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Antioxidant Properties of Fungal Endophytes Associated with the Three Medicinal Plants *Gliricidia sepium*, *Canna indica* and *Gardenia jasminoides*

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Authors' contributions

This work was carried out in collaboration between all authors. Author TEDC designed the study, corrected the first draft of the manuscript and supervised author SE. Author SE managed the literature searches, performed the isolation process protocols and wrote the manuscript. Author MB managed the experimental process on TLC and antioxidant assays, helped in editing of the manuscript and wrote the final draft. All authors read and approved the final manuscript.

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ABSTRACT

Fungal endophytes live within tissues of living plants without causing any disease. These fungi are recognized sources of natural, bioactive compound with potential applications in agriculture, medicine, and food.
Aims: This research study aimed to isolate and produce bioactive metabolites from fungal endophytes isolated from three Philippine medicinal plants and assess their antioxidant activities.
Study Design: The study used exploratory research where medicinal plants were selected

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because these were considered to contain a wide range of endophytes which could have varied secondary metabolites that exhibit biological activities.
Place and Duration of Study: Research Center for the Natural and Applied Sciences at the University of Santo Tomas, Manila and Center for Natural Sciences at Saint Mary's University, Bayombong, Nueva Vizcaya during June 2011 to October 2012.
Methodology: Medicinal plant leaf endophytes (MPLE) were isolated through surface sterilization of healthy leaves of *Gliricidia sepium*, *Canna indica* and *Gardenia jasminoides.* Selected MPLE were grown *in vitro* and extracted for their secondary metabolites. Assay of antioxidant activities was done using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) while total phenolics content was through the Folin-Ciocalteu method. The number of metabolites present was detected using thin layer chromatography.
Results: A total of **73** fungal strains belonging to 23 different morphospecies endophytes were isolated from the three host plants. *G. sepium* had the highest number of morphospecies (32) among the three medicinal plants studied. These fungal endophytes were identified as belonging to the genera Botrytis. *Curvularia, Aspergillus, Trichoderma, Penicillium,* and *Gliocladium.* Many MPLE were identified only as mycelia sterile The MPLE crude cultures showed antioxidant activities (75-90%RSA) and total phenolic content of 134 AAE/g. There were a maximum of 11 secondary metabolites present in the crude culture extracts belonging to the class of flavonoids, terpenoids, phenolics, fatty acids and coumarins.
Conclusion: Fungal endophytes are rich in tropical regions and the three Philippine medicinal plants studied are good hosts of fungal endophytes which showed antioxidant activities and secondary metabolites which can be harnessed as sources of pharmaceuticals and nutraceuticals.

Keywords: Medicinal plants; endophytic fungi; secondary metabolites; DPPH; total phenolics.

1. INTRODUCTION

Fungal endophytes colonize internal tissues of plants at some time of their life cycle without causing any apparent harm to their host plant [1]. These fungal endophytes have recently been tapped as sources of novel natural products [2]. Thus, it is not surprising to find new metabolites from these fungi. For example, *Diaporthe* sp. isolated from *Pandanus amaryllifolius* produced the new compounds, Diaportheone A and Diaportheone B [3]. Cryptocandin, an anticancer metabolites, was isolated from *Cryptosporiopsis quercina* [4], while4-epiradicinol was derived from *Curvularia* sp. [5]. Aureobasidins and botryodiplodin were also obtained from *Aureobasidium pullulans* and *Lasiodiplodia theobromae* [6]. Species of *Acremonium, Aspergillus, Fusarium,* and *Penicillium* isolated from plants were also reported to produce numerous metabolites [7,8]. Interestingly, these fungal endophytes were also extensively studied for their antioxidants and antimicrobial activities [9]. Still, many fungi awaits discovery for new antioxidants. Medicinal plants can therefore be hosts to many potentially new species of fungi [10]. For example, each of these medicinal plant species is host to a number of endophytic fungi: *Acacia* sp. for *Aureobasidium pullulans*, *Chaetomium* sp., and *Chaetomium globosum*; *Coffea arabica* for *Alternaria alternata*, *Colletotrichum crassipes*, *Myrothecium roridum*, *Leptosphaeria* sp., *Phoma herbarium*, *Cladosporium cladosporioides*, *Phomopsis stipata* and *Guignardia* sp.; and *Cephalotaxus mannii* for *Cladosporium* sp., *Acremonium* sp., *Trichoderma* sp., *Cephalosporium* sp [6,11,12]. Since there is an interaction of endophytes and its host plant, it is likely that we can find biological activities of endophytes when we isolate them from bioactive plants [9]. The leaves of *G. sepium* are used as an antihistaminic, antipyretic, expectorant, and diuretic drug. Extracts of *G. sepium* have been shown to have high anti-fungal activity [13]. *C. indica* has reported demulcent and diaphoretic activities. Leaves of *C. indica* are used to treat gonorrhea [14]. *G. jasminoides* is used to acute conjunctivitis, epistaxis, hematemesis, hematuria, pyogenic infection and ulcers of skin [15]. Thus, this research study isolated fungal endophytes from three known medicinal plants and assessed their antioxidant activities.

2. MATERIALS AND METHODS

2.1 Host Plants

Healthy and young leaves of three Philippine medicinal plants are collected from a local farm in San Miguel, Bulacan, Philippines. All plant materials were kept in ziplock plastic bags and

surface-sterilized within 24 hours. Voucher specimens of the collected medicinal plants were prepared and submitted to the Botany Division, Philippine National Museum, Manila for identification. The host plants were identified as *Gliricidia sepium* (Jacq.) Kunth. Ex Walp., *Canna indica* L. and *Gardenia jasminoides* Ellis.

2.2 Isolation and Identification of Fungal Endophytes associated with Medicinal Plants

Leaves of host plants were initially washed with tap water to remove dust and soil debris. After washing, leaf explants were cut from each of the young leaves using a flame-sterilized, one hole-puncher (6 mm in diameter). The leaf explants (30 explants per host plant) were then collected in sterile petri dishes and surface-sterilized following a slight modification of the protocol of Toofanee & Dulymamode [16]. Surface sterilization was done by immersing the leaf explants in 5% active sodium hypochlorite solution (commercially available bleach solution) for 2 minutes and then washed three times in sterile water for 3 minutes. To check if the fungi were indeed endophytes and not epiphytic fungi, another set of leaf explants was washed only three times with sterile water for 3 minutes. After surface-sterilization, all leaf explants (6 leaf explants per culture plate) were placed on culture plates pre-filled with Potato Dextrose Agar (PDA, Hi-Media) supplemented with 500 mg/L Streptomycin sulphate (Sigma). The addition of antibiotic prevents bacterial growth. All culture plates were sealed with parafilm and incubated at room temperature for 1-4 weeks. Isolation of fungal endophytes from the master plates, i.e. from plates with surface-sterilized leaf explants, was done by transferring hyphal tips from the leaf explants to freshly prepared PDA plates and subsequently sub-cultured until pure cultures were obtained. To ensure that the isolated fungi were indeed fungal endophytes, the fungi isolated from leaves washed only with sterile water were compared with isolated fungi from surface-sterilized leaves. Only fungal strains that were isolated from surface-sterilized leaves and did not grow from leaf explants washed only with sterile water were considered as medicinal plant leaf endophytes (MPLE) and used in the study. Aseptic techniques were done throughout the experiments to prevent the growth of unwanted microorganisms. Stock cultures of pure MPLE were all maintained on PDA slants and stored inside a refrigerator at the Pure and Applied Microbiology Laboratory, UST-RCNAS.

All isolated MPLE were identified based on their morphological and cultural characteristics. Initially, MPLE were grown on freshly prepared PDA plates for 3-5 days at room temperature. Following incubation, fungal colonies were described based on their colony appearance, e.g. texture, margin, color, reverse texture, and reverse color. For the spore and hyphal morphology of the isolated MPLE, spores and hyphal were fixed with lactophenol in clean glass slides and observed under a compound light microscope (Olympus model, 400-1,000x). Spore color, shape and arrangement as well as hypha morphology were all noted. Preliminary identification of the isolated medicinal plant fungal endophytes was done following comparison of their colony and spore shapes with published ID guides book [17]. Identities of the MPLE were confirmed by Prof. Dr. Irineo J. Dogma, Jr., Graduate School, University of Santo Tomas in Manila, Philippines.

2.3 Production Extraction and TLC Profiling of Secondary Metabolites from MPLE

Initially, 15 selected MPLE were grown on MEA slants for 7 days at room temperature (Table 1). Following culture, 10 mL sterile distilled water was poured on the slants and the spores and mycelia were dislodged using a flame-sterilized wire loop. For the production of secondary metabolites, this 10-mL spore and mycelial suspension was transferred aseptically to 100 mL Malt Extract Broth (MEB, Hi-media). Two 100 mL MEB in bottles were used for each of the fungal endophytes. All inoculated culture broths were incubated under stationary condition at room temperature for 4 weeks. This production of the secondary metabolites follows partly a published protocol Reino et al. [18]

Following culture of MPLE, extraction of secondary metabolites from the inoculated media was done following the procedure of Reino et al. [18]. In this procedure, 100 mL ethyl acetate (RCL Labscan) was added to each of the culture bottles and soaked overnight. After 14 hours, the fungal mycelia were filtered in filter paper and the ethyl acetate extract was decanted. The extracts from the same culture bottles were then pooled together. The culture filtrates per MPLE or fungal combinations were then concentrated *in vacuo* using a rotary evaporator (EYELA, OBS-2100). The crude culture extracts were transferred to pre-weighed

amber bottles, allowed to dry, and then, weighed to determine the product yield. Crude culture extracts of the fungal endophytes were also dissolved in methanol:acetone (1:1) and spotted on TLC plate using capillary tubes. The TLC plate was then run on ethyl acetate solvent system in a developing chamber [19]. Then, the TLC plates were sprayed with 10% ethanolic sulfuric acid and heated at 105ºC until separated spots were visible. Visible spots were then measured and Rf values were computed as follows:

$$Rf = \frac{\text{distance traveled by spot}}{\text{distance traveled by solvent front}}$$

The number of metabolites were then counted for each of the crude culture extracts and tabulated.

2.4 Screening of MPLE for Their Antioxidant Activities

2.4.1 DPPH on TLC assay

The crude culture extracts of MPLE cultures were initially spotted on silica gel TLC plates (Merck) and run in methanol:formaldehyde (2:8) solvent system. The TLC plates were then sprayed with 1,1-diphenyl-2-picrylhydroxyl (DPPH) reagent. Spot with clear zones against a violet background indicated the presence of antioxidants. To confirm further the presence of phenolic and non-phenolic antioxidants, another set of TLC plates were prepared as previously described and sprayed with $K_3(FeCN)_6$-$FeCl_3$ spray reagent. The presence of blue spots on the TLC plates indicated positive result for phenolic compounds.

2.4.2 Assay for radical scavenging activity (RSA)

To determine the radical scavenging activity of the MPLE, the crude culture extracts exhibiting greatest number of positive (>3) spots in the DPPH-TLC assay above were subjected to DPPH radical scavenging activity assay [20]. In this assay, the crude culture extracts were dissolved in methanol to a final concentration of 500 ppm. A 0.1mM DPPH solution in MeOH was also freshly prepared by diluting 1 ml DPPH stock solution (3.49 mg DPPH in 1 ml MeOH) to 100 ml MeOH. One ml of each of the crude culture extracts and 4 ml of the DPPH solution was mixed and incubated in the dark at 37ºC for 30 minutes. The reaction was done in triplicates for each crude culture extracts. The absorbance reading was monitored at 517 nm using APEL-100 UV-Vis spectrophotometer (PD-303UV, Japan). Finally, the ability to scavenge the DPPH radical was calculated using the equation below:

Equation 1

$$\% \text{ DPPH scavenging effect} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

where $A_{control}$ is the absorbance of the control (DPPH solution without the crude culture extract), and A_{sample} is the absorbance of the test sample containing the mixture of DPPH and the crude culture extract. The synthetic antioxidant Vitamin E was used as positive control.

2.4.3 Assay for total phenolic content (TFC)

The total phenolic content of the crude culture extracts were determined with the Folin-Ciocalteu method [21]. At first, a calibration curve was made at different concentrations (0.25, 0.5, 1.0, 2.0 & 4.0 mg/mL) of Ascorbic Acid using APEL-100 UV-Vis spectrophotometer (PD-303UV, Japan). These ascorbic acid solutions (volume of 1 ml) were placed in vials. To each vial, 200 µL of Folin-Ciocalteu reagent was added and the vials incubated at room temperature for 5 minutes. For this experiment, 20 ml of sodium carbonate (Na_2CO_3) with a concentration of 1 mg/ml was also prepared. Then, 200 µl Na_2CO_3 was added to the mixture of ascorbic acid and FC reagent. Immediately, 200 µL of these were transferred to cuvettes and read using the UV-Vis spectrophotometer at 680 nm wavelength. After standardizing the calibration curve, 1 mg of the crude culture extracts was dissolved in 1 ml distilled water. From each of the crude culture extracts, 100 µL was transferred to vials (in triplicates). To these, 200 µl of Folin-Ciocalteu reagent was added and incubated at room temperature for 5 minutes. Then, 200 µL Na_2CO_3 was added to the mixture of crude culture extracts and FC reagent and read using the APEL-100 UV-Vis spectrophotometer (PD-303UV, Japan) at 680 nm wavelength. Absorbance values of the crude culture extracts were compared with the calibration curve prepared using the ascorbic acid. The total phenolics were calculated based on the standard curve of the ascorbic acid and its linear regression as shown in this equation:

Equation 2

$$y = mx + b$$

where y represents the OD, m represents the slope of ascorbic acid, and b represents the y-intercept.

3. RESULTS AND DISCUSSION

The Philippines has more than 13,500 species of plants, 6,500 of which are endemic to the country while about 1,500 are considered as medicinal plants [16]. Thus, the country offers many host plants for fungal endophytes. The assemblages of fungal endophytes are dependent to its host's habitat, though some species exhibited specificity in their growth in one particular host or in one plant organ [1]. However, most mycologists agree that fungal diversity is very high in tropical forests. Fungal endophytes in particular are rich in tropical regions [22]. This led us to study the endophytic fungi associated with three Philippine medicinal plants and assess their antioxidant activities and secondary metabolic profiles.

In this study, *G. sepium* had 32 isolates of fungal endophytes. *C. indica* had 29 while *G. jasminoides* had 12 fungal endophytes. Most of the endophytic fungi that grew in *G. sepium* and *C. indica* were species of *Trichoderma*. Species of *Aspergilus* and *Penicillium* were noted less on the leaf explants than the other fungal endophytes. The results in this study showed that indeed medicinal plants are good hosts for fungal endophytes. Medicinal plants served as good hosts for fungal endophytes as reported in Japan [23]. Similar studies were reported in Thailand [6,7,11,24], Australia [25], Hong Kong [26] and the Philippines [3]. There are endophytes that are frequently isolated from any host plant while others are isolated only on specific genera of plants and only on specific collection sites [3]. The identified endophytes isolated from the three host plants studied were not host-specific to them and are found also in diverse hosts in different countries.

3.1 Secondary Metabolite Profiling of the MPLE

Secondary metabolites of fungal endophytes from medicinal plants are reported in many journals. Fungal endophytes had good relationship with their host plants by protecting it against pathogens and insects through the production of secondary metabolites [27]. In this study, secondary metabolic profiling of the crude culture extracts from 15 MPLE isolates was done using TLC with ethyl acetate as the solvent system and 10% ethanolic sulfuric acid as spray reagent. The highest number of compounds (11 visible spots) was recorded in *Trichoderma* sp. (MPL-S6) while one with mycelia sterile (MPL-S2*)* had the lowest number with only two visible spots (Table 2).

Table 1. Selected MPLE isolates used in the production of secondary metabolites and antibacterial assays

Code	Taxa	Host plants		
		G. sepium	***C. indica***	***G. jasminoides***
MPL-S1	*Botrytis* sp.	+	-	-
MPL-S2	mycelia sterila	-	+	+
MPL-S3	*Curvularia* sp.	-	+	+
MPL-S4	mycelia sterila	+	+	+
MPL-S5	*Aspergilus* sp.	+	+	-
MPL-S6	*Trichoderama* sp.	+	+	-
MPL-S9	*Curvularia* sp.	+	+	-
MPL-S10	*Gliocladium* sp.	-	+	-
MPL-S11	mycelia sterila	-	+	+
MPL-S12	mycelia sterila	+	+	+
MPL-S13	mycelia sterila	+	+	-
MPL-S14	*Trichoderma* sp.	+	-	-
MPL-S15	mycelia sterila	+	+	+
MPL-S17	*Penicillium* sp.	+	-	-
MPL-S18	mycelia sterila	+	+	-

[a] *(+) indicated that the species were isolated from that particular host plant/s while (-) means that the species were not isolated from that host plant/s. Note that if similar species were obtained from more than one host plants, only one species from one host plant was cultivated for the study*

To determine the types of secondary metabolites present, another solvent system methanol: formaldehyde (2:8) was used. Results showed the absence of alkaloids in the MPLE crude culture extracts. Most of the MPLE crude culture extracts have phenolic compounds, some fatty acids, and the presence of the coumarins, anthrones, terpenoids, and flavonoids. Species of MPL-S1 (*Botrytis* sp.) had flavonoids, MPL-S4 (mycelia sterila) had coumarins, anthrones, and flavonoids, while MPL-S5 (*Penicillium* sp.) had anthrones. MPL-S6 (*Trichoderma* sp.) had fatty acids and flavonoids, MPL-S9 (*Curvalaria* sp.) had anthrones, while MPL-S10 (*Gliocladium* sp.) had terpenoid, phenolics, fatty acids, coumarins, and flavonoids. Fatty acid and coumarins were detected in MPL-S11 (mycelia sterila) while terpenoids and phenolics were detected in MPL-S13 (mycelia sterila). MPL-S15 (mycelia sterila) showed the presence of terpenoids, phenolics, coumarines, anthrones, and flavonoids. MPL-S17 (*Penicillium* sp.) also had coumarins while MPL-S18 (mycelia sterila) had coumarins and anthrones. Also from other studies, the species of *Fusarium, Aspergillus,* and *Penicillium* isolated from *Lobelia nicotianifolia* showed various secondary metabolites including flavonoids [28]. The results of these findings are consistent with the findings of Schulz et al. [29] in the many decades of their screening of metabolites from endophytic fungi, that most of the compounds frequently isolated from endophytes were from the classes of phenols, benzopyranones, steroids, terpenoids, isocoumarines and flavonoids.

3.2 Antioxidant Activities of MPLE

Antioxidants comprise a range of substances that play a role in protecting biological systems against the deleterious effects of oxidative processes on macromolecules, such as proteins, lipids, carbohydrates, and DNA. Many of these substances which contribute to the prevention and treatment of diseases in which reactive species of oxygen are involved are natural molecules of plants. This protection can be explained by the capacity of the antioxidants of plants to scavenge free radicals. Radical scavenging activities by antioxidants in the plant extracts were evaluated using DPPH assay [30]. The reactive oxygen species play an important role related to the degenerative or pathological processes of various serious diseases, such as aging, cancer, coronary heart disease, Alzheimer's disease, neurodegenerative, disorders, atherosclerosis, cataracts, and inflammation [31]. In the study, the antioxidant activity was detected for selected MPLE cultures (Table 3). Results showed that high amounts of radical scavengers were noted in crude culture extracts of *Trichoderma* sp. (MPL-S9, 82%), *Penicillium* sp. (MPL-S17, 90%), mycelia sterila (MPL15, 78%), *Trichoderma* sp. (MPLS14, 89%). The results showed the electron donating ability of the crude culture extracts can be same in capacity with the commercial antioxidant, Vitamin E, and thus can serve as free radical inhibitors or scavengers. DPPH molecules can be correlated with the number of available hydroxyl groups. The significant DPPH scavenging potential of the endophytic fungal extracts may be due to hydroxyl groups present in their extracts. In the study of Pushpalatha [32] crude culture extracts of *Fusarium, Aspergillus, Penicillium,* and *Mucor* isolated from *L. nicotianifolia* had antioxidant activity. Several study showed fungal endophyte from medicinal plants to have high values of natural antioxidants, e.g. the fungal endophytes *Aspergillus niger* and *Alternaria alternata* from *Tabebuia argentea* [33], and *Aspergillus niger* and *Fusarium oxysporum* from *Crotalaria pallida* [34].

The total phenolic content of the crude culture extracts were also determined to show the relationship between its antioxidant activities and the phenolic content. MPL-S4 (mycelia sterila, TPC = 134 mg AAE/g sample) and MPL-S10 (*Gliocladium* sp., TPC=133 mg AAE/g sample) showed high phenolic compound contents as compared to other extracts. Other crude culture extracts also showed a good range of phenolic compounds between 11 to 134 mg AAE/g samples. In this study, there was no correlation between the total antioxidant capacity (%RSA) and total phenolic content (TPC). Most of crude culture extracts showed antioxidant activity and high amount of phenolic compounds after the DPPH assay. However, some of the crude culture extracts showed high antioxidant activities but low in total phenolic content. *Penicillium* sp. (MPL-S17) had high antioxidant activity which was the same with the control (Vit. E, 90%), but a low value in relation to the amount of phenolics (34.46 mg AAE/g). Also, MPL-S9 (*Curvalaria* sp., RSA = 81.9%, TPC = 34.46 mg/g) and MPL-S14 (*Trichoderma* sp., RSA = 89.2% TPC = 53.63 mg/g) showed high antioxidant activity but low phenolic content. However, MPL-S4 (mycelia sterila, RSA = 0%, TPC = 134 mg AAE/g), MPL-S10 (*Gliocladium* sp., RSA= 0%, TPC = 132.9 mg AAE/g) and

MPL-S6 (*Trichoderma* sp., RSA= 0%, TPC = 110.62 mg/g) had high phenolic content but did not show antioxidant activity (Table 4).

As previously noted, the phenolic contents are major antioxidant constituents of the fungal endophytes. These phenolic compounds can be used in wide range of applications, e.g. as therapeutic antioxidants, antimutagenic, anticarcinogenic, and free radical scavenging agents, and also used in decreasing cardiovascular complications [28]. Liu et al. [35] estimated the antioxidant activity of the fungal endophyte *Xylaria* sp. from *Ginkgo biloba* and also noted that the strong antioxidant capacity of the fungal endophyte was due to the presence of phenolics and flavonoids compounds. Also, in another study, graphislactone A, a phenolic metabolite isolated from the endophyte fungus *Cephalosporium* sp., showed free radical-scavenging and antioxidant activities [36]. These observations were also noted in the study.

Table 2. The number of secondary metabolites detected in crude culture extracts of MPLE

Taxa	Code	Number of metabolites
Botrytis sp.	MPL-S1	7
mycelia sterile	MPL-S2	3
Curvularia sp.	MPL-S3	9
mycelia sterile	MPL-S4	6
Penicillium sp.	MPL-S5	9
Trichoderma sp.	MPL-S6	11
Curvalaria sp.	MPL-S9	9
Gliocladium sp.	MPL-S10	8
mycelia sterile	MPL-S11	9
mycelia sterile	MPL-S12	8
mycelia sterile	MPL-S13	7
Trichoderma sp.	MPL-S14	8
mycelia sterile	MPL-S15	6
Penicillium sp.	MPL-S17	3
mycelia sterile	MPL-S18	2

Solvent systems used: Ethyl acetate, spray reagent: 10% EtOH-H_2SO_4

Table 3. Percent radical scavenging activity (%RSA) of the MPLE crude culture extracts

Code	Taxa/sample	Mean OD	%RSA
Vit E (+ control)	Vit E	0.252	89.9
MPL-S9	*Curvalaria* sp.	0.452	81.9
MPL-S17	*Penicillium* sp.	0.250	90.0
MPL-S15	mycelia sterile	0.558	77.7
MPL-S14	*Trichoderma* sp.	0.270	89.2

Table 4. The total phenol content of the medicinal plant leaf endophytes (MPLE) crude culture extracts

Taxa	Code MPLE	MPLE (mg AAE/g)[a]
mycelia sterile	MPL-S4	134
Curvularia sp.	MPL-S3	62.65
Trichoderma sp.	MPL-S6	110.62
Gliocladium sp.	MPL-S10	132.9
Penicillium sp.	MPL-S17	34.46
Curvalaria sp.	MPL-S9	34.46
Trichoderma sp.	MPL-S14	53.63
mycelia sterile	MPL-S11	11.66
mycelia sterile	MPL-S15	48.45

[a] *AAE = ascorbic acid equivalent (mg AAE/g sample)*

4. CONCLUSION

Endophytic fungi are sources of natural products which benefit humankind. A total of 73 endophytic fungi were isolated from three medicinal plants *G. sepium*, *C. indica* and *G. jasminoides.* The medicinal plant leaf endophytes (MPLE) were identified as belonging to the following genera: *Botrytis, Curvularia, Aspergillus, Trichoderma, Penicillium*, and *Gliocladium.* Many of the isolated MPLE did not produce any spores in culture and thus, were designated as mycelia sterila. Among the host plants, the highest number of species was noted in *G. sepium* followed by *C. indica* and *G. jasminoides.*

Thin layer chromatorgraphy detected the presence of up to 11 metabolites from the cultures of MPLE. Assay for antioxidant activities showed high phenolic and free radical scavenger contents in the MPLE crude culture extracts and that there is no correlation in the total phenolic content (TPC) and the total antioxidant capacity (%RSA) of the extracts. Thus there are compounds other than the phenols which contributed to the radical scavenging potential of the extracts. Results of the phytochemical analysis showed the presence of anthraquinones, coumarins, flavonoids, terpenoids, and phenolics. This study reports fungal leaf endophytes from Philippine medicinal plants as potential sources of bioactive secondary metabolites for pharmaceutical drugs.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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