Physicochemical properties, antimicrobial and antioxidant activity of ganoderma transparent soap

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Abstract. *Ganoderma lucidum* has been known as a cosmetic source. It contains bioactive ingredients such as triterpenoids and polysaccharides. The present study was to formulate transparent soap enriched with Ganoderma (*Ganoderma lucidum*) extract and investigate its antibacterial and antioxidant activity. Formulation of Ganoderma transparent soaps was made by hot process method. The results showed that Ganoderma transparent soaps have moisture content 21.8%, pH 9.22, total fatty matter 41.66%, free fatty acid 1.08% and chloride acid 5.57%. Antibacterial assay by agar well diffusion method showed that Ganoderma transparent soaps were more active against the *S. aureus* than the Gram-negative bacteria and even *C. albicans*. The antioxidant activity assay using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical showed that the IC50 of transparent soap was 1.53 mg/mL.

1. Introduction

Mushrooms have been recognized as a source of natural bioactive compounds. Mushrooms have been exploited for potential ingredients in the cosmetics industry. They have been known to be useful to the skin and hair. The typical ingredients such as phenolics, polyphenolics, terpenoids, selenium, polysaccharides, vitamins, and volatile organic compounds. These compounds are ideal candidates for cosmetics products because they show antioxidant, anti-aging, anti-wrinkle, skin whitening, and moisturizing effects [1]. *Ganoderma lucidum* (Fr.) Karst (Polyporaceae) is a medicinal mushroom which contains bioactive ingredients such as water-soluble polysaccharides, triterpenoids, adenosine, lectin, glycoproteins, vitamins, and germanium. Polysaccharides, especially -(1-3)-D-glucan in the *G. lucidum* fruiting body have been discovered to be medically active in some therapeutic effects such as antitumor, anti-inflammatory and antioxidant [2, 3]. Terpenoids were found in *G. lucidum* containing a wide range of terpenes and terpene derivatives, such as ganodermic and ganoderic acids, ganoderals, ganoderols, ganodermanontriol, lanostane, lucidone, and ganodermanondiol, Triterpenes are the most common antimicrobial compounds reported from *Ganoderma sp.* [1].

Nowadays, there is an increasing consumer demand for cosmetics comprising natural ingredients as healthier, organic, and ecological product [4]. Consumers are more and more refusing synthetic chemicals in beauty and cosmetic products. The damaging effects of synthetic surfactants, the environmental friendliness, and environmental sustainability of large – scale commercial soap making in the factory are being accentuated. A natural soap is prepared without a non-natural surfactant, with addition of functional ingredient from natural substance, such as essential oil or plant extract. It
typically run in small scale by small or home industry. A natural soap may be generally divided based on the production method into: a melt – pour soap, a hot process soap, and a cold process soap. The hot process soap is called a transparent or translucent soap. The soap has good detergency or cleansing power, good moisturizing effects, long-lasting fragrance, and less of irritant. The present study was to perform transparent soap formulation enriched with Ganoderma extract and investigate its antibacterial and antioxidant activity.

2. Materials and methods

2.1. Preparation of Ganoderma extract

The fresh mature fruiting bodies of G. lucidum were supplied by Sanggar Tani Media Agro Merapi, Sleman, Yogyakarta, and have been taxonomically verified at Pharmaceutical Biology Division, Faculty of Pharmacy, Universitas Gadjah Mada. Fresh fruiting bodies of G. lucidum were dried under direct sunlight then sliced and grinded into coarse powder mechanically and stored in airtight container before use. Samples were macerated in ethanol 70% (in a 1:15 ratio) at room temperature for 72 hours. After removal of residue by filtration, the residue was re-extracted with the same method. The filtrates were combined and evaporated to dryness under reduced pressure at 60°C. The resulting extracts were further used for determination of some quality control parameters and soap formulation. Characteristics of Ganoderma extract are summarized below (table 1).

| Parameters                          | Methods               | Description                                    | Acceptable criteria |
|------------------------------------|-----------------------|------------------------------------------------|---------------------|
| Organoleptic                       |                       |                                                |                     |
| Texture                            | Gravimetric           | Viscous, sticky                                |                     |
| Color                              | Gravimetric           | Blackish brown                                 |                     |
| Odor                               | Gravimetric           | Smoky, musty, woody, fungus – like             |                     |
| Taste                              | Gravimetric           | Mildly to extremely bitter                     |                     |
| Moisture content                   | Gravimetric           | 19.01 g/100 g                                  |                     |
| Total ash content                  | Gravimetric           | 15.30 g/100 g                                  |                     |
| Acid – insoluble ash content       | Gravimetric           | 0.104 g/100 g                                  |                     |
| Water – soluble extractive         | Gravimetric           | 79.20 g/100 g                                  |                     |
| Ethanol – soluble extractive       | Gravimetric           | 62.10 g/100 g                                  |                     |
| Water – soluble polysaccharides    | Spectrophotometric,   | 10.03 g glucose equivalent / 100 g of dry weight extract |                     |
|                                   | phenol-sulfuric [5]   |                                                |                     |
| Triterpenoids                      | Spectrophotometric [6]| 19.58 g ursolic acid equivalent / 100 g of dry weight extract |                     |
| Heavy metals residues              |                       |                                                |                     |
| Pb                                 | AAS                   | 0.00187 ppm                                    | ≤ 0.01             |
| Cd                                 | AAS                   | 0.00204 ppm                                    | ≤ 0.01             |
| Microbial contamination            |                       |                                                |                     |
| Total Plate Count                  | Spread plate          | 5 x 10^4 CFU/g                                 | ≤ 10^6             |
| Total Yeast – Mould Count          | Spread plate          | 5 x 10^4 CFU/g                                 | ≤ 10^4             |

2.2. Formulation of transparent soap

Firstly, virgin coconut oil, alcohol 96%, stearic acid, ricini oil, and citric acid were mixed while heated at temperature range about 50 to 60 °C until the mixture solution was clear. An aqueous solution of sodium hydroxide was added gradually to neutralized the batch while ensuring the temperature does not exceed about 85 °C. The mixture was kept at temperature between 80 to 85 °C for a moment to ensure the saponification reaction was complete. Aqueous mixture of sucrose and propylene glycol was added to the batch and mixed until the solution was clear. While continued mildly agitation, the batch was cooled to a temperature about 60 °C. Ganoderma extract was added to the batch and mixed
well. The homogenous mixture was decanted into molds and allowed to solidify for 24 hours. The cooled transparent soaps were removed from the molds and kept in a normal temperature for 2 weeks for aging process.

Table 2. Formula of Ganoderma transparent soaps

| Formula          | Proportion (%) |
|------------------|----------------|
| Stearic acid     | 7 - 11         |
| Citric acid      | 0.1 – 0.5      |
| Virgin coconut oil | 30 – 34     |
| Ricini oil        | 0.9 – 1.5      |
| Alcohol 96%      | 12 – 16        |
| Sodium hydroxide | 5 – 7          |
| Propylene glycol | 7 – 9          |
| Sucrose          | 9 - 13         |
| Aqudes           | 7 - 9          |
| Ganoderma extract | 4 – 8       |

2.3. Physicochemical properties of soap

Physicochemical properties of soap include pH, moisture content, total fat matter, free fatty acid content, and chloride content. The analysis of physicochemical properties used standard procedures as described by AOCS with slight modifications [7]. pH of 10 % solution of transparent soap was determined by means of a digital pH meter (Euthech PC 700). Moisture content was measured by moisture analyzer digital (AND MX – 50). Total fat matter and free fatty acids were analyzed with gravimetric and volumetric method. While chloride content was analyzed with argentometric methods.

2.4. Antimicrobial activity

One Gram – positive (Staphylococcus aureus) and two Gram – negative bacteria (Eschericia coli, Pseudomonas aeruginosa), and one fungus (Candida albicans) were used to assess the antimicrobial properties of ganoderma transparent soap. The microbial strains were obtained from Department of Agricultural Microbiology, Universitas Gadjah Mada, Indonesia. The antimicrobial activities were investigated by agar well diffusion [8, 9] using Nutrient Agar medium for the bacterial assay and Potato Dextrose Agar medium for the fungal assay. Bacterial inoculum (inoculum size was 10^8 cells/ml as per McFarland standard) was inoculated into the Nutrient Agar media) and fungal inoculum was inoculated into the Potato Dextrose Agar media. Test solution and control solution (Lifebuoy© transparent soap) at level concentration of 1%, 5%, 10%, 20% were introduced into the wells. Bacterial plates were incubated at 37 °C for 24 hours and fungal plates were incubated at 30°C for 48 hours. The experiment was performed 3 times under strict aseptic conditions.

2.5. Antioxidant activity

Antioxidant activity was analyzed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay according to the method of Shimada, et al. [10] with some modification. 2 mL of methanolic extract of soap at the different concentration level were added with 2 mL of DPPH solution (0.2 mM DPPH in methanol). The mixture was shaken, left to stand for 30 min in the dark place. The absorbance was measured at 517 nm, and methanol was used as the blank solution. The percent DPPH radical scavenging effect were calculated using the following equation (1), where A0 is the absorbance of DPPH solution without soap (control solution) and A1 is the absorbance of DPPH solution with soap (test solution).
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\text{DPPH scavenging effect (\%) = \left(\frac{A_0 - A_t}{A_0}\right) \times 100}
\] (1)

The antioxidant activity was stated as IC50 value. The IC50 value is the effective concentration at which the DPPH radicals were scavenged by 50%. The IC50 value was calculated by linear regression of plots, where the abscissa (x) represents the concentration of tested methanolic extracts and the ordinate (y) represent the average percent of scavenging effect. Effectiveness of antioxidant activity have an inverse correlation with IC50 value.

3. Results and discussions

The formula was made by mixing caustic soda with oil at high temperature for 40 to 50 minutes, and the heated mixture should be matured in a normal temperature for 2 weeks. Ganoderma transparent soaps have gloss – like clarity. Virgin coconut oil was used as a source of lauric acid (12 carbons) which was made into sodium laurate. Virgin coconut oil, ricini oil, and stearic acid as fatty acid basis provides lathering and washing properties [11]. Fatty acids with only 10 or fewer carbons are not used because they irritate the skin and have unpleasant odours [12]. The fatty acid should be counteracted with a polyol and or polyol blend. The occurrence of non-volatile polyols improves the clarity of the product and prevents shrinkage of the soap in storage and use. The sodium hydroxide was used as alkalis that reacts with glycerides to delivers further neutralizing activity for creation of optimum transparency. These are naturally different from soaps made from divalent metals which are not soluble in water such as calcium, magnesium, iron or aluminium [12]. Addition of sodium hydroxide must be controlled at the temperature not exceed 85 °C as the neutralization of the fatty acid is an exothermic reaction.

3.1. Physicochemical evaluation

The physicochemical properties of soap include moisture content, pH, total fatty matter, free fatty acid, and percentage chloride [13]. They are shown in table 3. The properties depend on several factors including the strength and purity of alkali, the kind of oil, and completeness of saponification.

| Parameters                  | Transparent soap | Control* |
|-----------------------------|------------------|---------|
| Moisture content            | 21.8 %           | 20.23%  |
| pH                          | 9.22             | 9.18    |
| Total fatty matter          | 41.66%           | 36.80%  |
| Free fatty acid             | 1.08%            | 0.08%   |
| Chloride content            | 5.57%            | 59.12%  |

* commercial transparent soap (Lifebuoy®)

The hardness and transparency of the finished soap are dependent on its total moisture content. This is the reason for the important role of water. There were some water sources in this formulation such as the caustic soda solution and the water produced throughout the neutralization reaction. The incorporation of free water also affects the final product. Typically, water addition of less than 5% total (not formed in situ or introduced by other ingredients) produce soap that is too hard and tends to form crystals which is related to the loss of clarity. While free water addition more than about 15% produce soap that is too soft. The transparency was sustained when the moisture content was higher than 17% but not exceed about 21% [14]. Higher moisture content will induce hydrolysis reaction of an excess water with un – saponified fat. The products of this reaction are free fatty acid and glycerol.

An appropriate pH range and the use of pH regulating agent are critical for achieving transparency. Regulating the pH within a range of 9.1 to 9.5 produces the desired end products [14]. Generating a pH outside the range will opacify the soap. The high pH expresses high percentage of unspecified and unsaponifiable substances as a result of incomplete alkaline hydrolysis. pH and free
fatty acid content are critical factor to get a suitable transparent soap. Citric acid causing the increasing of free fatty acid content and the reduce of pH value. pH value of Ganoderma transparent soap 9.22, while the free fatty acid content 1.08%. The transparency was maintained when the pH did not drop below 9.1 and the free fatty acid content did not pass 4.0% [14].

The total fatty matter is important characteristic indicating the quality of soap and it is specified in commercial soap [15]. The soap with the high total fatty matter gives more lather, lasts longer, cleansing skin better and more gently. Total fatty matter of Ganoderma transparent soap (41.66%) was higher than control (36.80%). The lower total fatty matter may be generated by the presence of unreacted NaOH in the mixture [16]. The percentage chloride level is important because excess quantity causes soaps to crack [17]. The chloride content of Ganoderma transparent soap (5.57%) was lower than control (59.12%).

3.2. Antimicrobial activity
Antimicrobial activities of Ganoderma transparent soap were determined by measuring the diameter of the zone of inhibition and the mean values are presented in Table 4. Ganoderma transparent soap and control at concentration level of 10% and 20% showed antibacterial activity against all microbial tested to a certain degree. Nevertheless, Ganoderma transparent soap and control were inactive against P. aeruginosa and C. albicans at concentration level of 1% and 5%. S. aureus was the most susceptible and C. albicans was the most resistant among all the microbial strains investigated in the present work. Ganoderma transparent soaps were more active against the S. aureus than the Gram-negative bacteria and even C. albicans.

| Soap     | Concentration (%) | Zone of inhibition (mm) |
|----------|-------------------|-------------------------|
|          |                   | S. aureus | E. coli | P. aeruginosa | C. albicans |
| Control  | 1                  | 15.91 ± 0.25 | 13.02 ± 0.66 | 0 | 0 |
|          | 5                  | 19.38 ± 0.19 | 17.49 ± 0.34 | 0 | 0 |
|          | 10                 | 20.93 ± 0.41 | 18.90 ± 0.24 | 9.90 ± 0.14 | 9.49 ± 0.19 |
|          | 20                 | 22.64 ± 0.39 | 20.27 ± 0.23 | 11.50 ± 0.12 | 11.34 ± 0.24 |
| Ganoderma| 1                  | 15.68 ± 0.48 | 15.87 ± 0.35 | 0 | 0 |
|          | 5                  | 21.07 ± 0.41 | 18.47 ± 0.43 | 0 | 0 |
|          | 10                 | 23.72 ± 0.25 | 19.65 ± 0.59 | 10.77 ± 0.32 | 10.09 ± 0.23 |
|          | 20                 | 25.22 ± 0.28 | 22.08 ± 0.39 | 12.81 ± 0.36 | 11.62 ± 0.30 |

*commercial transparent soap (Lifebuoy®)

3.3. Antioxidant activity
DPPH is a stable free radical substance that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. When the DPPH solution is mixed with an antioxidant, the dark color of it becomes lighter. The grade of discoloration indicated the inhibition potency of antioxidant compounds in the term of hydrogen or electron donating ability. Figure 1 shows the antioxidant activity of Ganoderma transparent soap which the inhibition percentage reaches 50% (IC50 value) was 1.53 mg/mL. Antioxidant activity is one of the functions provided by triterpenoids and polysaccharides of G. lucidum [18, 19].
Figure 1. The antioxidant activity of Ganoderma transparent soap

4. Conclusion
Physicochemical properties and antibacterial activity of herbal transparent soaps were comparable to control soap. Ganoderma transparent soap have moisture content 21.8%, pH 9.22, total fatty matter 41.66%, free fatty acid 1.08% and chloride acid 5.57%. Ganoderma transparent soaps were more active against the S. aureus than the Gram-negative bacteria and even C. albicans. The antioxidant activity assay showed that the IC50 of transparent soap was 1.53 mg/mL. The present investigation demonstrates that G. lucidum extract can be formulated as a transparent soap to add its functionality.

Acknowledgment
Authors are grateful to Indonesian Institute of Sciences for financially supporting this research through program Prioritas Nasional 2019.

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