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Antioxidant activity of methanol extracts from *Ganoderma lucidum* Karst. Mycelia

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Abstract. The study aimed to evaluate the effect of media cultivation on antioxidant activity of methanol extracts from *Ganoderma lucidum* mycelia. The effect of media was investigated in the submerged flask. Potatoes Dextrose Broth (PDB) and sweet corn were used as media, and cultivation incubation was carried out at 30°C. The results showed that the mean of mycelial dry weight, cultivated in different media were significantly different ($p < 0.05$). The mycelia dry weight from sweet corn (0.221 ± 0.004 g) was higher than PDB media (0.199 ± 0.008 g). Meanwhile to the metabolites content on the mycelia, the mycelia methanol extracts differed significantly ($p < 0.05$). The total polyphenols of mycelia methanol extracts from PDB (13.152 ± 1.89 GAE mg/g) was higher than mycelia methanol extracts from sweet corn (8.177 ± 0.44 GAE mg/g). In line with total triterpenoids of mycelia methanol extracts from PDB and sweet corn with values of 33.085 ± 2.95 mg/g and 11.199 ± 2.74 mg/g, respectively. The scavenging to DPPH radical of methanol extract of mycelia which cultivated on PDB (22.140 ± 3.25 %) higher than sweet corn media (12.840 ± 2.03 %) at 0.2 mg/mL. The scavenging activity was suggested related to the presence of polyphenols and triterpenoids.

Keywords : *Ganoderma lucidum*, sweet corn, antioxidant, mycelia, total polyphenol, total triterpenoids

1. Introduction

Ganoderma lucidum Karts. was known as medicinal mushroom has pharmaceutical and nutraceuticals effects [1]. This mushroom showed an anti-diabetic effect, neuroprotective effects, immunomodulatory, antitumor, antioxidant, hepatoprotective, anti-hypertensive, anticancer, and antimicrobial [2-4]. Triterpenoids, polysaccharides, proteins, sterol, lectins are metabolites in this mushroom that gave biological activities [5, 6].

Generally, this mushroom was cultivated on solid-state fermentation and take six months to form the fruiting body, and its quality is fluctuating. It is encouraging researcher to develop an efficient and effective method for enhancing the mycelia growth and its metabolites. One of the methods is a submerged culture. Most previous research reported that cultivating conditions affected the growth of *G. lucidum* and its metabolites such as polysaccharides and ganoderic acids [7-10]. Previously, our study obtained that the enhancement of water-soluble polysaccharides in the submerged culture was affected by temperature, pH, and media [11]. The pH and temperature for mycelia growing depend on the media. Modification of composition media and control of environmental conditions are important to increase mycelia production and its metabolites.



Previous research reported that corn had been used as a medium culture of *Neurospora* sp [12] and *Penicillium roqueforti* [13]. In this study, cultivation of *G. lucidum* on sweet corn which contains minerals and PDB media at temperature incubation (30 °C) was conducted to evaluate the effect of media cultivation toward the dry weight, total polyphenols, and triterpenoids. The antioxidant activity of methanol mycelia extracts on different media was also investigated.

2. Materials and methods

2.1. Materials

The mushroom was collected from Gubug Jamur Sitiram from Sleman, Yogyakarta. The *G. lucidum* was determined taxonomically at Pharmaceutical Biology Division, Faculty of Pharmacy, Universitas Gadjah Mada. The chemicals and microbiological materials that were used Potato Dextrose Agar (PDA), Potato Dextrose Broth (PDB), sweet corn, potassium dihydrogen phosphate, magnesium sulfate, sodium hydrogen phosphate, methanol, Folin-Ciocalteu reagent, sodium carbonate, distilled water, gallic acid, vanillin, perchloric acid, and ursolic acid.

2.2. Instrumentation

Antioxidant activity of methanol extracts was measured by Elisa reader (Thermo Scintific). Total polyphenols and triterpenoids content were analyzed by UV-Vis spectrophotometer (Dynamica type RB 10). Oven (Memert) was used for drying process of mycelia.

2.3. Cultivation and extraction

The mushroom *G. lucidum* was grown on PDA for seven days at 30 °C and then cultivated into 100 mL Erlenmeyer flasks, each flask containing 25 mL of media and incubated for 14 days. The effect of medium on antioxidant activity, total polyphenols, and triterpenoids of mycelia extracts was studied by growing fungal on PDB and sweet corn media at incubator temperature (30 °C). The sweet corn media contain 5% of sweet corn extract, 0.2% potassium dihydrogen phosphate, 0.05% magnesium sulfate, and 0.01% sodium hydrogen phosphate. Furthermore, the culture and mycelia were separated by filtration, and then the mycelia were dried in an oven at 50 °C until constant weight and ground to be pulverized mycelia. The powder of mycelia was extracted with 50 mL of methanol by sonication for one hour. The extracts were filtered through Whatman No 1 filter paper. The solvent was dried to obtain methanol extracts, MPDB (methanol mycelial extract from *G. lucidum* which cultivated in PDB) and MSC (methanol mycelial extract from *G. lucidum* which cultivated in sweet corn).

2.4. Total of Phenolic Content (TPC)

The TPC of extracts were determined using Folin-Ciocalteu reagent. The reaction mixture contained 500 µL (1 mg/mL) of extract, 500 µL of the Folin-Ciocalteu reagent, and 1.5 mL of 20% sodium carbonate. The final volume was made up to 10 mL with pure water. After 2h of reaction, the absorbance of samples were measured at 765 nm and gallic acid was used as standard [14].

2.5. Total Triterpenoids

Determination of triterpenoids on extracts was carried out using Lin et al. method with slight modification [15]. The mixture of 0.2 mL sample (1 mg/mL), 0.4 mL of vanillin 5% and 1 mL of perchloric acid solution were mixed and then incubated on a water bath at 60 °C for 45 minutes. The samples were then incubated at room temperature for 15 minutes and added with 5 mL acetic acid solution. The absorbance of samples was analyzed by UV-Vis spectrophotometer at 548 nm. The ursolic acid solution was used as a standard.

2.6. DPPH free radical scavenging assay

The samples were dissolved in methanol at various concentrations (0.1 mg/mL-0.8 mg/mL) and treated with DPPH (1 mM in methanol) and incubated for 30 min at room temperature in the dark. The absorbance was measured at 517 nm using Elisa reader. The ability of the samples to scavenge the DPPH radical was calculated using the following equation :

$$\% \text{ inhibition} = [(A_0 - A_1)/A_0] \times 100 \quad (1)$$

where A0 is the absorbance of the control and A1 is absorbance in the presence of the samples [16].

2.7. Statistical Analysis

Total polyphenols, triterpenoids, the weight of mycelia, and antioxidant activity were expressed as the mean (n=3). Data of total polyphenols, triterpenoids, and mycelia weight were analysed using independent t-Test.

3. Results and discussions

The statistical analysis result was suggested that the mean dry weight of *G. lucidum* mycelia cultivated in different media was significantly different. The mean of mycelia dry weight from sweet corn was higher than the mean of dry weight mycelia from commercial PDB media (Figure 1). The results indicated that sweet corn media which contain 5% of sweet corn extract, 0.2% potassium dihydrogen phosphate, 0.05% magnesium sulfate, and 0.01% sodium hydrogen phosphate was favorable for the cell growth than commercial PDB. The mushroom cell growth was better when a complex nutrient source added in media [17]. Sweet corn, also known as sugar corn, is a special low-starch variety (28%) with a higher sugar content (18%), most of which is sucrose and the protein content ranges from 10-15% [18]. Minerals in the culture media affected mushroom growth [19,20]. Similar results were obtained in the study of Curvetto et al. [21], the addition of limiting mineral components promoted mycelia growth rate of *P. ostreatus* up to 25%. Jonathan et al. reported that magnesium as the best element for enhancing the growth of mushroom. K_2HPO_4 could improve productivity through its buffering action, being favorable for mycelia growth [22].

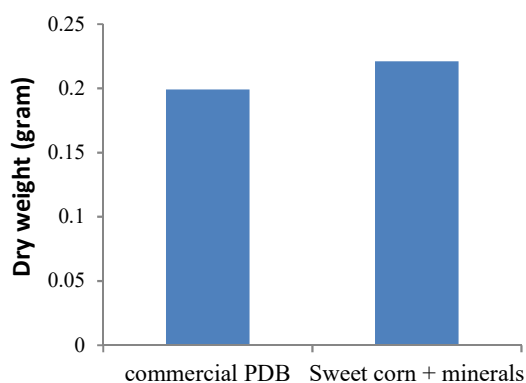


Figure 1. Dry weight (gram) of *G. lucidum* mycelia

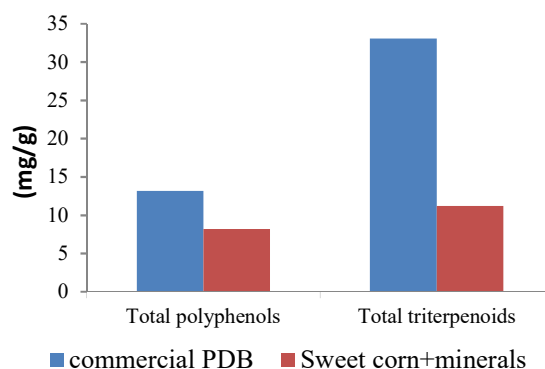


Figure 2. Total polyphenols (GAE, mg/g) and total triterpenoids (mg/g) of *G. lucidum* extracts values of dry weight, total polyphenols, and total triterpenoids are expressed as mean of three parallel measurements

On the contrary secondary metabolites from the extract of mycelia cultivated on sweet corn media lower than mycelia extract from commercial PDB (Figure 2). The mycelia extracts differed significantly ($p < 0.05$) in their mean of total polyphenols and triterpenoids. Commercial PDB was commonly used for preparing a liquid broth culture. The composition of commercial PDB was still better for polyphenols and triterpenoids production than sweet corn media.

This study showed that the scavenging activity increased with the concentrations of MPDB and MSC extracts (Figure 3). The DPPH radical scavenging effect of MPDB extract higher than MSC extract. The antioxidant activity of methanol extracts was suggested related to the presence of total polyphenols and triterpenoids on the mycelia. This result was in good agreement with those reported in previous research, the scavenging to DPPH radical correlates directly with the different polyphenols content [5, 23].

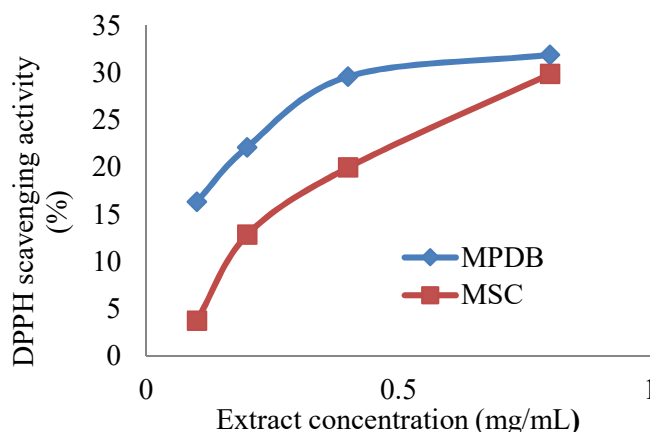


Figure 3. Scavenging effect on the DPPH radical test of methanol extracts from *G. lucidum* mycelia. The data represent the percentage of inhibition induced by increasing concentrations of MPDB and MSC

4. Conclusion

Cultivation media in the submerged flask affected the antioxidant activity of *G. lucidum* extracts. The sweet corn media which contain minerals was positively effected on cell growth of mushroom, contrary to the secondary metabolites and antioxidant activity. The optimum accumulation of metabolites and its antioxidant activity occurred when *G. lucidum* was cultivated on PDB media. The scavenging to DPPH radical was suggested related to the polyphenols and triterpenoids content on mycelia.

5. Acknowledgments

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6. References

- [1] Bishop K S, Kao C H, Xu Y, Glucina M P, Paterson R R, and Ferguson L R 2015 *J. Phytochem.* **114** 56-65
- [2] Nie S, Zhang H, Li W and Xie M 2013 *J. Bioac. Carbohydr. Dietary Fibre.* **1(1)** 10-20
- [3] Ferreira I C, Heleno S A, Reis F S, Stojkovic D, Queiroz M J, Vasconcelos M H and Sokovic M *J. Phytochem.* **114** 38-55
- [4] Li Y -b, Wang J -l and Zhong J -J 2013 *J. Process Biochem.* **48(2)** 331-339
- [5] Saltarelli R, Ceccaroli P, Iotti M, Zambonelli A, Buffalini M, Casadei L, Vallorani L, and Stocchi V 2009 *J. Food Chem.* **116(1)** 143-151
- [6] Batra P, Sharma A K and Khajuria R. 2013 *Int J. Med Mushroom* **15(2)** 127-143
- [7] Yang F -C, Ke Y -F and Kuo S -S 2000 *J. Enzyme and Microb. Technol.* **27(3)** 295-301
- [8] Yang F-C and Liao C -B 1998 *J. Process Biochem.* **33(5)** 547-553
- [9] Tang Y -J, Zang W, Liu R -S, Zu, L -W and Zhong, J-J 2011 *J. Process Biochem.* **46(1)** 404-408
- [10] Tang Y -J and Zhong, J -J 2002 *J. Enzyme Microb. Technol.* **31(1)** 20-28
- [11] Rosyida, V T, Hayati S N, Apriyana W, Darsih C and Poeloengasih C D 2017 in *IOP Conference Series: Earth and Environmental Science.* **101(1)** 012008
- [12] Chutrtong J 2015 *Procedia Social and Behavioral Science* **197** 797-800
- [13] Chang S -C, Wei Y -H, Wei D-L, Chen Y-Y, and Jong S-C 1991 *Appl Environ Microbiol.* **57(9)** 2581-2585
- [14] Yu L, Haley S, Perret J, Harris M, Wilson J, and Qian M 2002 *J. Agric. Food Chem.* **50(6)** 1619-1624
- [15] Lin M, Yu Z, Wang B, Wang, C H, Weng Y, and Koo MALCOLM. 2015. *Sains Malays*, **44** 1685-

1691

- [16] Indrianingsih A W, Tachibana S and Itoh K. 2015. *Procedia Environ Sci.* **28** 639-648
- [17] Wagner R, Mitchell D A, Lanzi S G, Lopes, A A M A and Berovic M 2003 *Food technol. biotechnol.* **41(4)** 371-382
- [18] Panzeri D, Cesari V, Toschi I and Pilu R 2011 *Seed Calorific Value in Different Maize Genotypes, Energy Sources, Part A: Recovery, Utilization, and Environmental Effects*, **33(18)** 1700-1705
- [19] Bellettini M B, Fiorda F A, Maieves H A, Teixeira G L, Avila S, Hornung P S, Junior A M and Ribani H R 2016 *Saudi J. Biol Sci.* 1 - 14
- [20] Jonathan S G and Fasidi I O 2001 *J. Food Chem.* **72(4)** 479-483
- [21] Curvetto N R, Figlas D, Devalis R and Delmastro S 2002 *J. Bioresour Technol.* **84**:171–176
- [22] Petre M and Teodorescu A 2009 *J. Ann. For. Res.* **52**: 129-136
- [23] Cilerdzic J, Stajic M and Vukojevic J 2016 *J. Curr. Pharm. Biotechnol.* **17(3)** 275-82