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PROXIMATE COMPOSITION AND BIOACTIVITIES OF HAIRY SAWGILL MUSHROOM, *Lentinusstrigosus* (BIL 1324) FROM THE PHILIPPINES

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ABSTRACT

This paper highlighted the proximate and mycochemical compositions, antioxidant activities, and antibacterial properties of the wild and cultured fruiting bodies of *Lentinusstrigosus*. The wild fruiting bodies had higher amounts of ash (6.70%), reducing sugar (4.60%), fiber (20%), potassium (4291.83 mg/100 g), phosphorous (2009.73 mg/100 g), and iron (24.69 mg/100 g).On the other hand,the cultured fruiting bodies contained higher amounts of crude protein (24.70%), soluble polysaccharide (33.57%), dietary fiber (52.03%), calcium (73.43 mg/100 g), and energy value (144.27 kcal/100g). Both samples contained saponins, alkaloids, flavonoids, anthraquinones, athrones, phenols, steroids, coumarins, and fatty acids. The extract of wild fruiting bodies of *L. strigosus* significantly recorded higher scavenging activity (with an EC_{50} value of 534.67µg/ml) when compared to the extract of the cultured fruiting bodies. The wild and cultured samples contained the phenolic contents of 79.70 and 78.80mg AAE/g sample, respectively. Antibacterial assay showed that the ethanolic extracts of both fruiting bodies exhibited antibacterial activities against *Staphylococcus aureus* and *Escherichia coli*. Therefore, *L. strigosus*is a natural source of functional food.

Keywords: *Lentinusstrigosus*, mushroom nutrients, antimicrobial, antioxidant, minerals

INTRODUCTION

Lentinusstrigosus, also known as *kabutengbalbon* by Filipinos, is a naturally occurring, wood-rotting edible mushroom that commonly found growing on fallen and rotten logs. It is characterized by its dense hairy velvety pileus and stubby stipe with pinkish tan to reddish-brown with violet tints in young fruiting body. This wild mushroom isacquired from the forest area of Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines and its mycelia are grown in different nutrient culture media. The fruiting bodies are successfully cultivatedusing formulated rice straw and sawdust based substrate**[1]**. In order to improve the biological efficiency of *L. strigosus*, optimization studies in reference to the nutritional and physical requirements for growth are conducted. Supplementation of its substrate using rice bran and corn gritstimulates the formation of primordia, produces the highest yield and biological efficiencyof *L. strigosus***[2]**. On this note, this new domesticated mushroom has a great potential in the emerging mushroom industry in the Philippines.

Mushrooms are natural sources of bioactive compounds with antioxidant, antitumor, antibacterial, immune-enhancing, anti-inflammatory, cardiovascular protective, radical scavenging, anti-hyper-cholesterolemic, anti-diabetic, antiviral, anti-parasitic, and antifungal activities and prevent arteriosclerosis and chronic hepatitis **[3-8]**. For the full realization of the valuable role of *L. strigosus* as functional and medicinal commodity, this study elucidated the proximate nutrient, mineral, and mycochemical compositions and demonstrated the anti-oxidant and antibacterial activities of the wild andculturedfruiting bodies of this newly recorded Philippine mushroom.

MATERIALS AND METHODS

Source of Mushrooms

The wild fruiting bodies of *L. strigosus*(Figure 1A) were collected from the forest area of Central Luzon State University, Science City of Munoz, NuevaEcija, Philippines.This mushroom was rescued and culturedin rice straw based substratesupplemented with rice bran in a growing house condition (Figure 1B). Both wild and cultured fruiting bodies of *L. strigosus* were air-dried and prepared for chemistry analysis and extraction.

Figure 1: Wild (A) and cultured (B) fruiting bodies of *L. strigosus*

Proximate Composition Analysis

The proximate compositions of the wild and cultured fruiting bodies of *L. strigosus* were analyzed.Crude protein, crude fat, ash, crude fiber, reducing sugar and moisture content (MC) were analyzed according to the guidelines of the Association of Official Analytical Chemist**[9]**.Kjeldahl method using the conversion factor N × 4.38 was used to determine the protein content whereasSoxhlet apparatus was used to determine the crude fat content. The ash content was analyzed using furnace at 550 °C. Reducing sugar content was determined using Munson-walker method. Total carbohydrate content was calculated as follows: total carbohydrates = 100 - (protein + fat + ash + MC). Total carbohydrate consists of reducing sugars (RS) and dietary fibre (DF), which consists of crude fibre and soluble polysaccharides (SP)**[10]**.Soluble polysaccharide content was then calculated by subtracting the crude fiber and reducing sugar contents from the total carbohydrate while dietary fiber was determined by adding the crude fiber and soluble polysaccharide contents. Energy value was computed following the formula of Ulziijargal and Mau**[10]**: Energy (kcal/100 g) = (RS × 4) + (fat × 9) + (protein × 4). The different minerals such as phosphorous, potassium, magnesium, calcium, iron, and zinc were also analyzed following the standard protocol for chemical analysis**[9]**. Values were expressed as mean ± standard deviation.The mycochemical screening of the fruiting bodies were carried out following the procedures described by Guevara et al. **[11]**. Three replicates were laid out for each test parameter.

Ethanolic Extraction of Mushroom

A total of 10 grams of air-dried fruiting bodies of each mushroom was extracted in 500 ml of95% ethanol (as solvent) for 48 hours soaking. Extracts were filtered using Whatman filter No.2 and concentrated to

dryness using rotary evaporator. The weight of the extract was determined and yielded 9.4% extract.

Radical Scavenging Activity Assay

The concentrated extract was used to make a stock solution and aliquot was taken to make 1000 ppm dilution and 1000 ppm cathechin as control (1 mg/ml). One ml of the prepared stock solution was mixed with 4 ml of 0.1 mM DPPH solution in separate plastic cuvette. Reaction was done in triplicate. The prepared mixtures were incubated in the dark at 37°C for 30 min. The absorbance readings were monitored at 517 nm using a UV VIS spectrophotometer. The ability to scavenge the DPPH radical was calculated using the formula:

$$\% \text{ Radical Scavenging Effect} = [(A_{control} - A_{sample}) / A_{control}] \times 100.$$

Estimation of Total Phenolic Content

The amount of total phenolics in the extracts was determined with Folin-Ciocalteu reagent **[12]**. Ascorbic acid was used as a standard and the total phenolics were expressed as mg/g Ascorbic Acid Equivalents (AAE). The different concentrations of ascorbic acid and 1 mg/ml concentration of mushroom extract were prepared in methanol. Each sample (0.5 ml) was introduced into test tubes and mixed with 2.5 ml of a 10 fold dilute Folin-Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. The tubes were covered with parafilm and allowed to stand for 30 min at room temperature prior to absorbance reading at 760 nm spectrophotometrically. All tests were performed in triplicate.

Determination of Antibacterial Property

The antibacterial activities of the extracts from the wild and cultured fruiting bodies of *L. strigosus*were determined following the paper disc diffusion method of Bauer et al. **[13]**. *Staphylococcus aureus* and *Escherichia coli* were cultured in 9 ml of nutrient broth (NB) medium and incubated at 37 °C. After 24 h, the turbidity of each bacterial culture was adjusted to equal that of 0.5 McFarland standard, which approximated 1.5×10^8 cells/ml. The bacterial suspension was spread using a sterile cotton swab on Mueller Hinton agar plate. Sixmillimetre diameter paper discs impregnated with 20 µL of 100 mg/ml of mushroom extract dissolved in 95% ethanol and streptomycin as standard were placed equidistantly on the medium. Plates were incubated at 37 °C, and the zones of inhibition were measured after 24 h. Each test was done in triplicate.

RESULTS AND DISCUSSION

Nutrient and Mycochemical Compositions of *L. strigosus*

The fruiting bodies of mushroom areimportant source ofprotein, carbohydrate,

minerals, crude fiber, and even fats. In this work, the proximate composition of the wild and cultured fruiting bodies of *L. strigosus* was analyzed and the results are presented in Table 1.The compositions of the two mushroom samples weresignificantly varied. Wild fruiting bodies had higher amounts of ash, reducing sugar, and fiber whereas the cultured fruiting bodies contained higher amounts of crude protein, soluble polysaccharide, and dietary fiber. On the other hand, the two samples were not statistically differed in moisture, total fat, and total carbohydrates. Energy value was found significantly higher in cultured fruiting bodies.The ash content is referred to the mineral fractions of the mushroom. Among minerals, potassium was the most abundant, followed by phosphorous and magnesium. Apparently, wild fruiting bodies had higher amount of potassium, phosphorous, and iron. Only calcium was found higher in cultured fruiting bodies. The magnesium and zinc content of the two samples were found statistically the same. Given these important nutrient compositions, *L. strigosus* is therefore considered a nutritious food source. In terms of mycochemical composition, the wild and cultured fruiting bodies of *L. strigosus* did not vary. Both samples contained saponins, alkaloids, flavonoids, anthraquinones, athrones, phenols, steroids, coumarins, and fatty acids. These valuable chemicals of mushroom are known to exhibit different functional and biological activities such as diabetes, hypertension, hypercholesterolemia and even cancer **[14]**. Tannin was not detected in both samples. Therefore, the nutrient compositions of *L. strigosus* could vary depending on the origin, whether wild or cultivated fruiting bodies.

Radical Scavenging Activity and Phenolic Content

Antioxidants are substances that inhibit the damaging effects of free radicals brought by oxidation. The ability of these substances to inhibit stable 2,2'diphenyl-1-1picrylhydrazyl (DPPH) free radicals was determined using radical scavenging assay. This present study evaluated the DPPH radical scavenging activity of ethanolic extracts of the wild and cultured fruiting bodies of *L. strigosus*. The results of radical scavenging assay and the EC_{50} value of the two extracts are shown in Table 2. Apparently, extract of wild fruiting bodies of *L. strigosus* significantly recorded higher scavenging activity (with an EC_{50} value of 534.67µg/ml) when compared to the extract of the cultured fruiting bodies. It is therefore noted that the antioxidant property of L. strigosus varied depending on the origin of the fruiting body.

Phenolic acids, flavonoids, carotenoids, tocopherol and ascorbic acids are the most common antioxidants contained in foods **[15]**. Phenol was detected in the mycochemical analysis of the present study. However, we also quantified the total phenolic content of the two mushroom samples in ascorbic acid equivalent using Folin-Ciocalteu method (Table 2). It can be seen that no significant difference was noted on the total phenolic contents of both fruiting bodies of *L. strigosus*. The wild and cultured samples contained the phenolic contents of 79.70 and 78.80mg AAE/g sample, respectively. In the previous study, the range of 23.74–30.16 mg/g total phenolic content of water extract of *Agrocybecylindracea* indicated its high antioxidant properties **[16]**.

Antibacterial Properties of *L. strigosus*

To further study the bioactivities of *L. strigosus*, evaluation of the antibacterial properties of the two sample extracts against two human pathogenic bacteria, *S. aureus* and *E. coli*, was also conducted. Table 3 shows the mean diameter zones of inhibition of the ethanol extracts of the wild and cultured fruiting bodies. Apparently, both extracts showed zones of inhibition against the two bacteria. Although extract of wild fruiting bodies had wider diameter in both bacteria, no significant difference was found between the two extracts at 5% level of significance. However, these diameters were higher when compared to ethanol, which showed no activity. These zones of inhibition strongly indicate the presence of antibacterial compounds in the ethanol extract of *L. strigosus*. This result is similar to our previous findings on the antibacterial activities of the ethanol extract of *Lentinustigrinus***[17]**, a relative of *L. strigosus*. The antibacterial activity of *L. strigosus* ethanol extracts could be explained by its mycochemicals, which are reported for their antimicrobial applications.

Table 1: Proximate nutrient and mycochemical compositions of the wild and cultured fruiting bodies of *L. strigosus*

Composition	Wild *L. strigosus*	Cultured *L. strigosus*
Nutrients		
Moisture (%)	11.97 ± 0.50	12.03 ± 0.21
Ash (%)	6.70 ± 0.40 *	4.03 ± 0.23
Crude Protein (%)	23.03 ± 0.35	24.70 ± 0.10 *
Total Fat (%)	3.13 ± 0.25	3.33 ± 0.32
Reducing Sugar (%), RS	4.60 ± 0.17 *	3.87 ± 0.21
Fiber (%)	20.00 ± 0.26 *	18.47 ± 0.25
Soluble Polysaccharide (%), SP [a]	30.57 ± 1.03	33.57 ± 0.35 *
Dietary Fiber (%), DF [b]	50.57 ± 1.05	52.03 ± 0.32 *
Total Carbohydrates (%)	55.17 ± 1.21	55.90 ± 0.26
Energy (kcal/100 g)[c]	138.73 ± 1.68	144.27 ± 3.43 *
Minerals		
Potassium (mg/100g)	4291.83 ± 17.82 *	3256.67 ± 25.66

Phosphorous (mg/100g)	**2009.73 ± 11.40 ***	**1657.17 ± 18.62**
Magnesium (mg/100g)	**617.20 ± 18.37**	**573.37 ± 15.96**
Calcium (mg/100g)	**49.67 ± 4.04**	**73.43 ± 3.70 ***
Iron (mg/100g)	**24.69 ± 5.32 ***	**12.03 ± 1.33**
Zinc (mg/100g)	**11.23 ± 2.16**	**10.99 ± 0.55**
Mycochemicals		
Saponins	**Present**	**Present**
Alkaloids	**Present**	**Present**
Tannins	**Absent**	**Absent**
Flavonoids	**Present**	**Present**
Anthraquinones	**Present**	**Present**
Athrones	**Present**	**Present**
Phenols	**Present**	**Present**
Steroids	**Present**	**Present**
Coumarins	**Present**	**Present**
Fatty Acids	**Present**	**Present**

Data presented as means ± SD (n = 3). * Represents significant difference of the wild and domesticated fruiting bodies of *L. strigosus*. [a]Soluble polysaccharide = Total – (RS + fiber); [b] DF, dietary fiber = SP + fiber; [c] Energy (kcal/100 g) = (protein × 4) + (fat × 9) + (RS × 4).

Table 2: Radical scavenging activities and total phenolic content of the wild and cultured fruiting bodies of *L. strigosus*

Sample	Radical Scavenging Activity (%)	EC_{50} (µg/ml)	Total Phenolics (mg AAE/g sample)
Wild *L. strigosus* Extract	**73.87 ± 0.31***	**534.67**	**79.70 ± 1.59**
Cultured *L. strigosus* Extract	**68.01 ± 0.16**	**588.52**	**78.80 ± 1.49**
Cathechin	**97.73 ± 0.33**	**2.71**	**-**

Data presented as means ± SD (n = 3). The concentration of ethanol extract of the mushroom fruiting body used was at 1 mg/ml. * Represents significant difference of the wild and domesticated fruiting bodies of *L. strigosus*.

Table 3: Antibacterial activities of ethanolic extracts of the wild and cultured fruiting bodies of *L. strigosus*ethanol extract against *S. aureus* and *E. coli*.

Sample	Diameter zone of inhibition*(mm)	
	S. aureus	***E. coli***
Wild *L. strigosus* Extract	**14.42 ± 0.18^{b}**	**10.93 ± 0.11^{b}**
Cultured*L. strigosus* Extract	**12.78 ± 0.09^{b}**	**9.91 ± 0.28^{b}**
Ethanol	**0.00 ± 0.00^{c}**	**0.00 ± 0.00^{c}**
Streptomycin	**31.52 ± 0.23^{a}**	**27.64 ± 0.16^{a}**

Data presented as means ± SD (n = 3). Treatment means with the same letter of superscript are not significantly different from each other at 5% level of significance using DMRT.
***The diameter of the disc used was 6.0mm. The concentration of ethanol extract of the mushroom fruiting body used was at 100 mg/ml in 95% ethanol.**

CONCLUSION

This present study demonstrated that the fruiting bodies of *L. strigosus* containvaluable nutrients which are required in healthy human diet. Moreover, this mushroom can also be a source of bioactive compounds with functional activities. The extracts of *L. strigosus* exhibitscavenging activity against DPPH radicals and antibacterial property against *S. aureus* and *E. coli*. However, the active components responsible for these activities are still unknown and which we need to elucidate and identify in future studies. It is also interesting to note in this study that the components and bioactivities of *L. strigosus*are greatly affected by the source or origin of the fruiting bodies (whether wild and cultured).

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