Antioxidant Evaluation of *Ganoderma lucidum* Extracts

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Abstract. *Ganoderma lucidum* is one of the many mushrooms utilized by the people as a medicine for cancer, asthma, diabetes and heart disease. The purpose of this experiment is to determine the antioxidant activity of the extracts from *G. lucidum* using different solvents. The simplicial powder of *G. lucidum* are subjected to maceration using water, methanol, ethyl acetate, methylene chloride, and n-hexane; and evaporated the solvent until a viscous extract was obtained. The methanol extract shows highest antioxidant activity with IC₅₀ value of 251.95 μg/mL.

1. INTRODUCTION

The use of plants as source of medicine in treating disease is an ancient practice. In fact, plants produce a diverse range of bioactive molecules as an antioxidant. These are chemical substances present in plants and include alkaloids, saponins, steroids, carbohydrates, glycosides, tannins, flavonoids and steroids, making them rich sources of different types of medicines. The presence of these substances is an indicator of the pharmacological property as well as the nutritive value of the plant [1], [2].

Free radicals can be produced from outside the body or from metabolic processes in the body when producing energy. Exposure to free radicals from outside can come from environmental pollution such as: contamination of pesticides, heavy metals and the use of preservatives and dyes [3]–[6]. Excess free radicals in living organisms result in chain reactions of free radicals that cause damage lipids, proteins and DNA, and cause damage to cells so that cell function decreases [7]. Damage caused by free radicals can lead to various diseases such as cancer, heart disease, cataracts, decreased immune system and diabetes [8]–[10].

In recent times, attention has been reverted back to plants as sources of therapeutic agents due to their higher properties. These include among others reduced cost, relative lower incidence of adverse reactions compared to modern conventional pharmaceuticals. In recent years much attention has been devoted to natural antioxidant and their association with health benefits [11]. Natural resources are potential sources of natural antioxidants. It produces various antioxidative compounds to counteract reactive oxygen species (ROS) in order to survive [12].

The genus *Ganoderma* has more than 400 species that live in tropical and subtropical regions of the world. *Ganoderma lucidum* generally grows throughout the year on dead wood or trees, such as oak, maple, elm, willow, and magnolia. Some species of the genus *Ganoderma* are generally used as medicine, including: *G. lucidum*, *G. luteum Steyaert*, *G. atrum* Zhao, *G. tsugae* Murrill, *G. applanatum Pat.*, *G. australe Pat.*, *G. capense* Teng, *G. tropicum Bres.*, *G. tenue* and *G. sinense*. Even the species *G. lucidum* has been known in China as an effective medicinal mushroom thousands of years ago [13]–[15].
G. lucidum commonly called lingzhi is a medicinal mushroom of the Polyporaceae family. It is a group of wood degrading mushrooms with hard fruiting bodies [13]. Traditionally, for generations G. lucidum have been used by people in China, Japan, Taiwan and other Asian countries to treat various diseases, such as: asthma, diabetes, heart disease, cancer and other chronic diseases [16]. Secondary metabolites of G. lucidum mushrooms are derived from groups of terpenoids, polysaccharides, proteins and glycoproteins. These compounds are known to have activities as anti-inflammatory, antioxidant, antidiabetic, antihypertensive, antimicrobial, immunostimulant, antitumor, anti-inflammatory, neuroprotective, AChE inhibitors, cardioprotective, antiherpetic, antimitagenic, antifungal and anti-HIV [17]–[25].

The present study is designed to determine antiradical activity fo G. lucidum extracts using DPPH assay. G. lucidum is one of the medicinal mushrooms with high antioxidant activity. Extraction of G. lucidum using a water solvent by ultrasonic method can produce polysaccharide extract with a inhibition value of DPPH of 53.62 % at a concentration of 47.87 mg/mL [26]. In addition, Joseph et al. extracted G. lucidum using chloroform extract was produced with a inhibition value of 58.81 % at a concentration of 300 mg/mL [27].

2. EXPERIMENTAL

2.1. Chemicals
Organic solvents including aqua DM, methanol, ethyl acetate, methylene chloride and, n-hexane. For the assay are used methanol from Merck in high grade, 2,2-Diphenyl-1-picyrylhydrazyl (DPPH) was purchased from Tokyo Chemical Industries (Tokyo, Japan), and gallic acid as positive control.

2.2. Preparation of G. lucidum Extract
G. lucidum were collected in Bantul, Central Java, Indonesia. The dried fruiting body of G. lucidum is cut into small pieces then grounded into powder using an electric milling machine. The powdered sample were extracted using maseration with water, methanol, ethyl acetate, methylene Chloride, and n-hexane for 24 hours at room temperature. After filtered through filter paper, the extracts were concentrated under reduced pressure using Rotary evaporator to give crude extract.

2.3. Antioxidant Activity
The antioxidant activities of G. lucidum were determined on the basis of the free radicals scavenging effect of DPPH free radical model as described Hidayati et al. (2017) with slight modification [4]. Each extract (10 mg) dissolved in 1 mL methanol to get extract solution. 33 μl each extract solution was added 1 ml DPPH radicals solution 6x10^{-5} M, then the mixture was shaken vigorously and left to stand for 20 min at room temperature in the dark. The absorbance of blank was then measured at 515 nm and The scavenging activity was expressed as IC50 (μg/mL). Gallic acid was used as the positive control. The percentage scavenging effect was calculated as:

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\text{Inhibition} \% = \left( \frac{(A_1 - A_2)}{A_1} \right) \times 100\%
\]

where A1 was the absorbance of blank and A2 was the absorbance in the presence of the extract.

3. RESULT AND DISCUSSION

3.1. Extraction of G. lucidum
The extraction process using 20 g G. lucidum powder with 300 mL various solvents provide varying extraction yield 0.60 g (3.02%) water extract; 0.64 g (3.19%) methanol extract; 0.34 g (1.71%) ethyl acetate extract; 0.28 g (1.40%) dichloromethane extract; and 0.13 g (0.67%) n-hexane extract.
3.2. Antioxidant Activity
The hydrogen atom or electron donation abilities of the corresponding extracts and standards were measured from the bleaching of the purple color methanol solution of DPPH. The antioxidant activity of various solution extracts of *G. lucidum* are given in Figure 1.

![Figure 1](image1.png)

**Figure 1** DPPH Scavenging Activity at concentration 319.46 µg/mL such as 1 (water); 2 (methanol); 3 (ethyl acetate); 4 (methylene chloride); 5 (n-hexane) and; 6 (gallic acid).

The *G. lucidum* n-hexane extracts has 8.77 % DPPH radical scavenging properties. While excellent activities were shown by the methanol 57.98 %, ethyl acetate 37.19 %, methylene chloride 27.10 % and water 21.58 %. The highest antioxidant activity of the methanol extract among the other extracts was suspected because methanol extract contained compounds that play active role as antioxidants. The methanol has more polarity and solubility. So that, in addition to get high yields of extracts, extracts of active antioxidants were also obtained [28]. Extraction with methanol is known to be obtained polysaccharides from *G. lucidum* where this component has good activity as an antioxidant [26][29]. Because methanol extract give highest antioxidant activity and more then 50 % of inhibition value, furthermore this extract was measured for IC$_{50}$. The presented curves were plotted between concentration and percentage of inhibition methanol extract was showed in Figure 2. Methanol extract gives IC$_{50}$ value of 251.95 µg/mL and gallic acid as positive control had IC$_{50}$ value of 0.4 µg/mL.

![Figure 2](image2.png)

**Figure 2** DPPH Scavenging Activity of Methanol Extract

4. CONCLUSION
Methanol extract from *G. lucidum* showed highest activity compared with other extracts as antioxidant. Methanol extract showed IC$_{50}$ value of 251.95 µg/mL, that include moderate activity as antioxidant.
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