an informal but relatively cohesive group. The extent of the members of this group is unclear as their medicinal uses vary from place to place. In the Philippines, plants with medicinal value were already documented as early as the 1950s. Comprehensive ethnobotanical surveys were also carried out in different parts of the country (Tantiado 2012; Abe and Ohtani 2013). Several research have elucidated various properties of medicinal plants, such as antibacterial (Penecilla and Magno 2011), anti-hyperglycemic (Villasenor and Lamadrid 2006) and antioxidant properties (Peteros and Uy 2010), among others.

The diversity of plants and their medicinal properties may mirror the diversity of fungal endophytes residing within them, and may even extend to the potential properties of the fungi themselves. Huang et al. (2008) for instance, have shown that certain fungal taxa were more likely to coexist with plants producing certain phenolic compounds. Radu and Kqueen (2002) posed the question of whether medicinal properties are produced by plants themselves or are consequences of

the fungal-plant association. From a biological standpoint, this may be hard to answer because growing a plant without any endophyte may be challenging. However, the fungi, even without the plant, may be harnessed to produce the same benefits as that of its host.

Therefore, this research is an initial species assessment of the fungal endophytes of ten medicinal plants located in the province of Albay, Philippines based on a previous medicinal plant survey by Mirandilla and Abalon (2013). The fungal endophytes of the medicinal plants used in this research have minimal documentation. For instance, cassava (*Manihot esculenta* Crantz.) and coconut (*Cocos nucifera* L.) only record bacterial endophytes (Melo et al. 2009; Rajendran et al. 2015), while research on the fungal endophyte of mango was limited only to *Colletotrichum* spp. (Vieira et al. 2015). Likewise, literature comparing the occurrence of foliar fungal endophytes in upland, lowland, and coastal areas are limited. The ten medicinal plants included in this study are the ten most used in the province, and thus, their occurrence in the sampling sites are guaranteed.

MATERIALS AND METHODS

Medicinal plants

Ten medicinal plants were collected in this study based on the previous assessment of their diversity and use in the province of Albay, Philippines (Mirandilla and Abalon 2013). The 10 medicinal plants shown in Table 1 are the most commonly used in the province of Albay.

Table 1. Medicinal plants in Albay, Philippines used in this study

Common Name	Scientific Name	Code Used in this Study
Papaya	*Carica papaya* L.	CP
Cassava	*Manihot esculenta* Crantz.	ME
Mango	*Mangifera indica* L.	MI
Jackfruit	*Artocarpus heterophyllus* Lam.	AH
Gabi	*Colocasia esculenta* (L.) Schott.	CE
Coconut	*Cocos nucifera* L.	CN
Malunggay	*Moringa oleifera* Lam.	MO
Oregano	*Origanum vulgare* L.	OV
Banana	*Musa paradisiaca* L.	MP
Sambong	*Blumea balsamifera* (L.) DC	BB

Sampling sites

Sampling was performed from August to September 2016. Four sampling areas were selected within the province of Albay to represent sites with adjacent upland, lowland, and coastal areas, namely the cities of Ligao, Legazpi, Tabaco, and the municipality of Pio Duran. Sites were qualitatively classified as upland, lowland, and coastal based on the characteristics of the area and on elevation. Upland areas refer to sites with hilly or inclined terrains, lowland areas refer to those close to residences and human settlements, and coastal areas refer to those proximate to the sea. One individual of each medicinal plant from each area was chosen. Leaf samples were collected from each of the medicinal plants and were taken immediately to the laboratory for plating. For those with big leaves, such as banana and coconut, a random portion of the entire compound leaf was cut out and placed in a sterile plastic bag.

Isolation of fungal endophytes

Ten mature leaves without apparent symptoms of disease, such as necrosis, chlorosis, and presence of external wounds and deformities, were selected from the fresh samples. The leaves were washed thoroughly with running water to remove any debris on the surface. After blot drying, a 0.64-cm diameter puncher was used to create one circular leaf disc per leaf at the mid-section of the blade not including the midrib. Each disc was sterilized following the methods of Torres and dela Cruz (2015) with modifications. Leaf discs were soaked in 95% ethanol for 1 minute, then transferred to a 0.5% NaOH (Zonrox®) solution for 2 minutes, and washed twice in sterile distilled water. Leaf discs were then blot dried prior to plating.

Leaf discs were plated on potato dextrose agar (PDA, Himedia). A leaf print, done by touching the leaf disc on the PDA, was made to ensure only endophytes were isolated. Plates with growth on the leaf print were discarded and not used in the study.

Identification of fungal endophytes

Identification of isolates were first based on morpho-cultural characteristics and compared to existing taxonomic keys, specifically that of Watanabe (2010). Isolates similar in cultural and morphological characteristics were grouped and a reference isolate was duplicated in tubes. Isolates or groups that exhibited differences in

morpho-cultural characteristics were separated into subgroups, and one reference isolate per subgroup was also sent for the sequencing of the *ITS* gene. Extraction of the genomic DNA from the culture and the sequencing of the *ITS* gene was performed by Macrogen, Korea. The primers ITS 1 5'-TCCGTAGGTGAACCTGCGG-3' and ITS 4 5'-TCCTCCGCTTATTGATATGC-3' were used for the amplification of the *ITS* gene, which is the universal DNA barcode marker for fungi (Schoch et al. 2012). The PCR reaction was performed with 20 ng of genomic DNA as the template: initial denaturation at 95ºC for 2 minutes; 35 cycles of 95ºC for 1 minute, 55ºC for 1 minute, and 72ºC for 1 minute; and a final extension at 72ºC for 10 minutes. The resulting nucleotide sequences were then cleared of noises and aligned using ChromasPro and Mega7 (Kumar et al. 2015). The identities of the isolates were determined by homology against the National Center for Biotechnology Information (NCBI) database using Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1990). Only hits with homology greater than 97% were considered. For isolates which obtained hits with less than 97% homology, their identities were assigned based on their morpho-cultural characteristics. All isolates are maintained at the Bicol University College of Science Department of Biology with duplicate deposits at the University of the Philippines – Los Baños Museum of Natural History Microbial Culture Collection.

Data analysis

The isolation rate (IR) of each fungal species was calculated using the following equation:

$$IR\ (\%) = \frac{Total\ number\ of\ occurence\ of\ species}{Total\ number\ of\ leaf\ discs\ plated} \times 100 \quad (1)$$

$$F\ (\%) = \frac{Number\ of\ [plant-site]\ species\ A\ occured}{Total\ number\ of\ \ [plant-sites]} \times\ 100 \quad (2)$$

where [plant–site] is defined as the number of medicinal plants sampled multiplied by number of locations (upland, lowland, and coastal). Differences in the number of unique isolates per location were statistically analyzed by the nonparametric Kruskall-Wallis test using the R programming software (R Core Team 2013). Significant difference was determined at an alpha value of 0.05.

RESULTS

A total of 120 isolates belonging to 17 fungal species were identified. Representative isolates are listed in Table 2 and are shown in Figure 1. Because of some morpho-cultural differences observed during the growth stage of the isolates, some species have numerous reference cultures. The counts of the isolates were then combined when appropriate to produce the succeeding tables in this study.

Table 2. Representative fungal endophytes isolated from medicinal plants in Albay, Philippines as identified through the sequencing of their *ITS* gene

Reference Isolate Code	Genbank Accession Number	Most Closely Related Fungal Species based on *ITS Sequence* Homology	% Homology	Identity
REF-ISO 1	KP132299.1	*Hormographiella aspergillata* strain IHEM 14649	97	*Hormographiella aspergillata*
REF-ISO 2	KF679356.1	*Fusarium solani* strain IHB F 2353	99	*Fusarium solani*
REF-ISO 3	JX535014.1	*Fusarium solani* strain MML4012	97	*Fusarium solani*
REF-ISO 4	KP724996.1	*Coprinopsis cinerea* isolate 21L06I2	99	*Coprinopsis cinerea*
REF-ISO 5	KP900278.1	*Colletotrichum gloeosporioides* strain SS1-CJS12	99	*Colletotrichum gloeospioides*
REF-ISO 6	JF710555.1	*Colletotrichum gloeosporioides* isolate OMC4 18S	98	*Colletotrichum gloeospioides*
REF-ISO 7	JX902434.1	*Colletotrichum gloeosporioides* isolate OORC30	99	*Colletotrichum gloeospioides*
REF-ISO 8	KT342872.1	*Colletotrichum gloeosporioides* strain DS01 18S	97	*Colletotrichum gloeospioides*
REF-ISO 9	FJ172224.1	*Colletotrichum gloeosporioides* isolate CG0305	99	*Colletotrichum gloeospioides*
REF-ISO 10	KF706658.1	*Aspergillus unguis* strain FS95	99	*Aspergillus unguis*
REF-ISO 11	FJ654485.1	*Aspergillus* sp. SV/09-11 18S	98	*Aspergillus* sp.1
REF-ISO 12	KP686465.1	*Aspergillus* sp. BAB-4665	98	*Aspergillus* sp.1
REF-ISO 13	JF436891.1	*Aspergillus* sp. YMCA 11	97	*Aspergillus* sp.2
REF-ISO 14	KP686456.1	*Aspergillus* sp. BAB-4649 1	96	*Aspergillus* sp.3
REF-ISO 15	KF923855.1	*Glomerella cingulata* isolate UOM P	99	*Glomerella cingulata*
REF-ISO 16	JX868760.1	*Glomerella cingulata* strain LVPEI.B431_11	99	*Glomerella cingulata*
REF-ISO 17	KF848941.1	*Phomopsis* sp. FZ100	98	*Phomopsis* sp.
REF-ISO 18	KT208382.1	*Phomopsis* sp. GXGLB-3	99	*Phomopsis* sp.
REF-ISO 19	GU066650.1	*Phomopsis* sp. 76CG/L	99	*Phomopsis* sp.
REF-ISO 20	GU066617.1	*Phomopsis* sp. 27LD/T	97	*Phomopsis* sp.
REF-ISO 21	KM362394.1	*Diaporthe* sp. C53-134	98	*Diaporthe* sp.
REF-ISO 22	FJ799937.1	*Diaporthe* sp. SAB-2009a strain Q3310 18S	99	*Diaporthe* sp.
REF-ISO 23	KP217184.1	*Daldinia* sp. P4.2	99	*Daldinia* sp.
REF-ISO 24	JX559584.1	*Guignardia mangiferae* strain LGMF1163	99	*Guignardia mangiferae*
REF-ISO 25	LM652417.1	*Microascus cinereus*	96	*Microascus cinereus*
REF-ISO 26	LN482516.1	*Aspergillus flavus*	99	*Aspergillus flavus*
REF-ISO 27	JQ763433.1	*Aspergillus flavus*	97	*Aspergillus flavus*
REF-ISO 28	KF577897.1	*Aspergillus flavus* strain A0628	97	*Aspergillus flavus*
REF-ISO 29	KF221065.1	*Aspergillus flavus* strain Bp5	97	*Aspergillus flavus*
REF-ISO 30	JN709035.1	*Aspergillus nomius* strain SGE19	96	*Aspergillus nomius*
REF-ISO 31	AB976023.1	*Aspergillus fumigatus*	97	*Aspergillus fumigatus*

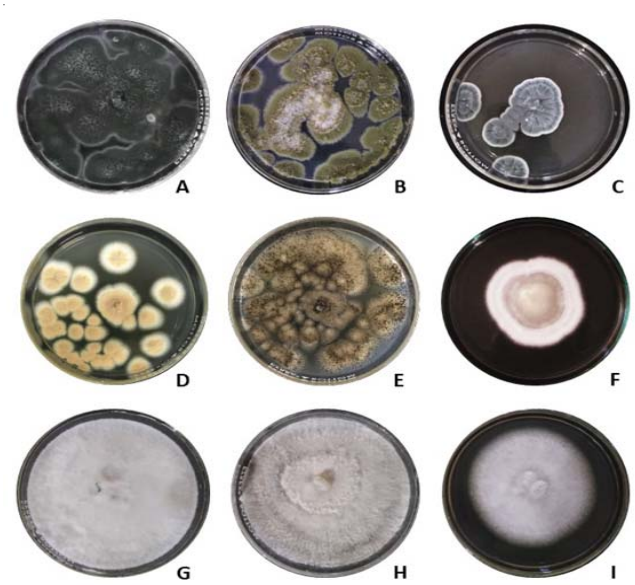

Figure 1. Representative fungal endophytes in medicinal plants from Albay, Philippines grown on potato dextrose agar (PDA) for eight days: (A) *Aspergillus fumigatus*, (B) *Aspergillus* sp.1, (C) *Aspergillus unguis*, (D) *Aspergillus* sp.2, (E) *Aspergillus* sp. 3, (F) *Colletotrichum gloeosporioides*, (G) *Phomopsis* sp., (H) *Diaporthe* sp., and (I) *Fusarium solani*.

Numerically, the upland area had the highest total endophytic count of 45 isolates among the sampling sites (Table 3). This, however, is not statistically significant compared to the coastal and lowland sampling areas. Medicinal plants from the upland, lowland, and coastal sampling sites constitute 37.5%, 35.8%, and 26.7% of the total isolates, respectively. No significant difference was detected in the number of fungal endophytes isolated from the same medicinal plants across different locations.

Table 3. Number of fungal endophytic isolates from medicinal plants in Albay, Philippines across different sampling locations

Location	Total Count										Total[ns]	%
	CP	ME	MI	AH	CE	CN	MO	OV	MP	BB		
Lowland	5	3	7	4	4	5	3	5	2	5	43	35.8
Coastal	5	1	2	2	4	3	5	3	3	4	32	26.7
Upland	3	7	4	7	2	2	4	6	3	7	45	37.5
Total	13	11	13	13	10	10	12	14	8	16	120	100

p-value = 0.2344

ns - no significant difference at 0.05 level of confidence

The total number of unique species (Table 4), which refers to the number of unique individual fungus regardless of the number of times it was isolated from the hosts, is highest in the upland area and lowest in the coastal area. The obtained values for the total number of unique species do not significantly vary from each other. Most species of fungi had overlapping occurrence both in sampling sites and the host plant.

Table 4. Number of unique species of fungal endophytes from medicinal plants in Albay, Philippines across different sampling sites

Location	CP	ME	MI	AH	CW	CN	MO	OV	MP	BB	Total^{ns}
Lowland	3	3	7	4	4	5	3	3	2	4	12
Coastal	4	1	2	2	3	3	4	3	3	3	10
Upland	2	5	3	5	2	1	4	4	2	7	14
Total	6	7	9	7	6	8	7	8	6	8	17

p-value = 0.3107

ns - no significant difference at 0.05 level of confidence

Glomerella cingulata was the most frequent among the fungal endophytes, having been isolated from all the medicinal plants sampled, and in 70% of the sampling areas (Table 5). The total isolation rate is 10%, 2.33% of which is contributed by *G. cingulata*. *Colletotrichum gloeosporioides* had an isolation rate of 1.67%, while the genus *Aspergillus* collectively forms 1.32% of the isolation rate. Isolation rate represents the probability of encountering the same species from a pool of isolates. A higher isolation rate means that the species is grown more frequently relative to the total number of isolates. Most isolates are from the phylum Ascomycota, although many are of the anamorphic form, such as *Aspergillus* spp., *Fusarium solani* (Mart.) Sacc., and *Colletotrichum gloeosporioides*. The basidiomycetes *Hormographiella aspergillata* and *Coprinopsis cinerea* (Schaeff.) Redhead, Vilgalys & Moncalvo may be anamorph and teleomorph of the same species (Surmont et al. 2002). Should this be the case, in combination, they account for 1.16% of the isolation rate. Seven species of fungi were absent from the coastal areas, while five and three species were not found in the lowland and upland areas, respectively. Six species of fungi, namely *Hormographiella aspergillata, Coprinopsis cinerea*, *Aspergillus unguis* Weill & L. Gaudin, *Aspergillus* sp.1, *Glomerella cingulata, Colletotrichum gloeosporioides*, and *Fusarium solani*, were consistently isolated from all sampling sites.

Table 5. Fungal endophytes of medicinal plants in Albay, Philippines

Species	Phylum	Number of Isolates	Upland	Coastal	Lowland	Total	Isolation Rate (%)
Hormographiella aspergillata	Basidiomycota	4	10.00	0.00	30.00	13.33	0.33
Coprinopsis cinerea	Basidiomycota	10	30.00	30.00	30.00	30.00	0.83
Aspergillus unguis	Ascomycota	6	20.00	30.00	10.00	20.00	0.50
Aspergillus sp. 1	Ascomycota	3	10.00	10.00	10.00	10.00	0.25
Glomerella cingulata	Ascomycota	28	50.00	70.00	90.00	70.00	2.33
Colletotrichum gloeosporioides	Ascomycota	20	70.00	10.00	50.00	43.33	1.67
Phomopsis sp.	Ascomycota	9	0.00	40.00	20.00	23.33	0.75
Diaporthe sp.	Ascomycota	12	50.00	40.00	10.00	36.67	1.00
Daldinia sp.	Ascomycota	5	20.00	0.00	30.00	16.67	0.42
Guignardia mangiferae A.J. Roy	Ascomycota	1	10.00	0.00	0.00	3.33	0.08
Microascus cinereus Curzi	Ascomycota	4	20.00	0.00	20.00	13.33	0.33
Aspergillus sp. 2	Ascomycota	1	10.00	0.00	0.00	3.33	0.08
Aspergillus sp. 3	Ascomycota	1	10.00	0.00	0.00	3.33	0.08
Aspergillus flavus Link	Ascomycota	3	0.00	10.00	20.00	10.00	0.25
Aspergillus nomius Kurtzman, B.W. Horn & Hesselt	Ascomycota	1	10.00	0.00	0.00	3.33	0.08
Aspergillus fumigatus Fresenius	Ascomycota	1	0.00	10.00	0.00	3.33	0.08
Fusarium solani	Ascomycota	11	30.00	30.00	40.00	33.33	0.92
Total		120				100.00	10.00

Total number of leaf discs plated = 1,200

Total number of [plant–site] = 30

DISCUSSION

Medicinal plants as source of endophytes

The fungi *G. cingulata* and *C. gloeosporioides*, the two most isolated species in this study, are common fungal endophytes of plants as they are also latent pathogens of many important fruits, such as mango, papaya, and avocado. In particular, for papaya, the *C. gloeosporioides* isolated in this study may be in its latency stage because no symptoms manifested on the leaf samples and symptoms of normal anthracnose disease often occur on the fruit. *F. solani,* likewise, was previously reported to be an endophyte of the medicinal plants *Alpinia calcarata, Bixa orellana, Calophyllum inophyllum, Catharanthus roseus*, and *Aquilaria sinensis*, all of which are plants known for their anticancer properties (Cui et al. 2011; Sunitha et al. 2013). Interestingly, *F. solani* isolated from *A. sinensis* exhibited antitumor properties, somehow mirroring the biological property of its host plant (Cui et al. 2011). Other endophytes isolated in this study form the first record of their isolation from specific medicinal plants.

It is now well known that all plants are in a symbiotic relationship with at least one fungal endophyte (Arnold et al. 2000; Rodriguez and Redman 2008; Rodriguez et al. 2009), translating to fungal species diversity (Arnold and Lutzoni 2007). Rodriguez et al. (2009) grouped together endophytes that primarily reflect differences in evolutionary relatedness, taxonomy, plant hosts, and ecological functions. Because of the importance of medicinal plants, especially in the Philippine context, it becomes an artificial plant group from where to isolate endophytic fungi. The medicinal benefits that may be derived from the plant may also theoretically be exhibited by the endophytes residing in them. Based on a survey by Mirandilla and Abalon (2013), the ten medicinal plants included in this study are the top ten medicinal plants used by households in the same sampling sites. Because of the importance of these medicinal plants to locals, their occurrence in the selected sampling areas is assured. Potential endophytes from these medicinal plants may also provide similar medicinal applications.

The diversity of fungal endophytes cannot be oversimplified. Just as there are a diverse group of plants to act as hosts, a variety of fungal groups could also grow in nearly all parts of the plant. Aside from the stem and leaves, the roots have been noted to also harbor fungal endophytes which can form mutualistic associations functionally similar to mycorrhizal symbiosis (Jumpponen 2001). A wide host population for fungal endophytes has been observed from the salt-tolerant species of mangroves (Suryanarayanan et al. 1998; Kumaresan and Suryanarayanan 2001; Ananda and Sridhar 2002), tropical forests (Suryanarayanan et al. 2002; Suryanarayanan et al. 2003), medicinal plants (Huang et al. 2008), and important agricultural crops including rice (Naik et al. 2009) and wheat (Dingle and McGee 2003). Environmental factors also affect fungal endophyte diversity as seen in studies on altitudinal and grazing gradients (Granath et al. 2007), as well as precipitation (Herrera et al. 2011).

Medicinal plants are important hosts to fungal endophytes because of their biological applications. Numerous studies have already explored medicinal plants and their associated fungal endophytes (Wiyakrutta et al. 2004; Li et al. 2005; Tejesvi et al. 2007), and almost all of these tackle their medicinal potentials. Whether the plants' medicinal value is a product of the symbiosis with its endophyte or is independent of the relationship is relative. It is also possible that the plants' medicinal value can be mirrored by the fungal endophyte, and thus, when isolated, the endophyte can also produce by itself the same active compounds in plants. The latter has already been shown to be true for many plants. For instance, *Taxomyces andreanae* Strobel, A. Stierle, D. Stierle & W.M. Hess produces taxol and taxane similar with its host, the Pacific yew (Stierle et al. 1993).

Considerations in sampling area

Location may be a big factor in determining the number of species, as well as the uniqueness of the fungal community. Location can alter the microclimate experienced by the host plant, thereby directly affecting the presence or absence of plant species, and their distribution and habit. Although not statistically observed in this research, many other studies suggest that geographical location can determine the species composition of fungal endophytes among plants. For instance, Mishra et al. (2012) noted that some species of fungi occur mostly exclusively at a particular season and in locations where emissions of certain gases and particulates are present. Similar observations were noted by Göre and Bucak (2007) when the number of fungal species that were isolated in leaves differed between sampling sites and the dominant fungi were site-dependent. This was not observed in this study primarily because of the small number of samples and the proximity of sampling sites. Specific weather conditions that can support any correlation were not recorded. Sánchez-Márquez et al. (2008) suggested that increasing the number of plants or locations would most likely reveal new endophytic species.

Endophytic relationship with plants

Any symbiotic relationship with the plant can be fluid, and therefore, fungal endophytes cannot be clear-cut mutualists all the time. Species such as *F. solani*, *C. gloeosporioides*, and *G. cingulata* are known pathogens of plants, and yet, are considered endophytes because of their location in the plant. Endophytism, by itself, should only be regarded as the location of the fungus and does not fully depict the fungus' interaction with the host. According to Faeth (2002), the predominant defensive mutualism perspective is a product of a long list of research involving agronomic grass cultivars and may not be the case among native grasses. The preference to look at plant-fungal endophyte interactions in the light of defensive mutualism may be because of the low genetic diversity and altered growing environment of domesticated grasses, and this concept should not be generalized. This defensive mutualism is rare even in introduced and domesticated grasses. Models suggest that host and fungus genotypes, as well as the environmental conditions, affect the direction of interactions (Faeth and Fagan 2002). Faeth and Sullivan (2003) also challenged defensive mutualism by showing that mutualistic asexual endophytes of native grasses are usually parasitic because of the negative effects on plant reproduction (number and mass of seeds and germination rate). Moreover, Sieber (2007) mentioned that evidence for such is mostly circumstantial but agreed that plants would probably not survive many environmental stresses

without the symbiosis with fungal endophytes. To prove unequivocally the positive impact of endophytes to its host, an endophyte-free control must be developed which, according to Sieber (2007), is a major problem. In addition, fungi can switch from quiescence to pathogenicity when factors are favorable for the fungi and unfavorable for the host. This switch is genetic, as observed in the genus *Colletotrichum* which causes anthracnose in cucurbits (Freeman and Rodriguez 1993). The switch later on leads to the maintenance of compatibility between *Colletotrichum* spp. and cucurbits, similar to that of a pathogen, as well as the survival of the host in what is known to be a balanced plant-endophyte status (Kogel et al. 2006). *Colletotrichum* spp. can switch from being pathogens to mutualists based on the host genotype, and thus, its ability to be an endophyte is not strictly confined (Rodriguez et al. 2005).

Potential biological applications of fungal endophytes

G. cingulata and *C. gloeosporioides*, two of the most isolated endophytes in this study, are known to have anticancer properties (Gangadevi et al. 2008), while *Phomopsis* spp. are known to produce terpenoids and isoflavonoids acting as antimicrobials (Redko et al. 2006; Nithya and Muthumary 2010). There is no literature stating the biological applications of *Hormographiella aspergillata* which is known to cause disease in humans (Surmont et al. 2002). *H. aspergillata* isolated from this study was not well characterized for this reason. *F. solani*, although known to be a plant pathogen, is also known to produce Taxol, an anticancer agent (Kusari et al. 2009). Because biological properties often differ due to location and host, the applications of the isolates from this research may be the subject of future investigations.

CONCLUSION AND RECOMMENDATIONS

Medicinal plants are good sources of fungal endophytes from which metabolites can be obtained with wide spectrum of applications. From this research, fungal endophytes from pre-selected medicinal plants were isolated and identified. A comparison of their occurrence across sampling sites and host plants displayed no significant difference. It is yet to be established whether a general mirroring of medicinal value can be observed from the sampled medicinal plants and their associated endophytes. The investigation of the biological activities of the isolates is recommended.

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