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Assessment of utilized species of macrofungi and evaluation of mycochemical composition and bioactive properties of *Phellinus robiniae* collected from Benguet Province, Philippines

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Abstract

Macrofungi have long been used as food, medicine, and other purposes. Several studies have been conducted on various macrofungi, however little is known about the wild useful macrofungi from the mountainous areas in the Philippines. Therefore, this study was conducted to document the diversity of wild-utilized macrofungi in Kabayan, Benguet, to come up with the species listing of wild-utilized macrofungi in the area and assessment of the mycochemical composition, anti-oxidant activity and cytotoxic property of the basidiocarps of *Phellinus robiniae*. Seven wild-utilized macrofungi were documented, all were identified morphologically, while four were subjected through molecular techniques to confirm identity; of the seven mushrooms identified, five are edible (*Hypholoma capnoides*, *Lentinula lateritia*, *Tremella fuciformis*, *Tremella mesenterica* and *Albatrellus confluens)* and two are medicinal (*Heterobasidion annosum* and *Phellinus robiniae)*. The identified mushrooms were: *Hypholoma capnoides* 92% (MF511082), *Lentinula lateritia* 99% (AF031192), *Tremella fuciformis* 94% (KY105682), *Albatrellus confluens*, *Heterobasidion annosum* 97% (KU645332), *Tremella mesenterica* and *Phellinus robiniae*. Assessment of bioactive properties of *P. robiniae* showed that the basidiocarp contains eleven bio-active secondary metabolites (essential oils, phenols, sugars, triterpenes, coumarins, anthraquinones, anthrones, tannins, alkaloids, flavonoids and steroids). This study also revealed that *P. robiniae* exhibited anti-oxidant activity with a DPPH radical scavenging activity of 34.59% and a total phenolic content of 49.42 mg GAE/g. Moreover, the ethanolic extract of *P. robiniae showed* an LC_{50} of 328.05 ppm which is considered as moderately toxic. This study provides baseline information on the utilized species of wild mushrooms that are found in the area.

Keywords: Antioxidant properties, cytotoxicity, molecular identification, mushroom, secondary metabolites

Introduction

From time immemorial, macrofungi are being used as a valuable food source and as traditional medicine around the world (Wasser 2002). This is due to its pleasant flavor, appealing texture (Ferreira et al. 2010; Chelela et al. 2014) and therapeutic properties which make them a good health promoting food. Macrofungi also contain minerals, vitamins, nutritive compounds, proteins, polysaccharide and low fat content (Giri et al. 2012). According to Chowdhury et al. (2015), mushrooms are attractive as a functional food and as a source for development of drugs and other nutraceuticals. It is known that they produce large and diverse varieties of secondary metabolites (Liu 2007; Oyetayo et al. 2013). These metabolites have health promoting properties in the body including antimicrobial, antiviral, anti-oxidant, anti-hypertensive, cholesterol lowering, cardiovascular diseases preventative, liver protective, anti-fibrotic, anti-inflammatory, anti-diabetic, anti-cancer activity (Younis et al. 2014) and immune stimulatory effect (Chowdhury et al. 2015).

The diversity of macrofungi in the Philippines is relatively high; Asia was roughly estimated to have about 10,000 to 25,000 mushroom species (Mueller et al. 2007). In Bifeng Gorge, Sichuan Province, China, 275 species of macrofungi has been recorded (Li et al. 2011). There are 60 species recorded in Malaysia (Bolhassan et al. 2012), 778 species in India (Swapna et al. 2008), while there are 176 species in Burma (Thaung 2007). In the Philippines, specifically in Central Luzon region, De Leon et al. (2013) reported 76 species of macrofungi in six Aeta tribal communities. In Mt. Malinao, Albay, Daep and Cajuday (2003) reported 9 Tricholomataceae, 3 Coprinaceae, 2 Pluteaceae, and 1 Auriculariaceae species. While in Mt. Apo in Mindanao 25 genera and 87 species of basidiomycetes were documented (Biadness & Tangonan 2003). According to Ellamar et al. (2009) 16 species of wild macrofungi were found in Camiling, Tarlac. Studies on identification and characterization of more macrofungi under various categories must be given attention for research for their possible benefits to the future researchers (Sun & Zhuang 2011).

Moreover, the search for safe and effective pharmacological substances has been increasing. In addition, the bioactive compounds obtained from macrofungi are perhaps the answer to novel pharmacological agents (Oyetayo et al. 2013). Some studies stated the presence of antioxidant activities of mushrooms (Mujic et al. 2010; Keles et al. 2011; Sudha et al. 2012; Kosanic et al. 2013; Kozarski et al. 2015) while others documented the different types of mycochemicals present in mushrooms possessing medicinal properties (Bustillos et al. 2014; Kalaw & Albinto 2014; Aquino et al. 2018). Additionally, mushrooms are reported to possess cytotoxic (Patel & Goyal 2011; Badshah et al. 2015; Ganeshpurkar et al. 2016; Haque et al. 2016) property as well. However, the pharmacological potential of some macrofungi, specifically the bracket or shelf like macrofungi are not yet explored. In fact, a large number of unknown species of macrofungi which may possess health promoting properties are not well studied (Oyetayo et al. 2013).

Hence, this study was conducted to document the diversity of wild utilized macrofungi and assessed the mycochemical properties, antioxidant and cytotoxic activity of *Phellinus robiniae* to serve as baseline information for other researchers in the future about these utilized macrofungi in Kabayan, Province of Benguet, Philippines.

Materials and Methods

Study Site

The wild macrofungi were collected at Barangay, Kabayan Barrio, Municipality of Kabayan, Benguet Province. The Barangay Kabayan is located on the eastern part of Benguet at 120°45" to 120°58" east longitude and 16°33" to 16°42" north latitude (Fig. 1). It is bounded on the north by Buguis by Buguias, south by Bokod, west by Atok while Tinoc, Ifugao and Kayapa, Nueva Vizcaya share the eastern border. The elevation is 1262.9 masl, air temperature is 20.5°C and the relative humidity is 80%.

Collection and Preservation of the Collected Macrofungi Samples

All the specimens collected were initially photographed in their habitat and representative specimens were collected. The

macrofungi were scraped off from the bark of trees where they were attached and for the ground and leaf litter macrofungi, they were dug carefully so as not to damage their bases. All the samples were labeled, wrapped in brown paper, and brought to the laboratory for identification. The collected samples were preserved by soaking in 95% ethanol. Voucher specimens were deposited at the Center for Tropical Mushroom Research and Development (CTMRD), Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines.

Identification of Collected Macrofungi

Morphological identification.

All the collected macrofungi were identified based on their macroscopic (basidiocarps) and microscopic (spore and hyphal morphologies) features. The mushroom's cap, gills, and stalk morphology were observed and recorded. A spore print was also prepared from fleshy mushrooms. This is to observe for microscopic features such as spore color, shape, and size. Identification and taxonomic classification were made by comparing these morphologies with published literature and works of Quimio (2001), Lodge et al. (2004), Tadiosa (2011), and Kuo (2007). Data obtained from the study were used to prepare a checklist of the utilized macrofungi found in the area.

Molecular verification.

The basidiocarps of the selected samples of macrofungi were frozen with liquid nitrogen. The frozen fruiting bodies were ground with a mortar and pestle, and approximately 1 g of each sample was collected and placed in the test tube, then added with 4 µL of proteinase K. The total genomic DNA of the macrofungi was extracted using a modified CTAB method.

The genomic DNA was diluted into a 1:100 ratio using sterilized distilled water. The nuclear rDNA containing the ITS regions was amplified by Polymerase Chain Reaction (2700 Thermal cycler) using the fungal specific primers ITS3-R F (5'ATCGATGAAGAACACAG3') and ITS4 (5'TCCTCCGCTTATTGATATGC3'). The PCR profile was used to amplify the target ITS region of the sample, this is composed of 35 initial denaturation at 95°C for 5 minutes, denaturation at 95°C for 30 seconds, 53°C annealing for 30 seconds, extension at 72°C for 45 seconds and final extension step of 72°C for 2 minutes. PCR components were made up of 2.5 µl of 10x PCR Buffer, 1.5 µl of 25 $MgCl_2$, 1.25 µl of 10 DNTP mix, 1.13 µl of ITS 3-R and ITS 4, 0.09 µl of Taq polymerase (KAPABiosystems, USA) and 15.4 µl of sdH_2O with a total volume of 25 µl together with 2 µl of DNA. The amplification product and 1 kb DNA ladder stained with 1 µl gel red (Biotium) were run for 30 minutes at 100 V on 1% agarose (prepared in 1x TAE) and were analyzed under gel photo documentation system (Labnet GDS-1302 Enduro Imaging System). Quantification of DNA was done using fluorometer. After the confirmation of the expected size of amplified fragments, the PCR products were sent to 1st BASE laboratory for PCR purification and sequencing procedure. The consensus sequences were used for Basic Local Alignment Search Tool (BLAST) analysis, related gene sequences were obtained from NCBI GenBank for identification.

Species Listing

The genera and species of the macrofungi that were observed in the area were listed and the number of each genera and species were recorded.

Mushroom sample

The fruiting bodies of *P. robiniae* were air-dried and pulverized using a blender. The sample was extracted and subjected to mycochemical screening and bioactivity assays.

Mycochemical screening

The mycochemical compositions of macrofungi were determined following the procedures of Guevara et al. (2005).

DPPH radical scavenging assay

The DPPH radical scavenging activity of the ethanol extract of P. robiniae was determined following the procedure of Kolac et al. (2006). A 100 µl of the test sample in ethanol was mixed with 5 µl DPPH solution (5 mg DPPH powder in 2 ml of ethanol) in 96-well microtiter plates. The mixture was vigorously shaken and left to stand for 30 minutes in the dark. The absorbance was measured, and the inhibition of DPPH free radicals was calculated using the equation: Percentage scavenging effect = $[(Ac_{ontrol} - A_{sample}) / A_{control}] \times 100$ where $A_{control}$ is the absorbance of the control and the A_{sample} is the absorbance of the test sample containing the mixture.

Analysis of phenolic content

The total phenolic content was estimated using Folin-Ciocalteu method as described by Hodzic et al. (2009). Fifty µl of sample solution was mixed with 500 µl of 10% Folin-Ciocalteu reagent (Folin:Methanol, 1:1, v/v). Fifty µl of 7.5% saturated sodium carbonate was added and kept in the dark for 1 h before taking the absorbance at 765 nm. The total phenolic content of the sample was expressed as mg of gallic acid equivalents (GAEs) per gram of sample.

Determination of cytotoxicity

The toxicity of mushroom extract was assessed using brine shrimp assay following the procedure of McLaughlin and Rogers (1998). The different concentrations (1, 10, 100, 500, 1000, and 10000 µg/mL) of extract were prepared in triplicate and ten (10) nauplii were exposed in each replicate. After 24 hours of exposure, the number of dead nauplii was recorded and the percentage mortality was computed. LC_{50} value was computed in order to determine the cytotoxicity level based on the rating of Alhadi et al. (2015).

Statistical analysis

Data were analyzed using Analysis of Variance (ANOVA) and the Duncan Multiple Range Test (DMRT) was used to compare the treatment means at a 5% level of significance. For cytotoxicity, the median lethal concentration LC_{50} was computed using probit analysis.

Figure 1. Map of Luzon showing the study site Kabayan, Benguet, Province.

Results and Discussion

Species listing

There were seven wild utilized macrofungi identified from the area. A checklist of macrofungi was arranged and listed where they are found, their substrate, elevation, local name, humidity, and utilization.

Albatrellacea

Albatrellus confluens (Alb. & Schwein.) Kol & Pouzar

Local name: Akipan

Substrate: Soil
Air temperature: 21.8°C
Humidity: 98%
Elevation: 2264 m
Coordinates: N 16°40'51.7"
E 120°52'29.4"
Utilization: Edible

The basidiomes are fleshy, stipitate, the pileus surface is glabrous to scaly, and the hymenophore poroid. Microscopically with simple septa or clamp connections;

basidiospores are smooth, broadly ellipsoid to subglobose, up to 12 µm long, non-amyloid to amyloid. Some species are edible and can be found in markets (Wang & Liu, 2002; Wang et al. 2004).

Bondarzewiaceae

Heterobasidion annosum (Fr.) Bref.

Local name: Tangila
Substrate: Soil
Air temperature: 21.5°C
Humidity: 97%
Elevation: 2255 m
Coordinates: N 16°40'51.7"
E 120°52'28.0"
Utilization: Medicinal

Fruitbody is brown, upper surface is corrugated that turns black with age; narrow round edge brackets; sometimes in tiers and occasionally resupinate; downy when young, then smooth but uneven or knobby. Individual brackets are 5 to 30 cm across and 1 to 2 cm thick with a wavy margin. The tubes are off-white and terminate in creamy white round pores that are spaced between 2 and 4 per mm. Spores are broadly ellipsoidal to subglobose, very finely warty, 4.5-6 x 3.5-4.5 µm; inamyloid with cream or pale yellow spore print (Kirk et al. 2008).

Hymenochaetaceae

Phellinus robiniae (Murrill) A. Ames

Local name: none
Substrate: Log
Air temperature: 21.5°C
Humidity: 88%
Elevation: 2252 m
Coordinates: N 16°40'51.1"
E 120°52'28.1"
Utilization: Medicinal

Basidiocarps perennial, resupinate to pileate, single or imbricate, sessile with decurrent pore surface; pore surface ferruginous brown to dull brown; pore 2-11 per mm; pores isodiametric more rarely irregular and angular and slightly split; upper surface pubescent to tomentose or becoming glabrous, often with a thin black cuticle, ferruginous to blackish brown; margin rounded and obtuse to acute, sterile below; hyphal system dimitic; generative hyphae hyaline to pale yellow, normally septate and thin-walled; skeletal hyphae yellowish to rusty brown, thick-walled with septa absent and wider than the generative hyphae; hymenal setae and tramal setae absent or present; basidiospores globose to cylindrical, smooth, hyaline to rusty brown, thin thick-walled, basidia hyaline, globose to clavate, 2-4 sterigmate. They are parasitic or saprobic species, some of them can be found in the boreal-temperature zone and most of them lives on hardwoods (Karadelev et al. 1998).

Omphalotacea

Lentinula lateritia (Berk.) Pegler

Local name: Kungbab
Substrate: Decaying Log
Air temperature: 21.5°C
Humidity: 88%
Elevation: 2252 m
Coordinates: N 16°40'51.1"
E 120°52'28.1"
Utilization: Edible

Pileus convex to applanate, firm-fleshy, initially smooth and glabrous, sometimes becoming squamose or fissured. Lamellae adnate or adnexo-sinuate, sometimes with a decurrent tooth, often soon separating from the stipe and becoming free, whitish, often discoloring brownish or vinaceous at maturity, crowded; edge entire or minutely serrulate. Stipe central to excentric, rarely lateral, cylindric or compressed, solid, limited to remnants on the pileal margin (Pegler 1983).

Strophariaceae

Hypholoma capnoides (Fr.) P. Kumm.

Local name: none
Substrate: Decaying Log
Air temperature: 21.5°C
Humidity: 88%
Elevation: 2252 m
Coordinates: N 16°40'51.1"
E 120°52'28.1"
Utilization: Edible

Macrofungi is brown when young and matured. Length 2.3 cm and width 1.2 cm. Spore bearing surface under cap is gills and for the pileus centrally attached. Cap of the carpophore size is umbonate, pileus color brownish, surface characters and zonation glabrous. Pileus margin regular, pileus cuticle is half peeling, texture of the fruiting body soft and flesh odor is unpleasant. Lamellae present, gill attachment adnexed, gill color

deep brown, shape and width is narrow, gill spacing crowded, lamellulae present, forking pattern unbranched (Rahaman et al. 2016).

Tremelleceae

Tremella fuciformis Berk.
Local name: none
Substrate: Decaying Log
Air temperature: 17°C
Humidity: 97%
Elevation: 2284 m
Coordinates: N 16°40'58.5"
E 120°52'28.9"
Utilization: Edible

The fruiting body 43–63 mm long and 35–42 mm broad, white, firm gelatinous, translucent, thin, grey to yellowish-grey colored when dry, repeatedly lobed or forked with margins flexuous to folded, sessile. Odor slightly fishy. It has white to crystal clear color. Substrate is decaying log. Basidiospores (6–) 6.5–8.4–9(–10) × (5–)5.5–6(–7) µm (Bera et al. 2018).

Tremella mesenterica Retz.
Local name: none
Substrate: Decaying Log
Air temperature: 19.1°C
Humidity: 97%
Elevation: 2269 m
Coordinates: N 16°40'59.2"
E 120°52'28.6"
Utilization: Edible

Fruit body 18–33 × 13–30 mm, folded into lobes, cerebriform, gelatinous, light yellow when fresh, becoming orange to greyish orange, horny and crust-like when dry. Sessile, odour mushroomy, basidiospores 14–16–18 × 14–15–16.5 µm (Bera et al. 2018).

The Identified wild utilized macrofungi is presented in Figure 2. Most of the collected species of wild utilized macrofungi from Kabayan, Benguet were Abarellaceae and Tremelleceae both with two species while Hymenochaetaceae, Omphalotaceae and Strophariaceae, had one species each family. A total of seven macrofungal samples were collected in Barangay Kabayan Bario, Kabayan Benguet. The identified wild utilized macrofungi belonged to 5 families, 6 genera and 7 species. The identified macrofungi were: *Albatrellus confluens, Heterobasidion annosum* (97%), *Hypholoma capnoides* (92%), *Lentinula lateritia* (97%), *Tremella fuciformis* (94%) (Table 1) *Tremella mesenterica* and *Phellinus robiniae*.

The phylogentic placement of the molecularly identified mushrooms is presented in Figure 3. The evolutionary history was inferred using the Neighbor-Joining method (Salitou & Nei 1987). The optimal tree with the sum of branch length = 22.69686360/28.66619997 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al. 2004) and are in the units of the number of base substitutions per site. This analysis involved 15/7 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 734/1679 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar et al. 2018).

Mycochemical constituents of P. robiniae

In this study, the mycochemical screening was carried out to detect the secondary metabolites present in the fruiting body of one medicinal macrofungi (*P. robiniae)* and results are presented in Table 2. Out of fifteen bioactive secondary metabolites tested in the fruiting bodies of *P. robiniae*, eleven were present namely: essential oils, phenols, sugars, triterpenes, coumarines, anthraquinones, anthrones, tannins, alkaloids, flavonoids and steroids. However, cardiac glycosides, saponins, fatty acids and terpenoids were not detected in the sample.

Antioxidant activity of P. robiniae

The radical scavenging activity of *P. robiniae* is found to be 34.59% (Table 3). This clearly indicates that *P. robiniae* has promising potential in the pharmaceutical industry because antioxidants perform important functions in preventing diseases related with free radicals. Furthermore, the fruiting body of *P. robiniae* contain 49.42 mg

GAE/g of phenolic compound per sample which strongly suggest that the fruiting bodies of *P. robiniae* could be a source of active phenolics as potent antioxidant.

Cytotoxic effect of P. robiniae

It can be seen that 10000 ppm registered the highest mortality rate with a mean of 100%, while the lowest mortality was noted in ≤10 ppm with a 0% mean mortality rate. Using analysis of variance (ANOVA), Table 4 shows that after 24 hours of exposure to the macrofungi extract, there was significant difference among treatment concentrations ≤10 ppm and ≥1000 ppm. However, comparison among means reveal that 1000 ppm and 10000 ppm are comparatively the same but significantly different from the rest of the treatments. Data presented herein was analysed to determine the median lethal concentration (LC_{50}) of the extract using probit analysis. Result showed that, P. *robiniae* ethanol extract exhibited an LC_{50} of 328.05 ppm (Figure 4) which is considered to be moderately toxic. This result implies that P. *robiniae* has great potential as source of toxic compounds that can be used in further evaluation of other biological activities such as anti-inflammatory, anti-microbial and anticancer, which needs to be further investigated.

Table 1. Molecularly verified macrofungi using BLAST with GenBank Accession Number.

Sample Code	Sample Identity	% Identity	GenBankAcc. N0.
WE02	*Hypholoma capnoides*	92%	MF511082.1
WE17	*Lentinula lateritia*	92%	AF031192.1
WE19	*Tremella fuciformis*	92%	KY105682.1
WE25	*Heterobasision annosum*	87%	KU645332.1

Table 2. Mycochemical composition of the *P. robiniae* fruiting body.

Mycochemical	Result
Essential oils	Present
Phenols	Present
Sugars	Present
Triterpenes	Present
Coumarins	Present
Anthraquinones	Present
Anthrones	Present
Tannins	Present
Alkaloids	Present
Flavonoids	Present
Steroids	Present
Cardiac Glycosides	Not detected
Saponins	Not detected
Fatty acids	Not detected
Terpenoids	Not detected

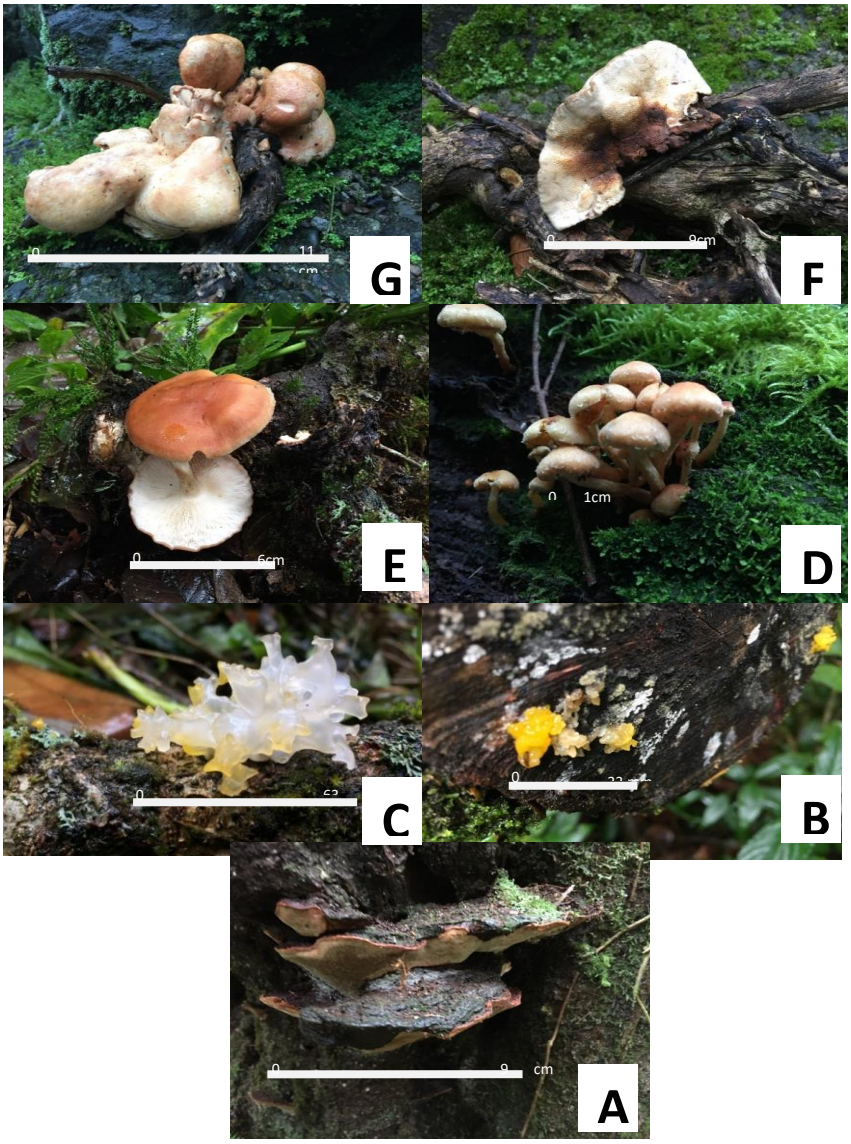

Figure 2. Useful macrofungi from Kabayan, Benguet: *Albatrellus confluens* (A), *Heterobasidion annosum* (B), *Lentinula lateritia* (C), *Hypholoma capnoides* (D), *Tremella fuciformis* (E), *Tremella mesenterica* (F) and *Phellinus robiniae* (G).

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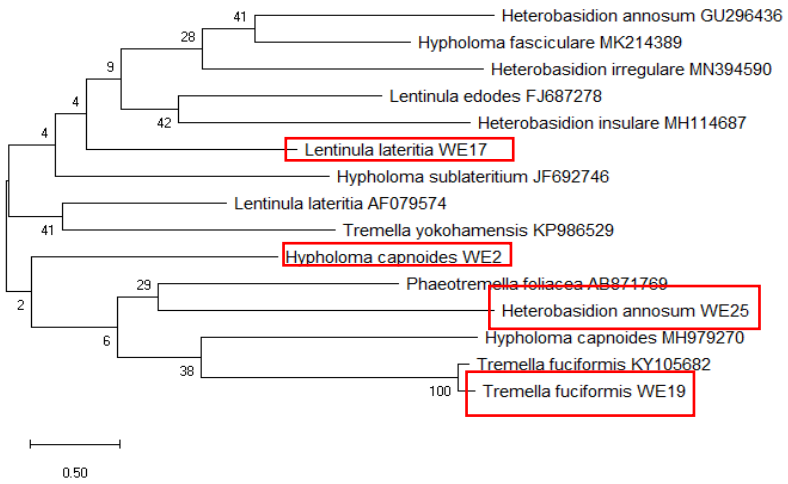

Figure 3. Phylogenetic tree based on maximum composite likelihood method showing the relationship of the collected samples with related species.

Table 3. Radical scavenging activity and total phenolic content of *P. robiniae.*

Extract	Radical Scavenging Activity (%)	Total Phenolic Content (mg GAE/g sample)
Phellinus robiniae	34.59	49.42
Cathechin (control)	57.14	

Table 4. Mean percentage mortality of *A. salina* nauplii after 24 hours of exposure to different concentrations of *P. robiniae* extract.

Concentration	Mortality
10000	100.00[a]
1000	93.33[a]
500	43.33[b]
100	23.33[bc]
10	0.00[c]
1	0.00[c]
Control	0.00[c]

* Means having the same letter of superscript in the same column are not significantly different at P ≤ 0.05 significance using LSD.

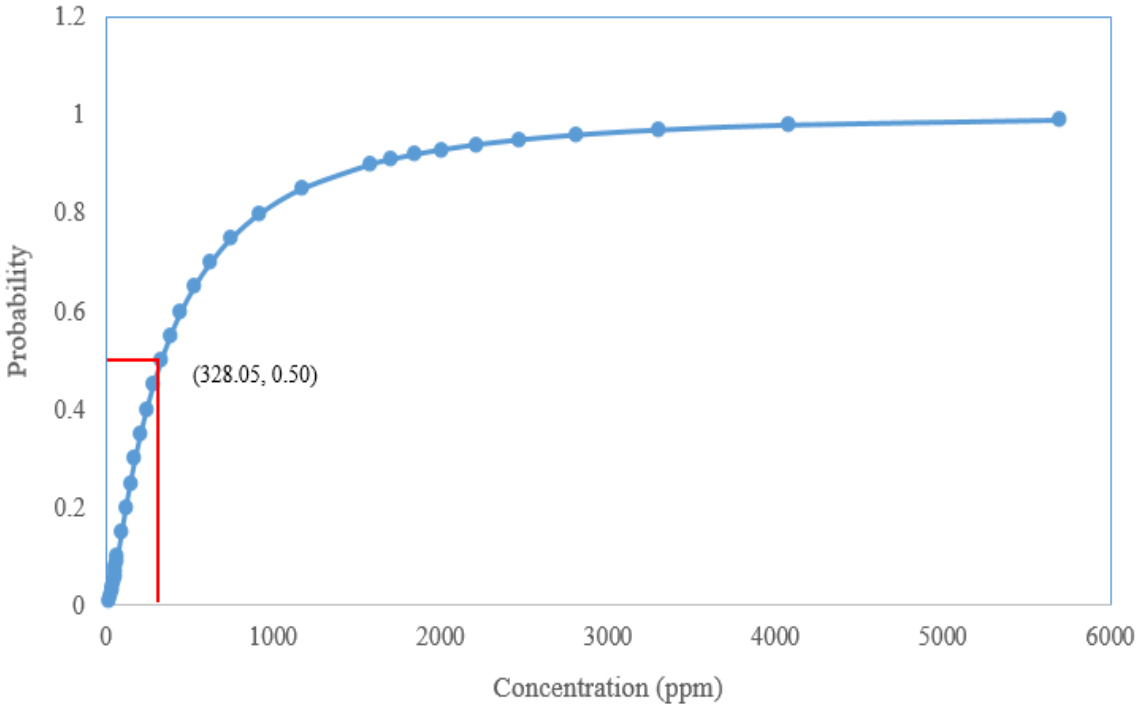

Figure 4. Point estimate of LC_{50} value of *P. robiniae* extract after 24 hours of exposure.

Discussion

The exhibited patterns of diversity of macrofungi were related largely to their substrates, host availability and the climate condition (Hou et al. 2012). Tadiosa et al. (2011) stated that the presence of leaf litters and the slightly acidic pH were favorable for the growth of macrofungi. Macrofungi play essential roles in maintaining forest ecosystems. A large number of macrofungi are decomposers or saprophytic. About one-tenth of macrofungal species form mycorrhizal associations whereas only minorities are parasites. As decomposers, macrofungi maintain forest health by returning organic materials to the soil, making the carbon source available to plants and other organisms (Senn-Irlet et al. 2007).

In Aurora province, Tadiosa et al. (2011) collected 684 fungi belonging to 107 species, 68 genera and 38 families. Sibounnavong et al. (2008) reported seven species of macrofungi during dry season in Puncan, Carranglan, Nueva Ecija. De Castro and Dulay (2015) collected a total of 20 species belonging to 17 genera and 15 families in multistorey agroforestry systems in Mt. Makiling Forest Reserve of Los Baños, Laguna, Philippines. Cruz et al. (2016) stated that in Banaue, Ifugao province, a total of 41 fleshy macrofungi were collected and 38 species were identified; 35 morphologically and 3 molecularly.

Molecular techniques had been used to adequately characterize and identify intra and inter specific characteristics of macrofungi (Zakaria et al. 2009). By determining nucleotide sequences of the mushroom, it may provide additional information that can enrich the GenBank database aiding molecular taxonomy and facilitating its domestication and characterization for human benefits (Das et al. 2013). In China, PCR-based approach was employed to identify *Boletus edulis* and *Verpa bohemica* using rDNA ITS sequences (Lian et al. 2008) In addition, eight wild macrofungi belonging to the genera Amanita, Astraceus, Termitomyces and Volvariella were characterized in the region (Das et al. 2013). In the black African countries, studies on macrofungi taxonomy using the molecular technique have not been widely investigated. In Kenya, Ojwang (2012) characterized seventy-one Pleurotus species collected from the wild whereas in Nigeria, literatures revealed that Oyetayo (2009, 2013), Bankole and Adekunle (2012) and Awala and Oyetayo (2015) have identified mushrooms. The samples were collected from Ekiti, Lagos, Ondo and Oyo states of the Southwestern Nigeria and characterized using the internal transcribed spacer polymerase chain reaction ITS-PCR). Modern molecular technique reduces the

challenges of inconspicuous nature, inconsistent morphology and indiscrimination among fungal species often associated with traditional method of nomenclature (Blackwell et al. 2006; Nilsson et al. 2011).

Moreover, mycochemicals are abundant in macrofungi, enabling them to perform various metabolic processes (Aquino et al. 2018). Essential oils, also known as volatile or ethereal oils, are aromatic oily liquids known for their antiseptic (bactericidal, virucidal, and fungicidal), medicinal properties, and fragrance (Bakkali et al. 2008). Thus, essential oils are used in embalmment, preservation of foods and as antimicrobial, analgesic, sedative, anti-inflammatory, spasmolytic, and local anesthetic remedies (Bakkali et al. 2008 as cited by Aquino et al. 2018). Phenolic fractions and anti-oxidant properties of macrofungi are related (Dulay et al. 2017). Aside from anti-oxidant properties, phenolics exhibit antimutagenic, antiviral, antibacterial, algicidal, antifungal, insecticidal, estrogenic, and keratolytic activities that may protect the organism from competing once in their biological environment (Dulay et al. 2017; Castellano et al. 2012 as cited by Aquino et al. 2018). On the other hand, triterpenes directly suppress the growth and invasive behavior of cancer cells (Dudhgaonkar et al. 2009). Dudhgaonkar et al. (2009) also reported the inflammatory activities of isolated triterpenes from *Ganoderma lucidum*, a bracket macrofungus. Also, coumarins are fragrant organic compounds used in the pharmaceutical industry for synthetic anticoagulants and fibrinolytics (Jose et al. 2004). It is also used as an edema modifier and stimulator for macrophages in degrading extracellular albumin (Aquino et al., 2018). Furthermore, anthraquinones, an important class of natural and synthetic compounds are highly crystalline solids and are ingredients in many dyes (Malik & Muller 2016). Medically, its derivatives can be used as antimalarial and antineoplastic (Aquino et al. , 2018). One of the most common medicinal uses of it is to bring relief to constipation through its laxative effects (Bolen, 2018). It is believed to increase the amount of fluid in the colon and stimulate colon contractions (Bolen, 2018). Subsequently, anthrone is a tricyclic aromatic ketone used in pharmacy and also as a laxative (Jose et al., 2004). It stimulates the motion of the colon and reduces water reabsorption. Macrofungi are being reported containing anthrones (Jose et al. 2004). Finally, tannins have been used against heart diseases because of its ability in scavenging free radicals (Bustillos et al., 2014). Also, flavonoids have been reported possessing anti-platelet, anti-turmor antioxidant, anti-inflammatory, antiallergic, antiviral (Haslam 1996; Edeoga & Eriata 2001) as well as anticarcinogenic activities and in reducing the aging process (Hilang & Ferraro 2000). Alkaloids were also found in the *P. robiniae*. Alkaloids have been reported to act as a powerful pain reliever and topical anaesthetic in ophthalmology and have stimulating effects and antipruritic action among other uses (Edeoga & Erita, 2001; De Leon et al., 2017). The presence of alkaloids in macrofungi indicates antibacterial activity as explained by Idowu et al. (2003). One of the most important biological properties of alkaloids is the toxicity against cells of foreign organisms (Nobori et al., 1994). This bioactivity has been widely studied for its potential use in the elimination and reduction of human cancer cell lines (Nobori et al., 1994). This suggests that the *P. robiniae* have a potential anti-cancer agent. The present investigation brings out adequate data on the mycochemical constituents present in *P. robiniae*. The presence of mycoconstituents make the mushroom possibly useful for treating different ailments and have a potential of providing useful drugs for human use. Further analysis of the active compound from the mushroom might lead to a potent therapeutic agent.

On the other hand, antioxidant compounds play an important role as a health protecting factor (Prakash 2001). They protect the human body against oxidative damage caused by free radicals (Dulay et al. 2015). At present, natural antioxidants are being extensively studied for their capacity to protect organisms and cells from damage brought about by oxidative stress (Kavishree et al., 2008). Mushrooms are currently found to possess an antioxidant activity, which is well correlated with their total phenolic content (Barros et al., 2007). Likewise, phenolic compounds possess scavenging ability because of their hydroxyl groups, making them one of the most significant bioactive compounds with antioxidant activity. Phenols were found to have activities against heart ailments, cancer, and the ability as anti-inflammatory. According to Oyetayo et al. (2009), mushrooms produce a wide range of secondary metabolites with high therapeutic value. It's been reported promoting health properties such as antioxidant, antimicrobial, and anticancer, among others. In addition, both macrofungi fruiting bodies and mycelium contain compounds with wide-ranging antioxidant and antimicrobial activities

(Oyetayo et al. 2009; Mau et al. 2004; Barros et al. 2007; Ferreira et al. 2010; Kosanic et al. 2013). Also, many studies demonstrated that the regular consumption of mushrooms or consumption of isolated bioactive constituents present in macrofungi is beneficial to health. The antioxidants present in dietary macrofungi are of great interest as possible protective agents to help the human body reduces oxidative damage without any interference. Macrofungi may thus be considered as functional food or nutraceutical product (Sudha et al. 2012). *P. robiniae* having a 34.59% radical scavenging activity and a 49.42 mg GAE/g of sample total phenolic contents, exhibits a higher scavenging activity and total phenolic contents as compared to other mushrooms such as *Lentinus tigrinus* (18.94%; 26.59 mg AAE/g), *Lentinus sajor-caju* (16.94%; 25.60 mg AAE/g) (Dulay et al. 2015), *Panaelous cyanescens* (14.84 ± 0.19%; 25.01 ± 0.01 mg AAE/g) (Bustillos et al. 2014), *Calocybe indica* (28.04 ± 0.41%) (Prabu & Kumuthakalavalli 2016) and *P. grammocephalus* (26.37%; 38.58 mg AAE/g) (Aquino et al. 2018). Finally, the result of the study is similar with the study of both Seephonkai & Chakuton (2011) and Chang et al. (2007), wherein macrofungi genus of *Phellinus* displays a strong antioxidant and free radical scavenging activities with high value of total phenolic content.

The results of the study wherein *P. robiniae* exhibited cytotoxic effect is in correlation to the cytotoxicity of various macrofungi species. Based on the study of Oyetayo et al. (2013) where three bracket macrofungi, *L. betulina, T. versicolor* and *C. polyzona* exhibited inhibitory activities against six carcinoma cell lines such as human glioblastoma (U251), human prostatic adenocarcinoma (PC-3), human chronic myelogenous leukemia (K562), human colorectal adenocarcinoma (HCT-15), human breast cancer cell line (MCF-7) and human lung adenocarcinoma (SKLU-1). Also, extract of *P. highking* was found out to have a remarkable anti-oxidant activity as well as cytotoxicity against brine shrimp larvae (Haque et al. 2016). Similarly, Kidukuli et al. (2010) reported varied cytotoxicity activities of macrofungi extracts against *A. salina* larvae. Subsequently, the result of the study is parallel to the result of Seklic et al. (2016), wherein *Phellinus linteus* extract significantly decreased the cell viability on both colon cancer cell lines such as HCT-116 and SW-480. Likewise, Haque et al. (2016) stated that the aggregation of phenolics and flavonoids in macrofungi might be amenable for its encouraging cytotoxic activity and the possible mechanism of cytotoxicity against brine shrimp nauplii due to poisonous effect on cell mitosis. Additionally, Martinez (2018) describe the cytotoxic activity of *Ganoderma applanatum,* a bracket macrofungus, as mildly toxic with an estimated LC_{50} of 979.33 ppm which is less toxic compared to the *P. robiniae*.

According to Aquino et al. (2018), Nanglihan (2018) and Nieves (2018), ethanol extract of *Polyporus grammocephalus, Trametes elegans* and extracts of *Thelephora* sp. exhibits a cytotoxic activity against brine shrimp larvae with an estimated LC_{50} value of 73.78 ppm, 32.57 ppm and 10.763 ppm, respectively. According to the rating of Alhadi *et al.* (2015), these values were considerably highly toxic. According to Garbi et al. (2015), the median lethal concentration value indicates the cytotoxicity level of one extract, in which the lower the LC_{50} value the higher the toxicity. Variation in the cytotoxic effect among macrofungi may be due to difference in levels of the bioactive compounds as well as the presence or absence of several chemical compounds which interfere with the bioactivity of the macrofungi (Chelela et al. 2014). Some macrofungi were observed to have higher cytotoxicity than other macrofungi species. The high cytotoxicity of some species proposes the reason why these macrofungi are usually not edible. Nonetheless, edible mushrooms are observed to have a certain degree of toxicity (Chelela et al. 2014). Furthermore, Chelela et al. (2014) also stated that lethality of brine shrimp nauplii to macrofungi extracts is an indication of the presence of potent and compelling cytotoxic components with merits to further investigation as an anticancer agent. Lastly, brine shrimp lethality assay shows the bioactivity of the extract which in most cases correlates reasonably well with cytotoxic and anti-tumor properties (Krishnaraju et al. 2005).

Conclusion

Seven wild utilized macrofungi were collected from Kabayan, Benguet. All species were identified morphologically and four verified molecularly. The morphologically identified macrofungi are *Tremella mesenterica, Phellinus robiniae, Albatrellus confluens* while the molecularly verified are; *Hypholoma capnoides, Lentinula lateritia,*

Tremella fuciformis, and *Heterobasidion annosum.* Two of the collected macrofungi ar medicinal (*H. annosum* and *P. robiniae*) while the remaining five are edible (*Hypholoma capnoides, Lentinula lateritia, Tremella fuciformis, Albatrellus confluens* and *Tremella mesenterica).* Furthermore *P. robiniae* contain eleven useful mycochemicals rich in bio-active secondary metabolites such as essential oils, phenols, sugars, triterpenes, coumarines, anthraquinones, anthrones, tannins, alkaloids, flavonoids and steroids. Essentially, the study also revealed *P. robiniae* having an anti-oxidant and cytotoxic properties. Thus, some useful drugs of therapeutic importance may perhaps be developed out of this research work.

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