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Proximate Analysis and Antioxidant Potential of *Schizophyllum Commune* Ethanolic Extract

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Abstract

Mushrooms are vital components of the forest ecosystems that play an important ecological role, economic advantage and provide nutritious food to humans. The study used the standard protocol to determine the proximate analysis of a wild and edible mushroom, *Schizophyllum commune.* The ethanolic crude extract was analyzed for the total phenolic content (TPC) using the Folin-Ciocalteu method and the total flavonoid (TFC) using aluminum chloride. The antioxidant potential was evaluated using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. Proximate analysis of the sample revealed that apart from moisture content (60.60±0.122%), it has a high amount of bioactive carbohydrates (25.15±0.005%), crude protein content (7.63±0.325%), ash content (6.17±0.001%), crude fiber (0.18±0.032%), and fat content (0.27±0.263%) which worth it to be nutritious food. The final concentration for TPC is 1.27±006 mgGAE/g and TFC is 17.18±0.054 mgRE/g. The extract exhibited a potential antioxidant activity with an IC_{50} = 1.39±0.005 mg/mL. The results suggest that the ethanolic crude extract of *S. commune* contains a natural antioxidant potential, which could be used for future applications in food, dietary supplements, and pharmaceutical industries.

Keywords: Antioxidant, DPPH, Flavonoid, Phenolic, Proximate analysis, *S. commune*

Introduction

Macrofungi are vital components of the forest ecosystems that play an essential ecological role, economic benefit and provide food nutritious to humans. They are indispensable components of the forest ecology, for they decompose large quantities of dead organic matter that maintain or enhance soil fertility (Tyler, 1992). Macrofungi are popular and valuable foods since they provide essential nutrients and healing properties. They have been valued as traditional sources of natural bioactive compounds and a wide range of valuable natural products with promising therapeutic agents (Devishree & Jain, 2017; Lindequist et al., 2005; Valverde et al., 2015; Zhong & Xiao, 2009).

Schizophyllum commune, also known as the split-gill mushroom, is a saprophytic macrofungus and an easily identifiable species since they attached themselves to deadwood or rotten logs (Volk, 2000). It is found predominantly during the rainy season. The cap is usually greyish to white with pale reddish to grey gills. The gills appear to be split and function to produce basidiospores on their surface, and they can dry out and rehydrate numerously during their growing season (Verma & Verma, 2017).

Interestingly, *S. commune* is consumed as food and sold or bartered by several people, particularly by several ethnic groups in Mexico (Quinonez-Martinez et al., 2014; Ruan-Soto, 2018; Ruán-Soto et al., 2006). It is considered a higher edible fungus in Nigeria and is being studied to

assess mass cultivation for its high nutritional value (Adejoye et al., 2007). In Congo, it is used mainly as food and provides income to local communities (Kamalebo et al., 2018). It is used in Japan to produce a cheese-like food product since it has both lactate dehydrogenase and milk-clotting enzyme activities (Okamura-Matsui et al., 2001). In India, consumption, and selling of wild edible mushrooms, including *S. commune,* were evident among ethnic groups (Bhaben et al., 2011). Food and Agriculture Organization (FAO) listed *S. commune* as wild edible fungi with medicinal properties (Boa, 2004).

The Philippines, as a tropical country, has rich mycological resources, including the *S. commune.* It is known to Visayans as *kuropdop* or *kulapdop*. It is underutilized nutritious food and under-discovered for its economic and pharmacological importance. Rural folks often ignore this type of mushroom due to a lack of information about its edibility and the unavailability of technology for production (Reyes et al., 2013). Due to social change, the younger generations were much less knowledgeable about mushroom utilization; thereby, nutritional and medicinal properties are being disregarded (Lee et al., 2009).

S. commune is considered a valuable food since it provides essential nutrients and physiologically significant bioactive substances. The antioxidant compounds in these mushrooms can scavenge the free radicals and protect or reduce the damage to the cells caused by the oxidation (Kozarski et al., 2015). The present study primarily focuses on the proximate analysis of the oven-dried *S. commune* and the determination of its total phenolic, flavonoid, and ethanolic crude extract's antioxidant potential. The result would benefit the present generation to appreciate this wild edible mushroom's nutraceutical potential and include it in their diet. Thus, it can be a source of alternative natural antioxidants instead of consuming synthetic antioxidants.

Materials and Methods

Collection, Identification, and Preparation of S. commune

Fresh samples of *S. commune* were collected with local folks' help in the forest of Minapasuk in the Northern part of Negros Island. The collected sample was kept using Ziploc® and labeled. Morphological identification was done using its macroscopic characteristics and compared with published literature and online identification keys mycological information site. The identified mushroom sample was cleaned from unwanted debris and transported to the Negros Prawn Producers Cooperative (NPPC) Diagnostic and Laboratory – a certified ISO/IEC 17025: 2005 in Bacolod City, Negros Occidental, Philippines.

S. commune was prepared for analysis by drying the sample in an oven for four hours at 104 ^{0}C (Okwulehie IC et al., 2017). The dried samples were pulverized using the mortar and pestle and kept in an air-tight container, placed in cool and dry storage until required for analysis.

Preparation of the Extract

The extract of the sample was prepared following the standard protocol with modification (Aguinaldo et al., 2004). 113.18 grams of oven-dried sample was macerated in 1.13 L of 95% ethanol for 48 hours. Using a rotary evaporator, the supernatant obtained from the decanted mixture was concentrated at 60oC and -80kPa. Then, the ethanolic extract was stored at 4^{o}C for further analysis.

Proximate Analysis

Oven-dried samples were analyzed for moisture content, carbohydrates, proteins, crude fibers, fats, and ash, using the standard methods (AOAC International, 2019; Puwastien et al., 2011)

Determination of moisture content

Moisture content was analyzed by gravimetric-oven drying at 105 ^{0}C. A 5-gram sample of *S. commune* was oven-dried at 105 ^{0}C for 3 hours and then cooled at the desiccator. The difference between the sample's weight before and after drying was used to determine its moisture content.

$$Moisture\ (\%) = \left(\frac{A-B}{B}\right)\ x\ 100 \quad (1)$$

where: A = weight of the sample before drying
B = weight of the sample after drying

Determination of total ash (oxidation at 550^{0}C)

The sample's total ash was determined by weighing one gram of the sample in a crucible and heated in a muffle furnace for about 6 hours at 550 ^{0}C until the sample was completely charred. Then cooled in a desiccator and weighed. The total ash content was calculated using the equation:

$$Ash\ (\%) = \frac{weight\ of\ ash}{weight\ of\ sample}\ x\ 100 \quad (2)$$

Determination of crude fiber (AOAC method)

The crude fiber was determined using moisture, and the fat-free sample was treated with 0.25N H_2SO_4 and 0.3N NaOH and washed with ethanol and ether. The sample was transferred to a crucible, dried overnight at 80 ^{0}C – 100 ^{0}C, and weighed using an analytical balance (W_1). The crucible was heated for 6 hours at 100 ^{0}C, cooled, and weighed again using the same analytical balance (W_2). The difference in weights represents the weight of the crude fiber.

$$Fiber\ (\%) = \left(\frac{100-(moisture+fat)}{weight\ of\ sample}\right) x100 \quad (3)$$

Determination of total fats (Soxhlet method)

The total fats were determined by the semi-continuous extraction (Soxhlet) method. About 5 grams of oven-dried sample was weighed and put into two separate thimbles, plugged with dried glass wool, and placed in a soxhlet extractor, extracted for about 4-6 hours using petroleum ether. After extraction, thimbles were removed, cooled in a desiccator, and weighed. The weight of fat can be determined by subtracting the weight of (thimble + glass wool + defatted sample) from (weight of the sample + thimble + glass wool).

$$Fat\ \% = \frac{weight\ of\ fat}{weight\ of\ sample}\ x\ 100 \quad (4)$$

Determination of total proteins (Kjeldahl method)

Total proteins were determined by boiling the 1-gram sample in a digestion flask with Kjeldahl catalyst and conc. H_2SO_4 until transparent. The solution was cooled down at room temperature and centrifuged to separate the mixture. The supernatant liquid was collected to determine total protein content using a 6.25 conversion factor (Mariotti et al., 2008). To calculate for % N on a wet and dry basis:

$$\%Nwet = \frac{(A-B\ x\ 1.4007)}{weight\ of\ sample}\ x\ 100 \quad (5)$$

where: A = vol. (mL) std. HCl x N of std. HCl
B = vol. (mL) std. NaOH x N of std. NaOH

$$\%Ndry = \frac{(5\ x\ Nwet)}{(100-\%\ moisture)}\ x\ 100 \quad (6)$$

To calculate the percentage of protein content:

$$\%\ Protein = \%N\ x\ 6.25 \quad (7)$$

Determination of nitrogen-free extract (NFE)

The nitrogen-free extract comprises all the nutrients not assessed by the prior proximate analysis methods: digestible carbohydrates, vitamins, and other non-nitrogen soluble organic compounds. NFE can be obtained by subtracting the percentages calculated for each nutrient from 100.

$$NFE\ (\%) = 100 - (moisture + fat + ash + fiber + protein) \quad (8)$$

Determination of Total Phenolic (TP) Content

The TP content of *S. commune* ethanolic extract was determined using the Folin-Ciocalteu method described by Dasgupta et al. (2014); Musci & Yao (2017); Rebelo et al. (2009); & Singleton et al. (1999).

A 1 mL extract was mixed with 5 mL deionized water, 1.5 mL of 2% sodium carbonate, and 1 mL of 1N Folin-Ciocalteu reagent. The solution was allowed to stand in a water bath for about 30 minutes at 40°C. Using a UV-Vis spectrophotometer (Optima SP 300), the absorbance was measured at 765 nm after incubation. Gallic acid was used as standard, and TP content was expressed as milligram gallic acid equivalent (mg GAE)/g of the extract.

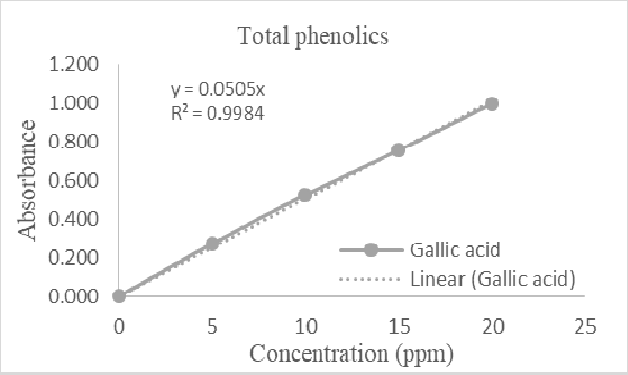

Figure 1. The concentration and absorbance of the standard (Gallic acid) to determine the total phenolic content of the *S. commune* extract. Values are the average of three replicates and are represented as mean ± SEM.

Determination of Total Flavonoid (TF) Content

The TF content was determined by the aluminum trichloride method using Rutin as a reference compound (Mathur & Vijayvergia, 2017; Zhao et al., 2018). 1 mL of the extract was added with 1 mL methanol and 1 mL 2% $AlCl_3$. The mixture was allowed to be incubated at room temperature for 1 hour. After incubation, the absorbance was measured at 415 nm using a UV-Vis spectrophotometer (Optima SP 300). The TF content was expressed as Rutin equivalent (mgRE) per g of dry weight.

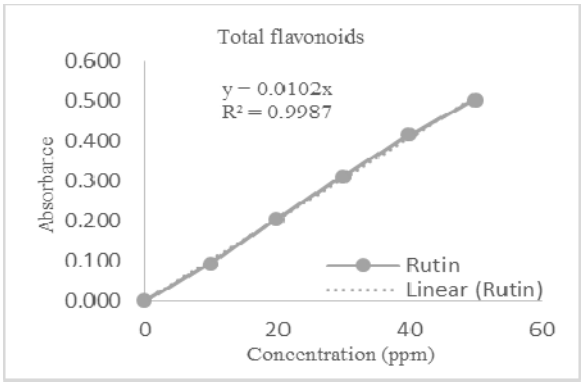

Figure 2. The concentration and absorbance of the standard (Rutin) to determine the total flavonoid content of the *S. commune* extract. Values are the average of three replicates and are represented as mean ± SEM.

Antioxidant Activity using DPPH

The DPPH radical-scavenging activity was quantitatively determined using the method described by (Okawa et al., 2001; Zhao et al., 2018).

A newly prepared DPPH stock solution was mixed with the *S. commune* extract at different concentrations. A control (Abs Control) was done containing methanol and DPPH solution. All solutions were kept warm at room temperature for one hour. The absorbance was measured at 517 nm. The free radical scavenging capacity of DPPH radical was evaluated by measuring the absorbance decrease at 517 nanometers using ascorbic acid as a standard. After the reading was completed, the percentage of inhibition of samples was calculated from obtained absorbance by the equation:

$$DPPH\ Inhibition\ (\%) = \frac{Abs\ control - Abs\ test)}{Abs\ control} x\ 100 \qquad (9)$$

Then, curves were constructed by plotting the percentage of inhibition against concentration in µg/mL. The equation of the curve was allowed to calculate the IC_{50} corresponding to the sample concentration reducing the initial DPPH absorbance by 50%. The smaller IC_{50} value corresponds to a higher antioxidant activity.

Statistical Analysis

Experimental data were obtained from three replicates. Statistical analyses (mean ± SEM, linear regression analysis) were carried out using MS Excel (Microsoft Office 2010 Professional).

Results and Discussion

Proximate analysis

The results of the proximate analysis of *S. commune* are shown in Table 1. Proximate analysis was done to quantitatively estimate the presence of nutrients in the sample.

The high moisture content (60.60±0.122%) indicates mushrooms are highly perishable and susceptible to enzymatic browning (Kumar et al., 2013). As one of the variable components of food, moisture content remains an essential constituent that affects the whole composition of the food (Greenfield & Southgate, 2003). *S. commune* is an excellent source of bioactive carbohydrates (25.15±0.005%), protein (7.63±0.325%), and fat (0.27±0.263%). The result was higher than other edible mushrooms and food sources, specifically *Agaricus sp, Boletus edulis, Volvariella, Macrolepiota,* Portobello, commercial and wild champignon, potatoes, cabbage, and spinach (Alvarez-Parrilla et al., 2009; E. Herawati et al., 2016; Elisa Herawati et al., 2021). The ash content (6.17±0.001%) is the basis for its mineral content. Knowledge of the nutritional value of the food we eat is necessary to encourage the increased consumption and cultivation of this nutritious mushroom. Additionally, other mushrooms, such as *M. dolichaula,* are a good source of carbohydrates (44.37%), protein (25.53%), fiber (14.17%), and ash (5.19%). This mushroom has higher lipid content (1.17%) and high fiber content (14.17%) compared to *S. commune* (Rizal et al., 2016). Both mushrooms can be excellent food sources because of their nutritional and culinary potential.

Table 1. The proximate Composition of *S. commune*

Parameter	% Composition
Moisture	60.60±0.122
Ash	6.17±0.001
Fiber	0.18±0.032
Fat	0.27±0.263
Protein	7.63±0.325
Carbohydrate	25.15±0.005

Values are the average of three replicates and are represented as mean ± SEM.

Some ethnic groups worldwide use wild edible mushrooms as a food source (Adejoye et al., 2007; Bhaben et al., 2011; Boa, 2004; Deka et al., 2017; Haro-Luna et al., 2019; Kamalebo et al., 2018; Okamura-Matsui et al., 2001; Quinonez-Martinez et al., 2014) since mushrooms have great potential for nutritionally functional food and can be easily added to the diet.

Total Phenolic (TP) and Total Flavonoid (TF) Content of S. commune ethanolic extract

The TP and TF content of *S. commune* ethanolic crude extract is shown in Table 2. The phenolic and flavonoid content of edible mushrooms is significant since both have a potent biological activity that functions as reducing agents and free-radical scavengers. Both compounds are essential in controlling cancers and other human diseases (Ghasemzadeh & Ghasemzadeh, 2011). The extract's total phenolic content was analyzed using the Folin-Ciocalteu method and expressed as a gallic acid equivalent of 1.267±006 mg GAE/g. The study result was deficient compared to *S. commune's* phenolic content cultivated using coconut water as a substrate with 25.52 mg AAE/g sample (Dulay et al., 2016). The result is relatively higher than the ten wild mushrooms collected from Selangor and Sabah forests, ranging from 0.015 to 0.075 mg GAE/g dried extract (Azieana et al., 2017). The phenolic compound found in *S. commune* is 12.5 ± 1.4 µg /mg of the sample, which is higher than the phenolic content of *Pleurotus ostreatus* (5.49 µg/mg) and *Pleurotus citrinopileatus* (8.62 µg/mg), and *Russula albonigra* (9.53 µg/mg)(Dasgupta et al., 2014). According to Islam (Islam et al., 2016), compared to 43 edible mushrooms consumed in China, the result is much lower than the stone ear (26.21 GAE/ g), porcino nero (11.93 GAE/g), and yellow bolete (10.62 mg GAE/g) but relatively higher than the phenolic content of tuckahoe (0.19 mg GAE/g), tremella (0.22 mg GAE/g), golden ear (0.80 mg GAE/g), Jew's ear (0.83 mg GAE/g), golden leaf (0.86 mg GAE/g), and mulberry yellow (0.93 mg GAE/g). Depending on species, eight edible mushrooms (*Agaricus bisporus, Boletus edulis, Calocybe gambosa, Cantharellus cibarius, Craterellus cornucopioides, Hygrophorus marzuolus, Lactarius deliciosus,* and *Pleurotus ostreatus*) contained 1 to 6 mg of phenolics per gram of dried mushroom; flavonoid content ranged from 0.9 to 3.0 mg per gram of dried matter (Palacios et al., 2011).

The *S. commune* ethanolic extract's total flavonoid content was analyzed using the aluminum trichloride method and expressed as rutin equivalent is 17.176±0.054 mg RE/g. The total flavonoid content is higher than that of the total phenolic content. Likewise, the study results suggest that *S. commune's* total flavonoid content is much higher than other mushrooms, as reported (Azieana et al., 2017; Ghasemzadeh & Ghasemzadeh, 2011; Sharififar et al., 2009). The flavonoid-rich mushrooms could be an excellent antioxidant source that would increase the overall antioxidant capacity and protect the organisms from lipid peroxidation (Dulay et al., 2016).

The values of phenolic and flavonoid content in this study varied significantly compared to several literature pieces due to extraction methods, the solvent used, the presence of different amounts of sugar, ascorbic acid, carotenoids, and geographical variations, and sources of the mushrooms (Aryal et al., 2019).

Table 2. The TP and TF Content of *S. commune.*

Extract	TP (mg GAE/g)	TF (mgRE/g)	IC_{50} mg/ml
S. commune	1.27±006	17.18±0.054	1.39±0.005

TP (total phenolic) content; TF (total flavonoid content); IC_{50} (half maximal inhibitory concentration). Values are means (± SEM) of the triplicate samples

DPPH Scavenging Activity of S. commune ethanolic extract

The DPPH radical scavenging activity of *S. commune* is shown in figure 3. The ethanolic crude extract of *S. commune* showed an increasing scavenging effect as the concentration increased. DPPH acted as free radical scavengers or hydrogen donors and was used to evaluate the extract's antioxidant activity. The extract's radical scavenging activity exhibited the IC_{50} value of 1.39±0.005 mg/mL. The IC_{50} value of the extract of *S. commune* is slightly higher than the ascorbic acid with IC_{50} = 0.017±0.012 mg/mL used as a standard; a lower IC_{50} value means high antioxidant activity. (Chandrawanshi et al., 2017) reported that *S. commune* ethanolic extract showed the most potent H_2O_2 scavenging activity with IC_{50} = 19.79 µg/ml and significant phenolic content IC_{50} =11.86 µg/ml compared to methanolic and hot water extract. A high correlation was observed between antioxidant capacity and total phenolic and flavonoid content with DPPH, R^2 = 0.75; H_2O_2, R^2 = 0.71 and DPPH, R^2 = 0.84; H_2O_2, R^2 = 0.66, respectively.

Similarly, *S. commune's* aqueous ethanol extract exhibited a most powerful reducing capability than methanol and aqueous methanol extract with 76.61 ± 1.79 mM Fe(II)/g crude extract (Razak et al., 2018). In the study conducted by Emsen et al. (2017)], *S. commune* has a maximum phenolic content (27.66 µg/mg) obtained from acetone extract; maximum flavonoid content (3.48 µg/mg) obtained from n-hexane extract; and the chloroform extract showed the highest DPPH capturing (7.652 mg/ml) and metal chelating (6.590 mg/ml) activities in the IC_{50} values. The result showed that it is possible to suggest that the ethanolic crude extract's phenolic and flavonoid contents were responsible for the antioxidant activity of *S. commune.*

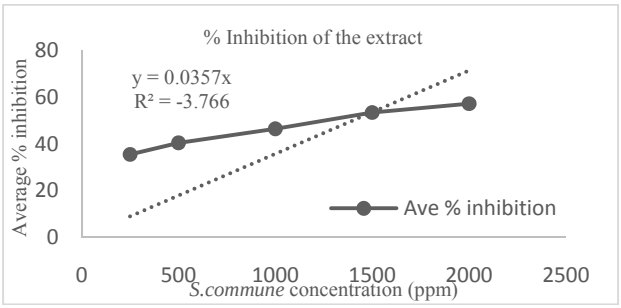

Figure 3. The concentration and average percent inhibition of *S. commune* extract. Values are the average of three replicates and are represented as mean ± SEM.

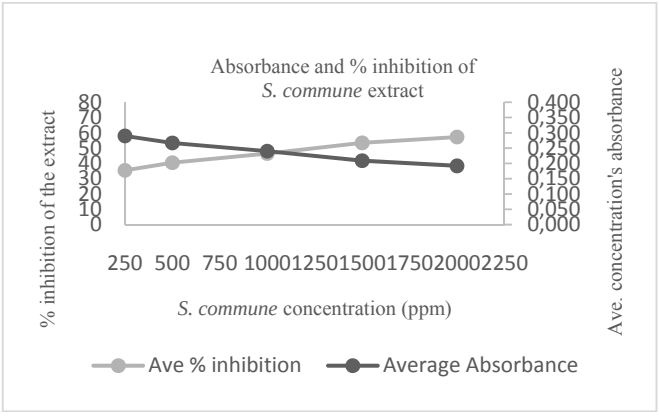

Figure 4 The percent inhibition and absorbance against the concentration of *S. commune* extract.

Conclusion

The proximate analysis, total phenolic, flavonoid, and antioxidant activity of the ethanolic crude extract of the wild edible mushroom, *S. commune*, were investigated. The result showed that *S. commune* is a profound nutritional and medicinal mushroom. The phenolic and flavonoid contents are a potential alternative natural antioxidant compound source. Further, with the established antioxidant activity and known therapeutic potential of the *S. commune*, phytochemical and biological characteristics of the compound present in the extract should be investigated for potential bioingredient of cosmeceutical and pharmaceutical products.

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