BU R&D Journal
21 (2): 19-27, December 2018 | ISSN (Print): 0016-4139
journal.bicol-u.edu.ph

GC-MS Analysis of Metabolites from *Aspergillus tamarii* and *Trichoderma* sp. Detected Promising Biological Compounds

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Abstract

Fifteen multicellular fungi previously isolated from rice paddies were evaluated for their antibacterial activities against test organisms using the agar plug method. Only seven of the fungal isolates showed activity against the test organisms *Escherichia coli*, *Bacillus cereus*, and *Staphylococcus aureus.* These seven fungi were subsequently grown on yeast extract sucrose agar (YES), potato dextrose agar (PDA), and Sabouraud dextrose agar (SDA) to determine their radial growth and time of sporulation over a period of seven days. The fastest sporulation was achieved by *Penicillium funiculosum* (three days), *Aspergillus tamarii* (five days), and *Trichoderma sp.* (six days). Spore suspensions (150 spores/mL) of these fungi were separately inoculated in 100 mL of Yeast Extract Broth (YEB), Potato Dextrose Broth (PDB), and Sabouraud Dextrose Broth (SDB), and cultured for 35 days in room temperature (24–27°C) at stationary condition. Culture filtrates were extracted with ethyl acetate (1:1 v/v) and used for herbicidal assay using *Vigna radiata* and for gas chromatography-mass spectrometry (GC-MS). No herbicidal activity was observed with water as control. Compounds identified (>90% similarity with National Institute for Standards and Technology [NIST] library) included bis (2-ethyl hexyl) phthalate (antifungal and anti-inflammatory), dehydromevalonic lactone (fragrance production), benzene acetaldehyde (fragrance production), and *n*-hexadecanoic acid (anti-inflammatory). Metabolomics data provide the basis for future optimization studies to generate utilizable bioactive compounds.

Keywords: *GC-MS, secondary metabolites, multicellular fungi, Aspergillus tamarii, Trichoderma sp.*

Introduction

Secondary metabolites are compounds produced by organisms that are not directly involved in normal growth, reproduction, and overall metabolism. These compounds, however, may play out significantly in ecological interactions. These low-weight molecules often have potent physiological activities (Keller *et al*., 2005), and among those produced by fungi, these compounds can be antibacterial, fungicidal, antitumor, immunomodulatory, and many more (Schulz *et al*., 1995; Zeng-Zhi *et al*., 2007; Xu *et al*., 2010).

Natural products from secondary metabolites are the single most productive source of leads for the development of drugs (Harvey, 2008). From where to mine them is a question of both logistical practicality and research novelty. Funding for harnessing active biological compounds from a multitude of sources depend on a sound bioprospecting method, a tool that screens potential sources before these sources are fully utilized for mass production and testing. Vis-à-vis this is a criterion that funnels down on source novelty, which refers to the uniqueness of the ecosystem from where to get the natural products and the general trend in research on that particular source. The balance between logistical practicality and research novelty is exemplified by the fungi as a novel source of active biological compounds.

Research trends on fungal novel compounds and secondary metabolites are relatively new compared to plant and microbial products. Unlike plants and bacteria, fungal species are relatively versatile in their choice of hosts and substrates, allowing them to thrive in a wide spectrum of ecosystem. However, Hawksworth and Lucking (2017) reported that science knows only about eight percent of the estimated 2.2–

3.8 million species of fungi and Suryanarayanan and colleagues (2009) said that only very few species have been cultivated and screened for drug production. The available known species can be a bottleneck to continuing research. Literature support, however, provides an opportunity to further studies since same species of fungi can produce different compounds at different conditions (Kokkomen *et al*., 2010) and bioactivities vary based on the biotopes they were isolated from (Schulz *et al*., 2002). Soil fungi are part of a group that plays various functions as saprobes, symbionts, and pathogens (Buee *et al*., 2009), and as such are a treasure trove of natural compounds. Their diversity differs in the type of soils and as seen in studies, with any amelioration to its nutrient content (Wallenstein *et al*., 2006), and disturbance (Cabello and Arambari, 2002). In the Philippines, the study on microfungal metabolites from soil microfungi is now gaining attention, albeit few researchers have fully ventured on investigations on this particular area of microfungal research. Guerrero and Alfante (2013) were able to isolate 117 microfungi comprising of seven genera and 23 species from rice paddies in Albay province, Philippines.

The discovery of novel compounds from microorganisms could be through the use of conventional biological assays. However, this approach is limited to the sensitivity of the assay. Thus, the use of more advanced instrumentation and analysis such as gas chromatography-mass spectrometry (GS-MS) allows for a better resolution of the presence of compounds based on metabolomics data (Luo *et al*., 2014).

The overall objective of this research was to determine the potential of locally isolated fungi as source of secondary metabolites for natural product discovery using culture-based assays and metabolomics approach through gas chromatography-mass spectrometry (GC-MS). Specifically, the study aimed to evaluate the antimicrobial and herbicidal activities of local fungi isolates, determine their growth and sporulation rates in solid and liquid media, and to identify the metabolites produced by the fungi after fermentation using GC-MS. Fungal species used in this study were from the culture collection maintained at the Bicol University College of Science as an output of a previous study conducted by Guerrero and Alfante in 2013. Same species are available at the University of the Philippines Los Baños Museum of Natural History.

Materials and Methods

Fungal Species

Fifteen species of filamentous multicellular fungi were used for preliminary testing and further trimmed down to only those with positive results in the preliminary assays: *Aspergillus parasiticus*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus oryzae*, *Aspergillus fumigatus*, *Aspergillus terreus*, *Aspergillus nidulans*, *Aspergillus tamarii*, *Penicillium glabrum*, *Penicillium variabile*, *Penicillium funiculosum*, *Penicillium rugulosum*, *Fusarium acuminatum*, *Trichoderma sp.*, and *Trichoderma viridae*. All fungal species were maintained on Potato Dextrose Agar (PDA) slants at 24–27 °C until further use.

Antimicrobial Assay

All 15 species were tested for their antimicrobial property following the agar plug method against the following test organisms obtained from the Philippine National Collection of Microorganisms (PNCM) at BIOTECH, University of the Philippines Los Baños: *Escherichia coli* BIOTECH 1825 (gram-negative rod), *Staphylococcus aureus* BIOTECH 1823 (gram-positive coccus), and *Bacillus cereus* BIOTECH 1509 (gram-positive rod). Antimicrobial activities were assessed based on the diameter of the zones of inhibition (ZOI) and classified as very active (>19 mm), active (14–19 mm), partially active (10–13 mm), and inactive (<10 mm) (Torres & dela Cruz, 2015). Fungi that gave active antimicrobial property were further grown on different media for secondary metabolites extraction.

Solid Media Culture

Subsequent cultures were grown on sets of three different solid culture media: Yeast Extract Sucrose Agar (YES), Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA). Diameter of radial growth and time of sporulation over a period of seven days were used to get the three best fungal cultures, which were then cultured in the liquid form of the same culture media.

Fermentation and Extraction of Crude Metabolites

Three fungal species were selected based on the rate of sporulation. Flasks containing 100 mL broth were inoculated with eight-day-old spore suspension (approximately 150 spore/mL) of the selected fungi. These were cultured in Yeast Extract Sucrose Broth (YESB), Potato Dextrose Broth (PDB), and Sabouraud

BU R&D Journal, Vol. 21, December 2018
ISSN (Print): 0016-4139
journal.bicol-u.edu.ph

Dextrose Broth (SDB), and allowed to grow for 35 days in room temperature (24–27 °C) at stationary condition. Mycelial mass was separated from the broth using Whatman filter paper no. 1. Weight of mycelia was used to determine which among the fungi were rapidly growing in the culture broth.

Culture broth with formed mycelial mat was mixed with ethyl acetate (1:1 v/v). The broth was mixed for two hours at 100 rpm and then left to soak for 24 hours. Ethyl acetate extract was separated from the broth using separatory funnel. The organic layer was then concentrated *in vacuo* and the resulting mixture was dried overnight. Dried extracts were used for herbicidal assay.

Herbicidal Activity

Germination setup was done using *Vigna radiata* seeds following the procedure of Kaveriammal and co-workers (2013) with some modifications. Commercially obtained seeds of *Vigna radiata* were washed thoroughly with distilled water and placed individually in wells layered with small filter paper. Each treatment was replicated five times, with four subreplicates each, for a total of 20 seeds per treatment. On the first day, each well was administered with 0.5 mL of extracts or water for control. Thereafter, seeds were given 0.5 mL of extracts every other day until day 10. Germination was recorded every day for ten days. Germination referred to the initial appearance of the radical by visual observation. It was calculated using the following formula (Carley & Watson, 1968):

$$germination\ percentage = \frac{(number\ of\ seeds\ germinate)}{(total\ number\ of\ seeds\ sown)} x\ 100$$

A separate setup was done using two-day pre-germinated *Vigna radiata* seeds. Ten seeds were individually placed in wells layered with moistened filter paper. Each seed received 0.5 mL of extracts daily for 10 days. Total length of seedling was measured daily and compared with the control treatment.

Gas Chromatography-Mass Spectrometry (Gc-Ms) Determination of Compounds from the Crude Fungal Extracts

The spectrometric analysis of the extracts was carried out using Schimadzu GCMS-QP 2010 Ultra at the Regional Center for Food Safety and Quality Assurance at Bicol University. Injection volume was 1 µL in 5 percent phenyl 95 percent dimethyl polysiloxane (30 m x 0.25 mm x 0.25 µm) column in a split mode. The oven temperature program was initially at 80 °C for 1 min at 8 °C/min then programmed to 110–240 °C at 10 °C/min, then to 240–280 °C at 12 °C/min, then, lastly at 20 min hold at 280 °C. Helium was the carrier gas at a flow rate of 1 mL/min. Injector temperature was at 240 °C. Mass spectra in the EI mode (70eV)were scanned from 40–500 *m/z* at a scan speed of 0.5 s. Data were analyzed using GCMS Labsolutions software and the mass spectra of compounds detected were compared with the library of the National Institute of Standard and Technology (NIST, USA/Wiley).

Statistical Analysis

Analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT) were used to statistically determine significant differences among treatments and against control groups. All values were written plus or minus the Standard Error of Mean (±SEM). *p* values of 0.05 or less were considered statistically significant.

Results and Discussion

The development of drugs from any microbial source starts from a preliminary assessment of the species' potential to produce bioactive compounds, either from a natural metabolic pathway or when induced to produce such by altering culture conditions. The advantage of using microbes, including fungal species, is the relative ease in handling and growing, as well as the minimal use of space and its ability to be manipulated to produce desired products. Eight out of 10 drugs developed in the past decade have fungal origin. Although it takes a big investment in terms of research and development, this figure is quite telling of how much can be mined from a plethora of fungal species in the ecosystem. Newman and Cragg (2012) highlighted the rapidly evolving recognition that many natural product drugs are of microbial origin and produced through microbial interactions with the host from where they were isolated. The discovery of penicillin from the fungi *Penicillium notatum* in 1928 was considered a breakthrough in antibiotic research which led to the first completed chemical synthesis of penicillin in 1957. The untapped biodiversity of fungi from various habitats can be equated to possible sources of novel metabolite-producing strains as well as novel compounds and structural leads.

Antimicrobial Assay

Among the 15 soil fungal isolates, seven showed antimicrobial potential against *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus* (Table 1). No *Aspergillus* species, with the exception of *A. tamarii*, showed any antimicrobial activity. *Aspergillus tamarii* was able to show partial activity against all three test organisms. Members of the *Penicillium* genus, with the exception of *P. rugulosum*, showed partial to moderate activity. *P. glabrum* was able to show partial activity against the gram-negative *E. coli* while *P. variabile* was able to show partial activity to both the Gram-positive test organisms. *P. funiculosum* was able show antimicrobial activity against all three test organisms, partially to both *E. coli* and *S. aureus* while moderate activity against *B. cereus*. Both *Trichoderma* species were partially active against the Gram-positive test organisms.

The production of antimicrobial compounds from microorganisms is strain-dependent and could be influenced by the cultivation conditions such as the culture media and nutrient supplements (Noaman *et al*., 2004). Further, the absence of antimicrobial activity could be also influenced by the ability of the secreted compound to diffuse into the culture medium to effect detectable zones of inhibition that would signify death of the test organisms.

Sporulation on Solid Media

Sporulation of the seven species of fungi with positive antimicrobial activities was observed over a period of seven days. Based on the sporulation, *Penicillium funiculosum* produced spores on day 3, *Aspergillus tamarii* on day 5, and *Trichoderma* sp. on day 6. All other species did not exhibit sporulation within the time frame.

The time it took the fungal species to produce additional mycelia and the consequent appearance of spores was an important basis for selection of which to culture in the liquid media. The sporulation process in microorganisms including fungi is correlated with the production of secondary metabolites, which may accumulate in the growth media. These metabolites can be those that activate sporulation, pigment production, or secretion of toxins (Calvo *et al*., 2002). The use of eight-day spore suspension ensures that the genetic requirements and biochemical pathways involved in the production of secondary metabolites are present in the selected fungal isolates. Furthermore, as the research was screening for the best species with time as a variable, the isolates *Penicillium funiculosum*, *Aspergillus tamarii*, and *Trichoderma* sp. were chosen to be cultured in liquid media.

Table 1. Antimicrobial activity of soil fungal isolates against test organisms through agar plug method

Fungi	Test Organisms		
	Escherichia coli	*Staphylococcus aureus*	*Bacillus cereus*
Aspergillus parasiticus	-	-	-
Aspergillus flavus	-	-	-
Aspergillus niger	-	-	-
Aspergillus oryzae	-	-	-
Aspergillus fumigatus	-	-	-
Aspergillus terreus	-	-	-
Aspergillus nidulans	-	-	-
Aspegillus tamarii	+	+	+
Penicillium glabrum	+	-	-
Penicillium variabile	-	+	+
Penicillium funiculosum	+	+	++
Penicillium rugulosum	-	-	-
Fusarium acuminatum	+	+	-
Trichoderma sp.	-	+	+
Trichoderma viridae	-	+	+

(-) – no activity (+) – partially active (++) – active

BU R&D Journal, Vol. 21, December 2018
ISSN (Print): 0016-4139
journal.bicol-u.edu.ph

Mycelial Growth on Liquid Media

After 35 days of stationary growth at room temperature, fungal growth in the three liquid media was measured by weighing the filtered mycelia. The mycelial weight of the fungi grown in SDB was significantly greater than those grown in PDB and YES (see Figure 1). This observation is similar to the findings in the study by Gebala and Sandle (2015) wherein SDB performed better in detecting and supporting the growth of fungi and yeasts as compared to PDA and Malt Extract Agar. Ikechi-Nwoga and Elenwo (2012) also noted that least growth was observed in PDA in the cultivation of filamentous fungi from foods as compared to other culture media, with soybean dextrose broth being the optimal media. Furthermore, they hypothesized that the soybean extract in the soybean dextrose broth provided more vitamins and minerals, which supported the proliferation of the fungi. The effects on growth of the components of the culture media used was not investigated in this current study, so it can only be assumed that the peptone in SDA provided more nitrogen and nutrients that favored the growth of fungi than just the potato infusion in PDA

Aspergillus tamarii was statistically consistent in terms of growth among the three media, although *P. funiculosum* and *Trichoderma sp.* far outgrew it in SDB. Statistically, all fungi had comparable mycelial mass in both PDB and YESB.

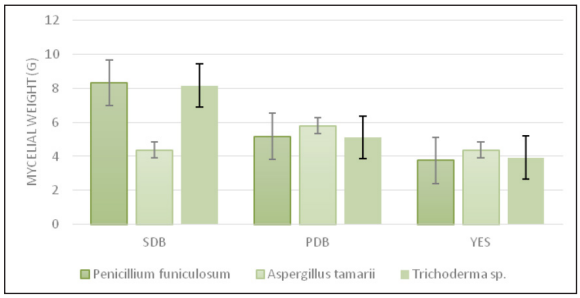

Figure 1. Mycelial Weight (in grams ± SEM) of *Penicillium funiculosum*, *Aspergillus tamarii,* and *Trichoderma sp*. grown in Sabouraud Dextrose Broth (SDB), Potato Dextrose Broth (PDB), and Yeast Extract Sucrose Broth (YESB) for 35 days

Herbicidal Activity

Only three isolates (*P. funiculosum*, *Trichoderma* sp., and *A. tamarii)*, which exhibited partially active to active antimicrobial activities, were chosen for the herbicidal activity since there is a reported good correlation between antimicrobial actions and herbicidal actions of certain naturally-derived drugs (Kos et al., 2013). This correlation is believed to be due to their overlapping effects against molecular targets or processes and these observations led to the discovery of the antibiotics Herbicidins A & B, as well as phosalacine (Arai et al., 1976; Omura et al., 1984).

Secondary metabolites extracted from the culture filtrates did not exhibit any significant inhibitory

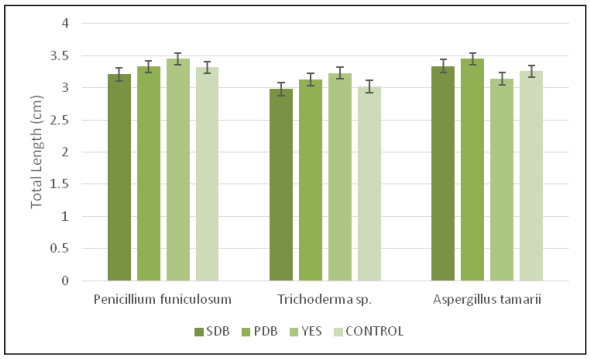

Figure 2. Mean Total Length (in cm ± SEM) of *Vigna radiata* seedlings administered with extracts from fungi grown on three different liquid media with water as control showed no herbicidal activity

effects on seed germination (p <0.05) (Figure 2). The germination rate of all treatments was 90–98 percent. In another setup using pre-germinated *Vigna radiata* seeds, extracts did not show any significant effects on further growth when applied to the seeds for seven days. Mean Total Length among treatments were comparable to the control group given water only.

While no herbicidal activity was observed for the fungal extracts, it was seen that the seedlings treated with fungal extracts were longer than those administered with water only, although the values are not statistically significant. There is a possibility then that the fungi promoted growth, instead of death, of the seedlings. The ability of some fungal strains to promote the growth of plants has already been investigated by several authors. One important role of PGPF is the secretion of organic and inorganic acids that solubilize partially soluble phosphates, making this nutrient available to plants (Whitelaw, 1999).

Gc-Ms Metabolomics Data

Among the three species that showed potential for biological activities, only *A. tamarii* and *Trichoderma* sp. were further analyzed for metabolite identification

using GC-MS because *P. funiculosum* is already a well-studied organism. *Aspergillus tamarii* in this study is also interesting as it is the only one among the *Aspergillus* species used in this study with activity against the test bacteria in the agar plug assay. *Trichoderma* sp. is also interesting because while it has the same antimicrobial profile as that of *T. viridae* in the agar plug assay, the possibility that the former is a new strain offers novelty to this isolate. Further, only extracts from PDB and SDB were used for GC-MS since these two culture media supported the good growth of the fungi as shown previously in Figure 1.

Analysis of the prominent peaks from GC-MS outputs showed that 19 and 17 compounds were detected from *A. tamari* grown in PDB and SDB, respectively, while 23 compounds were seen for *Trichoderma* in PDB and 17 from *Trichoderma* in SDB. While the composition of the four crude extracts in terms of the type of compounds were almost similar, the intensity of the peaks differed indicating the difference in the quantity of the compounds (see Figure 3). Although many compounds were detected, this study focused only on the compounds with high similarity in the database (>90 percent; see Table 2) since no chemical standard was used to verify the identity of the compounds particularly those with low similarity indices (<90 percent).

The most abundant and common compounds seen in the extracts is bis (2-ethyl hexyl) phthalate. Dehydromevalonic lactone was detected in extracts of *Aspergillus tamarii* in both SDB and PDB and in *Trichoderma* sp in PDB. The differences in the quantity and the presence or absence of some compounds can be attributed not just in the biology of the producing organism but also on the type of culture media to which the organisms were grown. Generally, greater intensities of compounds were observed for cultures grown in PDB than in SDB. In the investigation of the effect of culture media on growth and pigment production of *Fusarium monoliforme* KUMBF1201, for example, PDA supported the best growth and pigment production of this species among the eight culture media used (Pradeep & Begam, 2013).

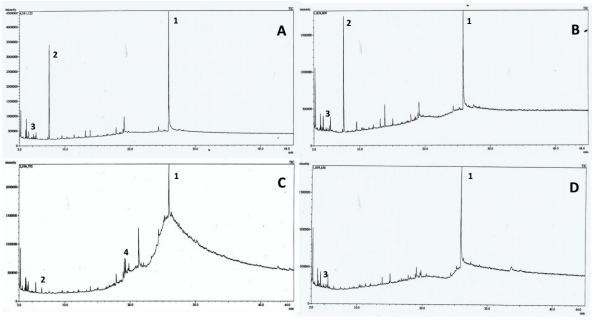

Figure 3. GC-MS chromatogram of ethyl acetate extracts of fungi cultivated for 35 days. A- *Aspergillus tamarii* in PDB; B- *A. tamarii* in SDB; C- *Trichoderma* sp. in PDB; D- *Trichoderma* sp. in SDB. Peaks: 1- Bis (2-ethyl hexyl) phthalate; 2- Dehydromevalonic lactone; 3- benzeneacetaldehyde; 4- *n*-hexadecanoic acid.

Bis (2-ethyl hexyl) phthalate, the most common and most abundant compound detected in the four culture extracts, is known to possess antimicrobial activities (Al-Bari *et al*., 2005). A marine fungus, *Cladosporium* sp. F14, was seen to produce the same metabolite that effectively inhibited larval settlement of *Bugula neritina* and *B. amphitrite* larvae, which designates its potential to be an antifouling compound (Shu-Hua *et al*., 2009). This compound is also known as Di-(2-ethylhexyl) phthalate (DEHP). *Aspergillus awamori* isolated from the Nile River was reportedly capable of inhibiting the growth of *Candida albicans* and *Sarcian lutea* and was also cytotoxic against some carcinoma cell lines (Lotfya *et al*., 2018).

Dehydromevalonic lactone was detected from the extracts of *A. tamarii* in PDB and SDB, as well as *Trichoderma* sp. grown in PDB. This compound was also

Table 2. Common compounds in the filtrates of Aspergillus tamarii and Trichoderma sp. after 35 days of growth as detected by Gas Chromatography-Mass Spectrophotometry (GC-MS) and compared with NIST library

Peak	Retention Time (min)	Similarity (%) in NIST database	Molecular weight	Formula	Putative identity
1	25.9	95	390	C24H38O4	Bis (2-ethyl hexyl) phthalate
2	7.9	93	112	C6H8O2	Dehydromevalonic lactone
3	5.1	93	120	C8H8O	Benzene acetaldehyde
4	19.3	90	256	C16H32O2	n-hexadecanoic acid

BU R&D Journal, Vol. 21, December 2018
ISSN (Print): 0016-4139
journal.bicol-u.edu.ph

reported from the fungi *Monascus ruber* (Vidyalakshmi *et al*., 2009) and from an unidentified fungal endophyte of ginger (Anisha & Radhakrishnan, 2017). This volatile compound is known to impart fragrance and has been isolated from the Soxhlet extracts of commercial cocoa powder (Krings *et al*., 2006). The presence of this compound in the extracts could make *Aspergillus tamari* and *Trichoderma sp*. in this study as likely sources of natural flavor and fragrance enhancers for food, feed, cosmetic, and pharmaceutical products. In fact, some fungi and their enzymes are utilized for their *de novo* production of odorous compounds. For example, *Trichoderma viridae* produces the coconut-flavored lactone, 6-pentyl-α-pyrone while *Kluyveromyces lactis* is also capable of producing fragrant terpenes, such as citronellol, linalool, and geraniol (Vandamme, 2003).

The volatile compound benzene acetaldehyde was detected in the culture extracts of *A. tamarii* in PDB and SDB, as well as *Trichoderma* sp. grown in SDB. This compound, commonly found in fragrant plants, was extracted from a strain of *Penicillium roqueforti* by Jelen (2003). This compound is also known to play a part in maggot therapy and production of penicillin G (Yu-Jing, 2010).

The presence of *n*-hexadecanoic acid (palmitic acid) was also detected only in the extracts of *Trichoderma* sp. grown in PDB. Commonly known as palmitic acid, hexadecanoic acid is one of the most common saturated fatty acid found in animals, plants, and microorganisms. A structural and kinetics study of *n*-hexadecanoic acid showed its anti-inflammatory property, particularly by competitive inhibition of phospholipase A2 (Vasudevan *et al*., 2012).

Acids, alcohols, and methyl esters were also detected in the metabolites but in lower amounts. The presence of acids in particular indicates the possibility of the assumption made for the plant-growth promoting activities of *A. tamari* and *Trichoderma* sp. since *de novo* production of acids could help in the solubilization and bioavailability of phosphates in the soil (Whitelaw, 1999).

While it was noted that there are differences in the presence and concentration of compounds detected among the three species grown in the two media as gleaned from the GC-MS spectra, no further correlation on the media components affecting the production of the compounds can be made since media optimization was not performed in this study.

While fungi are known as prolific producers of bioactive metabolites, particularly compounds with antimicrobial activities, the absence of or poor antimicrobial activities of the isolates used in this study does not necessarily mean that they do not have the ability to produce such. As seen in the GC-MS profile of metabolites, there is the presence particularly of Bis (2-ethyl hexyl) phthalate, which is a known antimicrobial compound. One factor that could be attributed to this is the low concentration of compounds produced, which is not enough to cause significant lethal effects on the test organisms.

Variation in culture conditions, such as pH, temperature, and the use of chemical inducers, could be further investigated to allow the production of more bioactive compounds or to increase the production of these compounds, some of which may even be novel. This One Strain Many Compounds (OSMAC) approach have been advocated by many scientists in search of novel compounds (Reen et al., 2015). In fact, many low-intensity peaks are seen in the GC-MS chromatogram (Figure 3), which could indicate that many compounds have been produced but in low amounts not enough to warrant a valid identification based on the database. Further studies that include the optimization of fermentation conditions and culture media, as well as the use of conventional culture-dependent approaches like bioassay guided-fractionation of metabolites coupled with genomics studies could elucidate and harness the potentials of these locally-isolated fungi for biotechnological applications, including the development of naturally-occurring food additives.

Conclusion and Recommendation

The profile of metabolites of *Aspergillus tamarii* and *Trichoderma* sp., as revealed in the GC-MS analysis of crude ethyl acetate extracts of the culture broths, showed promising potentials of these fungi for biotechnological applications including pharmaceuticals, agriculture, cosmetics, and food flavor enhancement even if there is low in vitro antimicrobial and herbicidal activities seen. Considering that these fungi were isolated from rice paddies, the possibility that they possess plant-growth promoting abilities as shown in the seed germination assay is not remote. Of particular interest is the presence of dehydromevalonic lactone and benzeneacetaldehyde, which are known fragrant compounds that can be produced and used as flavor

enhancers to address the current dependence of the food and cosmetics industries on synthetic flavor and fragrance enhancers. This study would serve as a basis for optimization studies involving fungi making use of the current culture collection available in Bicol University. Moreover, this study also highlights the empirical need to protect our vast ecosystems, which holds untapped biodiversity of ecological and industrial importance.

Acknowledgement

The researchers are grateful for the funding from the Bicol University Research and Management Development Division and Bicol University College of Science, through Dr. Lucy P. Estioko.

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BU R&D Journal, Vol. 21, December 2018
ISSN (Print): 0016-4139
journal.bicol-u.edu.ph

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