Manila Journal of Science ***13*** *(2020), pp. 74–88*

Preliminary Bioactivity Screening of Crude Extracts of Six Wild Macrofungi From Pine Forests in Benguet and Mt. Province, Philippines

Audrey Glenn L. Culliao,[1,*] Rhoda S. Lumang-ay,[2] Gloria Meryl T. Kingat,[3] Tomasa P. Colallad,[3] and Maria Victoria P. Canaria[2]

[1]Department of Medical Laboratory Science, School of Natural Science, Saint Louis University, Baguio City, Philippines
[2]Department of Pharmacy, School of Natural Science, Saint Louis University, Baguio City, Philippines
[3]Department of Biology, School of Natural Science, Saint Louis University, Baguio City, Philippines

*E-mail: aglculliao@slu.edu.ph

ABSTRACT

This study investigated the pharmacological significance of wild macrofungi collected from pine forests in Benguet and Mt. Province, Philippines, to help address a number of health-related conditions, such as oxidative stress, thrombosis, and bacterial infections. Six wild macrofungi were subjected to chloroform and ethanol extraction, and their crude extracts were screened for their total phenolic content (TPC), antioxidant, lethality, thrombolytic, and antibacterial activities. The ethanol extract of *Daedaleopsis confragosa* has the highest TPC at 49.28 ± 0.30 µg gallic acid equivalent (GAE)/mg extract and percent free radical inhibition activity at 74.59 ± 0.11%, which was comparable to the pure compound quercetin at 74.33 ± 0.32%. On the other hand, the ethanol extracts of *Scleroderma citrinum* and *Postia fragilis* have the most potent median effective concentration (EC_{50}) at 431.01 ± 17.82 and 469.63 ± 15.25 µg/mL. Only the ethanol extract of *Daedaleopsis confragosa* exhibited low toxicity (median lethal concentration (LC_{50}) = 565.90 µg/mL) while the rest of the test extracts are not toxic. Both chloroform and ethanol extracts of *Termitomyces eurrhizus* yielded the highest percent clot reduction values at 35.19 ± 0.13% and 32.41 ± 0.17%, respectively. This is the first study to report the thrombolytic activity of macrofungi extracts from the Philippines. The ethanol extract of *Lenzites betulina* gave the highest zone of inhibition (ZOI) against *Staphylococcus aureus* American Type Culture Collection (ATCC) 25923 (13.33 ± 0.58 mm) while the chloroform extract of *Daedaleopsis confragosa* gave the highest ZOI against *Escherichia coli* ATCC 25922 (12.33 ± 1.15 mm). These data indicated that the wild macrofungi tested could constitute a potential source of natural bioactive compounds in the production of pharmaceutical dosage forms or nutraceuticals.

Keywords: macrofungi, total phenolic content, antioxidant, lethality, thrombolytic, antibacterial, Philippines

INTRODUCTION

Bioactivity screening studies remain to be a fertile area for researches largely because of the ever-increasing demand for novel, potent, and safe drugs (Griffin, 2009). These studies that focus on the effects of an agent upon a living system are being done to address human health-related concerns such as cancer, cardiovascular diseases, and bacterial infections, which are among the top leading causes of morbidity and mortality worldwide (World Health Organization, 2019). To help address these diseases and their associated burdens, bioactivities that include antioxidant, lethality, thrombolytic, and antibacterial actions are being actively investigated from various natural sources (Barros et al., 2008; Keleş et al., 2011; Obodai et al., 2014; Ogidi & Oyetayo, 2016; Okhuoya et al., 2010; Subbulakshmi & Kannan, 2016; Toledo et al., 2016). Aside from the high demand for such agents, the continuous search for new drug sources is also being driven by the development of adverse effects brought about by the use of currently available drugs and the emergence of bacterial resistance to commonly used antibacterial agents, among others.

Bioactive compounds that are naturally occurring in living systems have been widely targeted as primary sources of diverse molecules in several drug discovery studies (Liu et al., 2017; Wong et al., 2013), and in several instances, the plant or fungal sources from where they are isolated have been incorporated in numerous herbal supplements and health foods (Cheung, 2013). The search for new sources of bioactive compounds has been directed towards screening for the bioactivities of phenolic compounds naturally occurring in macrofungi (Kouassi et al., 2016).

Macrofungi are a large group of fungal species that produce fruiting bodies that can be readily observed with the naked eye (Kirk et al., 2008). Macrofungal fruiting bodies are considered as significant nontimber forest products (Berch et al., 2007), particularly as important sources of food and medicine for humans. Fungal specimens collected from forests have been shown to demonstrate a myriad of biological activities (Ajith & Janardhanan, 2007; Wasser & Weis, 1999) and are being consumed as nutraceuticals (Ao et al., 2016). Globally, *Agaricus bisporus*, *Lentinus edodes*, *Pleurotus* spp., and *Flammulina velutipes* are the leading macrofungi being marketed as nutraceutical foods. *Ganoderma lucidum* and other *Ganoderma* spp., on the other hand, have commercially available preparations as an anticancer agent (Valverde et al., 2015). Some immunomodulating pharmaceuticals contain ß-glucans from macrofungi such as ganoderan from *Ganoderma lucidum*, grifolan from *Grifola fondosa*, and lentinan from *Lentinus edodes* (Ho et al., 2020). These applications all point to the potential of macrofungi as sources of bioactive agents.

However, there is a noted dearth in current literature on natural product researches involving Philippine macrofungi. Some of the recent research efforts in this field include one that screened for the antistaphylococcal and antioxidant properties of crude ethanolic extracts from wild macrofungi collected from Bataan Province (Gaylan et al., 2018). Ragasa (2018) reviewed and identified the chemical constituents of the dichloromethane extracts of commercially grown and wild Philippine macrofungi that may contribute to their anticancer activities. Aside from these, ongoing researches on bioactive agents from macrofungi in the Philippines are rather few and limited.

In Luzon in the Philippines, a continuous mountain range formed between the southwestern areas of Mt. Province and the northeastern areas of Benguet covers

some dense sections of pine forest land and is home to biodiverse macrofungi with unexplored or underexplored potential biological activities. Macrofungal species in these areas are typically being collected for consumption as food (Licyayo, 2018) but rarely or not at all for their biological activities. This study thus aimed to screen wild macrofungi for their phenolic contents and their potential antioxidant, lethality, thrombolytic, and antimicrobial activities as these may provide baseline data for the production of fungal natural products or as processed pharmaceutical and nutraceutical products.

MATERIALS AND METHODS

Wild Macrofungi Collection and Identification

Convenience wild macrofungi sampling from several pine forested collection sites in the municipalities of Bauko, Mt. Province, and Buguias and Mankayan, Benguet Province, was conducted during the months of September and October 2019 after being granted permission to do so by each of the local government units involved. The collection sites were at the following positions: 16°51.1660'N, 120°51.7750'E, 2250.8 masl; 16°51.2040'N, 120°7500'E, 2282.7 masl; 16°51.6690'N, 120°52.4320'E, 2211.1 masl; 16°51.6730'N, 120°52.4290'E, 2204.4 masl; 16°50.3740'N, 120°49.1730'E, 1739.8 masl; 16°50.3630'N, 120°49.1740'E, 1755.3 masl; 16°50.3110'N, 120°49.1760'E, 1761.3 masl; 16°51.0420'N, 120°47.2380'E, 1298.6 masl; and 16°51.0560'N, 120°47.2450'E, 1321.9 masl. Whole macrofungal specimens were taken, wrapped in paper bags, properly labelled, and transported to the Fr. Braeckman Museum of Natural History of the School of Natural Sciences, Saint Louis University, Baguio City, where identification and preservation of specimens were done. Identification of the macrofungal specimens was based on morphological characteristics as previously described (Ginns, 2017; Ginns, 2019; Gonthier & Nicolotti, 2007; Mortimer et al., 2014; Ostry et al., 2011). The following applicable characteristics were utilized in morphological characterization: pileus size, pileus shape, pileus color, pileus surface features, pileus margin, gill attachment, gill breadth, stipe location, stipe size, stipe shape, stipe color, and stipe surface features.

Six macrofungal species were selected and screened for their bioactivities, based on their abundance in all the collection sites, and include the following: *Albatrellus ellisii*, *Daedaleopsis confragosa*, *Lenzites betulina*, *Postia fragilis*, *Scleroderma citrinum*, and *Termitomyces eurrhizus*. Abundance of samples was operationalized to mean a minimum of 100 g of fresh mass collected from the identified sites. Other species present in the collection sites that were less than the cutoff mass were also collected but were excluded in the bioactivity screening assays.

Extract Preparation

All specimens were oven dried until there are no significant changes in their pre- and postdrying weights and then were cut into thin sections. Fifty-gram portions of each specimen were then exhaustively extracted via maceration in separate 50-mL portions of chloroform and 95% v/v ethanol with a 48-hr interval per extraction until no color was imparted unto the extracting solvent. All extracts were then pooled and filtered, and the extracting solvents were evaporated off in a water bath maintained at 40°C (Guevara, 2004). The crude extracts were utilized as is in the determination of antibacterial activity. For the determination of total phenolic content, antioxidant activity, extract lethality, and thrombolytic activity, the crude extracts were serially diluted with sterile distilled water to prepare the following working

solutions: 1000, 500, 250, 125, and 62.5 µg/mL.

Determination of Total Phenolic Content (TPC)

The TPC of the chloroform and ethanol extracts of the specimens was determined following a previously described procedure (Waterhouse, 2002) with modifications. Briefly, 1.0 mL of the diluted specimen extract (1000 µg/mL) was transferred in triplicate to separate tubes containing 5.0 mL of a 1/10 dilution of Folin–Ciocalteu reagent (Sigma-Aldrich, USA) in distilled water. Then, 4.0 mL of 7.5% w/v sodium carbonate solution was added. The tubes were then allowed to stand at room temperature for 60 min before absorbance at 765 nm was measured (Milton Roy, Spectronic 20D+) against reagent blank. Gallic acid (Sigma-Aldrich, USA) was used as standard, and the TPC of the specimens was expressed as gallic acid equivalent (GAE) in micrograms per milligram extract. The concentration of the phenolics in the specimens was derived from a standard curve of gallic acid at concentrations ranging from 3.906 to 62.5 µg/mL ($r^2 = 0.9952$).

Determination of Antioxidant Activity

The antioxidant activity of the different specimen chloroform and ethanol extracts was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH; Sigma-Aldrich, USA) assay as previously described (Molyneux, 2004). Two milliliters of each of the prepared dilutions was aliquoted into separate tubes containing 2 mL of 30-µM DPPH solution. The tubes were then incubated at 37°C for 30 min, and after incubation, the absorbance of each tube's contents was measured spectrophotometrically (Milton Roy, Spectronic 20D+) at 517 nm. All setups were done in triplicate. Control tubes containing all the reagents except the test solutions were also read. Quercetin (Sigma-Aldrich, USA) was used as positive control. Median effective concentration (EC_{50}) expressed in micrograms per milliliter of the extract was computed by linear regression using the calibration of the positive control. Inhibition of the free radical in percent was calculated using the following formula:

$$\%\ Free\ radical\ inhibition = \left(\frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absrobance\ of\ control}\right) \text{x } 100$$

Determination of Extract Lethality

The evaluation of lethality of the extracts was carried out using the methods of Meyer et al. (1982) with modifications. Brine shrimp (*Artemia salina*) cysts were hatched overnight in a 1000-mL beaker containing artificial seawater (ASW), prepared by dissolving 38 g of sodium chloride in 1 L of distilled water, at 37°C with continuous aeration and illumination. The newly emerged nauplii were then collected by pipetting, counted into sets of 10, and placed in separate wells of 24-well plates. Two milliliters of the prepared dilutions of the specimen extracts was then dispensed into each well and was properly labelled. Two-percent v/v ethanol and doxorubicin in ASW were used as positive controls, and ASW was set as the negative control. Three replicate wells were prepared for each extract concentrations and controls. After dispensing the appropriate solutions, the plates were then allowed to stand at room temperature for 24 hr. After standing, the number of dead nauplii in each well was counted and recorded. The median lethal concentration (LC_{50}) in micrograms per milliliter of the extract of each extract was then determined using probit analysis and

was interpreted following Clarkson's toxicity criterion (Clarkson et al., 2004).

Determination of Thrombolytic Activity

The thrombolytic assay of Prasad et al. (2006) was adapted with modifications in order to determine the thrombolytic activity of the specimen chloroform and ethanol dilutions. The protocol was reviewed and approved by the Saint Louis University Research Ethics Committee under protocol number SLU-REC 2019-060. Five hundred microliters of venous blood drawn from healthy and consenting volunteers was dispensed in a preweighed sterile microcentrifuge tube and incubated at 37°C for 45 min. After clot formation, the tube was spun down in a microcentrifuge for 1 min, and the serum was removed by pipetting without disturbing the clot. A tube was prepared for each dilution of the test specimen extracts, and all setups were done in triplicate. Each tube containing clots was then weighed, and the clot weight was obtained per tube. Then, 100 µL of each test dilution was added into each tube and was properly labelled. Reformulated streptokinase (Britton Streptokinase, Dae Han New Pharm) following the drug manufacturer's instructions was utilized as the positive control while distilled water served as the negative control. All tubes were incubated at 37°C for 90 min and spun down for 1 min, and all fluid released was pipetted off. The tubes were then reweighed. The difference obtained in weight taken before and after clot disruption was recorded. Thrombolytic activity was expressed as percent clot reduction using the following formula:

$$\%\ Clot\ reduction = \left(\frac{Clot\ (g)\ before\ treatment\ -\ Clot\ (g)\ after\ treatment}{Clot\ (g) before\ treatment}\right) \text{x } 100$$

Determination of Antibacterial Activity

Antibacterial activity of the specimen crude chloroform and ethanol extracts was performed by Kirby–Bauer disk diffusion technique according to the criteria set by the Clinical and Laboratory Standards Institute (CLSI; 2017). Inoculums from overnight cultures of *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 in tryptic soy broth (HiMedia, India) were suspended in sterile saline solution, and the density was adjusted by comparison with opacity standard on 0.5 McFarland solution. The test organisms were uniformly seeded over Mueller–Hinton agar (MHA; HiMedia, India) plates using a sterile cotton swab. The test crude extracts were impregnated into 6-mm-diameter filter paper discs and were directly applied onto the surface of the inoculated MHA plates. Cefaclor (30 µg; LiofilChem, Italy) and oxacillin (1 µg; LiofilChem, Italy) prepared discs were used as positive controls. All setups were prepared in triplicates. The plates were then incubated at 37°C for 24 hr. Diameters of the zone of inhibition around the discs were measured to the nearest millimeter using a ruler and interpreted as follows: less than 10 mm—inactive, 10–13 mm—partially active, 14–19 mm—active, and greater than 19 mm—very active (Guevara, 2004).

Data Treatment

Obtained data in each of the assays were tabulated and summarized in Microsoft Excel® 2013. Differences in measured bioactivities of the different macrofungal specimens were determined through analysis of variance followed by the Tukey post hoc test in SPSS® version 23. Probit analysis in the determination of the LC_{50} of the extracts was also carried out in SPSS® version 23.

RESULTS AND DISCUSSION

The wild macrofungi specimens that were selected based on their abundance in all the collection sites in pine forests in Benguet and Mt. Province, Philippines, were morphologically characterized and identified to be *Albatrellus ellisii* (Berk.) Pouzar [Russulales, Albatrellaceae], *Daedaleopsis confragosa* (Bolton) J. Schröt [Polyporales, Polyporaceae], *Lenzites betulina* (L.) Fr. [Polyporales, Polyporaceae], *Postia fragilis* (Fr.) Julich [Polyporales, Fomitopsidaceae], *Scleroderma citrinum* Pers. [Boletales, Sclerodermataceae], and *Termitomyces eurrhizus* (Berk.) R. Heim [Agaricales, Lyophyllaceae]. The noted morphological characteristics of the studied macrofungi are summarized in Table 1. Figure 1 shows some samples of the studied macrofungi.

Table 1. Morphological Features of Collected Wild Macrofungi From Pine Forests in Benguet and Mt. Province, Philippines

Species	Characteristics
Albatrellus ellisii	**Pileus size:** 20–160 mm; **pileus shape:** petaloid to spathulate; **pileus color:** mustard yellow to dark brown with characteristic green tinge upon bruising; **pileus surface features:** squamulose, rough scales; **pileus margin:** entire and some tuberculate; **gill attachment:** N/A; **gill breadth:** N/A; **stipe location:** eccentric to lateral; **stipe size:** 40–50 mm; **stipe shape:** cylindrical; **stipe color:** mustard yellow to dark brown; **stipe surface features:** squamulose
Daedaleopsis confragosa	**Pileus size:** 20–80 mm; **pileus shape:** applanate, dimidiate; **pileus color:** light to dark brown; **pileus surface features:** glabrous to velutinous, cork-like; **pileus margin:** entire; **gill attachment:** N/A; **gill breadth:** N/A; **stipe location:** sessile to reflexed; **stipe size:** N/A; **stipe shape:** N/A ; **stipe color:** dark brown; **stipe surface features:** velutinate
Lenzites betulina	**Pileus size:** 9–50 mm; **pileus shape:** applanate, flabelliform; **pileus color:** concentrically zonate, white to cream to grey; **pileus surface features:** tomentose to hispid, cork-like, tough; **pileus margin:** entire, even to regular; **gill attachment:** N/A; **gill breadth:** N/A; **stipe location:** sessile; **stipe size:** N/A; **stipe shape:** N/A; **stipe color:** N/A; **stipe surface features:** N/A
Postia fragilis	**Pileus size:** 20–50 mm; **pileus shape:** elongated, some dimidiate, imbricate habit; **pileus color:** white to cream to light yellow with characteristic red brown color upon bruising; **pileus surface features:**

	glabrous to tomentose; **pileus margin:** entire; **gill attachment:** N/A; **gill breadth:** N/A; **stipe location:** sessile; **stipe size:** N/A; **stipe shape:** N/A; **stipe color:** N/A; **stipe surface features:** N/A
Scleroderma citrinum	**(Pileus size) sporocarp:** 20–55 mm in diameter; **pileus shape:** N/A; (**pileus) peridium color:** brown to dark brown; (**pileus) peridium surface features:** squamulose with slight cracks; **pileus margin:** N/A; **gill attachment:** N/A; **gill breadth:** N/A; **stipe location:** sessile; **stipe size:** N/A; **stipe shape:** N/A; **stipe color:** N/A; **stipe surface features:** N/A
Termitomyces eurrhizus	**Pileus size:** 20–80 mm; **pileus shape:** applanate to umbonate; **pileus color:** light to dark brown; **pileus surface features:** pubescent; **pileus margin:** rimose; **gill attachment:** adnexed to free; **gill breadth:** narrow; **stipe location:** central; **stipe size:** 70–90 mm; **stipe shape:** cylindrical to clavate; **stipe color:** white to cream; **stipe surface features:** fibrillose

Note. N/A = not applicable.

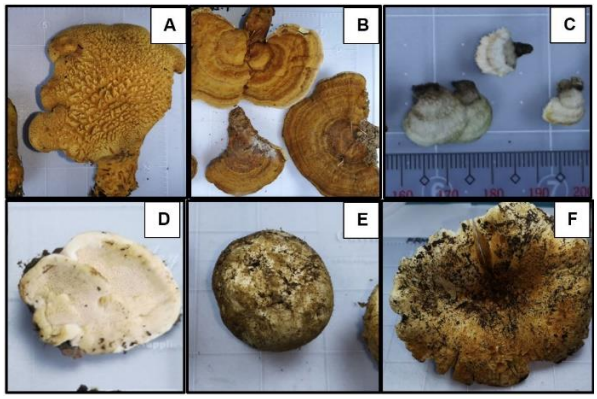

Figure 1. Macrofungi specimens collected and bioassayed: A = *Albatrellus ellisii*, B = *Daedaleopsis confragosa*, C = *Lenzites betulina*, D = *Postia fragilis*, E = *Scleroderma citrinum*, F = *Termitomyces eurrhizus*.

Chloroform and ethanol extracts of these macrofungi were prepared and screened for their total phenolic content (TPC) and bioactivities, particularly their antioxidant, lethality, thrombolytic, and antibacterial potentials.

The TPC of the macrofungal extracts and their antioxidant profile are shown in Table 2. *D. confragosa* ethanol extract (DCEE) has the highest TPC at 49.28 ± 0.30 µg GAE/mg extract followed by *S. citrinum* ethanol extract (SCEE) then *A. ellisii* ethanol extract (AEEE) at 33.54 ± 0.44 and 14.66 ± 0.21 µg GAE/mg extract, respectively, while *D. confragosa* chloroform extract (DCCE), *A. ellisii* chloroform extract (AECE), and *T. eurrhizus* ethanol extract (TEEE) had no detectable TPC as evidenced by absorbance readings that are less than 0. All of the test ethanol extracts have higher TPC than their chloroform extract counterparts for the same macrofungal specimen except for *T. eurrhizus*. Antioxidant profile was characterized by computing for percent free radical inhibition activity at a fixed concentration of 1000 µg/mL extract and median effective concentration (EC_{50}) in micrograms per milliliter. DCEE also has the highest percent free radical inhibition activity at 74.59 ± 0.11%, matching its highest measured TPC from among the test extracts, followed by *P. fragilis* ethanol extract (PFEE) at 61.45 ± 0.44% then by *L. betulina* ethanol extract (LBEE) at 52.55 ± 0.36%, although these two next values seem to be independent of their TPC. When compared to the percent free radical inhibition of the positive control quercetin, DCEE is comparable to that of quercetin at 74.33 ± 0.32%. SCEE and PFEE have the lowest EC_{50} at 431.01 ± 17.82 and 469.63 ± 16.25 µg/mL, which do not

significantly differ ($p > 0.05$), followed by DCEE at 557.74 ± 8.86 µg/mL, which is also comparable to that of PFEE ($p > 0.05$). When compared to the positive control, quercetin is significantly more potent than the prepared crude test extracts with an EC_{50} of 13.71 ± 0.08 µg/mL. Similar with the TPC, the antioxidant profiles of the ethanol extracts differ significantly from their chloroform extract counterparts, except for the EC_{50} of *L. betulina* with comparable values for both *L. betulina* chloroform extract (LBCE) and LBEE ($p < 0.05$).

Total phenolic content of the wild macrofungi was the primary component extracted, quantified, and screened in this study. Chloroform and ethanol extracts of six selected macrofungal specimens were prepared to compare whether the ethanol extracts yielded better bioactivity values than their chloroform extract counterparts as assumed functions of their extracted TPC, which was one of the particular active components extracted by ethanol but not, or to a lesser degree, extracted by chloroform. The polar nature of ethanol as compared to chloroform accounts for its ability to extract polyphenols more efficiently in addition to its smaller molecular size that allows it to penetrate cellular membrane to extract intracellular components (Tiwari et al., 2011). This was observed in the measured TPC of the test extracts wherein all ethanol extracts have higher TPC than the chloroform extracts. In *T. eurrhizus*, however, this trend was not observed, which may possibly be due to the chemical instability of the polyphenols present in this macrofungus that could have led to loss of its TPC during the drying and extraction procedures. In general, the presence of TPC ranging from 2.22 ± 0.20 to 49.28 ± 0.30 µg GAE/mg extract among the macrofungal test extracts, together with their observed antioxidant profiles, is in agreement with previously reported antioxidant compounds in species of macrofungi different from those included in this current study (Gan et al., 2013; Hussein et al., 2015; Tripathy et al., 2016; Yildirim et al., 2012). There was a noted dependence in the TPC and percent free radical activity and EC_{50} in some of the extracts, as in the case of DCEE that supports the antioxidant activities of the macrofungal polyphenols. Phenolic compounds had been reported to be well-correlated with antioxidant activity (Ferreira et al., 2005). Due to the hydroxyl groups in phenolic compounds present in these macrofungi, they have excellent free radical scavenging activity directly contributing to their antioxidative action. The noted antioxidative action of the macrofungi extracts can therefore have potential in the development of agents to reduce the risk of heart diseases, cancer, and some inflammatory diseases since antioxidants have been known to stabilize lipid peroxidation (Rodwell et al., 2015). Lipid peroxidation is a natural metabolic process that generates free radicals in vivo contributing to tissue damage associated to atherosclerosis, inflammations, and initial mutagenesis leading to cancer. Consumption of natural antioxidants sourced from macrofungi or their use when processed into pharmaceutical dosage forms may then help address these health concerns.

On the other hand, for some of the test extracts like PFEE and LBEE, their antioxidant activities may be due to other active compounds capable of exerting free radical inhibition but were not included in the current analysis. The results also suggest the possibility of polyphenolics' presence being taxonomically determined as seen in the predomination of members of the order Polyporales in TPC and antioxidant property, which is another avenue for further exploration. The relatively poor potency of the crude macrofungal test extracts as compared to the positive control quercetin may possibly be due to the presence of interacting compounds in the crude extracts as opposed to the pure quality of the control. It may be possible to have increased potency

of the semi-purified fractions or purified metabolites from the macrofungal extracts as possible interfering components get removed. Purification and isolation of the active compounds from the extracts are therefore recommended.

Table 2. Total Phenolic Content and Antioxidant Activity of Wild Macrofungi From Pine Forests in Benguet and Mt. Province, Philippines (Mean ± SD)

Test Extracts	Total Phenolic Content by Folin–Ciocalteu Method (µg GAE/mg Extract)	% Free Radical Inhibition Activity (at 1000 µg/mL Extract)	DPPH Radical Scavenging Activity (EC_{50}; µg/mL)
PFCE	5.40 ± 0.48^{f}	41.46 ± 0.46^{j}	2322.73 ± 54.51^{e}
PFEE	11.67 ± 0.46^{d}	61.45 ± 0.44^{b}	$469.63 \pm 16.25^{h,i}$
DCCE	ND	$43.45 \pm 0.34^{h,i}$	3852.73 ± 34.45^{a}
DCEE	49.28 ± 0.30^{a}	74.59 ± 0.11^{a}	557.74 ± 8.86^{h}
LBCE	2.40 ± 0.42^{g}	44.23 ± 0.28^{h}	854.19 ± 5.17^{g}
LBEE	4.44 ± 0.47^{f}	52.55 ± 0.36^{c}	767.67 ± 3.32^{g}
AECE	ND	47.77 ± 0.02^{e}	1083.74 ± 53.20^{f}
AEEE	14.66 ± 0.21^{c}	43.32 ± 0.31^{i}	3629.59 ± 30.51^{b}
SCCE	6.55 ± 0.14^{e}	46.44 ± 0.45^{f}	3819.50 ± 78.59^{a}
SCEE	33.54 ± 0.44^{b}	51.46 ± 0.11^{d}	431.01 ± 17.82^{i}
TECE	2.22 ± 0.20^{g}	45.33 ± 0.21^{g}	2745.15 ± 29.74^{d}
TEEE	ND	41.34 ± 0.10^{j}	3431.71 ± 49.36^{c}

Note. All bioactivity mean values that share a common letter superscript in each column are statistically similar; otherwise, these values differ significantly at $p < 0.05$. PFCE = *Postia fragilis* chloroform extract; PFEE = *P. fragilis* ethanol extract; DCCE = *Daedaleopsis confragosa* chloroform extract; DCEE = *D. confragosa* ethanol extract; LBCE = *Lenzites betulina* chloroform extract; LBEE = *L. betulina* ethanol extract; AECE = *Albatrellus ellisii* chloroform extract; AEEE = *A. ellisii* ethanol extract; SCCE = *Scleroderma citrinum* chloroform extract; SCEE = *S. citrinum* ethanol extract; TECE = *Termitomyces eurrhizus* chloroform extract; TEEE = *T. eurrhizus* ethanol extract; GAE = gallic acid equivalent; ND = not detected.

Only the ethanol extract of *D. confragosa* exhibited low toxicity with an LC_{50} of 565.90µg/mL while all the remaining test extracts have LC_{50} greater than 1000 µg/mL, which are considered nontoxic (Table 3). This value, however, coincides with the DPPH free radical inhibition EC_{50} of the same extract (DCEE). Of the six macrofungi specimens, *S. citrinum* and *T. eurrhizus* are edible with matching nontoxic LC_{50} values, while the remaining four specimens have no records of edibility among the locals in the collection provinces due probably to their organoleptic properties and not to their toxicity. Despite showing TPC and antioxidant activities that may be considered effective, the safety of the macrofungal test extracts if ever ingested as sources of polyphenols or antioxidant

nutraceuticals remains a major concern due in part to toxicities of some macrofungi. For the macrofungal test extracts in this study though, almost all of them are classified as nontoxic. DCEE with both DPPH EC_{50} and LC_{50} that is considered low toxic falling within the same range suggests the external application of the extract and probable unsuitability for ingestion.

Table 3. Lethality in *Artemia salina* Nauplii of Wild Macrofungi From Pine Forests in Benguet and Mt. Province, Philippines

Test Extracts	Median Lethal Concentration (LC_{50}; µg/mL)	Clarkson's Toxicity Criterion
PFCE	>10,000.00	Nontoxic
PFEE	1169.50	Nontoxic
DCCE	>10,000.00	Nontoxic
DCEE	565.90	Low toxicity
LBCE	2889.74	Nontoxic
LBEE	>10,000.00	Nontoxic
AECE	>10,000.00	Nontoxic
AEEE	9409.99	Nontoxic
SCCE	>10,000.00	Nontoxic
SCEE	>10,000.00	Nontoxic
TECE	1966.56	Nontoxic
TEEE	3041.81	Nontoxic

Note. PFCE = *Postia fragilis* chloroform extract; PFEE = *P. fragilis* ethanol extract; DCCE = *Daedaleopsis confragosa* chloroform extract; DCEE = *D. confragosa* ethanol extract; LBCE = *Lenzites betulina* chloroform extract; LBEE = *L. betulina* ethanol extract; AECE = *Albatrellus ellisii* chloroform extract; AEEE = *A. ellisii* ethanol extract; SCCE = *Scleroderma citrinum* chloroform extract; SCEE = *S. citrinum* ethanol extract; TECE = *Termitomyces eurrhizus* chloroform extract; TEEE = *T. eurrhizus* ethanol extract.

The ability of the extracts at a fixed concentration of 1000 µg/mL to lyse blood clot, expressed in percent clot reduction, is shown in Table 4. The chloroform and ethanol extracts of *T. eurrhizus* exhibited the highest percent clot reduction values at 35.19 ± 0.13% and 32.41 ± 0.17%, respectively, which both significantly differ ($p < 0.05$) from the thrombolytic activity of the streptokinase (positive control) at 66.73 ± 0.11%. The thrombolytic activities of *T. eurrhizus* chloroform extract (TECE) and TEEE are not correlated to their TPC. AEEE, LBEE, and SCEE also exhibited appreciable thrombolytic activities.

Some macrofungi toxins have been reported to prolong prothrombin time, which is a measure of blood clotting, pointing to the potential of macrofungi extracts as anticoagulant agents (Patowary, 2010). In this study, however, the reverse was done by looking into the thrombolytic activity of the macrofungal extracts. Thrombosis, when a thrombus or blood clot forms in the circulatory system due to failure of hemostasis, may cause vascular blockage and may lead to serious consequences such as myocardial or cerebral infarction. Thrombolytic agents that facilitate the lysis of these blood clots in vivo are routinely being used in the management of thrombosis.

Currently available thrombolytic drugs such as streptokinase, which was used as the positive control in this study, have shortcomings such as high cost and unwanted immune reactions, and so, attempts to look for new thrombolytic agents are significant in this field. The results of this current study reveal a promising thrombolytic activity in all the macrofungal test extracts with percent clot reduction at 1000 µg/mL of the extracts ranging from 10.28 ± 0.06% to 35.19 ± 0.13%. Thrombolytic activity however does not seem to be a function of the TPC of the test extracts. Studies on *T. eurrhizus* revealed high alpha-galactosidase contents, a novel protease with fibrin and fibrinogen degradation activity (Lu & Chen, 2012). The presence of the enzyme alpha-galactosidase in the *T. eurrhizus* samples used in the study may possibly be responsible for the noted thrombolytic activity of both the chloroform and ethanol extracts from this locally available macrofungi specimen. This is a promising avenue for further research as the raw material can be locally sourced and the active component in terms of thrombolytic activity can be explored and possibly processed as an alternative to commercially available agents.

For antibacterial activity screening (Table 4), the undiluted crude chloroform and ethanol extracts were utilized. LBEE, DCCE, and DCEE have partially active antibacterial activity against *S. aureus* ATCC 25923, while the rest of the test extracts are inactive. When tested against *E. coli* ATCC 25922, the same set of extracts also have partially active antibacterial activity. Both test organisms were found to be sensitive to the positive controls cefaclor (30 µg) and oxacillin (1 µg) with zones of inhibition at 32.67 ± 0.58 mm and 24.33 ± 0.58 mm, respectively. DCEE shows an antibacterial activity that may be dependent on its TPC, but DCCE and LBEE have antibacterial activities that are independent of their TPC. Phenolic compounds have been previously reported to exhibit antimicrobial activities (Ramawat & Merillon, 2013) via several proposed mechanisms of antimicrobial action such as bacterial enzyme inhibition, complexation with bacterial cell wall, and membrane disruption (Tiwari et al., 2011); thus, this current attempt to associate TPC with antimicrobial activity was done. In addition, DCEE's antibacterial activity correlated with its LC_{50}, but those of DCCE and LBEE do not.

The inactive to partially active antibacterial activities of the macrofungal test extracts make these a poor source of antibacterial agents. However, the mean zones of inhibition of the three extracts, namely, LBEE, DCCE, and DCEE, that inhibited the growth of both gram-positive and gram-negative bacterial test organisms are similar to a previous report (Lallawmsanga et al., 2016) on the antibacterial activities of methanolic extracts from wild macrofungi species that are different from the ones used in the study. The findings on the antibacterial activity of *D. confragosa* in ethanol extract though are supported by the finding of Gaylan et al. (2018).

Table 4. Thrombolytic and Antibacterial Activity of Wild Macrofungi From Pine Forests in Benguet and Mt. Province, Philippines (Mean ± SD)

Test Extracts	% Clot Reduction (at 1000 µg/mL Extract)	Zone of Inhibition of *S. aureus* ATCC 25923 (mm)	Activity Interpretation Against *S. aureus* ATCC 25923	Zone of Inhibition of *E. coli* ATCC 25922 (mm)	Activity Interpretation Against *E. coli* ATCC 25922
PFCE	19.68 ± 0.18^{e}	8.00 ± 0.00^{c}	Inactive	8.33 ± 0.58^{c}	Inactive
PFEE	15.24 ± 0.25^{g}	6.67 ± 0.58^{d}	Inactive	6.00 ± 0.00^{d}	Inactive
DCCE	20.57 ± 0.27^{e}	11.33 ± 0.58^{b}	Partially active	10.00 ± 0.00^{b}	Partially active
DCEE	17.71 ± 0.11^{f}	10.33 ± 0.58^{b}	Partially active	12.33 ± 1.15^{a}	Partially active
LBCE	10.28 ± 0.06^{h}	6.00 ± 0.00^{d}	Inactive	6.00 ± 0.00^{d}	Inactive
LBEE	22.50 ± 0.46^{d}	13.33 ± 0.58^{a}	Partially active	9.67 ± 0.58^{b}	Inactive
AECE	15.62 ± 0.31^{g}	6.00 ± 0.00^{d}	Inactive	6.00 ± 0.00^{d}	Inactive
AEEE	26.33 ± 0.24^{c}	8.33 ± 0.58^{c}	Inactive	6.00 ± 0.00^{d}	Inactive
SCCE	20.05 ± 0.71^{e}	6.00 ± 0.00^{d}	Inactive	6.00 ± 0.00^{d}	Inactive
SCEE	22.39 ± 0.10^{d}	6.00 ± 0.00^{d}	Inactive	6.00 ± 0.00^{d}	Inactive
TECE	35.19 ± 0.13^{a}	6.00 ± 0.00^{d}	Inactive	6.00 ± 0.00^{d}	Inactive
TEEE	32.41 ± 0.17^{b}	6.00 ± 0.00^{d}	Inactive	6.00 ± 0.00^{d}	Inactive

Note. All bioactivity mean values that share a common letter superscript in each column are statistically similar; otherwise, these values differ significantly at $p < 0.05$. PFCE = *Postia fragilis* chloroform extract; PFEE = *P. fragilis* ethanol extract; DCCE = *Daedaleopsis confragosa* chloroform extract; DCEE = *D. confragosa* ethanol extract; LBCE = *Lenzites betulina* chloroform extract; LBEE = *L. betulina* ethanol extract; AECE = *Albatrellus ellisii* chloroform extract; AEEE = *A. ellisii* ethanol extract; SCCE = *Scleroderma citrinum* chloroform extract; SCEE = *S. citrinum* ethanol extract; TECE = *Termitomyces eurrhizus* chloroform extract; TEEE = *T. eurrhizus* ethanol extract.

CONCLUSION

Chloroform and ethanol extracts from six macrofungal species were found to contain polyphenols and exhibited antioxidant, thrombolytic, and antibacterial activities, with nontoxicity to low-toxicity lethality. The ethanol extract of *D. confragosa* has the highest TPC at 49.28 ± 0.30 µg GAE/mg extract and percent free radical inhibition activity at 74.59 ± 0.11%, which was comparable to the pure compound quercetin at 74.33 ± 0.32%. On the other hand, the ethanol extracts of *S. citrinum* and *P. fragilis* have the most potent EC_{50} at 431.01 ± 17.82 and 469.63 ± 15.25 µg/mL. Only the ethanol extract of *D. confragosa* exhibited low toxicity (LC_{50} = 565.90 µg/mL) while the rest of the test extracts are not toxic. Both chloroform and ethanol extracts of *T. eurrhizus* yielded the highest percent clot reduction values at 35.19 ± 0.13% and 32.41 ± 0.17%, respectively. The ethanol extract of *L. betulina* gave the highest zone of inhibition against *S. aureus* ATCC 25923 (13.33 ± 0.58 mm) while the chloroform extract of *D. confragosa* gave the highest zone of inhibition against *E. coli* ATCC 25922 (12.33 ± 1.15 mm). Of particular interest was the noted appreciable thrombolytic activity of all the macrofungal extracts, and this study is the first to report on assessment of thrombolytic activity of macrofungi in the Philippines. Further investigations aimed at processing

or packaging these macrofungi or their extracts either into dosage formulations or as nutraceuticals for the management of oxidative stress-related conditions like heart diseases, cancer, and inflammatory diseases, as well as thrombosis and bacterial infections, are proposed future directions of this study.

Acknowledgments

The authors wish to offer their gratitude to the heads of the local government units of the collection sites—Mayor Abraham Akilit (Bauko), Mayor Ruben Tindaan (Buguias), and Mayor Frenzel Ayong (Mankayan)—for their kind permission for the collection of macrofungal specimens in their localities. Gratitude is also extended to Ms. Jennifer T. Buya of the Blood Bank of the SLU Clinical Laboratories for her help in blood extraction.

Disclosure Statement

No potential conflict of interest was reported by the authors.

Funding

This study was supported by a grant from Saint Louis University, Baguio City, under grant number FRMG2018.2.SNS.1.

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