ORIGINAL ARTICLE

Standardization of non-specific parameters of ethanolic extract of mushroom (*Ganoderma amboinense*)

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Abstract

Introduction: The purpose of this research to measure the quality of ethanolic extract of *Ganoderma amboinense* as source of herbal medicine. The quality parameters of the ethanolic extract of *G. amboinense* contain non-specific parameters. **Materials and Methods:** *G. amboinense* extract is made by maceration method using 96% ethanol to obtain a thick extract. Extracts are tested the moisture content, water content, ash content, acid-insoluble ash content, density, microbial contamination, and metal content. Metal content of lead (Pb) and cadmium (Cd) is determined using atomic absorption spectrophotometer. **Results:** This research resulted that the parameters of *G. amboinense* such as water content (7.95 ± 0.49%) v/w, total ash content of powders and extracts of 1.13 ± 0.12% and 2.13 ± 0.26%, acid-insoluble ash content of powders and extracts of 0.68 ± 0.25% and 1.12 ± 0.38%, the density of the extract (0.68 ± 0.25%), and the metal content of Pb extract (0.162 ± 0.01 ppm and Cd is not detection). **Conclusion:** The conclusion showed that the ethanol extract of *G. amboinense* has good non-specific parameters and it conducted in accordance to good extract quality requirements.

Key words: Extract, *Ganoderma amboinense*, mushroom, non-specific parameters

INTRODUCTION

Various compounds of active contained in *Ganoderma*. The compound active has the potential as antitumor and an anticancer, scarlet blood pressure, scarlet cholesterol levels in the blood, inhibitor the clumping platelets, protein immunomodulator, the release of histamine deterrent, and anti-HIV.[1] The compound active among others: Ganoderik, lusiderik, ganodermik, ganoderenik, ganolusidik, aplanosidik acid, polysaccharides, protein, amino acid, nucleotides, an alkaloid, steroid, lactone, fatty acids, and enzymes.[2] Herbal products from mushroom (*Ganoderma amboinense*) are determined by the quality of raw materials or extracts used. Extract as an ingredient and pharmaceutical products derived from crude drugs must meet the quality requirements that have been established to become standardized herbal medicine or fitofarmaka and to anticipate the distribution and use of plant extracts that do not meet the requirements.[3] Therefore, it is necessary to standardize the extracts and mushrooms powder to ensure that product (extract) has a value of a specific parameter constant to maintain the consistency of the content of the active compounds contained in extracts as well as comply with the standards BPOM RI regarding standard non-specific parameter extracts such as moisture content, total ash, acid-insoluble ash content, density, and the metal content of lead (Pb) and cadmium (Cd).[4]

MATERIALS AND METHODS

Identification *G. amboinense*

The results of mushroom identification carried out in Laboratory Biology, University Gadjah Mada showed that the mushroom was a species *G. amboinense*. Based on the systematics, the species *G. amboinense* has the genus *Ganoderma* and family Poliporaceae. *G. amboinense* was collected from Magelang, Indonesia, at November 2016 and all parts of the plant are used for this research.

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Powder from *Ganoderma amboinense*

G. amboinense is cut into pieces with a thickness of 1–5 cm. Cut into pieces of oven dried for 46 h at a temperature of 45°C. Drought simplisia characterized by fragility and easily broken. *G. amboinense* dried powder is then created.

G. amboinense Extraction

The extract was made by maceration using 96% ethanol. The maceration process was chosen because it was based on the optimization results of the extraction method which produced the highest yield with 96% ethanol. One part dry powder *G. amboinense* put in macerator with 10 parts solvent, soaked for 6 h while occasionally stirring, then allowed up to 2 h × 24 h. Maserati separated and the process is repeated 2 times with the type and amount of the same solvent. All Maserati collected and evaporated in a vacuum evaporator, after the solvent does not drip resumed in water bath to obtain a thick extract. The yield obtained is weighed and recorded.

Determination of Moisture Content

Moisture content from *G. amboinense* powder is using a halogen moisture analyzer.

Determination of Ash Content from *G. Amboinense* Powder and Extracts

The ash content is calculated on the total weight of the test material by gravimetric method, calculated in % w/w.[5]

Determination of Ash Content Insoluble Acid from *G. Amboinense* Powder and Extracts

Ash obtained in the determination of total ash is added 25 mL HCl. Acid-insoluble part is collected and filtered through ash-free filter paper and washed with hot water. Acid-insoluble ash content calculated on the weight of the test materials is calculated in % w/w.[5]

Determination of Bacterial Test

Total plate numbers test: The dilution solution is prepared by dissolving 0.9 g of NaCl into 100 mL of water. Then homogenized with a sample of 10 mL. Take as much as 1 mL and homogenize to the desired dilution.[6] Taken 100 µl pour on Mueller-Hinton media for each dilution. Then, flatten with the spreader until the liquid is evenly distributed on the Petri. Incubated at 37°C for 18–24 h. Calculated the number of bacterial colonies in each Petri at various dilutions.[7]

Escherichia coli (ATCC 25922) Test: A little extract plus 0.9% sterile NaCl then homogenized with stomacher (230 rpm 30 s), take 100 µl on TBX selective medium. Incubated at 37°C for 24 h and see colony growth.[7]

Determination of Water Content Extract

Assay of water with toluene distillation method calculated in % v/b.[5]

Determination of Density Extract

Pycnometer is weighed with a certain volume empty. Pycnometer filled with water and weighed so that the density of water can be set. Pycnometer then emptied and filled with 0.5 g of extract, then filled with water and weighed so that the density of the extract can be set.[5]

Determination of Metals Pb and Cd Levels

About 1 g sample of the extract was added HNO_3 (P):HCl (P) 1:3. Then heated until the volume is reduced by half over the hotplate. It is intended to vaporize as many organic substances are still there. The sample continues to be added HNO_3 (P):HCl (P) 1:3 until the solution is clear. Samples were destructed and measured the levels of Pb and Cd using atomic absorption spectroscopy (AAS).[8]

RESULTS AND DISCUSSION

Ganoderma has many active compounds that can be used in various disease treatments. Therefore, several studies on *Ganoderma* have been performed. The two species from Egypt were identified and the species status was confirmed as *Ganoderma* sp EGDA. and *G. resinaceum* EGM.[9] In addition, the types of *Ganoderma lucidum* have been analyzed.[10-12] While this study aims to standardize other types of *Ganoderma*, *G. amboinense* [Figure 1].

The maceration process in *G. amboinense* using ethanol solvent resulted in a rendement of 6.994%. This result is obtained from the moisture content of powder of 7.95%. The yield of ethanol extract was performed by standardization of the ethanol extract of *G. amboinense* covering several

Figure 1: *Ganoderma amboinense*

Table 1: Non-specific parameters of *Ganoderma amboinense*

Sample	Water content (%)	Total ash (%)	Ash-insoluble acid (%)	Density (g/mL)	Metal content (ppm)	
					Cd	Pb
Ganoderma amboinense powder	7.95 ± 0.49	1.13 ± 0.12	0.68 ± 0.25	0.133 ± 0.01	NA	NA
Ganoderma amboinense extract	7.66 ± 1.37	2.13 ± 0.26	1.12 ± 0.38	0.135 ± 0.03	ND	0.162 ± 0.00

NA: Not analyzed, ND: Not detected and samples testing are triplicated, Cd: Cadmium, Pb: Lead

parameters [Table 1] and also bacteria contamination test on *G. amboinense* ethanol extract [Figure 2].

Table 1 shows that the result of water content of the sample powder and extract ethanol was 7.95% and 7.66%, respectively. This result has met the requirements determined not >10% v/b. The total ash content of *G. amboinense* extract is more than *G. amboinense* powder. The ash content indicates the amount of minerals and other substances contained in the extract. The metal identification showed that the extract ethanol just contains Pb and Cd was not detected. Limit of metal contamination for Pb in the food is 0.25 ppm or mg/kg and limit metal contamination for Cd in food is 0.2 ppm or mg/kg.[4] In addition, the tests on the ethanol extract of *G. amboinense* and powder were carried out as standardization, i.e., the acid-soluble ash test and its density. The results showed that ethanol extract of *G. amboinense* and powder had acid-soluble ash, respectively, 1.12% and 0.68% with ethanol extract density of *G. amboinense* 0.135 g/mL.

The results of non-specific parameter testing on ethanol extract of *G. amboinense* showed that the ethanol extract of *G. amboinense* was in accordance with the standard specified by pharmacopeia. These results are also supported by bacterial assay results using test total plate numbers and *E. coli* methods [Figure 2].

Based on Figure 2 showed that the extract ethanol of *G. amboinense* is able to inhibit bacterial growth in either total plate numbers or *E. coli* testing. It can also serve as a basis that there are antibacterial properties of the ethanol extract of *G. amboinense*. This suggests that *Ganoderma* is able to inhibit bacterial growth. In *G. amboinense* extract, there is no microbial contamination. This is because in the extract contained terpenoids, where terpenoids have properties as antibacterial by reacting with porin (transmembrane protein) in the outer membrane in the cell wall of bacteria, forming a strong polymer bond resulting in damage to the porin. Damage to the porin leads to bacterial cells lacking nutrients, so bacterial growth is inhibited or dead.[13]

Other types of *Ganoderma* also have antibacterial activity that acetone extract from *G. lucidum* was able to inhibit the maximum bacteria by 31.6 mm.[14] Even organic extracts of the *Ganoderma lucidum* from Nigeria type exhibit antibiotic effects in *Pseudomonas syringae* and *Bacillus subtilis*.[15] The

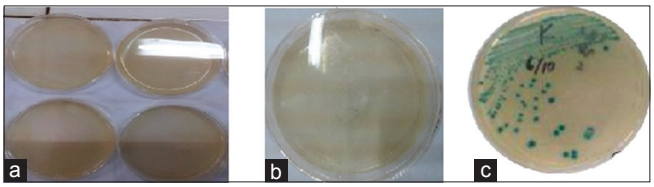

Figure 2: Bacterial testing of the ethanolic extract of *Ganoderma amboinense*: (a) Bacterial test with total plate numbers test, (b) bacterial test with *Escherichia coli*, (c) control positive of *E. coli* (ATCC 25922)

result of standardization of *G. amboinense* generally meets herbal standards in terms of non-specific parameters and bacterial content test. The standardization of *G. amboinense* encourages the process of identifying and testing its activity so that it can be more utilized in various drugs, cream, or some other products.

CONCLUSION

Non-specific parameter of *G. amboinense* is moisture content of powder (7.95 ± 0.49%), water content of extract (7.66 ± 1.37%) v/b, total ash content of powders and extracts of 1.13 ± 0.12% and 2.13 ± 0.26%, acid-insoluble ash content powders and extracts (0.68 ± 0.25 and 1.12 ± 0.38%), the density of the extract (1.12 ± 0.38) g/ml, and a metal content of Pb extract 0.162 ppm and Cd levels extract not detection. The ethanolic extract of *G. amboinense* can inhibit *E. coli* bacterial growth. Moisture content, water content, total ash, levels of acid insoluble ash, the density of the ethanolic extract, and the content of metallic Pb and Cd of *G. amboinense* meet the requirements of the extract was good.

REFERENCES

1. Dunham M. Potential of Fungi Used in Traditional Chinese Medicine: II *Ganoderma*. Available from: http://www.oldkingdom/UGprojects/Mark-Dunham/Mark-Dunhamhtml. [Last accessed on 2004 Apr 02].
2. Boh B, Hodžar D, Dolničar D, Berovič M, Pohleven F. Isolation and quantification oftriterpenoid acids from *Ganoderma applanatum* of Istrian origin. Food Technol Biotechnol 2000;38:11-8.

3. Faujan NH, Zulaikha AR, Maryam MR, FaujanHA. Comparative analysis of phenolic content and antioxidative activities of eight Malaysian tradisional vegetable. Malays J Anal Sci 2015;19:611-24.
4. Bpom RI. The Maximum Limits Microbes and Chemistry in Food: Regulation Head of BPOM RI No. HK.00.06.1.52.4011. Report; 2009.
5. Health Department. Papers Farmakope Herbs Indonesia 2008;1:150-5.
6. Hayati F, Wibowo A, Pinus J, Arde TN, Dian A. Standardization of *Ipomea reptans* poir leaf exhibition of cultivation in sardonoharjo, sleman and potential as antioxidant. J Indones Pharm Sci 2015;13:151-7.
7. Gunanti FZ, Witasari HA, Edityaningrum CA, Murrukmihadi MM. Determination of Non-Specific Standards Of Ethanolic Extract Carambola (*Averrhoa bilimbi* L.) leaf. Proceedings of Annual Scientific Meeting of Association of Indonesian Apothecary; 2016.
8. Health Department. Parameter a Common Standard Plant Extracts Medicine. The Ministry of Health of the Republic of Indonesia; 2000. p. 4-8.
9. El-Fallal AA, El-Sayed AK, El-Esseily SR. First record of two *Ganoderma* species from North East Nile Delta-Egypt. Mycosphere 2015;6:248-59.
10. Park YJ, Kwon O, Son E, Yoon D, Han W, Nam JY, *et al*. Genetic diversity analysis of *Ganoderma* species and development of a specific marker for identification of medicinal mushroom *Ganoderma lucidum*. Afr J Microbiol Res 2012;6:5417-25.
11. Kao CH, Jesuthasan AC, Bishop KS, Glucina MP, Ferguson LR. Anti-cancer activities of *Ganoderma lucidum*: Active ingredients and pathways. Func Foods Health Dis 2013;3:48-65.
12. Poomsing P, Pattanapanyasat K, Wongsinkongman P, Soonthornchareonnon N. Research and development of *Ganoderma lucidum* cultivated in Thailand. Mahidol Univ J Pharm Sci 2013;40:1-7.
13. Cowan M. Plant product as antimicrobial agent. Clin Microbiol Rev 1999;12:564-82.
14. Quereshi S, Pandey AK, Sandhu SS. Evaluation of antibacterial activity of different *Ganoderma lucidum* extracts. Peoples J Sci Res 2010;3:9-13.
15. Kamble R, Venkata S, Gupte AM. Antimicrobial activity of *Ganoderma lucidum* mycelia. J Pure Appl Microbiol 2011;5:1-4.

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