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AGARICS AND POLYPORE DIVERSITY SURVEY OF LA UNION'S MOLAVE FOREST AND THE α-AMYLASE AND α-GLUCOSIDASE INHIBITORY POTENTIAL OF *Fuscoporia torulosa* MFSLP-12

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ABSTRACT – Macrofungal species in the Philippines are dense and diverse, however, only a few studies focus on their taxonomy, density, and diversity due to lack of researchers specializing in this field and limited funding initiatives. A total of 108 samples were collected and identified, which were further classified into 56 morphospecies, of which 40 morphospecies were Polypores and 16 morphospecies were Agarics. The site was assessed to have high macrofungal diversity attributed to high species richness and evenness as indicated by Simpson's Diversity indices of 0.87202 and 0.85971; and Shannon-Wiener indices of 2.21849 for Agarics and 2.69332 for Polypores. To determine a possible beneficial health effect of macrofungi found in the area, the antihyperglycemic potential of the polypore species *Fuscoporia torulosa* MFSLP-12 was determined through *in vitro* α-amylase and α-glucosidase inhibition using its hexane, ethyl acetate, and methanol extracts. Methanol extract produced the strongest inhibition on α-amylase (38%) and α-glucosidase (56%) while hexane and ethyl acetate extracts showed weak to no inhibition. Compared to acarbose, its IC_{50} values were about nine folds higher for α-amylase and five folds higher for α-glucosidase. This promising result merits the elucidation of the active compound as a recommended future direction.

Keywords: Agarics, enzyme inhibition, Fuscoporia torulosa, Polypores, taxonomic diversity survey

INTRODUCTION

Quantitative estimation of macrofungal density determines the overall importance of each species in a community based on the sum of their relative frequency, relative density, and relative abundance (Mitchell, 2010). Macrofungal diversity in a given location is measured and described in terms of its species' richness, defined as the number of different species present in an area, and evenness, which compares the similarity of each species' population size (Balun, 2019). Indices such as Simpson and Shannon-Wiener are used by mycologists to determine the diversity measurement in specific locations.

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Shannon index is a diversity index for the estimation of evenness and richness in a given site (Brodie et al., 2003 as cited by Lim et al., 2010). In the Shannon index, the index value increases when both species richness and evenness increase. Using both parameters would provide the researchers with an easier understanding of the diversity of a given site. On the other hand, Simpson's index dictates the probability that a macrofungus acquired at different locations would be of the same species. It focuses on species dominance, which has an inverse relationship with diversity. This means that if the dominance of a species increases, the diversity of the place would decrease.

The Philippines is a diversity hotspot of macrofungi. The findings that will be presented in this paper are based on a follow-up study by Tadiosa and Arsenio (2014), one of the few studies conducted and published on macrofungal diversity in the country, which assessed the diversity of macrofungi in the Molave Forest of San Fernando, La Union, an important natural forest ecosystem in Ilocos Region. A total of 51 species of the wood-rotting Basidiomycetes belonging to 24 genera, 15 families, and four orders were collected. Polypores comprised 76% of total percentage composition of the fungal species while Agarics comprised 8% (Tadiosa and Arsenio, 2014). As a follow-up study, our research aimed to assess the current density and diversity of Agarics and Polypores in the said Molave Forest. Specifically, it aimed to identify and classify the collected species, determine the most important species of Agarics and Polypores through quantitative estimation of density, and evaluate the diversity of Agarics and Polypores in the site using Simpson's Diversity Index and Shannon-Wiener Index.

A possible health application was also investigated by determining the *in vitro* α-amylase and α-glucosidase inhibitory potential of a species found in the area, *Fuscoporia torulosa* MFSLP-12. *Fuscoporia torulosa* MFSLP-12 was chosen due to the following criteria: (1) sufficient fungal mass was available for subsequent extraction and mycochemical screening and (2) globally, no other known study evaluates its antihyperglycemic property.

Type 2 diabetes mellitus (T2DM) has already become a growing public health issue in the Philippines and its projected growth indicates its potential of becoming the number one metabolic disorder in the world (Alam et al., 2018). It is characterized by reduced production of insulin and insensitivity of cells to the regulatory action of insulin, both resulting in glucose accumulation in the blood. High blood glucose or hyperglycemia is usually associated with microvascular and macrovascular complications such as nephropathy, neuropathy, microangiopathy, macroangiopathy, retinopathy, and cataract, with some leading to premature mortality**.** Inhibition of key enzymes α-amylase and α-glucosidase is believed to be the best way to manage T2DM as these are the enzymes mainly involved in the hydrolysis of dietary carbohydrates into glucose and their inhibition decreases postprandial hyperglycemia (Chipiti et al., 2017). Currently used synthetic drugs that work through this approach produce unwanted side effects including abdominal distention, flatulence, meteorism, and diarrhea. Because of this, there is an established interest in the use of nutraceuticals and plant-based medicines in the treatment of postprandial hyperglycemia. These are considered non-cytotoxic, more effective, and less costly (Patel et al., 2012). *F. torulosa* has already been described to possess antimicrobial and antioxidant properties. In the study of Dulger et al. (2005), *F. torulosa* exhibited antimicrobial activities against some gram-positive and gram-negative bacteria, yeasts, filamentous fungi, and actinomycetes. In separate studies, methanol and ethanol extracts of *F. torulosa* were also revealed to have strong antioxidant ability (Hokmollahi et al., 2012; Seephonkai et al., 2011). Meanwhile, several species under its previous genus *Phellinus* have been reported to exhibit antidiabetic properties including *P. linteus, P. baumii, P. merrillii, and P. ribis* (De Silva et al., 2012). For the genus *Fuscoporia*, only the species *F. obliqua* was evaluated for antidiabetic property in published studies wherein it was found to reduce hyperglycemia in mice with T1DM. To our knowledge, this study

is the first study in the Philippines and one of the few international studies to establish the antihyperglycemic potential of *Fuscoporia torulosa*. It specifically aimed to determine the potency of hexane, ethyl acetate, and methanol extracts of *Fuscoporia torulosa* MFSLP-12 in inhibiting α-amylase and α-glucosidase activity *in vitro*; and identify the phytochemicals present in the extracts that showed enzyme inhibition.

MATERIALS AND METHODS

Field Sampling of Selected Sites in Study Area

The Molave Forest of San Fernando City, La Union is situated at 16.35° N, 120.66° E as shown. Sampling methods that were used in the study include the transect line method and the quadrat method. Four transect lines were established in four different sites. Each transect line comprised five quadrats with an area of 10 m x 25m and an interval of 50m wherein a total of 20 quadrats were utilized for the whole study as shown in Figure 1. The methods of sampling used in this study were based from Tadiosa and Arsenio (2014). However, the trail where the study was conducted was different from that study as per described through personal communication with Dr Edwin R. Tadiosa during the actual trek and sampling (2018). The observed changes were attributed to the modifications in landscape of the area brought about by additional infrastructures, such as roads and houses, where the previous trail was located.

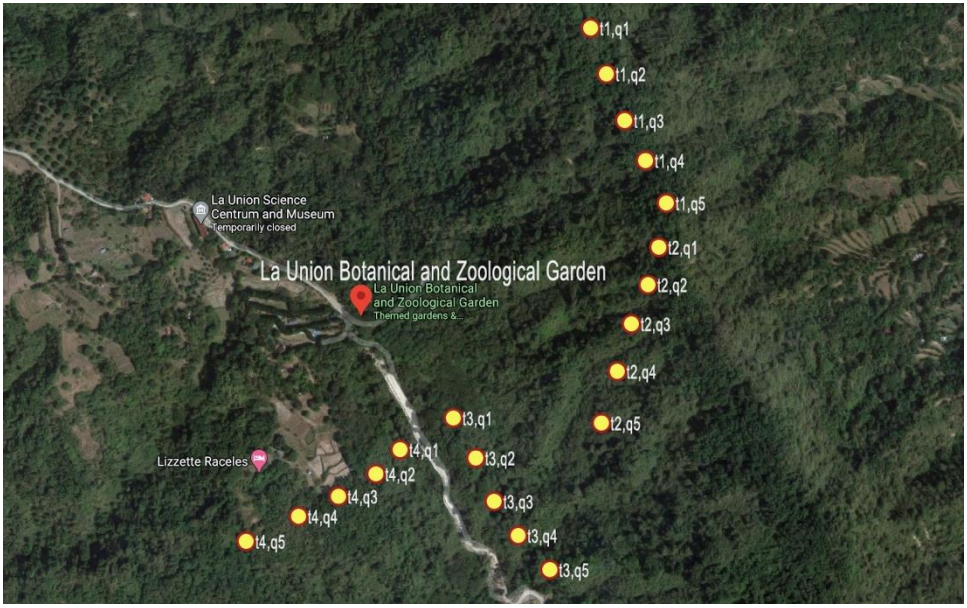

Figure 1. Satellite image of the Molave Forest of San Fernando, La Union, Philippines with the four transect lines and the five quadrats in each transect. Source: Google Earth Pro (2019)

Collection and Preservation of Fungi

The collection was conducted twice, for three days each, from November to December 2018. The species of Agarics and Polypores were collected in each quadrat along the transect lines with a minimum

of three specimens per species. Opportunistic sampling was integrated in the collection wherein the species outside the quadrat were not included in the diversity measurement and were only included in the species listing.

Woody species were collected using a bolo (weapon of Philippine origin described as a long, heavy, single-edged knife that is used for cutting vegetation) while fleshy species were collected using a knife or pair of scissors. Following collection, the Agarics were stored in Ziploc bags while the collected Polypores were wrapped in newspaper and were placed in a cooler with ice (approximately 10-12°C) during transport until it reached the laboratory. This is done to preserve the macromorphological characteristics and to preserve the structural integrity of the species that are needed for identification of both fungal groups. The fruiting bodies of the collected Agarics and Polypores were preserved by air-drying. For this study, the researchers relied on different documentation strategies, such as photographs and written description, for the identification of Agarics and Polypores.

The species of Agarics that were listed, documented, and identified in the data notebook were specific to macrofungi with fruiting bodies that were visible and accessible, and those with gills or lamellae underside its caps. The species of Polypores were specific to those with rigid and tough texture, shelf-like appearance, and pores located on the underside of the spore-bearing surface (hymenium). These species were photographed *in situ*. The documentation comprised codes, date, tentative identification, physical characteristics (such as form, texture, size, color, and odor), and other special features. The Collection Code used for each morphospecies was alphabetical for both Agarics and Polypores (MFSLP-001 as code for the first morphospecies of Polypores, and MFSLA-001 as the first morphospecies of Agarics at the Molave Forest of San Fernando City, La Union).

The Location and Sample Code used were based on an alphanumeric identification code with the first letter corresponding to the transect (t), the second letter for quadrat (q) and the last letter for the sample (s). A specimen labeled "t1 q1 s1" is the first sample in the first quadrat of transect one. If a similar morphospecies was found in the same quadrat of the same transect, it was also labeled "t1 q1 s1." Both Agarics and Polypores used the code (t_ q_ s_) and the only difference was the color of the string tied in the code, which was blue for Polypores and yellow for Agarics.

Identification of Macrofungi

Macrofungal samples were identified based on their observed macroscopic characteristics such as their gills (Agarics), fruiting bodies (Agarics and Polypores), and sporophores (Polypores). Spore prints were used for easier identification of Agaric species. It was done by removing the stalk from the cap then, placing the cap gill side down on a white paper for several hours with a bowl covering the cap to maintain its moisture for the spores to be discharged.

The samples were identified using reference books such as The Book of Fungi by Roberts and Evans (2011), Illustrated Philippine Fungi by Quimio (1998), and Mushrooms & Other Fungi by Lawrence and Harniess (2007). One of the co-authors, Dr. Edwin R. Tadiosa, a resident mycologist in the National Museum of Natural History, verified the identifications.

Determination of the Most Important Species

The density of macrofungi can be measured and evaluated through the data from the quadrat method. These include many factors that are important in ecological studies such as density, relative

density, abundance, relative abundance, frequency, relative frequency, importance value, and importance percentage. The formulas used for density, abundance, and frequency were based on Krishnappa et al. (2014) and are shown in Table 1.

Table 1. Formulas for quantitative estimation of density.

Formula	Definition	Equation
Density	Total number of fruiting bodies per species in all transects divided by the number of transects.	$\frac{Total\ number\ of\ fruiting\ bodies\ per\ species\ in\ all\ quadrats}{Total\ number\ of\ quadrats\ studied}$
Relative Density		$\frac{Density\ of\ species\ A}{Total\ density\ for\ all\ species} x100$
Abundance	Total number of fruiting bodies in all transects over the number of transects where the species occur.	$\frac{Total\ number\ of\ fruiting\ bodies\ per\ species\ in\ all\ quadrats}{Number\ of\ quadrats\ of\ species\ occurrence}$
Relative Abundance		$\frac{Abundance\ of\ species\ A}{Total\ abundance\ for\ all\ species} x100$
Frequency	The extent of dispersion of each species in a given site. The value is often presented in percentage form.	$\frac{Number\ of\ quadrats\ of\ species\ occurrence}{Total\ number\ of\ quadrats\ studied}$
Relative Frequency		$\frac{Frequency\ value\ of\ species\ A}{Total\ frequency\ values\ for\ all\ species} x100$
Importance Value	The importance value takes into consideration the sum of the three parameters: relative density, relative dominance and relative frequency wherein the average of this importance value would give an estimation of the importance percentage of a species.	$Relative\ density + Relative\ abundance + Relative\ frequency$
Importance Percentage		$\frac{Importance\ value}{3}$

Evaluation of Macrofungal Diversity

Diversity of macrofungi in the study site was estimated using Simpson's Diversity Index and Shannon-Wiener Index. The formulas used for the diversity indices based on Curtis & Mcintosh (1950) are shown in Table 2.

Table 2. Formula of Simpson's Diversity Index and Shannon-Wiener Index.

Simpson's Diversity Index	***Shannon-Wiener Index***
$D' = 1 - \sum_{i=1}^{S} \frac{n_i(n_i - 1)}{N(N-1)}$	$H' = -\sum_{i=1}^{S} (P_i(lnlnP_i)$
Where: S = the number of species ni = the abundance of the nth species N = the total abundance of each species	Where: S = the number of species Pi = the proportion of the ith species

Description of Relationship between Diversity and Physical factors

During the study, the following physical factors present in the study site at the time of collection were acquired: latitude, altitude, temperature, and the amount of rainfall. The latitude and altitude were determined using a global positioning system (GPS) while the temperature, humidity, and precipitation were acquired using the Philippine Atmospheric, Geophysical, and Astronomical Services Association (PAG-ASA) website with the link http://bagong.pagasa.dost.gov.ph.

Extraction of Fuscoporia torulosa MFSLP-12 samples

Fuscoporia torulosa MFSLP-12 were cleaned and air dried for one week at ambient temperature prior to extraction. Dried specimens were stored in airtight plastic bags with 2-3 packs of silica beads (2 grams each) to prevent accumulation of moisture. Extraction protocol by Su et al. (2013) was followed with some modifications. About 100 grams of dried sample were subjected to exhaustive and sequential extraction with hexane, ethyl acetate, and methanol. First, it was soaked in an Erlenmeyer flask with 250 mL of hexane (99+%, analytical grade, Chemline Scientific) for 2 days at 12-hour, 4-hour, and another 4-hour interval. Between intervals, the solution was filtered and refilled with the same solvent. The filtrates were subjected to rotary evaporation. Final products were pooled in a small vial and evaporated to dryness in a water bath. The vial was refrigerated at 4°C until further use. Following extraction with hexane, same procedures were conducted using ethyl acetate (99+%, analytical grade, Chemline Scientific) and methanol (99+%, analytical grade, Chemline Scientific). Stock solutions of 5 mg/ml were prepared by dissolving the extracts in a 0.02 mM phosphate buffer with pH 6.9.

Assay for α-Amylase and α-Glucosidase Inhibition

The α-amylase assay was carried out according to the method described by Banerjee et al. (2017) with minor modifications. In brief, 25 µl of varying concentrations of each extract (0.125, 0.25, 0.5, 1, 2 mg/ml) was added to 50 µl of α-amylase (0.275mg/ml). The solution was incubated at 37ºC for 20 minutes. Prior to another incubation at 37ºC for 10 minutes, 25 µl of starch solution (1%) was added. Then, 10 µl of DNS color reagent was added and the solution was immersed in a boiling water bath for five minutes to

stop the reaction. Each solution was diluted with 550 µl of distilled water. Finally, 200 µl of the final mixture was transferred to a 96-well plate and the absorbance was measured at 540 nm using an 96-well Microplate Reader (BMG LabTech, Japan).

To prepare for the α-glucosidase assay, extraction of α-glucosidase was first performed following the method of Hodoniczky et al. (2012). About 200 mg of rat intestinal acetone powder was dissolved in 6 ml of 0.9% NaCl solution. The solution was vortexed for three minutes prior to sonication for five minutes at approximately 20ºC. The solution was vortexed again for one minute followed by centrifugation for 30 minutes at 4ºC and 8000 rpm (Centrifuge Hermle Z326K; Rotor 220.78 V05). The pellet was discarded while the supernatant served as enzyme stock solution and was kept on ice until further use. Due to the solubility limitations of *α*-glucosidase, the estimated concentration is <200mg/6mL.The assay proper was conducted according to the method described by Telagari and Hullatti (2015) with few modifications. In a 96-well plate, 50 µl of 0.2mM phosphate buffer (pH 6.9), 10 µl of α-glucosidase, and 20 µl of varying concentrations of each extract (0.125, 0.25, 0.5, 1, 2 mg/ml) were mixed and incubated at 37°C for 15 minutes. Then, 20 µl of 5mM pNPG was added prior to another incubation at 37ºC for 20 minutes. Finally, 50 µl of 0.1M Na_2CO_3 was added. Absorbance was measured at 405 nm.

For both assays, acarbose at various concentrations (0.125 to 2 mg/ml) was used as standard while a mixture containing buffer in place of extract or acarbose served as negative control. Blank solution of each reaction mixture was prepared by replacing the enzyme and starch solution with equal volumes of buffer. Results were expressed as percent inhibition, which was calculated using the formula:

$$\% \, Inhibition = \frac{\Delta A_{control} - \Delta A_{extract}}{\Delta A_{control}} \times 100$$

Where: ΔA is the absorbance of the reaction mixture containing enzyme subtracted by the absorbance of the blank.

Preliminary Mycochemical Screening

The same extracts of *Fuscoporia torulosa* MFSLP-12 (hexane, ethyl acetate, and methanol extracts) were subjected to qualitative mycochemical screening of reducing sugars, alkaloids, glycosides, steroids, mycosterols, terpenes, terpenoids, quinones, anthraquinones, cyanins, coumarins, flavonoids, saponins, phenols, and tannins according to the methods described by Tiwari et al. (2011). The method described is composed of the following tests: Benedict's Test and Fehling's Test for the detection of reducing sugars; Wagner's Test, Hager's Test, and Dragendorff's Test for the detection of alkaloids; Modified Borntrager's Test and Keller-Kiliani Test for glycosides; Libermann Burchard Test to detect steroids and phytosterols; Salkowski's Test for detection of terpenes and terpenoids; Sulfuric Acid Test for the detection of quinones; Hydrochloric Acid Test for anthraquinone; Alkaline Reagent Test for coumarins, Alkaline Reagent Test for flavonoids; Froth Test for the detection of saponins; and Ferric Chloride Test for phenols and tannins.

Statistical Analysis

SPSS statistical package was used for statistical analysis. For α-amylase inhibition, results were expressed as mean ± SE for triplicate determinations whereas for α-glucosidase inhibition, results were expressed as mean ± SE for duplicate determinations. IC_{50} values were calculated using GraphPad Prism 7 statistical package. SE is standard error of the mean.

The data for α-amylase inhibition were analyzed by one-way analysis of variance. Assumptions for the test included normality and homogeneity of variance of the data which was done using the Shapiro-Wilk test and Levene's Statistic, respectively. Since the homogeneity of variance was not met according to Levene's Statistic, Games-Howell Test was used as the post-hoc test to determine significant differences between the means of samples and standard.

Since the data obtained for α-glucosidase was only in duplicates, it was analyzed using the Kruskal Wallis test which is a nonparametric alternative for ANOVA. Then, the Mann-Whitney U test was performed to identify the significant differences between the samples.

RESULTS AND DISCUSSION

Identification of Macrofungi

A total of 1,064 Polypore individuals were counted, collected, identified and classified into 40 different morphospecies as shown in Table 3. The species were classified into ten different families. The most abundant by number of individuals (fruiting bodies) was *Microporus xanthopus* (Fr.) Kuntze MFSLP-18 (18.37%) with 363 individuals, followed by *Hexagonia tenuis* (Hook) Fr. MFSLP-15 (5.14%) with 72 individuals, and *Corticium* sp. MFSLP-5 (4.78%) with 65 individuals (see Plate 1). Other species of Polypores that were identified are shown in Table 3.

Table 3. Importance percentage per species of Polypores encountered in San Fernando's Molave Forest.

Morphospecies	Importance Percentage (%)	Morphospecies	Importance Percentage (%)
Microporus xanthopus (Fr.) Kuntze MFSLP-18	18.36652	*Polyporus badius* (Pers.) Schwein. MFSLP-24	1.24255
Corticium sp. MFSLP-6	5.36565	*Stereum rugosum* (Pers.) Fr. MFSLP-33	1.24255
Microporus xanthopus (Fr.) Kuntze MFSLP-18	18.36652	*Polyporus badius* (Pers.) Schwein. MFSLP-24	1.24255
Corticium sp. MFSLP-6	5.36565	*Stereum rugosum* (Pers.) Fr. MFSLP-33	1.24255
Radulomyces confluens (Fr.) M. P. Christ. MFSLP-32	5.25715	*Polyporus* sp. MFSLP-26	1.23816
Hexagonia tenuis (Hook). Fr. MFSLP-15	5.13815	*Fomitiporia punctata* (Pilat) Murrill MFSLP-11	1.16829
Earliella scabrosa (Pers.) Gilb. & Ryvarden MFSLP-9	5.04015	*Fuscoporia torulosa* (Pers.) T. Wagner & M. Fisch *MFSLP-12*	1.16829
Corticium roseum Pers. MFSLP-3	4.947	*Ganoderma applanatum* (Pers.) Pat. MFSLP-13	1.16829
Corticium sp. MFSLP-5	4.78426	*Microporus* sp. MFSLP-17	1.13405
Corticium sp. MFSLP-4	4.76761	*Polyporus* sp. MFSLP-28	1.13405
Corticium polygonoides P. Karst. MFSLP-2	4.28063	*Pulcherricium* sp. MFSLP-31	1.13405
Meruliporia incrassata (Berk. & M.A. Curtis) Murrill MFSLP-16	3.82341	*Favolus alveolaris* (DC.) Quel. MFSLP-10	0.95868

Table 3 (Continued). Importance percentage per species of Polypores encountered in San Fernando's Molave Forest.

Morphospecies	Importance Percentage (%)	Morphospecies	Importance Percentage (%)
Poria sp. MFSLP-30	3.3041	*Phellinus* sp. MFSLP-22	0.95868
Poria sp. MFSLP-29	2.91508	*Stereum sp.* MFSLP-34	0.80854
Trametes hirsuta (Wulf) Lloyd. MFSLP-38	2.30702	*Hexagonia* sp. MFSLP-14	0.70004
Trametes villosa (Sw.) Kreisel MFSLP-39	1.93688	*Daedalea quercina* (L.) Pers. MFSLP-8	0.59154
Trametes gibbosa (Pers) Fr. MFSLP-37	1.89357	*Phellinus gilvus* (Schwein.) Pat. MFSLP-19	0.59154
Daedalea ambigua Berk. MFSLP-7	1.86452	*Polyporus alveolaris* (DC.) Bondartsev & Singer MFSLP-23	0.59154
Phellinus linteus Berkeley & M.A. Curtis MFSLP-20	1.69354	*Thelephora terrestris* Ehrh. MFSLP-35	0.59154
Trametes flavida (Lev.) Zmitr., Wasser & Ezhov MFSLP-36	1.51765	*Trametes versicolor* (L.) Pilat. MFSLP-40	0.59154

(a)*Microporus xanthopus* (Fr.) Kuntze **(b)***Hexagonia tenuis* (Hook.) Fr. **(c)** *Corticium* sp. 2

Plate 1. The top three most abundant species of Polypores based on the number of individuals.

A total of 266 Agaric species were counted and collected and were identified and classified into 16 different morphospecies as shown in Table 4. The species were classified into eight different families. The most abundant by number of individuals was *Mycena* sp. (Pers.) Roussel MFSLA-7 (16.45%) with 36 individuals, followed by *Panus rudis* Fr. MFSLA-10 (14.86%) with 32 individuals, and *Schizophyllum commune* Fr. MFSLA-11 (10.47%) with 21 individuals (see Plate 2)**.** Other species of Agarics that were identified are shown in Table 4.

(a) *Mycena* sp. 1(Pers.) Roussel **(b)** *Panus rudis* Fr. **(c)** *Schizophyllum commune* Fr.

Plate 2. The top three most abundant species of Agarics based on the number of individuals.

Table 4. Importance percentage per species of Agarics encountered in San Fernando's Molave Forest

Morphospecies	Importance Percentage (%)	Morphospecies	Importance Percentage (%)
Mycena sp. (Pers.) MFSLA-7	16.45459	*Marasmiellus* sp. Murrill MFSLA-5	4.07934
Panus rudis Fr. MFSLA-10	14.85778	*Collybia* sp. (Fr.) Staude MFSLA-1	2.48253
Schizophyllum commune Fr. MFSLA-11	10.46657	*Coprinus* sp. Pers. MFSLA-2	2.48253
Marasmiellus sp. Murrill MFSLA-4	8.86976	*Mycena* sp. (Pers.) MFSLA-8	2.48253
Marasmiellus sp. Murrill MFSLA-3	8.47056	*Stropharia sp*. (Fr.) Quel. MFSLA-12	2.48253
Marasmiellus sp. Murrill MFSLA-6	7.27295	*Tricholoma* sp. Fries MFSLA-14	2.48253
Panaeolus semiovatus (Sowerby) S. Lundell & Nannf MFSLA-9	6.87375	*Tricholomopsis rutilans* (Schaeff.) Singer MFSLA-15	2.48253
Stropharia sp. (Fr.) Quel. MFSLA-13	5.27695	*Volvariella* sp. Speg. MFSLA-16	2.48253

This study focused on Agarics and Polypores only while the study of Tadiosa and Arsenio (2014) focused mainly on wood-rotting Basidiomycetes. A difference in the frequently occurring species in the site was observed between the 2014 and the present study. *Ganoderma applanatum* (Pers.) Pat., *Schizophyllum commune*, *Hexagonia tenuis*, and *Polyporus xanthopus* Fr. were the species recorded both in the 2014 and present survey while *Ganoderma lucidum* (Curtis) P. Karst., the most frequently occurring fungi during the 2014 survey, was not found in any transect in the duration of the present survey. The differences in frequently occurring macrofungi and abundance of macrofungi between the mentioned years may be attributed to the limited time that the study was conducted, the previous study by Arsenio and Tadiosa (2014) was conducted for 12 months compared to our study which was only conducted for two months. According to Salerni et al. (2002), there is a need to study a specific site for around ten years or more in order to determine different successions present in the site.

Determination of the Most Important Species

The importance value measures the overall impact of a certain species in a given site. Three community attributes are needed for the computation of the importance value: relative density, relative

abundance, and relative frequency (Krishnappa et al., 2014).

Relative density measures the number of fruiting bodies of a single species in all the quadrats divided by all the number of quadrats. It determines the predominant species that contributes to the competition of species in the site. High competition for resources of highly dense species limits the growth and survival of other species (Krishnappa et al., 2014). The species with the highest relative density recorded was *Microporus xanthopus* MFSLP-18 for Polypores and *Mycena* sp. MFSLA-7 for Agarics. This means that these species predominate the study site in terms of competition for resources due to the high number of fruiting bodies in all the quadrats.

The relative abundance, on the other hand, provides the total number of fruiting bodies in all the quadrats for a species over the number of quadrats where the species occur. It focuses on the degree of closeness and dispersion of the same species per area of occurrence. The species with the highest relative abundance recorded was *Corticium* sp. MFSLP-5 for Polypores and *Mycena* sp. MFSLA-7 for Agarics. This means that species were more clumped and therefore have a high number of fruiting bodies per area of occurrence.

Lastly, relative frequency is defined as the number of quadrats where a species occurs divided by the total number of quadrats. This measures the degree of uniformity with which individuals are distributed, and therefore reflects the possibility of encountering a certain organism in a given site (Krishnappa et al., 2014). The species with the highest relative frequency recorded was *Microporus xanthopus* MFSLP-18 for Polypores. For the Agarics, all species had the same value of relative frequency. This means that these species are evenly distributed in all quadrats of the site.

The species with the highest importance value is the most important species present in the site or a given ecosystem due to the high average of the three parameters that are all ecologically significant. A high value in any of the parameters will not automatically equate to a high value in importance value because the most important species of a given site is dependent on the totality of the density measurements (Dash, 1993). The implication of the most important species is the determination of what species define the study site.

As seen in Table 3, *Microporus xanthopus* MFSLP-18 was the most important species for Polypores and therefore defines the overall Polypores that thrive in the site. The morphology of *M. xanthopus* was first described by Corner (1932) and the morphological features of *M. xanthopus* make spore dispersal easier. The fruiting bodies are known to be funnel-shaped and have concentric zones of varying shades of brown. The foot of the stem of *M. xanthopus* has a distinct yellow color and the pores present on the underside are minute. Large samples are seen in damp areas with a dark shade whereas smaller samples can be seen during dry periods (Corner, 1932). It is one of the well-known and most studied wood-rotting fungi and has the potential to be used for pollutant purification and soil bioremediation (Lyngdoh and Dkhar, 2014). *Microporus xanthopus* also has the ability to thrive in most of the climates uniformly, which may explain the highest sum of its relative density and relative frequency. In fact, *Microporus xanthopus* was absent only in transect 3, which was the transect closest to road-widening. The absence of *Microporus xanthopus* MFSLP-18, therefore, may be used as an indicator of man-made disturbances.

As shown in Table 4, *Mycena* sp. MFSLA-7 was the most important species for Agarics and was the indicator and representative of all the Agarics in the study site. *Mycena* sp. has a cone or bell shape. It has a thin stem that is often white, gray, or brown in color. They are often seen growing in clusters on rotten stumps and soil (Kuo, 2007).

Majority of macrofungal species are observed to be distributed from all over the globe and are often associated with lignin, cellulose, and hemicellulose degradation in forests. The distribution of *Mycena* sp. can be attributed to the pH of the soil. Specific *Mycena* species thrive on acidic soils whereas some thrive on alkaline soils. The acidity or alkalinity of the soil can also determine the type of vegetation that may thrive in a forest. Majority of plant species thrive at optimum soil pH because most nutrients become easily available for plant use, which in turn can increase the number of substrates or hosts for macrofungal growth, hence, higher diversity. The ability of *Mycena* sp. MFSLA-7 to thrive and absorb more nutrient-rich molecules from the soil in varying pH as compared to other Agarics may provide an explanation as to the observed highest sum of relative density and relative abundance for the species (Tyier, 1991).

Evaluation of Macrofungal Diversity

Simpson's index measures the probability that two individuals of Agarics and Polypores randomly selected from the sample area belong to the same category. The values range from zero to one. As the value approaches one, the species richness increases. The value obtained for both the Agarics and Polypores showed a high species diversity, with a value of 0.8720 for the Agarics and 0.8597 for the Polypores as shown in Table 5.

Table 5. Simpson's index value and Shannon Wiener index values of Agaric and Polypore species in San Fernando's Molave Forest.

Macrofungi	***Simpson's Diversity Index (D')***	***Shannon Wiener Index (H')***
Agarics	0.8720	2.2185
Polypores	0.8597	2.6933

Shannon-Wiener Index measures both richness and evenness of Agarics and Polypores. The range that can be obtained from computing the index is from 0 to 4 and as the values approach 4, the species diversity increases (Smith and Wilson, 1996). Using this formula, it showed that both Agarics and Polypores species were diverse with a value of 2.2185 for the Agarics and 2.6933 for Polypores as shown in Table 6.

Table 6. The IC_{50} for α-amylase and α-glucosidase inhibition by *Fuscoporia torulosa* MFSLP-12 extracts and acarbose.

Sample	**IC_{50} (mg/ml)**	**IC_{50} (mg/ml)**
	α -amylase	**α -amylase**
Acarbose	0.217±4.357	0.217±4.357
Methanol	1.978±0.775	1.978±0.775
Ethyl acetate	Cannot be determined	Cannot be determined
Hexane	No inhibition	No inhibition

Using the Simpson's Diversity Index, it can be seen in Table 5 that the Agarics were more diverse in the site than the Polypores. Using the Shannon-Wiener Index, the Polypores were more diverse in the site than Agarics as shown in Table 5. The Shannon-Wiener Index takes into account both species richness

and evenness, whereas the Simpson's Diversity Index takes into account only species evenness (Kerkhoff, 2010). Both diversity indices are widely used and are easier to calculate in comparison to other diversity indices as explained by Yeom and Kim (2011). However, there are also disadvantages to each. The index value of Shannon-Wiener is influenced more by species richness than species evenness, making the index an insensitive measure of the distribution of species in the site. On the other hand, Simpson's Diversity index is influenced more by the abundance and evenness of species in the site (Magurran, 1988). Having a low value in Simpson's index means that there is an abundance of species while having a value closer to 1 means that each species is evenly distributed. That is why there is a need to use at least two diversity indices for comparative studies in order to take into account both species richness and evenness with reduced bias (Kerkhoff, 2010).

The Agarics and Polypores in the Molave Forest of San Fernando, La Union are diverse as implied by the high richness and evenness of both groups, wherein no specific species dominates the place. This may be attributed to the physical factors present in the site.

Inhibition of α-Amylase and α-Glucosidase by Fuscoporia torulosa MFSLP-12 Extracts

Among the species collected, *Fuscoporia torulosa* MFSLP-12 was chosen for the *in vitro* determination of α-amylase and α-glucosidase inhibitory potential. As previously mentioned, the availability of sufficient fungal mass for subsequent extraction and mycochemical screening, and the absence of studies that evaluate the species' antihyperglycemic property, were the bases for choosing *F. torulosa* MFSLP-12 for testing. Out of the three extracts of *F. torulosa* MFSLP-12 tested, the methanol extract showed statistically significant results. It produced the highest inhibition in both α-amylase and α-glucosidase activities as seen in Figures 2 and 3. At the highest concentration used (2mg/ml), it inhibited α-amylase by 38% (SEM: 2.840), and α-glucosidase by 56% (SEM: 1.087). It was about 23% and 8% less effective α-amylase and α-glucosidase inhibitor, respectively, than acarbose. Moreover, it produced a dose-dependent inhibition on both enzymes. These data suggest that although methanol extract produced weaker inhibition than acarbose, it may efficiently inhibit the two enzymes at high concentrations. Hexane extract did not inhibit α-amylase in all concentrations, but it inhibited α-glucosidase in all concentrations. However, it was less efficient than methanol extract and it did not display a dose-dependent inhibition. Ethyl acetate extract only inhibited α-amylase activity at 0.25 mg/ml, but it inhibited α-glucosidase in all concentrations. Similar to hexane extract, it was less efficient than methanol extract and it did not display a dose-dependent inhibition. It is possible that this observation is due to stochastic effects of ethyl acetate solvent. However, this study cannot confirm this at this point. Values obtained reveal that the methanol extract can strongly inhibit α-amylase activity while hexane and ethyl acetate extracts were weak α-glucosidase inhibitors even at high concentration.

To further evaluate the inhibitory potency of the extracts against α-amylase and α-glucosidase, IC_{50} values were obtained and results were shown in Table 6. Sörme et al. (2003) stated that IC_{50} values of test samples in a specific assay should be discussed in relation to a reference compound in the same experiment, because these values may vary from one assay to another due to certain conditions. In this study, IC_{50} values of 1.978 mg/ml (against α-amylase) and 1.270 mg/ml (against α-glucosidase) were obtained from methanol extract. Compared to acarbose ($IC_{50:}$ 0.261 mg/ml), methanol extract was a less potent α-amylase inhibitor by about nine folds and a less potent α-glucosidase inhibitor by about five folds. This indicates that although methanol extract can efficiently inhibit α-amylase and α-glucosidase activities, acarbose was still the more potent inhibitor since lesser amount of acarbose compared to methanol extract is needed to elicit the desired response. This supports the results from Figure 2 and Figure 3 where methanol

extract was less efficient than acarbose in inhibiting enzyme activity in all concentrations. However, acarbose is a pure compound, while methanol extract is a crude mixture. Further purification may elicit lower IC_{50} values. An additional implication for the IC_{50} of the methanol extract is its stronger effect on α-glucosidase than α-amylase, which was supported by the percent inhibition obtained from methanol extract at its highest concentration (α-amylase:38%; α-glucosidase:56%). For hexane and ethyl acetate extracts, no dose-dependent inhibitory effects were observed, hence, IC_{50} cannot be determined by the statistical analysis employed.

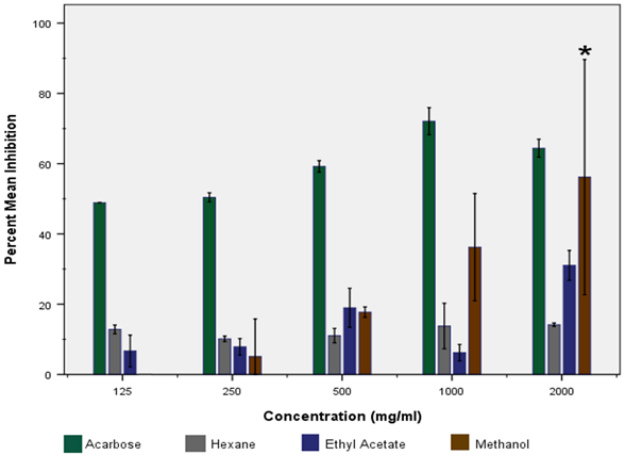

Figure 2. Percent inhibition of α-amylase by acarbose and the three *Fuscoporia torulosa* MFSLP-12 extracts at varying concentrations. Asterisk (*) denotes no statistically significant difference with acarbose at same concentration ($p>0.05$).

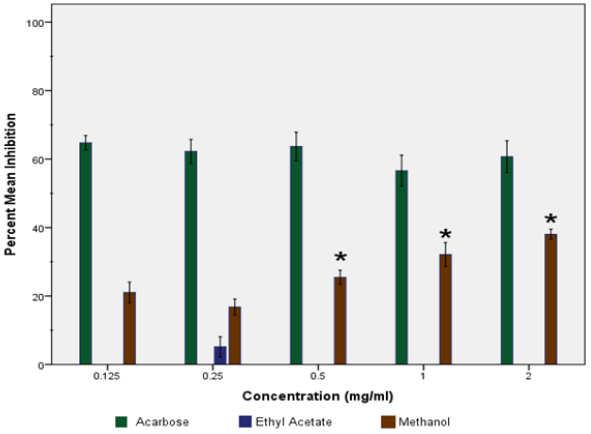

Figure 3. Percent inhibition of α-glucosidase by acarbose and the three *Fuscoporia torulosa* MFSLP-12 extracts at varying concentrations. Asterisk (*) denotes no statistically significant difference with acarbose at same concentration ($p>0.05$).

Effective treatment of type 2 diabetes mellitus entails moderate α-amylase inhibition with high α-glucosidase inhibition (Gowri et al., 2007). However, the standard drug acarbose strongly inhibits both enzymes. High inhibition against α-amylase results in gastrointestinal pain caused by abnormal bacterial fermentation of undigested carbohydrates in the colon (Hanefeld, 2007). In this study, the methanol extract produced a strong α-glucosidase inhibition and a slightly weaker α-amylase inhibition as supported by its IC_{50} values (1.270 mg/ml for α-glucosidase; 1.978 mg/ml for α-amylase). This indicated that methanol extract of *Fuscoporia torulosa* MFSLP-12 has the potential to reduce postprandial hyperglycemia while presenting less gastrointestinal side effects usually caused by excessive α-amylase inhibition.

Mycochemicals Present in the Extracts

Although IC_{50} value was only obtained from the methanol extract, the hexane and ethyl acetate extracts still displayed enzyme inhibition as seen in Figure 2 and Figure 3. In our study, solvents of different polarities (polar, semi-polar, non-polar) were used. Ethyl acetate is considered a semi-polar solvent in many studies (Rahardo et al., 2018; Yanuarti et al., 2017; Yeni et al., 2014). Therefore, mycochemical screening was conducted on all extracts to determine the mycochemicals present that may have contributed to the observed inhibition. The methanol extract of *Fuscoporia torulosa* MFSLP-12 composed of polar compounds showed the strongest α-amylase and α-glucosidase inhibition while the hexane extract (non-polar) and the ethyl acetate extract (semi-polar) showed significantly weaker to no inhibition of both enzymes. Similar results were obtained by Tulin et al. (2017) where compounds extracted from highly polar solvents strongly inhibited α-glucosidase while compounds extracted from non-polar solvents showed low to no inhibition. They even suggested that non-polar components in the ethanol extract may have masked the activity of α-glucosidase inhibitors.

As presented in Table 7, reducing sugars, alkaloids, cyanin, tannins, and phenols were the mycochemicals present in at least one of the extracts. While reducing sugars and alkaloids were detected in all extracts, cyanin was only present in the ethyl acetate extract, and phenols and tannins were only present in the methanol extract.

Table 7. Mycochemicals present in the three fruiting body extracts of *Fuscoporia torulosa* MFSLP-12 as determined by qualitative tests.

Phytochemical Constituent	Hexane	Ethyl acetate	Methanol
Reducing sugar	+	+	+
Alkaloids	+	+	+
Tannin	-	-	+
Phenol	-	-	+
Cyanin	-	+	-
Quinone	-	-	-
Anthraquinone	-	-	-
Terpenes and Terpenoids	-	-	-
Coumarin	-	-	-
Flavonoid	-	-	-
Saponin	-	-	-
Steroids and Phytosterols	-	-	-
Glycosides	-	-	-

+: detected; -: not detected

Most of the mychochemicals detected in the methanol extract of *Fuscoporia torulosa* MFSLP-12 have shown inhibition against *α*-amylase and/or *α*-glucosidase based on literature. These were tannins, phenols, and alkaloids. In a study by Lin et al. (2016), tannins and phenols were described to inhibit both *α*-glucosidase and *α*-amylase. Laoufi et al. (2017) even stated that the presence of phenols and tannins had a direct relationship with the inhibition of α-glucosidase and α-amylase activities. The ability of tannins to strongly bind to carbohydrates and proteins allows it to inhibit α-amylase and α-glucosidase. Moreover, hydroxide groups in tannins participate in hydrogen bonding with human α-amylase (Sales et al., 2012). Meanwhile, if phenols in the methanol extract also contributed to enzyme inhibition, chlorogenic acid seems to be the most plausible cause of inhibition. Phenolic compounds of *Fuscoporia torulosa* were already described in two separate studies and their findings revealed that *F. torulosa* contains the following phenols: benzoic acid, catechin, chlorogenic acid, gallic acid, *p*-coumaric acid, syringic acid, and trans-2-hydroxycinnamic acid (Bal et al., 2017; Deveci et al., 2019). Among the phenols listed, only chlorogenic acid is confirmed to be an α-amylase and α-glucosidase inhibitor (Kalita et al., 2018).

Alkaloids, which were present in all extracts, have also been described to have inhibitory effects against α-glucosidase (Laoufi et al., 2017). Zafar et al. (2016) revealed two alkaloids, oriciacridone F and O-methylmahanine, to demonstrate comparable inhibition with acarbose *in silico.* For reducing sugars, which were also detected in all extracts, no antihyperglycemic effects have been established. In fact, Gowri et al. (2007) even suggested that reducing sugars may accelerate α-amylase activity. Moreover, when medicinal properties of plants, or in this case macrofungi, are considered, presence of carbohydrates are generally not valued (Dash et al., 2017). For the cyanins that were only present in the ethyl acetate extract, no direct relationship with enzyme inhibition has been reported yet.

While the presence of the compounds in the three extracts of *Fuscoporia torulosa* MFSLP-12 suggests that they may be responsible for the observed key enzymes inhibition, their specific effect on the activities of α-amylase and α-glucosidase cannot be confirmed in this study because only qualitative data were obtained. Moreover, this study cannot confirm whether the observed strong enzyme inhibition was a result of synergistic, antagonistic, or additive effects among the compounds present.

CONCLUSION

A total of 16 species of Agarics belonging to eight families were collected accounting for 266 collections. In addition to this, 40 species of Polypores belonging to eight families were collected with a total of 1064 number of collections. The most important Agaric species was *Mycena* sp. 1 with an importance percentage of 16.45% and the most important Polypore species was *Microporus xanthopus* with an importance percentage of 18.34%. High species diversity of Agarics and Polypores at the Molave Forest of San Fernando City, La Union is an indication that no single macrofungal species dominates and that the physical factors present are sufficient to permit the growth of these species.

For the α-amylase and α-glucosidase inhibitory potential of the hexane, ethyl acetate, and methanol extracts of the chosen polypore species, *Fuscoporia torulosa* MFSLP-12, the methanol extract showed the maximum inhibitory activity on both α-amylase and α-glucosidase. Using the highest concentration, it inhibited α-amylase by 38%, and α-glucosidase by 56%. Compared to acarbose, its IC_{50} values were about ninefold higher for α-amylase and about fivefold higher for α-glucosidase. The slightly weaker α-amylase inhibition by methanol extract implies that it may present lesser side effects compared

to currently used synthetic drugs, hence, its potential to be an antihyperglycemic agent. Tannins, phenols alkaloids, cyanin, and reducing sugars were the mycochemicals present in at least one of the extracts. It will be interesting to discover their contribution to the observed activities.

RECOMMENDATIONS

Macrofungal studies should be conducted for a long period, ranging from monthly to annually in a span of ten years, in order to see more types and species of macrofungi. The study should be conducted during the wet season in order to get the most number of individuals present and to better estimate the diversity and density measures in a given site. It is also recommended that taxonomic and diversity studies be conducted in other regions of the country so that there can be an assessment of the overall macrofungal diversity of the Philippines. Molecular biology-based approaches in species identification may also be considered to provide confidence in the identification and address biases created from phenotypic characterization.

Meanwhile, to establish the effects of each compound found in the methanol extract of *Fuscoporia torulosa* MFSLP-12 on its ability to inhibit α-amylase and α-glucosidase activity, further study is needed. It is also important to determine whether the enzyme inhibition was a result of synergistic, antagonistic, or additive effects among the compounds present. These may be accomplished by separation and isolation of these compounds and testing their effects on α-amylase and α-glucosidase activities individually. If there was no synergistic or antagonistic effect between the compounds present, isolation and purification of the extract components would allow concentrating of only the important compounds that can inhibit the enzymes. This may result in stronger inhibitory effect, suggesting that semi-purified to purified compounds may even be more potent than the crude extracts. A subsequent cytotoxicity test on isolated compounds is also recommended to determine if it is safe for *in vivo* and clinical trials. If the bioactive compounds from *F. torulosa* MFSLP-12 are developed into antihyperglycemic drugs, its cultivation would be the most efficient way to obtain sufficient amounts of these compounds. Several studies have already shown that *F. torulosa* can be cultured in different media (Gorka et al., 2017; Campanile and Luisi, 2004; Tomsovsky and Jankovsky, 2007).

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STATEMENT OF AUTHORSHIP

Dr. Balolong, Dr. Tadiosa, and Mr. Mancera initiated the concept. Dr. Yu added the metabolic screening concept. Dr. Tadiosa supervised the field experiment and verified the identities of the collected macrofungi species. Ms. Lazo, Mr. Salazar, Ms. Alcantara, and Mr. Calupitan prepared the conceptual framework, did literature search, conducted the field experiment, and identified the macrofungal isolates. All authors contributed to the preparation of the manuscript.

REFERENCES

Alam, F., Islam, M. A., Kamal, M. A., and Gan, S. H. (2018). Updates on managing type 2 diabetes mellitus with natural products: towards antidiabetic drug development. *Current Medicinal Chemistry* 25: 5395-5431.

Bal, C., Akgul, H., Sevindik, M., Akata, I., and Yumrutas, O. (2017). Determination of the anti-oxidative activities of six mushrooms. *Fresenius Environmental Bulletin*, 26(10): 6246-6252.

Balun, R. (2019). The Importance of Species Diversity to the Ecosystem. Retrieved April 14, 2019, from https://sciencing.com/importance-species-diversity-ecosystem-6508788.html

Chipiti, T., Ibrahim, M. A., Singh, M., and Islam, M. S. (2017). In vitro α-amylase and α- glucosidase Inhibitory and Cytotoxic Activities of Extracts from *Cissus cornifoliaplanch* parts. *Pharmacognosy Magazine* 13(50): 329-333.

Corner, EJH. (1932). The Fruit-body of *Polystictus xanthopus*Fr. *Annals of Botany* 46: 71-111 & Plate V.

Curtis, J. T., & Mcintosh, R. P. (1950). The interrelations of certain analytic and synthetic phytosociological characters. *Ecology* 31(3): 434-455.

Dash, M.C. (1993). *Fundamentals of ecology*, Tata McGraw-Hill Publishing Limited, New Delhi.

Dash, S. P., Dixit, S., and Sahoo, S. (2017). Phytochemical and biochemical characterizations from leaf extracts from *Azadirachta indica*: An important medicinal plant. *Biochemistry & Analytical Biochemistry* 6: 323.

Deveci, E., Tel-Çayan, G., and Duru, M. E. (2019). Evaluation of phenolic profile, antioxidant and anticholinesterase effects of *Fuscoporia torulosa*. International Journal 6(1): 79-89.

Gowri, P. M., Tiwari, A. K., Ali, A. Z., and Rao, J. M. (2007). Inhibition of α-glucosidase and amylase by bartogenic acid isolated from *Barringtonia racemosa*Roxb. seeds. *Phytotherapy Research* 21(8): 796-799.

Hanefeld, M. (2007). Cardiovascular benefits and safety profile of acarbose therapy in prediabetes and established type 2 diabetes. *Cardiovascular Diabetology* 6(1): 20.

Hodoniczky, J., Morris, C. A., and Rae, A. L. (2012). Oral and intestinal digestion of oligosaccharides as potential sweeteners: A systematic evaluation. *Food Chemistry* 132(4): 1951-1958.

Hou, W., Li, Y., Zhang, Q., Wei, X., Peng, A., Chen, L., and Wei, Y. (2009). Triterpene acids isolated from *Lagerstroemia speciosa*leaves as α-glucosidase inhibitors. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives* 23(5): 614-618.

Kalita, D., Holm, D. G., LaBarbera, D. V., Petrash, J. M., and Jayanty, S. S. (2018). Inhibition of α-glucosidase, α-amylase, and aldose reductase by potato polyphenolic compounds. *PLoS One* 13(1): e0191025.

Kerkhoff, L. V., (2010). *Measuring biodiversity of ecological communities* [Worksheet].

Krishnappa, M., Swapna, S., & Syed, A. B. R. A. R. (2014). Diversity of macrofungal communities in Chikmagalur District of Western Ghats, India. *In Proceedings of the 8th International Conference on Mushroom Biology and Mushroom Products (ICMBMP8).*

Kuo, M. (2007). Key to major groups of mushrooms. Retrieved April 24, 2019, from http://www.mushroomexpert.com/major_groups.html

Laoufi, H., Benariba, N., Adjdir, S., and Djaziri, R. (2017). In vitro α-amylase and α-glucosidase inhibitory activity of *Ononis angustissima*extracts. *Journal of Applied Pharmaceutical Science* 7: 191-198.

Lin, D., Xiao, M., Zhao, J., Li, Z., Xing, B., Li, X., Kong, M., Li, L., Zhang, Q., Liu, Y. and Chen, H. (2016). An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. *Molecules* 21(10): 1374.

Lyngdoh, A. & Dkhar M.S. (2014). Wood-rotting fungi in East Khasi Hills of Meghalaya, Northeast India, with special reference to *Heterobasidion perplexa* (a rare species - new to India). *Current Research in Environmental & Applied Mycology* 4: 117–124.

Magurran, A. E. (1988). *Ecological diversity and its measurement.* Princeton University press.

Mitchell K. (2010) Quantitative analysis by the point-centered quarter method. Hobart and William Smith Colleges. *arXiv preprint arXiv:1010.3303*.

Rubilar, M., Jara, C., Poo, Y., Acevedo, F., Gutierrez, C., Sineiro, J., and Shene, C. (2011). Extracts of Maqui *(Aristotelia chilensis*) and Murta (*Ugni molinae*Turcz.): sources of antioxidant compounds and α-Glucosidase/α-Amylase inhibitors. *Journal of Agricultural and Food Chemistry* 59(5): 1630-1637.

Salerni, E., Laganà, A., Perini, C., Loppi, S., & Dominics, V. D. (2002). Effects of temperature and rainfall on fruiting of macrofungi in oak forests of the Mediterranean area. *Israel Journal of Plant Sciences 50*(3): 189-198.

Sales, P. M., Souza, P. M., Simeoni, L. A., Magalhães, P. O., and Silveira, D. (2012). α-Amylase inhibitors: a review of raw material and isolated compounds from plant source. *Journal of Pharmacy & Pharmaceutical Sciences* 15(1): 41-183.

Sengupta, S., Mukherjee, A., Goswami, R., & Basu, S. (2009). Hypoglycemic activity of the antioxidant saponarin, characterized as α-glucosidase inhibitor present in *Tinospora cordifolia*. *Journal of Enzyme Inhibition and Medicinal Chemistry* 24(3): 684-690.

Smith B, Wilson J.B. (1996). A consumer's guide to evenness measures. *Oikos* 76: 70–82.

Sörme, P., Kahl-Knutsson, B., Wellmar, U., Magnusson, B. G., Leffler, H., and Nilsson, U. J. (2003). Design and synthesis of galectin inhibitors. *Methods in Enzymology* 363: 157-169.

Tadiosa, E. R., and Arsenio, J. S. (2014). A taxonomic study of wood-rotting Basidiomycetes at the Molave Forest of San Fernando City, La Union Province, Philippines. *Asian Journal of Biodiversity* 5(1).

Telagari, M., and Hullatti, K. (2015). In-vitro α-amylase and α-glucosidase inhibitory activity of *Adiantum caudatum*Linn. and *Celosia argentea* Linn. extracts and fractions. *Indian Journal of Pharmacology* 47(4): 425.

Tiwari, P., Kumar, B., Kaur, M., Kaur, G., and Kaur, H. (2011). Phytochemical screening and extraction: a review. *Internationale Pharmaceutica Sciencia* 1(1): 98-106.

Tulin, E. K. C. B., Loreto, M. T. P., and Tulin, E. E. (2017). Alpha-Glucosidase Inhibitory Activity and Fractionation of Bioactive Compounds from bark Extracts of Sibucao (Caesalpinia sappan L.) In the Philippines. *Pharmacognosy Journal* 9(3): 356-360.

Tyier, G. (1991). Ecology of the genus *Mycena*in beech (*Fagus sylvatica*), oak (*Quercus robur*) and hornbeam (*Carpinus betulus*) forest of S Sweden. *Nordic journal of botany* 11(1): 111-121.

Yeom, D. J., & Kim, J. H. (2011). Comparative evaluation of species diversity indices in the naturaldeciduous forest of Mt. Jeombong. *Forest Science and Technology* 7(2): 68-74.

Zafar, M., Khan, H., Rauf, A., Khan, A., and Lodhi, M. A. (2016). In Silico study of alkaloids as α-glucosidase inhibitors: hope for the discovery of effective lead compounds. *Frontiers in Endocrinology* 7: 153.