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Evaluation of Nutrient Content and Antioxidant Activity of Wood Ear Mushroom (*Auricularia auricula-Judae*) in the Addition of Reeds (*Imperata cylindrica* (L.) Beauv) as a Cultivation Medium

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ABSTRACT

The nutritional content of mushrooms depends on the type of substrate in the growing medium. Lignocellulose is one of the substrates used for fungal growth media. In this study, the effect of adding dried reeds (*Imperata cylindrical* (L.) Beauv) on Sengon (*Paraserianthes falcataria* (L.) Nielsen) sawdust-based media was investigated for wood ear mushroom (*Auricularia auricula-Judae*) fruiting body. Reeds have been added to Sengon sawdust as wood ear mushroom cultivated medium with variations of S0, S1, S2, S3, and S4 (0%, 25%, 50%, 75%, and 100% of reeds, respectively). S0 was designed as the negative control and S4 as the positive control. The evaluation was carried out against the C/N ratio, yield, biological efficiency (BE), nutritional content, and antioxidant activity of fruiting body mushrooms after 10, 20, and 30 days planted. The highest C/N ratio and BE were obtained from the S1 medium. The lowest moisture content was obtained from the S0 medium. The lowest fat content was obtained from the S4 medium. The best result of nutritional analysis includes the following; the highest total carbohydrate, ash, crude fiber, and crude protein content was obtained from S3, S4, S3, and S0. Furthermore, the best result of antioxidant activity against ABTS and DPPH was obtained at methanol extract of fruiting body mushrooms from S1 medium, compared with ascorbic acid as the positive control. This study has shown that adding reeds to the cultivated medium influences the growth, nutritional content, and antioxidant activity of wood ear mushrooms.

1. Introduction

Nowadays, *Auricularia auricula* is one of the four most important edible mushrooms in the world which is mainly grown in China and East Asia with production reaching 420,000 tons/year (Yan *et al.* 2004). *A. auricula* has a high nutritional value because containing high carbohydrates, amino acids, minerals, and vitamins and can be processed into various foods (Fan *et al.* 2006). *A. auricula* fruiting body is rich in carbohydrates, proteins, and minerals (Ca, P, and Fe). 100 g of dried *A. auricula* fruiting body contains 18.3 g of protein, 18.9 g of carbohydrate, and 50 g of fiber (Khaskheli *et al.* 2015). Some previous studies have shown that *A. auricula* contains melanin pigment (Shujing Sun *et al.* 2016; Zou *et al.* 2013), phenolic compounds, and flavonoid compounds. Polysaccharides in the *A. auricula* fruiting body have been known as antitumors, anticoagulants, anti-glycolipidemic, anti-cholesterol activities, and antioxidants both *in vitro* and *in vivo* (Chen *et al.* 2008; Tang *et al.* 2016; Yoon *et al.* 2003; Zou *et al.* 2010).

In nature, *A. auricula* grows on dead logs (Chang and miles 2004). *A. auricula* was the first edible mushroom planted artificially in China and the world. The Chinese classic agricultural book 'Tan Ben Cao' written in A.D. 600, explains the method of its planting (Cheung 1997; Song *et al.* 1998; Ukai *et al.* 1983). Besides, *A. auricula* is widely used, one of which is herbal medicine in China and Asia, which causes an increase in the demand for *A. auricula* mushrooms while its natural habitat was decreased. Therefore, research regarding *A. auricula* cultivation was conducted to determine the most

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suitable lignocellulosic substrate for cultivating medium (Onyango *et al.* 2013). In general, *A. auricula* mushrooms are cultivated on wood sawdust media. However, the availability is running low, so it is necessary to look for other alternatives. Reeds are one of the lignocellulosic substrates that can be used as an alternative mushroom cultivating medium (*Imperata cylindrica* (L) Beauv). In the Southeast Asian region, reeds can be found around 35 million ha, and around 8.5 million ha are scattered in Indonesia. So far, reeds are used as a raw material for medicines, raw materials for paper, and fertilizer, and the rest is cut and discarded because it inhibits the growth of the primary plan.

Reeds containing α-cellulose 40.22%; holocellulose 59.62%; hemicellulose (pentose) 18.40%; and lignin 31.29%. Cellulose was found to be strongly bound with hemicellulose and coated by lignin to form the lignocellulose complex (Kartikasari *et al.* 2013). Reeds as a substrate medium in mushroom cultivation have been carried out in a previous study that used *Pleurotus ostreatus* (Ardianti 2015; Thohari 2016). In this study, reeds were used as a cultivated medium for the *A. auricula* mushroom and evaluated the nutrient content and antioxidant activity of the *A. auricula* mushroom in the variation of reeds addition on Sengon (*Paraserianthes falcataria* (L.) Nielsen) sawdust as the main component of mushroom growing media. The objective of the paper is to provide essential insights on the effect of adding dried reeds in the Sengon sawdust as the planting media substrate on the nutritional content of mushroom and their bioactivity which can be used to determine the most suitable planting media to improve the nutraceutical quality of the *A. auricula* mushroom.

2. Materials and Methods

2.1. Materials

Reeds used in this study were obtained from Sukoharjo, Central Java, Indonesia. Reed was collected without roots, dried, and then grounded into a powder. Sengon sawdust was obtained from Magelang, Central Java, Indonesia. Other chemicals were obtained from Merck Chemicals. *A. auricula* mushroom F1 seeds were purchased from Ngadiluwih mushroom house, Karanganyar, Indonesia.

2.2. Preparation of Cultivation Medium

The cultivation medium was Sengon sawdust and powdered reeds with comparison as shown in Table 1. The mixtures were supplemented with rice bran as carbon sources and zelomite as pH buffer by 10% (w/w) and 2% (w/w), respectively. The cultivated medium was fermented for 24 hours before being put into baglog and sterilized at 100°C, 1 atm for 12 hours. F1 inoculation of *A. auricula* mushroom was carried out after the media in cold conditions.

Table 1. Variations of cultivation medium

Variant	Composition (%)	
	Reeds	Sengon sawdust
S0	0	100
S1	25	75
S2	50	50
S3	75	25
S4	100	0

Baglogs were put into the incubation room with a temperature of 28-30°C and 90-95% air humidity during the somatic phase, until mycelium growth was completed (Onyango *et al.* 2013). Next, baglogs were perforated and transferred into a mushroom cage to begin the reproductive phase (fruit body development). The temperature and humidity of the mushroom cage range between 25-30°C and 95%.

2.3. Determination of Cultivating Medium C/N Ratio

Samples were taken as much as 10% of the total of each variation of the sterile cultivation medium. The total carbon content of this sample was determined by the Walkey-Black method (Sharmistha and Marschner 2016). While the total nitrogen content was determined by the Kjeldahl method (Mlangeni *et al.* 2013), and the C/N ratio was analyzed with the following equation 1.

$$\frac{C}{N}\text{ Ratio} = \frac{\%\ \text{Total C content}}{\%\ \text{Total N content}} \quad (1)$$

2.4. Growth of *A. auricula* Mushroom

The duration of growth of the *A. auricula* mushroom was calculated to start from perforation of baglog. Yield and biological efficiency (BE) were determined according to previous studies (Onyango *et al.* 2011). The yield and BE were calculated based on equations 2 and 3, respectively.

$$\text{Yield} = \frac{\text{weight of fresh fruiting body}}{\text{weight of medium}} \times 100\% \quad (2)$$

$$\text{BE} = \frac{\text{weight of fresh fruiting body}}{\text{weight of used medium}} \times 100\% \quad (3)$$

2.5. Determination of Nutritional Content

Water and ash contents of *A. auricula* mushrooms were determined with the gravimetric method (Alam *et al.* 2011). Meanwhile, the determination of carbohydrate content was determined with the Luff Schroorl Method, while the protein content was determined by the Kjeldahl method (Smiderle *et al.* 2012). Besides, the fat content was determined by Soxhlet extraction (AOAC 1995; SNI 1992), and the content of crude fiber was determined with acid-base hydrolysis.

2.6. Extraction of *A. auricula* Fruiting Body and Determination of Antioxidant Activity

Extraction was carried out by maceration method using methanol, ethyl acetate, and *n*-hexane solvents with a ratio of 1:30 (w/v) for 72 hours and a stirrer every 12 hours (Shah and Li 2014). Antioxidant activity was carried out with the DPPH (Zou *et al.* 2015) and ABTS methods (Zeng *et al.* 2012).

2.7. Statistical Data Analysis

All data values were average of triplicate determination expressed with standard deviation (SD). Significant differences between the various treatments were analyzed by ANOVA using SPSS 17 for Windows, with the significance level estimated at 5%.

3. Results

3.1. C/N Ratio of Cultivation Medium

The results of the analysis C/N ratio of the cultivation medium was presented in Figure 1. The more reeds were added, the more the nitrogen content was increased and the carbon content decreased. So, in general, the ratio of C/N decreased with the increase of reeds. The C/N ratio in the S1 variant (65.76±1.16) was not significantly different from the control medium (64.42±6.79). Meanwhile, the variants of S2 (44.47±7.32), S3 (38.35±0.69), and S4 34.72±0.51) were significantly different.

3.2. Yield and Biological Efficiency

The yield and biological efficiency of *A. auricula* mushrooms in various culture mediums were shown in Table 2. In general, the more addition of reeds, yield, and biological efficiency was decreased. The addition of reeds in the S1 variation had the highest yield and biological efficiency, 21.45±2.69% and 38.97±4.04%, respectively. Meanwhile variant S3 cultivation media had the lowest yield at 11.13±1.31% and S4 cultivation media had the lowest biological efficiency at 23.58±2.18%.

3.3. Nutritional Content

The nutritional contents of *A. auricula* fruit bodies were shown in Table 2. Nutritional content involves water content, ash content, carbohydrate totals, total fat content, crude protein content, and crude fiber content. The addition of reeds as a cultivated medium did not significantly change the water content of the fruit bodies. However, in general, the addition of reeds increased ash content, protein content, carbohydrate content, crude fiber content, and decreased fat content.

The optimum condition of nutrient content obtained from this research was 86.61±0.25% for

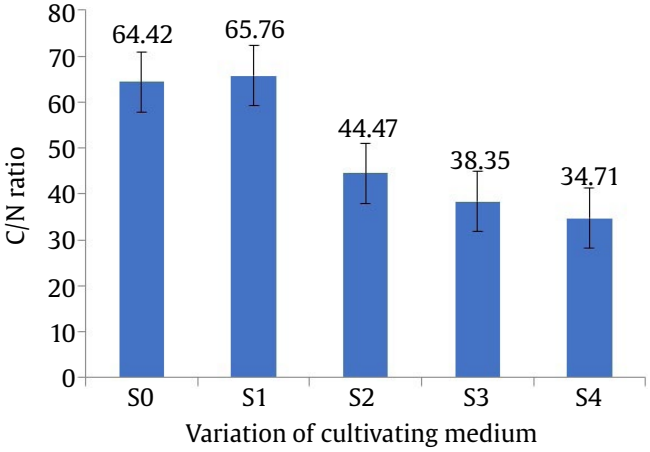

Figure 1. C/N ratio of cultivation medium

Table 2. Nutritional content of *A. auricula* fruiting body mushroom

Variation	Yield (%)	Biological Efficiency (%)	Water content (%)	Ash content (%)	Total carbohydrate (%)	Total protein (%)	Total fat (%)	Crude fiber (%)
S0	17.87±1.99^{a}	32.40±3.40^{a}	86.61±0.30^{a}	0.53±0.30^{a}	10.84±1.17^{a}	7.49±0.49^{a}	1.82±0.06^{a}	49.22±0.04^{a}
S1	21.45±2.60^{bf}	38.97±4.04^{bf}	88.34±1.02^{bd}	0.44±0.17^{ab}	13.97±1.60^{ad}	6.25±0.53^{bd}	2.06±0.03^{bf}	49.68±0.77^{ac}
S2	12.77±0.89^{cgj}	25.86±3.50^{cgj}	87.40±0.65^{ade}	0.59±0.20^{abc}	20.43±3.53^{bef}	6.19±0.88^{cdf}	1.71±0.02^{cgj}	51.72±0.55^{acd}
S3	11.13±1.31^{dhkl}	23.63±5.14^{dhjk}	89.39±0.25^{cdfg}	0.60±0.33^{abcd}	16.03±0.51^{cdgi}	7.43±0.4^{aegh}	1.46±0.03^{dhkm}	55.15±6.95^{bcde}
S4	12.44±1.00^{eijl}	23.58±2.18^{eijk}	87.58±0.46^{adeh}	0.90±0.16^{abcd}	12.92±1.27^{adhi}	6.89±0.12^{adfh}	1.36±0.06^{eiln}	54.13±0.25^{acde}

*The same letter in one column shown that the data were not significantly different (ANOVA, p>0.05), n = 13 for yield and BE; n = 3 for proximate analysis

water content (S0), 0.90±0.16% for ash content (S4), 20.43±3.53% for total carbohydrate (S2), 7.49±0.49% for crude protein (S0), 2.06±0.03% for total fat (S1), and 55.15±6.95% for crude fiber (S3).

3.4. Antioxidant Activity

Antioxidant test results were presented in Table 3. The inhibitory value of extracts was compared with positive control of ascorbic acid. In this test, the positive control showed the results of antioxidant activity of 98.92±0.03% for ABTS and 92.89±0.48% for DPPH. The addition of reeds to the cultivated medium caused the antioxidant activity of the methanol, ethyl acetate, and *n*-hexane extracts had been higher than the antioxidant activity of the mushroom fruiting bodies grown on the control cultivated medium (S0). Similar to the ABTS test, the test results of the highest inhibitory value of the extract against DPPH were the methanol extract and the S1 variant, except for the ethyl acetate extract the highest test value was the S4 variant. The optimum antioxidant activity in the S1 media variant based on the ABTS test was 63.17±0.06% for methanol extract, 58.26±0.26% for ethyl acetate extract, and 51.85±0.03% for *n*-hexane extract. Meanwhile, based on the DPPH test, the optimum inhibition value was 57.62±0.05% for the methanol extract (S1 variant of medium), 48.39±0.11% of the ethyl acetate extract (S4 variant of the medium), and 43.82±0.10% of the *n*-hexane extract (S1) variant of the medium. All extract variants had antioxidant activity compared with ascorbic acid as the positive control.

4. Discussion

4.1. The C/N Ratio of Cultivating Medium, Yield, and Biological Efficiency

The C/N Ratio is the ratio of the mass of carbon and the mass of nitrogen in the substrate, which the ideal C/N ratio was 30-40% (USDA 2011). If the C/N ratio was limited (high N content), the emission of nitrogen will be increased through the volatilization of ammonia (denitrification). Denitrification could inhibit the proliferation of microorganisms. On the other side, degradation of substrate medium would occur more slowly in the presence of a high C/N ratio (low N element content). This was caused by the lack of nitrogen content. The lack of nitrogen content was one of the inhibiting factors for cell growth (growth-rate limiting factor) (Alexander 1994).

Composition and nutrition of growth media affect growth, yield, quality, nutritional composition, and bioactive compounds of mushroom (El Sheikha and Hu 2018). Variation of the addition of reeds into the cultivation media caused differences in carbon and nitrogen content and different C/N ratios. The results of the C/N ratio were shown in Figure 1. If more reeds were added into the cultivation media, then the nitrogen content was higher and the carbon content was lower. In general, if the content of reeds was higher in the cultivation media, then the C/N ratio would be lower. The highest C/N ratio was obtained at S1 variant (65.76±1.16%). Meanwhile, the lowest C/N ratio was obtained at S4 (34.71±0.51%). The C/N ratio of S1 variant was not significantly different from the control cultivation media (Variant S0). In this case, variant S1 can be used as an alternative cultivation media.

In this study, the duration for mycelium and fruit bodies of *A. auricula* mushroom growth varies in each cultivation medium variant (detailed data not shown). The average duration for mycelium growth ranges from 28-34 days from inoculation. The best result of mycelium growth and yields of *A. auricula* mushroom fruiting body was obtained at the S1 variant. Evaluation of *A. auricula* fruiting body yield was expressed in terms of yield and

Table 3. Antioxidant activity of *A. auricula* fruiting body extract

Variation	ABTS			DPPH		
	Methanol	Ethyl asetate	*n*-hexane	Methanol	Ethyl asetate	*n*-hexane
S0	58.16±0.09	52.10±0.33	46.40±0.45	52.88±0.75	39.26±0.72	43.02±0.19
S1	63.17±0.06	58.26±0.26	51.85±0.03	57.62±0.05	44.94±0.43	43.82±0.10
S2	60.10±0.10	54.25±0.09	47.72±0.36	53.16±1.02	45.06±0.31	35.71±0.78
S3	59.64±0.12	50.84±0.09	48.98±0.28	53.24±0.42	41.11±0.12	39.71±0.44
S4	61.48±0.17	56.58±0.67	50.86±0.37	52.77±0.10	48.39±0.11	37.24±1.45
Control	98.92±0.03			92.89±0.48		

*The positive control used was ascorbic acid with a concentration of 400 ppm, the extract concentration used for ABTS and DPPH tests were 400 ppm

biological efficiency. The yield was the ratio of the total weight of the crop to the weight of the dry cultivation medium. Biological efficiency was the ratio of the total weight of the crop to the weight of the cultivation medium used. The highest yield was obtained in variant S1 cultivation media, which was 20.45±2.69%.

Meanwhile, the lowest yield was obtained at variant S3 cultivation media, which was 11.13±1.31%. The cultivation media of variants S2, S3, and S4 have low yields. This was possible because of the lack of carbon source (low C/N ration) in the cultivation media which can support the growth and development of *A. auricula* mushrooms. Based on previous studies, the cellulose content of the cultivation media and the production of enzymes from the mushroom was very important. They affected the yield of the mushroom fruiting body (Onyango *et al.* 2011). The cellulose degradation by enzymes in mushrooms would take a longer duration if cellulose in the used substrate medium was more complex.

Biological efficiency (BE) was used to determine the efficiency of the nutrient cultivation medium used to grow the mushroom. Variant S1 cultivation media had the highest biological efficiency at 38.97±4.04%, while variant S4 cultivation media had the lowest biological efficiency at 23.58±2.18%. Based on the biological efficiency, among the five variations of cultivation medium, variant S1 cultivation media was the most efficient for the cultivation of *A. auricula* mushrooms.

4.2. Nutritional Content Analysis

The ideal drying technique for the mushroom fruit body can provide time efficiency, lower energy consumption, and lower drying costs, as well as obtain the desired final product quality (Hu *et al.* 2020). For nutritional content analysis, *A. auricula* fruiting body mushroom yields were dried using the freeze-drying technique. This technique was chosen to minimize the damage of essential nutrients in the sample. Evaluation of water content in an agricultural product was critical because it relates to the product's texture, quality, handling, and shelf life. Meanwhile, ash content was important because it showed mineral content in the product.

Based on Table 2, the S3 variant had the highest water content (89.39±0.25%) while the S0 (86.61±0.30) variant had the lowest water content. For ash content, the S4 variant had the highest value (0.9±0.16%). In general, if more reeds were added to the cultivation media, the ash content of the *A. auricula* mushroom would be higher. It was made possible by the higher reeds content, then the minerals contained in the cultivation medium would be higher. However, compared with the S0 control, variants S1, S2, S3, and S4 were not significantly different. That was, the ash content of the *A. auricula* mushroom fruit did not change significantly with the addition of reeds into the cultivation medium. Meanwhile, research by Kadnikova *et al.* (2015) showed that the ash content of *A. auricula* mushrooms commonly sold in the Kharbin market, China was 3.6%. However, the planting media used was not determined in Kadnikova's study. Determining other nutrient content was carried out on total carbohydrate, protein, fat, and crude fiber content.

Carbohydrates are one of the primary nutrients in the body of the *A. auricula* mushroom. Determination of carbohydrate content was done using the Luff-Schrool method. This test was based on reducing the Luff-Schrool reagent by reducing sugars (monosaccharides, lactose, and maltose). The rough carbohydrate content of *A. auricula* mushroom fruiting body was different in each media variation. The highest carbohydrate content was found in variant S2, which was 20.43±3.53%, while the lowest carbohydrate content was obtained in the control media S0, 10.84±1.17%. In this case, the cultivation media that most supported the production of *A. auricula* mushrooms with the best carbohydrate content was the variant S2 which was the cultivation media containing 50% reeds. However, judging from the yield, biological efficiency, and carbohydrate content of the mushroom fruiting body, the best alternative planting media possible to be used in the cultivation of *A. auricula* were S1 (25% reeds) and S2 (50% reeds).

Crude protein content in food was protein obtained by determining the total amount of nitrogen. Protein is one of the essential nutritional contents in mushrooms. That is why mushrooms were widely used as an alternative source of vegetable protein. The protein content represents the number of nutrients in meeting the need for amino acids, wherein amino acids, namely aspartate acid and glutamate acid provide a savory taste in the body of mushroom fruit (Ardianti 2015). Compared

to other types of mushrooms, the protein content in the body of *A. auricula* mushroom was somewhat lower (Afiukwa *et al.* 2015; Okoro and Achuba 2012). S3 variant mushroom had the highest crude protein content compared to S1, S2, and S4 variants, although it was not significantly different from the protein content in the S0 control variant of mushroom. This result was proportional to the nitrogen content in the planting media where the nitrogen content in the variant S3 and S4 cultivation media was higher than in the variants S1 and S2. In another study, the total protein content was 12.5%. The study did not know the growing media used because *A. auricula* was obtained from a local supermarket in China (Kadnikova *et al.* 2015). Meanwhile, Zhu (2017) had determined the protein content of *A. auricula* grown on a mixture of woodchip and cottonseed hull at 13.0%.

Determining the total fat content in the body of the *A. auricula* mushroom was very important because mushrooms were known as low-fat food. Fat content was closely related to lignin content in the cultivation media (Badu *et al.* 2011). Correspondingly, the more weeds added, the lower the lignin content in the growing media so that the fat content of the *A. auricula* mushrooms produced would be lower. Judging from the total fat content, *A. auricular* mushroom variant S4 was the lowest (1.36±0.06%) and more recommended than all other variants.

In addition to water, ash, carbohydrate, fat, and protein content, what needs to be analyzed is the level of crude fiber in *A. auricular* fruit body because fiber was important in human digestion. Dietary fiber was known to prevent disease, one of which was colon cancer (Kunzmann *et al.* 2015). The existence of fiber that was not hydrolyzed into the growth substrate of decay bacteria in the colon. *A. auricular* mushroom variant S3 (55.15±6.95) body had the highest crude fiber content and has no significant difference compared to the crude fiber content in the S0 control variant (49.22±0.04%). The results of dietary fiber analysis were similar to the study by Zhu (2017) on *A. auricula* grown on woodchip and cottonseed hull, which was 51.85%.

4.3. Antioxidant Activity

The existence of these bioactive components is in demand as a functional food because of its potential benefits for human health (Abdelshafy *et al.* 2021). The *A. auricula* was rich in polysaccharides, flavonoids, and polyphenolic compounds, which have antioxidant activity (Boonsong *et al.* 2016; Bahadori *et al.* 2019; Fan *et al.* 2006; Mingyi *et al.* 2019). In addition to the nutritional content, the important thing to do was to analyze the effect of different plant media on the antioxidant activity of the *A. auricula* mushroom fruiting body. Antioxidant activity was carried out with two test methods, ABTS and DPPH. Antioxidant testing was carried out on three types of *A. auricula* mushroom body extracts. It was methanol, ethyl acetate, and *n*-hexane extracts. Antioxidant test results were presented in Table 3. The inhibitory value of extracts was compared with positive control of ascorbic acid. The ABTS test showed that the highest inhibitory value was methanol extract for all sample variants (58.16-63.17%). The variant which had the highest inhibitory value was the S1 variant of methanol (63.17±0.06%), ethyl acetate (58.26±0.26%), and *n*-hexane (51.85±0.03 %) extracts. Meanwhile, Zeng (2012) study showed that the *in vitro* ABTS test results for *A. auricula* Polysaccharides (AAP) were 43.5-81.7% at various concentrations. Similar to the ABTS test, the test results of the highest inhibitory value of the extract against DPPH were the methanol extract (52.77-57.62%) and the S1 variant, except for the ethyl-acetate extract, the highest test value was the S4 variant.

The two inhibitory tests show that chemical components that have more influence on free radicals were polar components in the methanol extract variant group and the chemical components that have the least inhibitory effect on free radicals were non-polar components in the extract variants *n*-hexane.

In conclusion, the addition of Reeds to the cultivation medium significantly affected the growth, nutrient content and antioxidant activity of *A. auricula* mushrooms. The addition of reeds to the cultivation medium as much as 25% increased the yield to 21.45±2.60%, biological efficiency to 38.97±4.04%, and antioxidant activity of methanol extract 63.17±0.06% in the ABTS test and 57.62±0.05% in the DPPH test. Meanwhile, the nutrient content of mushrooms increased in the cultivation medium with the addition of reeds by 75%, such as an increase in total carbohydrates (16.03±0.51%), crude fiber content (55.15±6.95%), ash content (0.60±0.33%) and fat content (1.46±0.03%). Based on this research,

the nutrient content and antioxidant activity of the *A. auricula* mushroom can be increased with the addition of reeds by 25% and 75% into the cultivation medium. Based on this study, the addition of 25% reeds is recommended for commercial production.

Conflict of Interest

The authors declare that they have no conflict of interest.

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