Antioxidant Potential of Ethanol and Ethyl Acetat Extract of *Ganoderma* sp. Mycelium

**Nuniek Ina Ratnaningtyas, Purnomowati, Endang Sri Purwati, Aisyah Tri Septiana, Nuraeni Ekowati, Adi Supriyadi**

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Faculty of Biology, Universitas Jenderal Soedirman, Indonesia

**Abstract**

*Ganoderma* sp. Banyumas 1 isolate referred as *Ganoderma* sp. is a new discovered isolate from Banyumas, Central Java, Indonesia expected to have a potential properties of antioxidant of medicinal mushroom. This study aimed to determine the antioxidant potential and the appropriate solvent for it's extracting from *Ganoderma* sp. This research result showed that ethyl acetate was able to extract as many as 15.57%, while ethanol was only able to extract 3.87% active compounds from dried 28 days old *Ganoderma* sp. mycelium cultivated in the Mushroom Complete Medium (MCM). Extract of ethyl acetate (non-polar) extraction of mycelium of *Ganoderma* sp. had a potential character as an antioxidant source and performed a better result than from ethanolic (polar) extraction as shown in the IC50 value. Extract from ethyl acetate extraction had an average IC50 value smaller than from ethanolic extract (581.80 < 1285.67). Extract from ethyl acetate extraction resulted in a higher amount of phenol than that ethanolic extract (29.23 < 57.67). Inhibition percentage of both extracts at 65% was known to occur at concentration of 1000 ppm for ethyl acetate extract and 2000 ppm for ethanolic extract. An important finding was that ethyl acetate can be used as appropriate solvent for extracting antioxidant compound better than ethanolic. In conclusion, the mycelium extract of *Ganoderma* sp. extracted with ethyl acetate and ethanol as solvent is potential to be used as a source of natural antioxidants. This research result has benefit in developing potency of local resources as herbal resources.

**How to Cite**

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INTRODUCTION

Ganoderma sp. isolate is a local isolate from Banyumas, Central Java isolated by the researchers. Since a long time ago, it has been placed in the top rank of medicinal mushrooms and so called as “king of herbal medicine” (Jaellani, 2008). Modern pharmacological studies showed that Ganoderma sp. could be used in healing several diseases. Previous study by Senggoro (2005) was investigating the activity and bioactive compound from Ganoderma sp. mycelium grown on solid medium containing cellulose from palm fruit. Some mushrooms are known to contain medicinal compounds and so called as medicinal mushrooms e.g. Ganoderma lucidum. This mushroom is known to be potentially used as a medicament especially its basidiospores, mycelium and fruiting body.

According to Yue et al., (2008), G. lucidum has been used as a traditional medicine in China for more than 2,000 years. Wachtel-Galor et al., (2004) stated if G. lucidum could maintain the consumer’s health. Analysis of Ganoderma lucidum by Stamets (2000) both qualitatively and quantitatively reported that Ganoderma lucidum contain different bioactive compounds which depend on its growth medium and conditions. Different growth media attracts the mushrooms to produce different amount of bioactive compounds (Stamets, 2000). The mycelium of Ganoderma sp. isolate is extracted using a macerations method applying ethanolic and ethyl acetate solvents. Solvents were chosen based on components of the samples.

Antioxidant activity can be identified using either polar solvents or non-polar solvents such as alkaloids and triterpenoids (Zazouli et al., 2016). Information of source of antioxidant and its effective solvent is essential. However, studies of antioxidant compounds from fungi are still very limited, especially the extraction of antioxidant compounds from dry filtrate culture of Ganoderma sp. which isolated with different solvents. Recent research by Ekowati et al. (2017) studied different macro fungi which was Pleurotus ostreatus extract and its potential as anti-cervical cancer cells, not it’s antioxidant potential. Ganoderma sp. originating from several different cultivation locations is thought to have different abilities to produce bioactive compounds as the result of biosynthetic processes. Its ability is either influenced by genes or affected by physical and chemical environment factors. Examining antioxidant ability may lead to the discovery of superior strains. It is necessary to assess the potency of each strain to produce bioactive compounds that can be used as an antioxidant. It is also important to examine the most appropriate solvent in extracting dried isolate of Ganoderma sp. Current research aimed to study potential of Ganoderma sp. B1 isolate in producing antioxidants and to determine the solvent with the highest amount of antioxidant. This research was expected to give some scientific information about active compounds of Ganoderma sp. isolate especially the antioxidants content and the possibility of the use of Ganoderma sp. isolate as an antioxidant source.

METHODS

This research applied an experimental quantitative descriptive. Potential of antioxidant components obtained from Ganoderma sp. Banyumas 1 isolate determined using DPPH test, based on the observed color changes using spectrophotometer. It was assumed that the change in colour was due to the ability of mycelium extract of the mushroom in trapping the free radicals. Data of colour change were then analyzed by checking the IC50 value, followed by drawing a graph of percentage of the DPPH trapped by the mycelium. Total phenolic compounds, flavonoids, and terpenoids were also examined. In order to know the differences of those two groups, a statistical analysis was applied using an independent sample T-test. Two variables called dependent (antioxidant capacity) and independent (type of solvents) were noted as secondary parameters. Main parameter were total phenolic compounds and test of antioxidant capacity applying the DPPH. Data obtained were then drawn as curve of relations of antioxidant capacity content with free radicals trapped in the extract. Supporting parameters were yields of mycelium extract of Ganoderma sp. and its flavonoids and terpenoids contents measurement.

Preparation of mycelium extract of Ganoderma sp.

Dried myelia were macerated using ethanol at 1:9 ratio for 1 x 24 hours. Extraction and sieving were done in triplicate to get first, second and third levels of extract using Whatman paper no. 41. First and second level which contain ethanolic extracts were then mixed, sieved and placed in a vacum pump then being evaporated. Similar steps were done to the extract which contain ethyl acetate.

Test of Antioxidant Capacity Using a DPPH (Sheikh et al., 2009), Total Phenolic Compounds (Matanjun et al., 2008), Flavonoid Content
and Terpenoid Compounds. The test of antioxidant capacity using DPPH was done by dissolving solid extract in methanol at 125, 250, 500, 1000, and 2000 ppm concentrations. As many as 2ml of each extract concentration was mixed in 2ml DPPH 0.16 mM in methanol, vortexed for 1 minute and left for 30 minutes then measured for their absorbance in a spectrophotometer at 517 nm wavelength. Control was divided into two; negative control wth DPPH and methanol as much as 4 ml volume and positive control which is solution of negative control with addition of α tocopherol. Reduction in absorbance that represented increase ability of solution in trapping the free radical DPPH was calculated using formula applying a regression equation in percentage. Meanwhile, the test of total phenolic compounds was done by preparing a standard solution by weighing 0.025 g tannic acid in 25 ml methanol, then sequentially dissolved for the concentration of: 0 ppm, 25 ppm, 50 ppm, 75 ppm, 100 ppm, and 125 ppm. Two ml of each concentration was then placed in reaction flasks containing 5 ml free ionic water and 0.5 ml reagent Folin ciocalteau (10x). Solution was then vortexed and added with 1 ml Na₂CO₃ 5% and re-vortexed. Absorbance was measured at 750 nm wavelength.

Data of samples analysis were taken from 1 ml of each concentration that was then added by 5 ml free ionic water and 0.5 ml Folin-ciocalteau (10 x dissolving). The tested solutions then were vortexed and left for 1 minute and added by 1ml Na₂CO₃ 5% solution, vortexed and left for another 30 minutes in a dark room for absorbance measurement under 750 nm wavelength. Then the test of flavonoid content was done by adding 1 ml extract by some drops of strong H₂SO₄ to change its color. The present of flavonoid component was represented by color change into orange or cream. The test of terpenoid compounds was done by dissolving 1 ml extract in 10 ml chloroform and added with 10 drops of acetic acid anhydride and 3 drops of strong H₂SO₄ and left for several minutes. Positive reaction was represented by the change of solution’s color into soft red.

**Analysys Method**

Data from several tests were then analyzed using SPSS statistical program while quantitative data were analyzed by an independent T-test. Qualitative data from both flavonoid, and terpenoid tests were described by comparing the data taken from baseline and after being treated or even between the treatments.

**RESULTS AND DISCUSSION**

Regenerated mycelium of *Ganoderma sp.* isolate were grown in an MCM (Mushroom Complete Medium) for 28 days on an orbital shaker and then weighed. As much as 12.79 g constant dry weight of 319.07 g fresh weight was prepared for ethanolic extract. Meanwhile, fresh weight for mycelium prepared for ethyl acetic extraction was 753.41 g and reached a constant weight at 28.83 g. Dried mycelium of each preparation was grinded and extracted by macerating in two different solvents to get the crude extract weight as well as its yield of mycelium extract. Weight of crude extract derived from ethanol solvent was 1.99 g with 16.69% of mycelium extract, while crude extract derived from ethyl acetate solvent was 0.79 g with 3.87% mycelium extract. A statistical analysis using an independent T test ($T_{calculated}$: 3.259 > $T_{table}$: 2.776) showed that the two solvents producing different amount of extract (Table 1). Percentage of yield of mycelium extract of ethanolic extraction was higher than that of ethyl acetic which might be because the component contained in the mycelium of *Ganoderma* sp. isolate have more polar characteristics than non-polar ones (Figure 1).

Septiana and Asnani (2013) stated that selection for solvent for extraction process have to be based on it's compounds polarity. Agarwal et al. (2012) reported that bioactive compounds in the mycelium of *Ganoderma lucidum* were polysaccharides (β-D-glucans, heteropolysaccharides and glycoprotein) which were dissolved in water- and triterpenoid. Gowrie et al. (2014) reported that *Ganoderma lucidum* contains alkaloids, carbohydrate, saponin, protein, amino acids, phytosterols, fats, triterpenoid, flavonoid, phenolic compounds and tannin which might be used as antiinflammation, antibacteria, anticancer and antioxidants. Padmasari et al. (2013) stated that saponin, flavonoid, oils, alkaloids, tannin and glycosides were dissolved in ethanol solvent.

| Table 1. Fresh weight, dry weight, crude extract weight and yield of *Ganoderma sp.* mycelium extract |
|---------------------------------------------------------------|
| Extraction solvents | Weight (g) | Yield of Mycelium Extract (%) |
|---------------------|------------|-------------------------------|
| Ethanol             | 1.99       | 16.69 a*                     |
| Ethyl Acetic        | 0.79       | 3.7  b*                      |

*different letters showed a significant difference*
Figure 1. Colony of *Ganoderma* sp. Banyumas 1 isolate on Potato Dextrose Agar, 15 days at room temperature (27-29°C), reverse white to brownish.

**Phytochemistry**

Phenolic compounds play a significant role in attacking free radicals. Kinsella et al. (1993) stated that this compound can be used as antioxidants due to its ability to attack free radicals and peroxides radicals leads to slowing down lipid oxidations. Flavonoid is one among those phenolic compounds contained in some plants and mushrooms. Flavonoids are the most common compound in plant’s tissues and being produced as secondary metabolites (Redha, 2010). Pietta (2000) reviewed flavonoids as antioxidant and assessed the antioxidant capacity in relation with their chemical structures critically. Owing to the unique characteristics of flavonoids, they are likely to be radical scavengers, reducing agents, hydrogen donors, singlet oxygen quenchers and/or metal chelators to reduce the amount of free radical in the body. In order to know the flavonoids content of the mycelium of *Ganoderma* sp. isolate, a further test in form of qualitative test was conducted (Table 2).

The table shows that mycelium of *Ganoderma* sp. isolate extracted by both ethanol and ethyl acetate contains flavonoids and terpenoids as represented by the color change. However, the change of the color noted in this study was categorized as weak because both solvents have a closed-fraction in polarity. Dielectric constant of ethanol is 24.5 and ethyl acetate is 6.0 which means that both solvents are able to extract both polar and non-polar compounds from its substrates. Buchari & Sulaeman (2003) stated that constant dielectric is one among those parameters used to judge the polarity level of a particular solvent, the higher dielectric constant means the more polarity.

| Qualitative test | Ethanol | Ethyl acetate | Remarks          |
|------------------|---------|---------------|------------------|
| Flavonoid        | +       | +             | Orange or cream  |
| Terpenoid        | +       | +             | Pink             |

Remarks: - : absent, + : weak, ++ : strong, +++ : very strong

Qualitative test of this study showed that type of solvent did not affect flavonoid and terpenoid compounds which extracted. This might be because both compounds have a specific structure which has both polar and non-polar character at almost balance amount. Terpenoids for example contain both polar and non-polar characters in which the amount of non-polar is higher than that of polar ones. The total extract resulted from non-polar solvent tend to be more soluble than polar solvent. Terpenoid which was dissolved in polar solvent might be in form of globule with a polar character in its outer part (Septiana & Assani, 2012). Ganoderic acid is a bioactive compound which derived from lanosterol of *Ganoderma lucidum* fruiting body and was reported to have pharmacologic activity in form of triterpenoids (Trigos & Medellin, 2011). Figure 2 shows the structure of flavonoid and ganoderic acid:

![Structure of (a) flavonoid (Redha, 2010); (b) ganoderic acid (Trigos and Medellin, 2011).](image)
Total Phenol

Analysis of total phenol was done as Cio-calteau method by measuring total phenolic compound quantitatively. Table 3 shows the analysis results of total phenolic compounds of mycelium of *Ganoderma* sp. mycelium extracted with ethanol and ethyl acetate.

**Table 3.** Results of total phenolic compounds contain in mycelium of *Ganoderma* sp. mycelium analysis by ethanol and ethyl acetate.

| Total phenol (mg/g) | Ethanol extraction | Ethyl acetate extraction |
|--------------------|-------------------|------------------------|
|                    | 29.23 a           | 57.67 a                |

*different letters show a significant difference.*

A statistical analysis applying independent sample T test showed if average value of total phenolic compound resulted from both solvents did not significantly different ($T_{calculation} = -12.960 < T_{table} = 2.776$). The result showed that ethyl acetate extraction produces 57 mg/g phenol and ethyl acetate extraction produces 29.23 mg/g phenol. Mushrooms are known to produce phenolic compounds as secondary metabolites in form of polyketides, terpenes and steroid, and also ascorbic acids, flavone, beta carotene and lycopene which can be used to count total phenolic compound of the mushrooms (Phunita & Rajasekaran, 2014).

Phenol is a chemical compound characterized by its aromatic ring with one or more hydroxyl groups. Phenol which found in the food might be divided into two namely simple phenol and folic acids (P-cresol, 3-ethyl phenol, 3,4-dietetyl phenol, hydroxyquinone, vanillin, and gallic acid), derivate of hydroxy cinnamic acid (p-coumarate, caffeic, phenolic acid and chlorogenic acid) and flavonoids (catechin, proanthocyanin, anti-cyanidin, flavone, flavanol and glycosides). Phenolic compounds are able to slowing down the rate of lipid oxidation by donating hydrogen atom to free radicals (Widiyanti, 2006).

Current study results were parallel to those resulted by Mau *et al.* (2002) who reported that fruiting body of several medical mushrooms contained total phenolic compounds as follows: *Coriolus versicolor* (23.28 mg/g), *G. lucidum* (47.25 mg/g), *G. tsugae* (51.28 mg/g), and *G. applanatum* (55.96 mg/g). Applying different solvent to extract bioactive compound of *Schizophyllum commune*, reported if extraction using ethyl acetate produced higher amount of total phenol than extracted with dichloromethanol or water which are known as polar solvents. Phenolic compound contains in mycelium of *Ganoderma* tend to be dissolved in non-polar solvents. It might be because its phenolic compound, in structure, it does not belong to simple phenol. Phenol structure varies from simple phenolic acid with on 1 ring, biphenyl and flavonoids which have 2 or more phenolic rings (Vattem *et al.*, 2005). The simple phenol contains in Ganoderma are catechol dan hydroquinon (Castellano *et al.*, 2012).

**Capacity of trapping DPPH radicals**

DPPH has widely used as a component to test chemical compound characterized by their ability of trapping free radicals or as hydrogen atom donor. Electron contained in DPPH radicals could be absorbed maximally under 517 nm wavelength by producing violet color (Septiana and Asnani, 2013). Antioxidant compound would donate proton to the DPPH radicals to slowing down its ability in absorbing the light and change the color from violet to yellow (Kalyoncu *et al.*, 2010). Average percentage of *Ganoderma* sp. mycelium extract ability on DPPH radicals blocking (Table 4).

**Table 4.** Blockage of the DPPH radicals by mycelium extracted by ethanol and ethyl acetate

| Concentration | Ethyl acetate (%) | Ethanol (%) |
|---------------|------------------|-------------|
| 125           | 22.60            | 8.32        |
| 250           | 42.68            | 16.56       |
| 500           | 61.09            | 30.75       |
| 1000          | 65.02            | 52.60       |
| 2000          | 63.55            | 66.14       |

It can be concluded that extract which derived from ethyl acetate extraction produce better blocking activity than ethanolic extraction. At 125 ppm extract of ethyl acetate shows a blocking score of 22.60% in compared with 8.32% due to the present of extract from ethanolic extraction. Both extract, however, show a gradual increase in blocking when concentration is also increased and keep increasing up to a certain concentration (Figure 3).
Figure 3. shows if blocking percentage DPPH radicals by ethanol extract was significantly increased and reached its maximum level at 66.14% at concentration of 2,000 ppm. On the other hand, the highest blocking level of ethyl acetate was 65.02% reached at the concentration of 1,000 ppm but at a higher concentration (2,000 ppm) its blocking ability was gradually decrease. Tocopherol which was treated as a positive control reached a constant increase in blocking activity. Dewi and Murtini (2007) added that antioxidant at higher concentration caused a higher activity but at a particular level its antioxidant activity would decrease. Extract of ethyl acetate extraction showed a better activity in blocking DPPH radicals than extract from ethanol extraction (Table 5).

Table 5. Analysis of extract of ethanol and ethyl acetate extraction of *Ganoderma* sp. isolate on blocking DPPH radicals

| Average value of IC<sub>50</sub> (ppm) | Extract from ethyl acetate extraction | Extract from ethanol extraction | Control (α tocopherol) |
|-------------------------------------|---------------------------------------|---------------------------------|-----------------------|
| 581.80 a                           | 1285.67 a                             | 1.37 x 10<sup>-5</sup>         |

*different letters show significant differences

It might be then concluded if (H0) was accepted which means that mycelium of *Ganoderma* sp. isolate has potential character to be used as a source of natural antioxidant.

A statistical analysis applying an independent sample T-test show that the average level of IC50 of both solvents did not significantly different (T<sub>calculation</sub> = 1.94 < T<sub>table</sub> = 2.77). The average level of IC50 of ethyl acetate extraction was still high (581.80 ppm) or (0.58 mg/ml) while the extract from ethanolic extraction had 1285.67 ppm or 1.28 mg/ml. α tocopherol which was used as a positive control in blocking the DPPH radicals, showed the highest score on blocking the DPPH radicals with average of 1.3 x 10<sup>3</sup> ppm, but this level is still lower than that one shown by extract of mycelium of *Ganoderma* sp. isolate extracted by both ethanol and ethyl acetate. It might be because of varies mechanisms of antioxidants in blocking the DPPH radicals. The DPPH method is a methodology which represent the number of antioxidant by releasing its hydrogen atoms to the DPPH in order to stabilized it.
tion of non-polar extract of *Ganoderma lucidum* contains more than 130 isolated triterpenoids, some of lanosterol derivate and showed a pharmacological activity and as antioxidant or anticancer, like ganoderic acids, ganoderols, ganolucidic acids, lucidones and lucidicenic acids. This result show that mycelium extract of *Ganoderma* sp. from ethyl acetate extraction had better antioxidant activity than from ethanolic extraction, due to its compound that non polar and had a better performance in blocking DPPH radicals. This research result has benefit in developing potency of local resources as herbal resources.

**CONCLUSION**

Based on the result and discussion before, it can be concluded that mycelium extract of *Ganoderma* sp. is having potential of antioxidant as shown by average percentage on blocking DPPH free radicals of both solvents that reached 65%. The extract from ethyl acetate extraction (non-polar) of *Ganoderma* sp. mycelium isolate had a potential character as antioxidant source better than the extract from ethanolic extraction as shown in the IC50 value of both types of extracts. Extract from ethyl acetate extraction had an average IC50 value smaller than ethanolic extract 581.80 < 1285.67. Extract from ethyl acetate extraction resulted in higher amount of phenol than that ethanolic extract 29.23 < 57.67.

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