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Research Article

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Mycochemical Profile of Mycelia and Fruiting Body of *Panaeolus cyanescens* and its Optimal Submerged Culture Conditions for Antioxidant Properties

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ABSTRACT

Edible mushrooms are being recognized for their mycochemical values which enormously contibute to the different bioactivities. This work highlighted the mycochemical compositions of the mycelia and fruiting body of P. cyanescens and its antioxidant property as influenced by culture media and pH levels. Mycochemical screening revealed that saponins, alkaloids and tannins were detected in both mycelia and fruiting body while flavonoid was only found present in the fruiting body. Mycelia had higher amounts of crude fiber (7.99 ± 0.01%), carbohydrates (59.45 ± 0.01%), moisture (12.40 ± 0.14%), and crude fat (2.91 ± 0.06%) while fruiting body had higher amounts of ash (5.61 ± 0.01%), crude protein (17.89 ± 0.00%) and energetic value (321.04 ± 0.03 kcal). Coconut water medium at pH 5 significantly recorded the highest mycelia weight (5.5 ± 0.15 g), percentage radical scavenging activity (14.84 ± 0.19%) and total phenolics (25.25 ± 0.01 mg AAE/g sample). Hence, P. cyanescens is another promising source of nutraceuticals.

Key words*: Panaeolus cyanescens, submerged culture, antioxidant, mycochemicals, coprophilous.*

INTRODUCTION

A number of wild mycological genetic resources in the Philippines remains to be unharnessed. One of these diverse groups of mushroom is coprophilous basidiomycetes that commonly found growing on the dried dung of ruminants in the pasture areas of Central Luzon. Ilocanos called these mushrooms as *oong takki* meaning dung mushroom. *Panaeolus cyanescens*, one of the coprophilous fungi, is characterized by a light brown fruiting body when young, fading to whitish gray at maturity and blue or greenish blue stain appears when any part of the fruiting body is damaged which indicates the psilocybin content[1]. This mushroom contains psychoactive alkaloids psilocin (0.48%) and psilocybin (0.11%), as well as serotonin (0.072%), urea (1.8%), and baeocystin (0.02%)[2]. Psilocybin from *P. cyanescens* has demonstrated promising therapeutic benefits such as acute reduction in obsessive-compulsive disorder (OCD) symptoms[3]. In addition, this compound also showed great potential to patients suffering from depression, anxiety, schizophrenia, and obsessive-compulsive disorder[4].

Mushrooms are abundant sources of a wide range of various useful compounds including, alkaloids, terpenoids, phenols, steroids, and nucleotides, which are shown to exhibit bioactivities such as diabetes, hypertension, hypercholesterolemia and even cancer[5]. Apart from these functional activities, the antioxidant properties of wild mushrooms have also been extensively studied by many researchers. The most common medicinal mushroom, for instance, is the *Ganoderma lucidum.* Its antioxidant activity is primarily due to its terpenoids, phenolic and polysaccharide polypeptide contents,[6] which are reported to stimulate nitric oxide synthase activation, interlekin-12 production, iron chelating and free radical scavenging properties[7].

In addition, these antioxidants play an important role in preventing the oxidative stress which contributes to cell damage, generating of cancer cell and brain cell aging[8].

Although previous works on the coprophilous mushrooms particularly *P. cyanescens* were focused on the therapeutic effects, information about their antioxidant properties is very minimal. Hence, this present study elucidated the mycochemicals of mycelia and fruiting bodies and evaluated the antioxidant activity of submerged culture of *P. cyanescens* as affected by the different culture media and pH levels.

MATERIALS AND METHODS

Preparation of culture inoculant

The *P. cyanescens* culture was aseptically transferred into sterilized potato dextrose agar (PDA) plates and incubated at 30°C for 7 days to allow growth of the mycelia. The mycelial discs were prepared using flame sterile cork borer (10 mm diameter size) in revived culture which were used as inoculant in mycelia and fruiting body production.

Mycelial mat production

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

Fruiting body production

Rice straw, the substrate used, was soaked in water tank for 7 days and then washed with running water, hauled out from the tank, piled and covered with sacks to maintain moisture content. The pile was turned everyday for three consecutive days until materials become pliable and dark brown in color. After the decomposition process, the rice straw was chopped about 2-3 inches in size and 7 parts of it was mixed with 3 parts of sawdust (v/v) with 65% moisture content. Two hundred grams of formulated substrates were placed in a glass container. Fifty containers were prepared. The bottles were covered with polypropylene sheets, secure with rubber band and sterilized at 121°C, 15 psi for 45 minutes. The sterilized substrates were allowed to cool before inoculation of mycelia discs. The inoculated bottles were incubated at 30°C to allow full ramification of mycelia on the substrate. Once completely colonized, the emergence of fruiting bodies was allowed. Matured fruiting bodies were harvested and air-dried for 3-4 days for mycochemical analyses.

Mycochemical analyses of *P. cyanescens*

The chemical screening of the aqueous extracts of fruiting body and mycelia were carried out following the procedures described by Sofowora[9] and Harborne[10]. Three replicates were laid out for each test parameter. Results were compared with distilled water as control and determined based on the color/intensity of the reaction[11].

The nutritional compositions of the air-dried fruiting body and mycelia of *P. cyanescens* were also analyzed. Crude protein, crude fat, crude fiber, ash, and moisture content (MC) were analyzed according to the guidelines of the Association of Official Analytical Chemist[12]. 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).

Influence of nutritional factors

The influence of nutritional factors on the antioxidant activity of *P. cyanescens* was evaluated using different broth culture media: coconut water from mature coconut (*Cocos nucifera*), rice bran D1 (class A) 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).

Broth media were adjusted to pH 6 and 100 ml of each was dispensed into microwavable plastic container. These were sterilized using an autoclave at 121C, 15 psi for 30 minutes prior to inoculation of the mycelial discs. Each set-up was comprised of three replicates. The inoculated broth media were incubated at 30°C under alternating light and dark condition to allow mycelial growth. After 10 days, the mycelial mats were harvested and weighed. The mycelia and spent were mixed and subjected to a blender to homogenize for antioxidant analysis.

Influence of pH level

The most appropriate culture medium with optimum antioxidant activity was used to evaluate the antioxidant activity as affected by pH level. The best broth medium was adjusted to varying pH levels (6.0-8.0) and 100 ml of each pH was poured into microwavable plastic container and sterilized using an autoclave. These were inoculated with mycelial discs and incubated at room temperature. The weight of mycelial mat was determined. The mycelia and spent were homogenized for antioxidant analysis.

DPPH radical scavenging activity

Ethyl acetate (10 ml) was added into each cultured broth to extract the antioxidant compounds. The ethyl acetate soluble portion was concentrated under reduced pressure and the concentrates were dissolved in ethanol. 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.*[13] 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.

Total phenolic content

The total phenolic content was estimated using Folin-Ciocalteu method of Slinkard and Singleton[14] 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 and nutritional contents

The term mycochemical refers to a classification system of mycological chemicals. These mycochemicals are chemicals that fungi particularly mushrooms produce to perform metabolic functions and also to protect themselves, however, in plants it is called phytochemicals. The mycochemical composition of the mycelia and fruiting body of *P. cyanescens* is presented in Table 1. Out of six mycochemical constituents screened, three were found present in mycelia while four were detected in the fruiting body. Saponins, alkaloids and tannins were detected in both samples while flavonoid was only found present in the fruiting body. With its important compositions, this basidiomycete could demonstrates an enormous potential for different bioactivities. Alkaloids were found to be in appreciable amount in *P. cyanescens*. The same is true with the study conducted by Adebayo *et al.*[15] that alkaloids are present in the purified mycelial mat extract of *P. pulmonarius.* One of the most biological properties of alkaloids is the toxicity against cells of foreign organisms. This bioactivity has been widely studied for their potential use in the elimination and reduction of human cancer cell lines[16].

Another important mycochemical compositions are saponins and tannins. Saponins are potent antioxidants that neutralize free radicals to prevent disease and stimulate the production of antibodies, which help in fighting bacterial and fungal infections[17.] Other pharmacologic effects include haemolytic, anti-inflammatory, antifungal, anti-bacterial, anti-viral, ichthyotoxic, cytostatic and antieoplastic activities[18].

Tannins exhibit antioxidant properties related to their scavenging activity which have been used against heart diseases[19]. The presence of flavonoids was detected with the yellow coloration of the ethanolic extract of fruiting bodies of mushroom sample. Menaga *et al*.[20] reported that flavonoids are present in moderate concentrations in methanolic, ethanolic, and aqueous extracts, but not present in hexane and ethyl acetate extracts. It has been found that flavonoids exhibit antioxidant, anti-inflammatory, antiallergic, antiviral, as well as anticarcinogetic activities[20]. However, cardiac glycosides and phlobatannins were not detected in both samples.

Table 1. Compositions of mycelia and fruiting body of *P. cyanescens*

Composition	Mycelia	Fruiting body
Mycochemicals		
Flavonoids	Absent	Present
Phlobatannins	Absent	Absent
Cardiac glycosides	Absent	Absent
Tannins	Present	Present
Saponins	Present	Present
Alkaloids	Present	Present
Nutrients (%)		
Moisture	12.40 ± 0.14	11.72 ± 0.01
Ash	5.20 ± 0.00	5.61 ± 0.01
Crude protein	12.06 ± 0.03	17.89 ± 0.00
Crude fiber	7.99 ± 0.01	4.54 ± 0.01
Crude fat	2.91 ± 0.06	1.70 ± 0.28
Total carbohydrate	59.45 ± 0.01	58.56 ± 0.01
Energy value	312.23 ± 0.03	321.04 ± 0.03

Mushrooms are rich in protein, minerals, and vitamins, and they contain abundant essential amino acids[21]. In the present study, the proximate composition of air dried mycelia and fruiting body of *Pleurotus cyanescens* were analyzed and the amount of crude protein, crude fiber, crude fat and carbohydrates as well as moisture content, ash content and energy value (kcal) were determined. The nutritional composition of *P. cyanescens* mycelia and fruiting body is also presented in Table 1. It can be seen that the mycelia *P. cyanescens* contained higher amount of moisture, crude fiber, carbohydrates, crude fat than its corresponding fruiting body. On the other hand, fruiting body had higher ash, crude protein, and energetic value than mycelia.

The crude protein of the fruiting body of *P. cyanescens* (17.89%) is better than the content of *P. ostreatus* (14.03%),[22] and near to the crude protein of *P. pulmonarius* grown on the different substrates[23]. This amount of protein makes this mushroom an ideal food to human nutrition. Protein content of mushrooms were reported to vary according to genetic structure of species, and physical and chemical differences in growing medium[24]. On the other hand, *P. cyanescens* contained 59.45% (in mycelia) and 58.56% (in fruiting body) of the total carbohydrates. This carbohydrate content is higher compared to *C. comatus* ranging from 32% to 39.07%[25] and ten mushroom species ranging from 8.00% (*M. procera*) to 32.50% (*P. ostreatus*)[22]. The carbohydrate content of mushrooms represents the bulk of fruiting body accounting for 50% to 65% on dry weight basis[26]. Result showed that *P. cyanescens* is a very good source of carbohydrates.

Dietary fiber helps in digestion process. Although result is lower compared with wild Nigerian mushrooms namely, *P. ostreatus* (20.36%), *H. erinaceus* (16.76%), *C. odora* (12.72%), *L. amethysta* (15.72%) and *C. cibarius* (13.64%),[22] the considerable amount of fiber in this study suggest that *P. cyanescens* can also be a potential source of dietary fiber. Mushrooms are known to be low calorie food with very little fat and are highly suitable for obese persons[27]. According to Ulziijargal and Mau, [28] the fat content of mushrooms ranges from 1.1% to 8.3% in terms of dry weight. The amount of crude fat content of *P. cyanescens* containd 2.91% in mycelia and 1.70% in fruit which is higher compared to *C. comatus* ranging from 0.53% to 0.55%[25] but significantly less fat when compared to Nigerian mushrooms studied by Egwim *et al*.[22] ranged from 1.29% (*H. erinaceus*) to 14.29% (*C. odora*).

The energy value of obtained in the mycelia and fruiting body of *P. cyanescens* are higher compared to energy value of *C. comatus* (262.92 kcal) and *G. lucidum (*138.33 kcal).[28] This result revealed that *P. cyanescens* is a good source of energy. Many factors may be involved in the difference of nutritional composition of mushrooms cultivated in different substrates. Adenipekun and Gbolagade[29] mentioned that growth yield and quality of mushroom depends on the status of their nutritional sources such as; C:N ratio, vitamins, phytohormones, macro and microelements. Kalač[30] added that nutritional profiles of mushrooms are directly affected with their moisture content depending on their harvesting time, maturation period and environmental conditions (humidity, temperature, growing period, storage condition etc.).

Mycelial weight and antioxidant activity of *P. cyanescens* on the different culture media and pH levels

The effect of the different broth media on the antioxidant activity of *P. cyanescens* was determined in this present work. Table 2 depicts the mycelial weight, percentage radical scavenging activity, and phenolic content of the *P. cyanescens* grown on the different culture media and varying pH levels. The highest mycelial weight was significantly registered in coconut water, which is statistically comparable with rice bran broth. On the other hand, corn grit broth had the lowest mycelia weight. This efficient growth on coconut water medium could be explained by its nutritious composition that stimulated the growth of *P. cyanescens*. Its unique chemical composition includes sugars, amino acids, vitamins, minerals and phytohormones[31]. Similar with the growth response, coconut water had the highest percentage radical scavenging activity (14.40 ± 0.19%) and total phenolic content (25.19 ± 0.07 mg AAE/g sample). In contrast, corn grit significantly recorded the lowest percentage scavenging activity and phenolic content. These significant findings strongly dictate that the antioxidant properties of *P. cyanescens* are affected by the culture media, thus, this bioactivity is culture media dependent.

pH greatly affects the enzymatic activity of the mushroom species. Since coconut water showed the most efficient growth and highest antioxidant activity, this medium was used in evaluating the optimum pH level. Coconut water at pH 5 significantly recorded the highest mycelia weight (Table 2) which indicates that pH 5 is the optimum pH for the efficient growth of *P. cyanescens*. This observation supports the findings of Chang and Miles[32] that basic medium could denature the mycelia and become functionless. In terms of activity, pH significantly affects the antioxidant property. pH 5 registered the highest percentage radical scavenging activity (14.84 ± 0.19%) and phenolic content (25.25 ± 0.01 mg AAE/g sample) which is statistically comparable to pH 6. On the other hand, pH 8 showed the lowest activity. These results of the present study indicate that *P. cyanescens* contain bioactive compounds with antioxidant properties which influenced by the type of media and level of pH. This findings support pervious works that mushrooms demonstrated antioxidant activities. Such mushrooms include *Pleurocybella porrigens, Volvariella esculenta, Ganoderma lucidum, Pleurotus ostreatus* and *Lentinus squarossolus*[33,34]. Moreover, the methanolic extracts from *Leccinum scabrum* showed the most potent radical scavenging activity (97.96%) while *Boletus edulis had the highest total phenolics*, among the 24 mushroom species evaluated by Keles *et al* [35].

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

Treatment	Mycelial weight (g)	RSA (%)	Total phenolics (mg AAE/g sample)
Culture broth			
Coconut water	5.9 ± 1.60^{a}	14.40 ± 0.19^{b}	25.19 ± 0.07^{a}
Potato broth	4.0 ± 0.76^{bc}	10.74 ± 0.51^{c}	22.71 ± 0.13^{b}
Rice bran broth	5.6 ± 0.93^{ab}	13.18 ± 0.19^{b}	25.04 ± 0.01^{a}
Corn grit broth	3.8 ± 0.50^{c}	08.64 ± 0.33^{d}	22.42 ± 0.04^{c}
Cathechin (+)		31.34 ± 1.92^{a}	
pH level			
pH 5.0	5.5 ± 0.15^{a}	14.84 ± 0.19^{b}	25.25 ± 0.01^{a}
pH 6.0	3.4 ± 0.36^{b}	14.06 ± 0.51^{bc}	24.92 ± 0.04^{ab}
pH 7.0	3.5 ± 0.10^{b}	12.85 ± 0.51^{cd}	24.39 ± 0.01^{ab}
pH 8.0	2.6 ± 0.25^{c}	11.74 ± 0.51^{d}	23.36 ± 1.25^{b}
Cathechin (+)		28.02 ± 1.92^{a}	

(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.)

CONCLUSION

Altogether, *P. cyanescens*, being coprophilous and reported to be hallucinogenic mushroom, provides important nutrients and healthful benefits. Its mycochemical components which contribute to its bioactivity are essential to the nutraceutical industry. The free radical scavenging activity and phenolic content of this mushroom, commonly used to estimate antioxidant activity, could bring novel perspectives in finding cures for the different types of diseases associated with oxidation. However, better insights and understanding on the amino acid, fatty acid composition, and other bioactivities as well as the ability to be cultivated for commercial scale must be carried out in the future studies.

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