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Proximate composition and antioxidant activity of *Panaeolus antillarium*, a wild coprophilous mushroom

Dulay RMR[1*], Cabalar AC[2], De Roxas MJB[2], Concepcion JMP[2], Cruz NE[2], Esmeralda M[2], Jimenez N[2], Aguilar JC[2], De Guzman EJ[2], Santiago JQ[2], Samoy JR[2], Bustillos RG[2], Kalaw SP[1] and Reyes RG[1]

[1]Center for Tropical Mushroom Research and Development, Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines
[2]Nueva Ecija University of Science and Technology – San Isidro Campus, San Isidro, Nueva Ecija, Philippines

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Abstract

Panaeolus antillarium remains to be underutilized mycological resource in the Philippines. Thus, this present work established the nutraceutical and funtional attributes of this coprophilous mushroom. The mycochemical and proximate nutrient composition were analyzed and its antioxidant activity as affected by the different media and pH levels was studied. Radical scavenging activity and total phenolic content were used as parameters for antioxidant property. Extracts of *P. antillarium* contained appreciable amounts of alkaloids, saponins, and cardiac glycosides. Nutrient composition analysis revealed that mycelia had higher amounts of crude fiber (7.05 ± 0.04), crude fat (1.96 ± 0.06), moisture (11.85 ± 0.01) and total carbohydrate (61.12 ± 0.01) than its corresponding fruiting bodies. On the other hand, the fruiting bodies had higher amounts of ash (5.26 ± 0.03), crude protein (16.77 ± 0.01), and energy value (321.49 ± 0.04) than its mycelia. Potato broth significantly had the highest mean volume loss (14.5 ± 2.50 ml), mycelia weight (4.5 ± 0.65 g), scavenging activity (16.06 ± 0.51%) and phenolic content (25.07 ± 0.02 mg AAE/g sample). However, varying pH levels of potato broth did not significantly affect the mycelial growth, but pH 7.0 recorded the highest scavenging activity (17.39 ± 0.19%) and total phenolic content (25.11 ± 0.01 mg AAE/g sample). Herein, these significant data suggest that *P. antillarium* is another potential source of substances and nutrients with functional attributes such as antioxidant which strongly infuenced by different media and pH levels.

Keywords – *Panaeolus antillarium* – proximate analysis – mycochemicals – antioxidant – coprophilous mushrooms

Introduction

Functional foods are enriched or modified foods which are consumed as normal diet to provide healthful benefits (Patel et al. 2012). Mushrooms are considered as functional foods because they contain substances that might be used directly in diet and promote healthiness. They

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Corresponding Author: Rich Milton R. Dulay – e-mail – richmiltondulay@yahoo.com

are rich in protein, carbohydrates, minerals, vitamins, unsaturated fatty acids, phenolic compounds, tocopherols, ascorbic acid and carotenoids that fit the definition of food supplements (Reis et al. 2011). The medicinal benefits derived from mushrooms such as antifibrotic, anti-inflammatory, antidiabetic, antimicrobial activities, anti-tumor, anti-HIV, anti-Alzheimer, anti-malarial, blood sugar lowering, cholesterol reducing, and liver protectant have been previously reported (Smith & Sullivan 2003, De Silva et al. 2012a, De Silva et al. 2012b, De Silva et al. 2013).

In addition to their medicinal importance, mushrooms have also been reported to exhibit significant antioxidant and free radical scavenging activities. These include *Agaricus bisporus*, *Hericium erinaceus*, *Flammulina velutipes*, *Lentinus edodes*, *Pleurotus eryngii*, *Pleurotus ostreatus*, *Agaricus blazei*, *Sparassis crispa*, *Phellinus linteus*, *Ganoderma lucidum*, and *Inonotus obliquus* (Fu et al. 2002, Lee et al. 2007). Antioxidants address various diseases by protecting the cells from oxidative stress; scavenging free radicals, chelating catalytic metals and halting lipid peroxidation chain reactions such as atherosclerosis, Alzheimer's disease, hepatopathic, nephropathic, retinopathic damage, cancer, adult respiratory distress syndrome and diabetes (Chennupati et al. 2012).

Panaelous antillarium is widely distributed in tropical countries such as the Philippines which is commonly found on the dung of cattle, carabao, goat, and horses, thus, it is considered as coprophilous or dung loving mushroom. The fruiting body is whitish to grayish at first and then becoming dark to mottled black once it matures. Its bitter and rancid taste makes it unworthy for eating but it is considered an edible species. The production technology of this coprophilous species has been successfully established by Bustillos et al. (2014) using the different formulations of the dried dung of ruminants in an aseptic cultivation condition.

With the increasing demand of functional foods, it is necessary to establish new sources such as wild edible mushrooms which could possibly provide beneficial effects to human health. Thus, this study was conducted to elucidate the different mycochemical and nutritional attributes of the mycelia and fruiting bodies of *P. antillarium* and to investigate the optimum submerged cultural conditions of this mushroom on the different indigenous broth media and various pH levels for its antioxidant activity based on the radical scavenging activity and total phenolics.

Materials and methods

Source and preparation of *P. antillarium* inocula

A pure culture of *P. antillarium* was obtained from the culture collections of the Center for Tropical Mushroom Research and Development (CTMRD), Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines and was aseptically transferred into sterilized potato dextrose agar (PDA) plates. Culture plates were incubated at 30 °C to allow growth of the mycelia for 7 days. Mycelial discs as inocula were prepared using flame sterile cork borer (10 mm diameter).

Mycelial mat and fruiting body production

The mycelia mat of *P. antillarium* was mass produced in a lawn culture using coconut water from newly cracked mature coconut (*Cocos nucifera*) as culture medium. A 100 ml of the medium was dispensed into microwavable plastic container, sterilized using an autoclave at 121C, 15 psi for 30 minutes and inoculated with mycelial discs. The 50 lawn culture containers were incubated at 32°C to allow mycelial growth. The mycelial mat was harvested and air-dried for analyses.

The mass production of fruiting bodies was done in aseptic cultivation using rice straw and sawdust (7:3 v/v) based formulation with 65% moisture content. Two hundred grams of formulated substrates were placed in a glass container, covered with polypropylene sheets, secured with rubber band and sterilized at 121°C, 15 psi for 45 minutes. Fifty sterilized substrates were inoculated with mycelia discs of *P. antillarium*, incubated at 32°C. Once completely colonized, the emergence of fruiting bodies was allowed. Matured fruiting bodies were harvested and air-dried for 3-4 days for analyses.

Proximate composition analyses

The chemical screening of the aqueous extracts of mycelia and fruiting bodies were carried out following the procedures described by Sofowora (1993). The different mycochemicals namely; alkaloids, cardiac glycoside, flavonoids, saponins, tannins and phlobatannins were analyzed. Results were compared with distilled water as control and determined based on the color/intensity of the reaction. Three replicates were laid out for each test parameter.

The crude protein, crude fat, crude fiber, ash, and moisture content (MC) were analyzed according to the guidelines of the Association of Official Analytical Chemist (AOAC 2002). The Soxhlet apparatus was used to determine the crude fat content, and the furnace at 550 °C for the ash. The crude protein was determined by Kjeldahl method, using the conversion factor N × 4.38. Total carbohydrate content was calculated as follows: total carbohydrates = 100 - (protein + fiber + fat + ash + MC). Energy value was computed as follows: energy value (kcal/100 g) = 4 × (g of protein + g of carbohydrates) + 9 × (g of fat).

Submerged cultivation of *P. antillarium* on different media and pH levels

The effect of the different broth culture media namely: coconut water from mature coconut (*Cocos nucifera*), rice bran decoction (50g of *Oryza sativa*/L of water), local yellow corn grit decoction (50g of *Zea mays*/L of water) and potato sucrose broth (250g of *Solanum tuberosum*/L of water+10g of white table sugar) on the antioxidant activity of *P. antillarium* was evaluated. Broth media (100 ml) were dispensed into microwavable plastic container, sterilized in an autoclave at 121C, 15 psi for 30 minutes, inoculated mycelial discs, and incubated at 32°C for 10 days. The mycelial mats were harvested and weighed and the volume loss of culture spent was measured. Culture spent and mycelia were homogenized using a food processor and then ethyl acetate (10 ml) was added into each cultured broth to extract antioxidant compounds. The ethyl acetate soluble portion was concentrated under reduced pressure and the concentrates were dissolved in ethanol for antioxidant analysis.

The most appropriate culture medium with optimum antioxidant activity was used to evaluate the influence of various pH levels on this bioactivity. The best broth medium was adjusted to varying pH levels (6.0–8.0), sterilized in an autoclave, inoculated and incubated. The weight and volume loss of mycelial mat and culture spent were respectively gathered and the antioxidant activity was determined.

DPPH radical scavenging activity assay

The free radical scavenging activity of the samples was estimated using the stable 2,2'-diphenyl-1-1picrylhydrazyl (DPPH) radical following the standard method of Shimada et al. (1992) with modifications. A 100 µl of test sample in ethanol was added with 5 µl DPPH solution (5 mg DPPH powder in 2 ml of ethanol) in 96-well microtitter plates. The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was then measured at 517 nm. The inhibition of DPPH free radicals was calculated.

Estimation of total phenolic content

The total phenolic content was estimated using Folin-Ciocalteu method of Slinkard & Singleton (1977) with modifications. Sample solution (50 µl) was mixed 500 µl of 10% Folin-Ciocalteu reagent (Folin:Methanol, 1:1, v/v). After 2 min, 50 µl of 7.5% saturated was added and kept in the dark for 1h before absorbance was taken at 765 nm. A calibration curve was obtained using various concentrations of ascorbic acid. The total phenolic content of the sample was expressed as mg of ascorbic acid equivalents (AAEs) per gram of sample.

Data were analyzed using Analysis of Variance (ANOVA) in SAS Statistical Program. Means were compared using Least Significant Difference (LSD) at 5% level of significance.

Results and Discussion

Mycochemical composition

Mycochemicals are naturally occurring constituents of fungi particularly mushroom. There are various researches have been done to prove the bioactivities of such constituents. The mycochemical screening of *P. antillarium* mycelia and fruiting bodies was conducted in the present study. Table 1 presents the mycochemical components of the mycelia and fruiting bodies of *P. antillarium.* Apparently, among the six mycochemicals tested, three were detected in traceable and appreciable amounts in both mycelia and fruiting bodies which include saponins, cardiac glycosides and alkaloids. These mycochemicals exhibit significant human healthful benefits. For instance, saponins can inhibit the growth of cancer cells, boost immune system and energy, lower cholesterol, act as natural anti-inflammatory, antibiotic, and anti-oxidant, and can reduce the uptake of certain nutrients including glucose and cholesterol at the gut through intralumenal physicochemical interaction (Aberoumand 2012, De Silva et al. 2013). This substance can also found in other mushrooms such as *Lentinula edodes*, *Flammulina velutipes*, *Agaricus bisporus*, *P. ostreatus* and *Ganoderma lucidum* (Ogbe & Obeka 2011, Prasad & Sethi 2013).

Table 1 Mycochemical and nutrient compositions of *P. antillarium* mycelia and fruiting bodies.

Composition	Mycelia	Fruiting body
Mycochemicals		
Tannins	-	-
Saponins	+	+
Flavonoids	-	-
Phlobatannins	-	-
Cardiac glycosides	+	+
Alkaloids	+	+
Nutrients (%)		
Moisture	11.85 ± 0.01	11.24 ± 0.00
Ash	4.78 ± 0.01	5.26 ± 0.03
Crude protein	13.25 ± 0.07	16.77 ± 0.01
Crude fiber	7.05 ± 0.04	5.05 ± 0.01
Crude fat	1.96 ± 0.06	1.53 ± 0.03
Total carbohydrate	61.12 ± 0.01	60.16 ± 0.08
Energy value	315.10 ± 0.03	321.49 ± 0.04

(In table: Values are the Mean ± SD. (+) present, (-) absent)

Cardiac glycosides which are responsible in cardioactivity and increase the function of myocardial circulation were also present in *P. antillarium* but absent in *Agaricus bisporus*, *Bunapi shimeji*, and *Flammulina velutipes* (Prasad & Sethi 2013). Similarly, these are present in *Cantharellus cibarius, Termitomyces robustus, Termitomyces manniformis, Pleurotus ostreatus, Pleurotus pulmonarius, Auricularia* sp., *Hericium erinaceus, L. deliciousus* and *Ganoderma* sp. (Unekwu et al. 2014, Ogbe et al. 2009).

In addition, *P. antillarium* had appreciable amounts of alkaloids which has been reported to act as powerful pain reliever and topical anaesthetic in ophthalmology, and has stimulating effects and antipuretic action among other uses (Edeoga & Enata 2001). The presence of alkaloids in the mushroom indicates antibacterial activity. Other mushrooms like *P. tuberregium*, coral mushroom, *A. bisporus* and *L. sajor- caju* also contain alkaloids (Mondal et al. 2013, Afiukwa et al. 2013). On the other hand, flavonoids, phloabatannins and tannins were not detected in *P. antillarium*. The result of the present study confirms the previous work of Mondal et al. (2013) who reported that most mushrooms are regarded as lower source of flavonoids.

Nutritional composition

Mushrooms are well-balanced food stuff that have sufficient vitamins and minerals and contains a good source of amino acids. Nutritionally, it has unique flavor and aromatic properties, and rich in protein, fiber, carbohydrates, vitamins and minerals and cholesterol free (Menaga et al. 2012). In the present study, the proximate composition of *P. antillarium* was determined to establish its name in nutraceutical industry. The proximate nutrient composition of the air-dried mycelia and fruiting bodies of *P. antillarium* is depicted in Table 1. It can be seen that mycelia contained higher crude fiber, crude fat, moisture and total carbohydrate than its corresponding fruiting bodies. On the other hand, the fruiting bodies had higher ash, crude protein, and enegy value than its mycelia.

Protein is an important component of mushrooms. Kayode et al. (2013) reported that the mushroom protein content depends on the composition of the substratum, size of pileus, harvest time and species of mushrooms. The protein content of mushroom is said to be twice of vegetables and four times of that of orange and significantly higher than wheat (Prasad & Sethi 2013). In the study of Reis et al. (2011) mushrooms are reported to be a good source of protein, and some investigators have even contended that the amino acid compositions of mushrooms are comparable to animal proteins.

The carbohydrate content of *P. antillarium* is higher than the values of *P. sajor-caju*, *P. ostreatus* and *P. eryngii* (37.72%, 37.87% and 39.85%, respectively) and *Coprinus armamentariums* (24%) (Kayode et al. 2013). According to Ashraf et al. (2013) mushrooms is a potential source of total carbohydrates (42.62% - 66.78%) depending upon the species. Furthermore, Kayode et al. (2013) stated that the carbohydrate content of mushrooms represents the volume of fruiting bodies accounting for about 50 to 65% on dry weight basis. With regards to crude fiber, it can be noted that the value observed in this study is lower as compared to previous study done by Musieba et al. (2011) in *P. citrinopileatus* (20.78), *P. sajor-caju*, (26.28%), *P. ostreatus* (24.53%) and *Pleurotus djamor* (22.03%) (Ashraf et al. 2013). Fibers have an important defensive action in colorectal carcinoma and a basic part of a healthy diet (Manjunathan & Kaviyarasan 2011).

The fat contents of basidiocarp of *Lentinus squarrosulus, Tricholoma giganteum* and *Russula albonigra* and *Amanita ceasarea* in g/ 100g dry tissue (Kayode et al. 2013, Giri et al. 2013) are higher than the value obtained in the present study. Thus, *P. antillarium* is recommended as good source of food supplement for patients with cholesterol associated diseases due to its low fat content. On the other hand, the ash content of *P. antillarium* is within the range from 5 to 12% in a dry basis (Parashare et al. 2013). The ash content is a measure of the total amount of minerals present in a food. The ash constitutes to the minerals like potassium, phosphorus, or magnesium in addition to calcium, copper, iron and zinc (Reis et al. 2011).

The moisture content observed in this study was significantly lower than those in *P. tuberregium, L. edodes* and *A. bisporus* (Ikewuchi & Ikewuchi 2008, Reis et al. 2011). However, energetic values of both mycelia and fruiting bodies of *P. antillarium* were found high suggesting that this mushroom could be an important source of energy for human.

Antioxidant activity of the *P. antillarium* as influenced by different media and pH levels

The effect of the different media namely: coconut water, potato broth, rice bran broth, and corn grit broth were evaluated in this study. Table 2 shows the volume loss of media, weight of mycelia and antioxidant properties of *P. antillarium* grown on the different culture media and pH levels after 2 weeks of incubation. Potato broth significantly produced the highest volume loss (14.5 ± 2.50 ml) and mycelia weight (4.5 ± 0.65 g). On the other hand, rice bran broth registered the lowest mean volume loss and mycelia weight. The results of the present study indicate that potato broth was the most suitable medium that favored the mycelial growth of *P. antillarium*.

Similarly, among the different broth evaluated, potato broth also registered the highest scavenging activity with a mean of 16.06 ± 0.51% and highest phenolic content with a mean of 25.07 ± 0.02 mg AAE/g sample. This effective scavenging activity and high phenolic content could

be attributed to its efficient growth on potato broth. On the other hand, coconut water significantly recorded the lowest scavenging activity (9.30 ± 0.66%) against DPPH radicals and phenolic content (20.91 ± 0.02 mg AAE/g sample).

Table 2 Mycelial growth and antioxidant activity of *P. antillarium* as affected by different media and pH levels after 2 weeks of incubation.

Treatment	Volume loss (ml)	Mycelial weight (g)	RSA (%)	Total phenolics (mg AAE/g sample)
Culture broth				
Coconut water	05.7 ± 0.58^{b}	0.6 ± 0.72^{c}	09.30 ± 0.66^{d}	20.91 ± 0.02^{d}
Potato broth	14.5 ± 2.50^{a}	4.5 ± 0.65^{a}	16.06 ± 0.51^{b}	25.07 ± 0.02^{a}
Rice bran broth	05.3 ± 0.58^{b}	0.3 ± 0.23^{c}	11.96 ± 0.88^{cd}	21.12 ± 0.10^{c}
Corn grit broth	12.3 ± 2.89^{a}	3.0 ± 0.45^{b}	13.62 ± 0.33^{bc}	24.74 ± 0.05^{b}
Cathechin (+)			26.91 ± 0.01^{a}	
pH level				
pH 5.0	10.3 ± 1.60^{a}	2.7 ± 1.46^{a}	14.17 ± 0.51^{c}	24.20 ± 0.01^{c}
pH 6.0	11.7 ± 0.45^{a}	2.0 ± 1.32^{a}	14.40 ± 0.51^{c}	24.00 ± 0.04^{d}
pH 7.0	10.0 ± 2.20^{a}	3.9 ± 0.25^{a}	17.39 ± 0.19^{b}	25.11 ± 0.01^{a}
pH 8.0	12.3 ± 0.46^{a}	2.9 ± 0.57^{a}	15.06 ± 0.38^{c}	24.32 ± 0.01^{b}
Cathechin (+)			28.02 ± 0.01^{a}	

(In Table: Treatment means with the same letter of superscript are not significantly different from each other at 5% level of significance using LSD. RSA: radical scavenging activity; AAE: ascorbic acid equivalent.)

After establishing the optimum nutritional requirement for successful mycelia growth and highest antioxidant activity, the pH (acidity and basicity) of the potato broth was evaluated. It can be seen that at 5% level of significance, potato broth with varying pH levels did not significantly affect the mycelial growth of *P. antillarium*. These results suggest that *P. antillarium* has a wide range of pH requirement, thus, pH of the medium is not critical for mycelia growth. However, the pH levels influenced the antioxidant property of the *P. antillarium*. Apparently, the optimum pH requirement for *P. antillarium* to efficiently perform as antioxidant was at pH 7.0, having a percentage scavenging activity of 17.39 ± 0.19% and total phenolic content of 25.11 ± 0.01 mg AAE/g sample. These results obtained strongly suggest that the antioxidant activity is dependent on the medium and pH of the medium where this mushroom is being grown. Alam et al. (2008) enumerated several factors that influenced the properties of mushrooms. These include the differences among strains, composition of growth substrate, method of cultivation, stage of harvesting and specific portion of the fruiting bodies used for analysis.

Moreover, the presence of phenolic compounds in *P. antillarium* strongly suggests its antioxidant activity. Aside from being antioxidant, phenolic compounds also exhibit antimutagenic, antiviral, antibacterial, algicidal, antifungal, insecticidal, estrogenic and keratolytic activities that may serve to protect the organism from competing ones in their biological environment (Castellano et al. 2012). This significant antioxidant activity of *P. antillarium* showed in the present study is equivalent to other mushrooms such as *Agaricus bisporus*, *Ganoderma lucidum*, *Phellinus rimosus*, *Pleurotus florida*, *Pleurotus pulmonarius*, *Volvariella volvacea*, *Thelephora ganbajun*, *Thelephora aurantiotincta*, *Boletopsis grisea* (Ajith & Janardhanan 2007, Liu et al. 1997).

Finally, aside from being a good source of mycochemicals such alkaloids, saponins, and cardiac glycosides and nutrients like protein, minerals, carbohydrates, fibers and energy, *P. antillarium* also exhibits antioxidant property which directly influenced by media and pH. Furthermore, evaluation of other bioactivities of *P. antillarium* is highly recommended.

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