Phytochemical properties and antioxidant activity of wild-grown and cultivated *Ganoderma lucidum*

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**Abstract.** The most biologically active compounds of medicinal mushroom *Ganoderma lucidum* can be classified into polysaccharides and terpenoids. Most of these biological compounds are supposed to associate with its antioxidant activity. Both of wild grown and cultivated *G. lucidum* have been commercially in demand in Indonesia during the past years. Due to their different growing conditions, the wild-grown and cultivated *G. lucidum* may contain different levels of effective chemical components which affect their quality and medicinal efficacy. This present study was carried out to determine the differences between wild-grown and cultivated *G. lucidum* which might be useful in exploring the characteristic of chemical compounds of *G. lucidum* regarding its antioxidant activity. The physicochemical evaluation was determined using gravimetric method. The phytochemical evaluation includes water – soluble polysaccharides, phenolic, and terpenoids content. The antioxidant activity was evaluated by measuring the radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay. Cultivated *G. lucidum* from Godean has the highest water – soluble polysaccharides (29.86±2.42 GE, mg/g dw) and phenolic content (5.07±0.39 GAE, mg/g dw) among other studied samples. Whereas, cultivated *G. lucidum* from Gunung Kidul has the lowest water – soluble polysaccharides (21.65±2.45 GE, mg/g dw) and phenolic content (3.21±0.87 GAE, mg/g dw). Both of wild grown *G. lucidum* have higher terpenoids content compare to all of cultivated *G. lucidum*. The cultivated – Godean revealed the highest DPPH scavenging activity (the lowest IC50, 344.15±9.57 µg/mL) among of the studied samples. Hence, the results suggested that *G. lucidum* contained high metabolites compounds and has a potential natural source of antioxidants.

1. **Introduction**

*Ganoderma lucidum*, one of polypore macro fungi growing in decomposing woods, commonly known as lingzhi, has been used as a therapy for the prevention and treatment of various diseases for centuries in Asia. The regular consumption was believed to preserve human vitality and to promote health and longevity. A number of studies on the aqueous or ethanol extracts of *G. lucidum* have discovered that the mushroom has anti-tumor, hepatoprotective, anti-inflammatory effects and shows activities in the immune, cardiovascular and central nervous systems [1,2]. Most of these therapeutic activities are believed to have association with its antioxidant activity [3]. *G. lucidum* contains bioactive components such as terpenoids, steroids, phenols, glycoproteins and polysaccharides [4]. Numerous publications have shown that the most pharmacologically active compounds of *G. lucidum* can be generally divided into triterpenes and polysaccharides [4,5]. *G. lucidum* was introduced to Indonesia in the 1990s and its
large-scale cultivation was started in 1999. Both of wild grown and cultivated *G. lucidum* has been commercially in demand in Indonesia during the past several years. Due to their different growing conditions, the wild-grown and cultivated *G. lucidum* may comprise different levels of effective chemical compounds which affect their quality and medicinal efficacy. This current study aimed to determine the differences between wild and cultivated *G. lucidum* which might be useful in exploring the characteristic of chemical compounds of *G. lucidum* regarding its antioxidant activity.

2. Materials and Methods

2.1. Mushroom materials

The mushrooms were dried in the sun with a sufficient amount of air flow to prevent molding. The dried wild – grown *G. lucidum* were collected from Cianjur and Gunung Kidul, Indonesia. While, the dried cultivated *G. lucidum* were collected from Kaliurang, Godean, and Gunung Kidul, Indonesia. They have been taxonomically verified at Pharmaceutical Biology Division, Faculty of Pharmacy, Universitas Gadjah Mada. The voucher specimen was retained in the Research Unit for Natural Products Technology, Indonesian Institute of Sciences. The ground dried mature fruiting bodies of *G. lucidum* were stored in airtight container for further analysis.

2.2. Preparation and extraction

Samples were macerated in ethanol 70% (1: 15) at room temperature for 72 hours. After filtration, the residue was re-extracted with the same method. The filtrates were combined and dried under reduced pressure at 60° C. The resulting extracts were further used for determination of some parameters.

2.3. Physicochemical evaluation

Determination of physicochemical characteristics include total ash content, acid insoluble ash, water soluble extractive, and ethanol soluble extractive using gravimetric method [6,7].

2.4. Determination of water-soluble polysaccharides

The water-soluble polysaccharides were determined with phenol-sulfuric acid colorimetric assay as glucose with hydrolyze polysaccharides into glucose monomer [8-11]. Samples (0.50 g) were extracted with hot water at 95°C and hydrochloric acid (HCl) 2 M in water bath (Memmert) for two hours. Filtrates were separated by filter paper and transferred on the centrifuge tube 10 mL. Then, 1 mL of filtrates were added with 5% phenol 0.5 mL (Merck) and 2.5 mL of concentrated sulfuric acid (H₂SO₄) (98% v/v) (Merck). The mixtures were shaken for 2 minutes and incubated using water bath at 100 °C for 15 minutes. The water-soluble polysaccharides were analyzed quantitatively by measuring the absorbance at 490 nm using UV/Vis spectrophotometer (Dynamica Halo RB-10). The blank solution contained 1 mL of distilled water, 0.5 mL of 5% phenol and 2.5 mL of H₂SO₄ (98% v/v). The standard glucose (Sigma, Milwaukee, WI, USA) was used as a standard solution. The results are expressed as mg glucose equivalent (GE) in gram dry weight (dw) basis.

2.5. Determination of total of phenolic content

The total of phenolic content of extracts was determined using Folin – Ciocalteu reagent [12]. The reaction mixture contained 500 µL (1 mg/mL) of samples, 500 µL of the Folin – Ciocatue reagent (Merck), and 1.5 mL of 20% sodium carbonate (Merck). The final volume was made up to 10 mL with aquadest. After two hours of incubation in a dark at room temperature, the absorbance of samples were measured by UV/Vis spectrophotometer (Dynamica Halo RB-10) at 765 nm and a gallic acid (Merck) was used for calibration purposes [13], and the results are presented as milligram gallic acid equivalent (GAE) in gram dry weight (dw) basis.
2.6. Determination of the total terpenoid content

The determination of total terpenoids was performed according to the colorimetric method of Lin et al. (2015) with slight modification [14]. The samples were added with 0.4 mL of vanillin – glacial acetic acid (5% w/v) (Merck) and 1.0 mL of perchloric acid solution (Merck). The tubes were then placed in a water bath (Memmert) at 60°C for 45 min. Then the mixed solution was cooled and diluted with 5 mL of acetic acid solution (Merck). The absorbance was measured at 548 nm against blank solution using UV/Vis spectrophotometer (Dynamica Halo RB-10). The standard ursolic acid (Sigma, Milwaukee, WI, USA) was used as a standard solution. The results are expressed as milligram ursolic acid equivalent (UAE) in gram dry weight (dw) basis.

2.7. DPPH Radical Scavenging Assay

The antioxidant activity of mushrooms methanolic extracts was evaluated by measuring the radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay [15]. The 2 mL methanolic extracts of dried G. lucidum powder were added with DPPH (Merck) solution at different concentrations of 0.25, 0.5, 1.0, 1.5, and 2.0% w/v. Each of mixtures were then shaken and left to stand in the dark at room temperature for 30 minutes. Quercetin was used as a positive control. The absorbances were measured by UV/Vis spectrophotometer (Dynamica Halo RB-10) at 517 nm. The antioxidant activities presented as IC50 values. The IC50 values is the effective concentration of samples at which the DPPH radicals were inhibited by 50%. The IC50 value were calculated by linear regression, where the abscissa (x) represents the level concentration of tested samples and the ordinate (y) represents the average percentage of inhibitory effect.

2.8. Statistical analysis

All assays were carried out in triplicate. The data is represented as means ± standard deviation (SD). Analysis of variance and Duncan's test were used for determination of statistical significance and p < 0.05 were regarded as significant.

3. Results and Discussion

3.1. Physicochemical evaluation

Physicochemical evaluation is used for the preliminary identification of the natural drug to know the significance of physical and chemical properties of the substance being analyzed in terms of their observed activities and especially for the determination of their purity and quality. Physical properties are often exhibited as observables. The total ash values provide an overview of inorganic composition and other impurities in dried mushrooms. Ash content parameters related to purity and contamination. The dried mushrooms are heated at the temperature at which organic compounds and their derivatives are destructed and evaporated leaving only mineral or inorganic elements. The highest and the lowest total ash value were found to be 13.88 % of cultivated – Kaliurang and 7.63 % of cultivated – Godean, respectively. While, the highest and the lowest acid – insoluble ash value were found to be 2.11 % in cultivated – Kaliurang and 0.64 % in wild grown – Cianjur, respectively.

The acid-insoluble ash value indicates the presence of silicaceous substances. The determination of extractable matter refers to the amount of constituent in material which is extracted by specific solvents. Polar compounds tend to be more attracted to and are more soluble in polar solvents. It is indicating the nature of constituents of the raw material, and also helps in detecting low grade material. Water-soluble and ethanol soluble extractive values plays an important role in evaluation of crude drugs. Less extractive value indicates addition of exhausted material, adulteration or incorrect processing. The highest water – soluble and ethanol extractive value were found in cultivated – Kaliurang (71.19 % and 59.49 %, respectively). Whereas, the lowest water – soluble and ethanol extractive value were found to be 45.25 % in wild – Gunung Kidul and 4.18 % in wild grown – Cianjur, respectively. Even though wild grown – Gunung Kidul has the lowest water – soluble extractive value, it has higher ethanol soluble
extractive value compare to cultivated – Gunung Kidul and cultivated – Godean. All the results were tabulated in Table 1.

**Table 1. Physicochemical properties of wild grown and cultivated G. lucidum.**

| G. lucidum       | Total ash (%) | Acid insoluble ash (%) | Water soluble extractive (%) | Ethanol soluble extractive (%) |
|------------------|---------------|------------------------|-------------------------------|-------------------------------|
| Wild grown       |               |                        |                               |                               |
| Gunung Kidul     | 9.12 ± 2.23  | 0.90 ± 0.21            | 45.25 ± 4.45                  | 27.2 ± 2.37                   |
| Cianjur          | 10.43 ± 1.45 | 0.64 ± 0.11            | 55.67 ± 3.67                  | 4.18 ± 1.17                   |
| Cultivated       |               |                        |                               |                               |
| Gunung Kidul     | 10.34 ± 1.40 | 0.83 ± 0.17            | 57.22 ± 2.23                  | 12.18 ± 1.67                  |
| Kaliurang        | 13.88 ± 2.33 | 2.11 ± 0.67            | 71.19 ± 3.11                  | 59.49 ± 2.15                  |
| Godean           | 7.63 ± 1.11  | 0.75 ± 0.25            | 54.62 ± 3.17                  | 12.26 ± 1.85                  |

**3.2. Phytochemical evaluation**

The results of phytochemicals evaluation of G. lucidum samples are presented in Tables 2. Water – soluble polysaccharides were the most abundant metabolite compound in G. lucidum, followed by terpenoids, and phenolic, based on the samples. Cultivated G. lucidum from Godean has the highest water – soluble polysaccharides (29.86 GE, mg/g dw) and phenolic content (5.07 GAE, mg/g dw) among other studied samples. Whereas, cultivated G. lucidum from Gunung Kidul has the lowest water – soluble polysaccharides (21.65 GE, mg/g dw) and phenolic content (3.21 GAE, mg/g dw). There was very interesting that both of wild grown G. lucidum have higher terpenoids content compare to all of cultivated G. lucidum. Wild grown G. lucidum are grow spontaneously in self-maintaining populations in natural or semi-natural ecosystems (usually in the forest and oil palm plantation) and can exist independently of direct human action. Cultivated G. lucidum have arisen through human action (composting, spawning, casing, pinning) and are grown for their produce in mushroom farm. Metabolites in mushroom could be affected by physical variations, ecological conditions, terrestrial variations, genetic factors and evolution. Another research showed that production of metabolites was influenced by environmental factors such as humidity, temperature, intensity of light, supply waters, minerals, and CO$_2$ [16].

**Table 2. Phytochemical properties of wild grown and cultivated G. lucidum.**

| G. lucidum       | Water-soluble polysaccharides (GE, mg/g dw) | Phenolic content (GAE, mg/g dw) | Terpenoids content (UAE, mg/g dw) |
|------------------|--------------------------------------------|---------------------------------|----------------------------------|
| Wild grown       |                                            |                                 |                                  |
| Gunung Kidul     | 22.45 ± 1.08                              | 4.18 ± 0.67                     | 23.72 ± 2.17                     |
| Cianjur          | 23.90 ± 2.39                              | 4.13 ± 0.54                     | 23.14 ± 2.39                     |
| Cultivated       |                                            |                                 |                                  |
| Gunung Kidul     | 21.65 ± 2.45                              | 3.21 ± 0.87                     | 18.71 ± 2.85                     |
| Kaliurang        | 23.11 ± 3.23                              | 3.90 ± 0.72                     | 20.42 ± 2.52                     |
| Godean           | 29.86 ± 2.42                              | 5.07 ± 0.39                     | 22.80 ± 2.43                     |

**3.3. Antioxidant activities**

Effectiveness in antioxidant activities inversely corresponded with IC$_{50}$ value. The scavenging capacities of wild grown and cultivated G. lucidum in the DPPH free radical scavenging activity assay ranged between 344.15±9.57 and 686.05±12.11 µg/mL. Quercetin was used as positive controls in this experiment and exhibited IC50 of 10.55±2.23 µg/mL. The antioxidant activity of G. lucidum decreased in order: cultivated Godean > wild Gunung Kidul > wild Cianjur > cultivated Kaliurang > cultivated Gunung Kidul. The cultivated – Godean revealed the highest DPPH scavenging activity (the lowest IC50, 344.15±9.57 µg/mL) among of the studied samples. Both of wild grown G. lucidum have higher
DPPH scavenging activity than a sample from cultivated *G. lucidum* from Gunung Kidul and Kaliurang. This bioactivity was believed to be associated with the different contents of polysaccharides (β-1,3-glucans) and triterpenes (ganoderic acids and others) in each of the samples [17,18].

| Table 3. Antioxidant activities of wild grown and cultivated *G. lucidum*. |
|--------------------------------------|
| **G. lucidum** | IC50 (μg/mL) |
| Wild grown | | |
| Gunung Kidul | 381.16 ± 13.52 |
| Cianjur | 387.17 ± 15.47 |
| Cultivated | | |
| Gunung Kidul | 686.05 ± 12.11 |
| Kaliurang | 457.25 ± 10.44 |
| Godean | 344.15 ± 9.57 |
| Quercetin | 10.55 ± 2.23 |

4. Conclusion

Physicochemical, phytochemical includes water – soluble polysaccharides, phenolic, terpenoids content, and the antioxidant activity of 2 wild grown and 3 cultivated *G. lucidum* were evaluated. The present investigation demonstrates that the physicochemical, phytochemical and antioxidant properties of wild grown and cultivated *G. lucidum* were significantly different. Cultivated *G. lucidum* from Godean has the highest water – soluble polysaccharides and phenolic content. Both of wild grown *G. lucidum* have higher terpenoids content compare to all of cultivated *G. lucidum*. The cultivated – Godean revealed the highest DPPH scavenging activity among of the studied samples. Overall, the presence of primary and secondary metabolites in *G. lucidum* suggested that the mushroom has potential natural source of antioxidants.

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