The elevation effect on water-soluble polysaccharides and DPPH free radical scavenging activity of Ganoderma lucidum K

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Abstract. Water soluble polysaccharide is one of the important phytochemical in Ganoderma lucidum K. Phytochemicals in the plants, microorganisms, and plants were affected by internal and external factors. The objective of the research was to evaluate the effect of elevation on the water-soluble polysaccharides and its DPPH radical scavenging activity. We found that the water-polysaccharides in mushroom from Godean (elevation <100 mamsl) (35.28 ± 0.31%) higher than Kaliurang (elevation 800 mamsl) (25.17 ± 1.85%). The DPPH free radical scavenging activity of Ganoderma lucidum K from Godean (IC₅₀ 11.5 ± 0.29 mg/mL) higher than Kaliurang (IC₅₀ 14.4 ± 0.27%).

1. Introduction
Ganoderma lucidum K known as Lingzhi in Chinese and Reishi in Japanese [1]. G. lucidum is one the mushrooms that used as medicine in Asian countries. Several researches showed that metabolites contained in this mushroom exhibited specific biological activities, such as anti-diabetic effect, neuroprotective effects, immunomodulatory, antitumor, antioxidant, hepatoprotective, anti-hypertensive, anticancer, and antimicrobial [1-5]. Bioactive compounds found in G. lucidum include polysaccharides, triterpenes, protein, amino acids, mineral elements, vitamin, and phenols [6]. Two bioactive compounds, polysaccharides, and triterpenes are recently considered as the important constituent in this mushroom.

Several researches have been reported the important of polysaccharides from G. lucidum. Water-soluble polysaccharides from four strain G. lucidum showed antibacterial activity and the most sensitive bacteria toward polysaccharides was Micrococcus luteus ATCC 10240 with MIC of 0.63-1.25 mg/mL [7]. Water soluble polysaccharides of this mushroom demonstrated that polysaccharides reduced cerebral infarct area by oral administration in rats (100, 200, and 400 mg/kg) [8]. Polysaccharides of G. lucidum with a molecular weight of 5.2 kDa showed more effective in free radical scavenging and Fe²⁺ chelating than polysaccharides with a molecular weight of 15.4 kDa [9].

Metabolites in plants could be affected by corporal variations, ecological conditions, terrestrial variations, genetic factors and evolution, and political/social conditions [10, 11]. Gua et al. reported that secondary metabolites in Scutellaria baicalensis were influenced by internal and external
environmental. Temperature affected the metabolites in *S. baicalensis* positively and chemical constituents high latitude lower than in high latitude [12]. Another research showed that production of secondary metabolites was influenced by environmental factors such as humidity, temperature, intensity of light, supply waters, minerals, and CO$_2$[13]. Elevation factor where the plant was grown also affected the biochemical composition in plants significantly. The research reported that increasing elevation effected significantly the variation of metabolites and increased the chloronogenic acid and fat concentrations on the traditional cultivar of coffee bean. Variation of chloronogenic acid and fat in Arabica hybrids were little effected by increasing elevation [14]. Those results indicated that the important environmental on metabolites production and encourage this research to know the effect of elevation factor on water-soluble polysaccharides and the antioxidant activity of *G. lucidum* extract.

2. Materials and methods

2.1. Materials
The *G. lucidum* was determined taxonomically at Pharmaceutical Biology Division, Faculty of Pharmacy, Universitas Gadjah Mada. The mushroom was cultivated in same media containing sawdust, bran, and dolomite in different elevation, Merapi mountain side, Kalirang (800 maml) and lowland at Godean, Sleman (<100 mamsl). Furthermore, the mushroom was dried under direct sunlight and grounded to be powder.

2.2. Water-Soluble Polysaccharides Analysis
The water-soluble polysaccharides were determined based on the color reaction of polysaccharides and their derivatives with phenol and concentrated sulfuric acid [15]. Samples (100 mg) was extracted using 5 mL HCl 2 M at 100°C in the water bath (Memmert) for two hours. The filtrate and fruit bodies of *G. lucidum* were separated by filter paper, and then the filtrate was transferred into the centrifuge tube 5 mL. Further, 1 mL of filtrate was added with 5% phenol 0.5 mL and 2.5 mL of H$_2$SO$_4$ (98% v/v) (Merck). The mixture was then shaken for 2 minutes and incubated using water bath at 100°C for 15 minutes. The water-soluble polysaccharides concentration of *G. lucidum* was analyzed by UV-Visible spectroscopy (Dynamica Halo RB-10 spectrophotometer) at 490 nm. The blank solution contain 1 mL of aquadest, 0.5 mL of 5% phenol (Merck) and 2.5 mL of H$_2$SO$_4$ (98% v/v) (Merck). The standard glucose (Sigma, Milwaukee, WI, USA) was used as a standard solution.

2.3. DPPH Radical Scavenging Assay
The DPPH assay was conducted to find the radical scavenging activity of *G. lucidum* from two different elevations of land following the Shimada, et.al method [16] with some modification. The 2 mL methanol extracts of dried *G. lucidum* were added with 2 mL of DPPH solution at concentrations of 0.25, 0.5, 1.0, 1.5, and 2.0% w/v. The mixture was then shaken and incubated at room temperature in the dark for 30 minutes. The samples and control were analyzed by ELISA at 517 nm. The equation 1 was used to calculate the percent DPPH radical scavenging effect, where A0 is DPPH solution absorbance without sample and A1 is DPPH solution absorbance with sample *G. lucidum*.

$$\% \text{ inhibition} = \left[ \frac{A_0 - A_1}{A_0} \right] \times 100 \text{ .................1}$$

The antioxidant activities expressed as IC$_{50}$ values for comparison. Effectiveness in antioxidant activities inversely corresponded with IC$_{50}$ value. The IC$_{50}$ value is the effective concentration at which the DPPH radicals were inhibited by 50%. The IC$_{50}$ value was measured by linear regression of plots, where the abscissa (x) describe the concentration of tested methanolic extracts and the ordinate (y) describe the average percentage of inhibitory effect.
3. Results and discussions

The chemical compositions and concentrations in plants, fungi, and microorganisms were influenced by internal and external environmental. In this research, the mushroom *G. lucidum* was grown in different elevation and its effect on water-soluble polysaccharides was evaluated. Elevation difference related to the change of the temperature and intensity of light in the field [14]. The temperature in Kaliurang is 24-27 °C, lower than the temperature in Godean (29-33 °C). Relative humidity in Kaliurang (85-95%) higher than that Godean (75-85%), and the light intensity in Kaliurang (300-500 lux) lower than that Godean (400-600 lux). The physical geographic characteristics data are shown in Table 1.

| Physical geographic characteristics | Merapi mountainside, Kaliurang | Lowland at Godean, Sleman |
|-------------------------------------|-------------------------------|---------------------------|
| Elevation                           | 800 mamsl                     | <100 mamsl                |
| Temperature                         | 24 - 27 °C                    | 29 - 33 °C                |
| Relative humidity                   | 85 - 95%                      | 75 - 85%                  |
| Illuminance/light intensity          | 300 - 500 lux                 | 400 - 600 lux             |

The results showed that water-soluble *G. lucidum* polysaccharides from Godean higher than *G. lucidum* mushroom from Kaliurang, shown in Table 2. The difference of polysaccharides concentration indicated that variation of elevation effected phytochemical production in mushrooms. The difference of phytochemical in elevated variations related to temperature, relative humidity, and light intensity. Variation of temperature can affect the regulation of metabolic in plant [17]. This results in line to previous research which the metabolites concentration were influenced by temperature and light. The research reported that biomass and ginsenoside production in *Panax ginseng* were influenced by variations of temperature and light quality [18]. Another research reported that leaf senescence and secondary metabolites of *Panax quinquefolius* root increased with elevation of temperatures. Increasing temperature to a 5°C affected the *Panax quinquefolius* [17].

Antioxidant activities of this mushrooms related to phytochemical in *G. lucidum*, mainly water-soluble polysaccharides. The result exhibited DPPH free radical activity of *G. lucidum* from Kaliurang and Godean with IC$_{50}$ of 14.4 mg/ml and 11.5 mg/ml, respectively. This is suggested that concentration of metabolites for antioxidant in both mushrooms are proportional with water-soluble polysaccharides level.

| No | Samples | Water-soluble polysaccharides (%) | % inhibition at 10 mg/mL | IC$_{50}$ value (mg/mL) |
|----|---------|-----------------------------------|--------------------------|------------------------|
| 1. | GM      | 25.17 ± 1.85                      | 34.81 ± 5.54             | 14.4 ± 0.21            |
| 2. | GG      | 35.28 ± 0.31                      | 47.66 ± 3.94             | 11.5 ± 0.29            |

GM : *G. lucidum* was grown in Merapi mountain side, Kaliurang
GG : *G. lucidum* was grown in Lowland at Godean, Sleman
4. Conclusions
The chemical constituents on *G. lucidum* was influenced by external factor. Increasing of elevation decreased the water soluble-polysaccharides on *G. lucidum* as well as DPPH free radical scavenging activity of *G. lucidum*.

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