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Original Article

Mycochemicals, antioxidant and anti-diabetic properties of Philippine sawgill mushroom *Lentinus swartzii* (Higher Basidiomycetes)

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Abstract

Lentinus swartzii is a new record of successfully domesticated Philippine basidiomycetous mushroom. This paper highlights the chemical compositions, antioxidant and anti-diabetic properties of mycelia and fruiting body extracts of *L. swartzii*. The compounds present in the ethanolic extracts were determined using thin layer chromatography (TLC), and the biological properties were assessed using stable 2,2-diphenyl-1-1picrylhydrazyl (DPPH) and unstable nitric oxide scavenging activity assays and α-amylase inhibition assay. Mycelia had essential oil, triterpenes, sugars, tannins, flavonoids, fatty acids and phenols, while the fruiting body had the same except fatty acids and sugars. Mycelia ethanolic extract at 1000 µg/mL exhibited scavenging effects against DPPH (35.29%) and nitric oxide (36.04%), contained 20.25 mg gallic acid equivalent (GAE)/g sample and showed high inhibitory activity against α-amylase (81.98%). On the other hand, the fruiting body ethanolic extract at 1000 µg/mL scavenged 43.69% of DPPH and 31.75% of nitric oxide, contained 16.92 mg GAE/g sample and exhibited high inhibitory activity against α-amylase (71.08%). Therefore, *L. swartzii* mycelia and fruiting body could be valuable sources of bioactive compounds with antioxidant and anti-diabetic activities.

Keywords: *Lentinus swartzii*, Mushroom biomass, Anti-diabetic, Antioxidant, Bioactive compounds

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Introduction

Mushrooms have been traditionally and seasonally utilized all over the world because of their nutraceutical and pharmaceutical importance. As food, they are rich in valuable nutrients and contain various bioactive metabolites responsible in the management and prevention of different diseases. Medicinal mushrooms contain polysaccharides, proteins, and their complexes, phenolic compounds, lipid components and terpenoids, which act as antioxidant, immunomodulatory, anti-inflammatory and hypoglycemic actions (Ferreira et al., 2009; Borchers et al., 2008; Padilha et al., 2009; Hu et al., 2006;

Puttaraju et al., 2006).

The antioxidant and anti-diabetic properties of various mushrooms have been claimed in several studies. For instance, Boonsong et al. (2016) reported the antioxidant activities of five edible mushrooms such as *Volvariella volvacea, Lentinus edodes, Pleurotus eous, Auricularia auricular* and *Pleurotus sajor-caju*. Ethanolic extracts of mycelia from *Coprinus comatus* and four strains of *Pleurotus ostreatus* also showed antioxidant properties (Vamanu, 2014). Some Philippine wild and edible mushrooms such as *Pycnoporus saguineus, Pleurotus cystidiosus, Trametes elegans, Polyporus grammocephalus, Ganoderma lucidum* and *Pleurotus djamor* have also been reported for their significant antioxidant properties (Nanglihan et al., 2018; Aquino et al., 2018; Bustillos et al., 2018; Garcia et al., 2020; Mendoza et al., 2020). Moreover, Sanchez (2016) reported the different mushrooms with antioxidant activities and their antioxidant compounds including phenolics, polysaccharides, carotenoids, tocopherols, ascorbic acid and ergosterol. On the other hand, the anti-diabetic activities of ethanolic extracts of *G. lucidum, L. edodes, Tremella fuciformis, Agrocybe aegerita, Grifola frondosa, Russula sanguinea, Hericium erinaceus* and *Auricularia auricular-judae* in enzyme-based assay have been demonstrated (Wu and Xu, 2015). The anti-diabetic effect of mushrooms has been linked to polysaccharides, dietary fibers, protein complexes, and other bioactive compositions (Lo and Wasser, 2011).

Lentinus species are naturally-occurring mushrooms in the Philippines and are considered edible and medicinal. However, only few species are recorded and documented including *Lentinus sajor-caju, Lentinus strigosus, Lentinus tigrinus, Lentinus squarrosulus* and *Lentinus swartzii*. The cell lines of *L. swartzii* were recently rescued, and its optimum cultural conditions for growth and production of biomass are currently under investigation in our laboratory. On the other hand, the production technologies and biological activities of the first four above-mentioned species have been reported. For instance, *L. tigrinus* and *L. strigosus* extracts exhibit hypoglycemic, antibacterial and antioxidant activities (Dulay et al., 2017; Dulay and Pamiloza, 2018; Dulay et al., 2014) while *L. sajor-caju* extract showed anti-hypertensive activity in spontaneously hypertensive rats (Eguchi et al., 2014). Given the significant properties of the relative species, it is therefore of our hypothesis that *L. swartzii* might also contain valuable compounds and exhibit several biological activities. To the best of our knowledge, no work has been done on the chemical and biological profiling of this *Lentinus* species. Thus, this current study aimed to elucidate the chemical compositions and evaluate the antioxidant and anti-diabetic activities of *L. swartzii* mycelia and fruiting body extracts in our intention to establish the position of this wild mushroom for proper utilization in pharmacological applications.

Material and Methods

Source and mass production of mushroom

L. swartzii mycelia and fruiting bodies (Figure 1) were obtained from the Bioassay Laboratory, Department of Biological Sciences, College of Science, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines. Mycelia were mass produced under submerged culture condition using coconut water medium for 15 days. On the other hand, the established production technique of Dulay et al. (2012) was followed for the fruiting body production using rice seeds as grain spawn and 7 parts of rice straw with 3 parts of sawdust as basal substrate in fruiting bags in growing house conditions. Mature fruiting bodies of mushrooms (usually 3 days after primordia formation) were harvested and air-dried for 5 days.

Table-1. Chemical compositions of mycelia and fruiting body of *Lentinus swartzii*.

Chemicals	Mycelia	Fruiting body
Essential oil	+	+
Triterpenes	+	+
Sugars	+	0
Tannins	+	+
Flavonoids	+	+
Fatty acids	+	0
Phenols	+	+

(+) positive, (0) not detected

Extraction

The air-dried mycelia and fruiting bodies were pulverized using food blender. Twenty grams of each sample were individually soaked in 95% ethanol for 48 hours in the dark at room temperature. After soaking, these were filtered (Whatman #2) and concentrated using a rotary evaporator at 40°C until dryness. Extracts were stored in a refrigerator until

used for the assays. As required in the assay, an aliquot of the concentrated crude extract was re-dissolved in an appropriate solvent.

Figure-1. Mycelial culture (A) and mature fruiting bodies (B) of *Lentinus swartzii*

Chemical composition analysis

The screening of the different groups of compounds present in the mushroom extracts was employed following the methods of Guevara (2005). A thin layer chromatography (TLC) was used to detect the presence of the different secondary metabolites of the mushrooms. TLC was performed in vertical glass chamber with ethyl acetate. The different mycochemicals were detected as spots in thin layer chromatography (TLC) through the use of UV light, hot plate, and several reagents used for a typical visualization of the secondary metabolites. Vanillin-sulfuric acid was used to determine the presence of phenols, sterols, fatty acids, triterpenes, and essential oil. Methanolic potassium hydroxide was used to visualize anthraquinones, coumarins, and anthrones, while potassium ferricyanide-ferric chloride was used to test phenolic compounds and tannins. Alkaloids and flavonoids were detected using Dragendorff's reagent and antimony (III) chloride, respectively.

Evaluation of DPPH radical scavenging activity

The method of Kolak et al. (2006) on the DPPH scavenging activity determination was followed with modifications. One mL of each extract and catechin at 1000 µg/mL were prepared and separately mixed with 4 mL of 0.1 mM DPPH solution and incubated at 37°C for 30 min in the dark condition. After incubation, a UV VIS spectrophotometer (Spectrumlab 752S, Hinotek Instrument Co., LTD, China) was used to read the colorimetric absorbance at 517 nm. The percentage radical scavenging activity was computed using this equation: % RSA = $[(A_c - A_s) / A_c] \times 100$, where, A_c is the absorbance of the control and A_s is the absorbance of the tested sample.

Phenolic content analysis

The Folin-Ciocalteu method described by Sunita and Dhananjay (2010) was carried out to determine the phenolic content of the mushroom extracts. The phenolic content was expressed as mg/g gallic acid equivalents (GAE). A 0.5 mL of extracts and gallic acid was separately added into 2.5 ml of a ten-fold diluted Folin-Ciocalteu reagent and 2 ml of 7.5% sodium carbonate, and subsequently stand for 30 min at 30°C. Absorbance was measured at 760 nm in the UV VIS spectrophotometer and the phenolic content was computed.

Nitric oxide scavenging assay

Griess reaction was carried out in micro scale volumes. Extracts at 1000 µg/mL with sodium nitroprusside (10 mM, 2 mL) in phosphate buffer saline were incubated at room temperature for 150 min. After 30 min, 1 mL of Griess reagent (0.33% sulfanilamide in 20% glacial acetic acid, 0.5 mL and 0.1% NED, 1.0 mL) was added into 0.5 mL of the incubated solution and was incubated at room temperature for 30 min. Absorbance reading was determined at 546 nm. The percentage nitric oxide scavenging activity was calculated using the equation: % NOS = $[(A_c - A_s) / A_c] \times 100$, where, A_c is the absorbance of the control, and A_s is the absorbance of the tested sample. This method was followed after Bueno et al. (2013).

Alpha-amylase inhibition assay

The method of α-amylase inhibition assay was followed after Watcharachaisoponsiri et al. (2016). Different concentrations of the extracts and acarbose (100 µL) were placed in microcentrifuge tubes. A 200 µL porcine pancreatic amylase was added and incubated for 20 min at 37 ºC. Afterwards, 100 µL of 1% starch solution was added and incubated for 10 min at 37 ºC. A 200 µL DNSA was mixed and kept for 5 min in a boiling water bath to stop the reaction. Mixture was diluted in 2.2 mL of distilled water prior to absorbance reading at 540 nm. Blank tubes were prepared by replacing the enzyme solution with 200 µL in distilled water. Negative and positive controls were also prepared in the same manner. The percentage inhibitory activity of the extracts and acarbose was determined based on the absorbance of each replicate and blank.

Statistical analysis
All tests were replicated three times. One-way analysis of variance was used to analyze the data and treatment means were compared at 0.05 level of significance using Tukey's HSD. Statistical Analysis Software (SAS) System Version 9.0 was used for analysis. Values were presented as mean ± standard deviation.

Results

Chemical compositions
The results of TLC analysis are presented in Table 1. *L. swartzii* mycelial extract contained seven groups of compounds such as essential oil, triterpenes, sugars, tannins, flavonoids, fatty acids and phenols, while only five groups were found present in the fruiting body extract.

Scavenging activity and phenolic content of mushroom
Table 2 shows the scavenging activities and phenolic contents of mycelia and fruiting body extracts. Both extracts showed moderate scavenging activity against DPPH free radicals and contained phenolic compounds.

Table-2. DPPH and nitric oxide scavenging activity of *Lentinus swartzii* extracts and their phenolic content.

Extract	Scavenging Activity (%)		Phenolic content (mg GAE/g of sample)
	DPPH	Nitric oxide	
Mycelia	35.29 ± 1.28^{c}	36.04 ± 5.54^{b}	20.25 ± 0.42
Fruiting body	43.69 ± 1.68^{b}	31.75 ± 8.48^{b}	16.92 ± 0.43
Control (+) [a]	60.50 ± 2.95^{a}	76.61 ± 13.2^{a}	-

Each value represents mean ± SD of triplicate tests (n=3). Means having the same letter of superscript are not significantly different from each other at 5% level of significance. The concentration of extracts used in scavenging activity was 1000 µg/mL. [a] Catechin and gallic acid were used as positive controls in DPPH and nitric oxide scavenging assays, respectively.

Fruiting body extract showed higher scavenging activity, while mycelial extract had higher phenolic content. The nitric oxide scavenging activities of *L. swartzii* extracts are also presented in Table 2. Noticeably, both mycelia and fruiting body extracts at 1000 µg/mL scavenged 36.04% and 31.75% nitric oxide, respectively. However, these were not statistically comparable with the scavenged nitric oxide of control gallic acid.

Alpha-amylase inhibitory activity
Figure 2 presents the α-amylase inhibitory activities of *L. swartzii* fruiting body and mycelial extracts. Both mycelia and fruiting body extracts showed inhibitory activity against alpha-amylase. Surprisingly, the inhibitory activity of mycelial extract at 1000 µg/mL was statistically comparable with the acarbose. Although the fruiting body extract also showed high inhibitory activity, it is significantly lower when compared to acarbose.

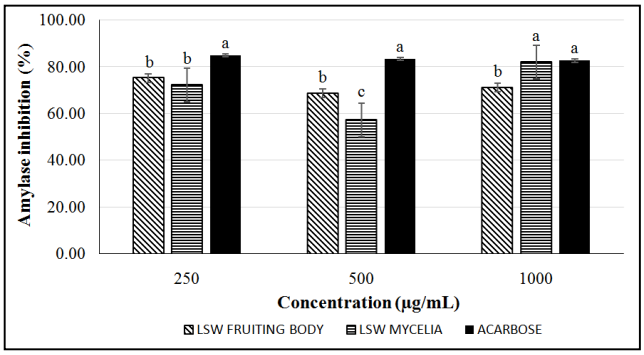

Figure-2. Inhibition activity of *Lentinus swartzii* extracts compared to acarbose against porcine pancreatic α-amylase at different concentrations (µg/mL). Each value represents mean ± SD of triplicate tests (n=3). Means having the same letter of superscript in each concentration are not significantly different from each other at 5% level of significance.

Discussion

Mushrooms are rich in a variety of compounds, which possesses many health benefits. The chemical components of mycelia and fruiting body extracts of *L. swartzii* were analyzed using thin layer chromatography. Essential oil, triterpenes, sugars, tannins, flavonoids, fatty acids, and phenols were found present in both extracts except sugars and fatty acids in fruiting body extract. The above-mentioned compound groups have been reported for their anti-diabetic, anti-hypertensive, anti-hypercholesterolemic, anti-inflammatory, antioxidant, antimicrobial, analgesic, sedative, spasmolytic, and anticancer properties (Kavishree et al., 2008; Dudhgaonkar et al., 2009; Dulay et al., 2017; Bakkali

et al., 2008; Lindequist et al., 2005). It is presumed that the mycelia and fruiting body would contain the same chemical compositions, but surprisingly, fatty acids and sugars were not present in fruiting body extract. This could be accounted to the nutritional compositions of the medium and substrate used in mass production. The mycelia were mass produced in coconut water, which contain sugars and fatty acids whereas the fruiting bodies were grown in formulated sawdust and rice straw, which basically composed of lignin and cellulose. Previously, we reported that the production of mycelia and antioxidant properties of *L. sajor-caju* and *L. tigrinus* are influenced by the liquid media used (Dulay et al., 2015). Therefore, the type of substrate or media is an important factor that plays major role on the chemical attributes and functional activities of mushroom.

Free radicals cause oxidation, which are responsible to various physiological diseases and aging. Thus, any compounds, substances, or extracts that could eliminate free radical could be used as alternative remedy to several diseases. The DPPH scavenging activity of mycelia and fruiting bodies of *L. swartzii* was investigated in this study. Interestingly, extract of fruiting body recorded higher activity than the extract of mycelia. Although both values were not statistically comparable with the effect of catechin, the values imply the promising potential of mycelia and fruiting body of *L. swartzii* as natural source of antioxidants. Studies have reported the successful isolation of the active antioxidant compounds. For instance, gallic acid and protocatechuic acid were isolated in *Ganoderma lucidum*, *Ganoderma applanatum*, *Coriolus versicolor*, *Panus tigrinus*, *Pleurotus ostreatus*, *Laetiporus sulphureus*, *Flammulina velutipes* and *Meripilus giganteus* (Karaman et al., 2010), while variegatic acid was isolated in *Boletus* species (Vidovic et al., 2010). Moreover, the hexane and acetonitrile extracts of *Pleurotus djamor* and *L. tigrinus* exhibited scavenging activity (Dulay et al., 2017). Accordingly, it is therefore necessary to isolate and identify the active fraction of the *L. swartzii* extracts responsible for the above-mentioned bioactivity.

Phenolic content is one of the most important antioxidants (Barros et al., 2008). The phenolic content of both extracts was quantified in gallic acid equivalent using Folin-Ciocalteu method. Cheung et al. (2003) and Jung et al. (2008) showed the positive correlation of antioxidant activities and phenolic contents useful mushrooms such as *Inonotus xeranticus*, *Phellinus linteus*, *Lentinula edodes*, and *Volvariella volvacea*. However, in the present work, the correlation of the two was not confirmed. The fruiting body extract had higher scavenging activity but lower phenolic content and the mycelial extract had lower scavenging activity but higher phenolic content. These observations could possibly be accounted to other compounds, which are also antioxidant agents including β-glucan, tocopherols, niacin, flavin, pyridoxine, ascorbate, shikimate, malate, fumarate, monoterpenoid, diterpenoid, lipids, hydrophobins and trace elements such as selenium (Yim et al., 2010; Aggarwal et al., 2012).

Nitric oxide is involved in various processes in the human system. However, high concentration of this unstable free radical can be toxic and associated several diseases; thus, inhibition of over production of this free radical is of great interest (Wang et al., 2005). Results obtained from this study showed *L. swartzii* mycelial and fruiting body extracts also exhibited inhibitory activities against nitric oxide. Similarly, the nitric oxide scavenging activities were also confirmed to other mushrooms including *Trametes versicolor*, *Calvatia gigantea*, *Gymnopilus junonius*, *Cortinarius* sp., *Tricholoma equestre*, *Tricholoma* sp., *Mycena* sp., *Coprinus comatus*, *Amanita muscaria* and *Pleurotus florida* (Ragupathi et al., 2018; Menaga et al., 2013). Our results suggest the promising potential of both extracts of *L. swartzii* as natural source of antioxidants.

Alpha-amylase is involved in the degradation of carbohydrate compounds and its absorption which affects the blood glucose levels. *L. swartzii* mycelial extract at 1000 µg/mL showed inhibitory activity against α-amylase, which was comparable to acarbose. Many mushrooms species have been reported to exhibit hypoglycemic effect. Ma et al. (2013) reported that *Trametes gibbosa* extract decreased the plasma glucose levels, total cholesterol and triacylglycerol concentrations implying its anti-diabetic properties. Dulay et al. (2014) reported the hypoglycemic eff ect of lyophilized water extract of fruiting bodies of *L. tigrinus* in alloxan-induced mice. In addition, Liu et al. (2012) revealed that aqueous extracts of *Stropharia rugoso-annulata*, *Craterellus cornucopioides*, *Catathelasma ventricosum*, *Clitocybe maxima*, and *Laccaria amethystea* showed potent inhibitory activity against α-glucosidase. Moreover, a water-soluble polysaccharide from *Auricularia auricula-judae* fruiting bodies exhibited hypoglycemic activity (Yuan et al., 1998). The results obtained in this study strongly

suggest a very potent anti-diabetic property of *L. swartzii* extracts even in the crude form.

Conclusion

The present work has shown that the mycelia and fruiting body of *L. swartzii* could be source of natural mycochemicals accountable to the antioxidant and anti-diabetic properties. Comparing the two extracts, mycelia grown in coconut water showed better activities than the fruiting body cultivated sawdust and rice straw substrate, which strongly indicate that the chemical attributes and bioactivities are not only dependent on the species type, strain type, culture media type, extraction solvent, but also the type of mushroom biomass as source of extract. Thus, submerged cultivation for mycelial biomass production as source of bioactive fungal metabolites is more advantageous and highly recommended. Since our presented data are results of the enzyme-based assay, it is necessary to further evaluate in-vivo and assess other pharmacological properties. Isolation and characterization of the compounds responsible for the significant bioactivities must also be carried out.

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Contribution of Authors

Austria AB: Conceptualization of the study, literature review, experimental design and data collection, manuscript writing

Dulay RMR: Conceptualization of the study, statistical analysis and data interpretation, manuscript writing

Pambid RC: Research design and methodology assessment, manuscript critiquing, final reading and approval