Analysis of Metabolite Levels, Secondary Minerals and Aloe Vera Formulation from Kalimantan Indonesia

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Abstract: Products from natural ingredients such as aloe vera must be high quality and meet standardization aspects. Determination of secondary metabolites and mineral levels is part of the standardization of natural product products. The dosage formula needs to be optimized to get the best formula based on evaluating the physical properties of the gel preparation. This study aimed to analyze the highest mineral and metabolite content between aloe vera from South Kalimantan and West Kalimantan, as well as the optimum formula for aloe vera gel based on its physical properties. The research method used is to perform sample preparation, extraction, analysis of minerals and secondary metabolites by spectroscopy. The data were analyzed descriptively, and the results showed that the content of iron, calcium, and zinc originating from West Kalimantan was 0.314 mg/g; 93.42 mg/g; 0.059 mg/g, while South Kalimantan 0.064 mg/g, 53.24 mg/g, 0.032 mg/g. The total phenolic, flavonoid, and anthraquinone levels from West Kalimantan were 0.512%, 1.31%, respectively, 2.28%, while those from South Kalimantan were 0.321%, 1.12%, 1.14%. The best formula for aloe vera gel is formula three, which has a darker color and meets the requirements of the physical properties of the gel. This study concludes that the highest mineral content in aloe vera comes from West Kalimantan, the highest secondary metabolite content also comes from West Kalimantan. And the best formula is the third formula with 20% natural dyes. Suggestions for further research are to examine the mineral content and secondary metabolites of aloe vera in various places in Indonesia so that the best aloe vera can be known.

Keywords: Aloe vera; secondary metabolites; mineral levels; gel; teeth

INTRODUCTION

Aloe vera has a unique texture because it is in the form of a gel with various nutrients. Aloe vera has antibacterial, anti-inflammatory, wound healing, anti-acne, moisturizing, and disclosing properties (Marhaeni, 2020). Aloe vera can be used directly, but it must be made in pharmaceutical dosage forms to become a product. Aloe vera can be formulated in a gel dosage form (Buulolo & Syamsul, 2016). Gel preparations have various advantages, including having an attractive texture, high stability, and containing a high water phase to be comfortable to use. Natural ingredients formulated in dosage forms must undergo a series of tests to ensure that all elements can be mixed. In addition, gel preparations must meet various aspects of the physical evaluation of the practice so that they can last a long time and are of high quality.

Natural ingredients to be developed into a product must meet the aspects of standardization based on the Indonesian Herbal Pharmacopoeia. Standardization of
natural product products is a series of processes and tests that include specific and non-specific parameters (Rizki et al., 2021). On particular parameters, the raw material must be known the content and levels of secondary metabolites and minerals in it. This material needs to be measured to ensure that the natural ingredients have optimal secondary metabolites and mineral content (Rizki, 2020). Optimal secondary metabolites and minerals will provide the efficacy and quality of these natural products.

Natural ingredients such as aloe vera are developed into a disclosing agent product. Disclosing agents work by giving color to the bacteria in dental plaque so that there is a difference in color between teeth and dental plaque. Several disclosing agents use synthetic dyes, including iodine, gentian violet, erythrosine, basic fuchsin, and three-tone disclosing agents (Purbaningtyas et al., 2020). The benefits of disclosing agents are so that patients can do an independent assessment of dental plaque, increase awareness of the importance of cleaning dental plaque, and find out where there is plaque so that cleaning is optimal (Fasoulas et al., 2019). This research is the first step to analyze the optimal formula of aloe vera so that it is expected to be a candidate disclosing agent.

Based on previous studies, aloe vera found in India contains mineral substances such as magnesium, phosphorus, potassium, calcium, barium, boron, sodium, zinc, manganese, and aluminum. The highest content in aloe vera is potassium (Pawar & Kamble, 2013). In another study, it was also known that aloe vera contains the most increased potassium, magnesium, sodium, and zinc (Rajendran et al., 2007). The secondary metabolite content analysis results showed that aloe vera ethyl acetate extract had the highest total phenol content of 4.088 g GAE/mg and total flavonoids of 12,376 g QE/mg (Mulyanita et al., 2019). Research that analyzes the mineral content and secondary metabolites in aloe vera from West Kalimantan and South Kalimantan is still limited, so the purpose of this study was to examine the highest mineral and metabolite content between aloe vera from South Kalimantan and West Kalimantan, as well as the optimum formula for aloe vera gel based on its physical properties.

MATERIALS AND METHODS

This type of research uses an analytic observational method with an analytical design using the purposive sampling technique. Aloe vera samples were taken from 2 areas in the Provinces of South Kalimantan and West Kalimantan. Sample preparation, extract making, analysis of mineral content, secondary metabolites, and formulation of gel dosage forms was carried out at the Banjarmasin Pucuk Sirih Herbal Medicine Factory, which already has a certificate of Good Traditional Medicine Manufacturing Practices (CPOTB) from the Food and Drug Supervisory Agency (BPOM).

The equipment used is an oven (Vinco), sieve no. 20, silica gel plate 254, capillary tube, dropper, volume pipette (Iwaki), proppipet (Vital), UV spectrophotometry (Genesys), atomic absorption spectrophotometry (ASC-700), analytical balance (Pioneer), vortex (Jeio Tech), and water bath (SMIC). The chemicals used were technical ethanol, gallic acid pa, quercetin pa, anthraquinone pa, iron pa, calcium pa, and zinc pa, which were purchased from chemical distributor CV. Viana Berkah Chemical.

This study used mangosteen peel extract as a dye. Aloe vera leaves and mangosteen peel were washed with running water and weighed. The inside of the aloe vera leaf was taken to obtain a gel which was then considered and dried in a drying
cabinet at 50°C for 72 hours. Aloe vera and dried mangosteen peel were weighed, then extracted using 70% ethanol by maceration method for 3x24 hours. Then filtered, the solution was evaporated using a rotary evaporator and a water bath to obtain a thick extract. The aloe vera extract was then analyzed to determine the secondary metabolites and mineral content levels.

Determination of secondary metabolite levels using UV-Vis spectrophotometry method to determine the levels of phenolics, flavonoids, and anthraquinones. In the conclusion of phenolics using gallic acid standards, while flavonoids using catechins, and for anthraquinones using anthraquinones. Determination of mineral content using an atomic absorption spectrophotometer to determine the levels of calcium, iron, and zinc using the standards of each of these metals. The determination of mineral content obtained absorbance values which were then analyzed using linear regression with a standard curve to get levels in milligrams in gram samples. The results of determining the levels of secondary metabolites also used linear regression to obtain grades in the form of a percentage of weight per weight. The test was carried out with three repetitions (triple).

Aloe vera extract, which has the highest levels of secondary metabolites and minerals, is then made in the form of a gel. The aloe vera powder was weighed and mixed with mangosteen rind extract to form a gel disclosing agent. Three kinds of aloe vera gel formulas were made: formula 1 using 10% mangosteen peel extract, formula 2 using 20% mangosteen peel extract, and formula 3 using 40% mangosteen peel extract. The three gel formulas were then evaluated physically, namely:
1. Organoleptic test in the form of smell, taste, shape, and color
2. Homogeneity test using the precise glass method under sunlight
3. Testing the degree of acidity (pH) using a pH meter
4. Testing viscosity (thickness) using a Brookfield viscometer
5. Testing dispersion using ballast with millimeter paperback

RESULTS AND DISCUSSION

Aloe vera flesh has a unique characteristic because it is semi-solid and soft texture. Aloe vera meat originating from West Kalimantan has a slightly tough texture compared to South Kalimantan. Aloe vera extract has a gel-like texture. There was almost no organoleptic difference between the aloe vera extract from West Kalimantan and South Kalimantan. The difference is only in other parameters in the physical evaluation of aloe vera gel.

The extract obtained was then analyzed for its mineral content in iron, calcium, and zinc. The mineral levels detected were only iron, calcium, and zinc. These three minerals play a role in strengthening teeth, so they can be another benefit for revealing ingredients from natural ingredients. The intermediate results from West Kalimantan and South Kalimantan are presented table1.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Iron (mg/g)</th>
<th>Potassium (mg/g)</th>
<th>Zinc (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aloe Vera from West Kalimantan</td>
<td>0.314</td>
<td>93.42</td>
<td>0.059</td>
</tr>
<tr>
<td>Aloe Vera from South Kalimantan</td>
<td>0.064</td>
<td>53.24</td>
<td>0.032</td>
</tr>
</tbody>
</table>
The table results show that iron, calcium, and zinc content is different between those from West Kalimantan and South Kalimantan. The table shows that the highest iron content in aloe vera from West Kalimantan, in calcium also from West Kalimantan, is more elevated, similar to the results of the dominant zinc content from West Kalimantan.

Aloe vera extract was also analyzed for levels of secondary metabolites contained in it. The metabolites analyzed were phenolic, flavonoid, and anthraquinone groups. The results of the determination of secondary metabolite levels are presented in table 2.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Phenolic (%b/v)</th>
<th>Flavonoids (%b/v)</th>
<th>Anthraquinones (%b/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aloe Vera from West Kalimantan</td>
<td>0.512</td>
<td>1.31</td>
<td>2.28</td>
</tr>
<tr>
<td>Aloe Vera from South Kalimantan</td>
<td>0.321</td>
<td>1.12</td>
<td>1.14</td>
</tr>
</tbody>
</table>

The test results showed that the highest content of aloe vera was anthraquinone, followed by flavonoids, and finally phenolic. The highest was aloe vera from West Kalimantan when compared with the results.

Aloe vera extract from West Kalimantan is then made in the form of a gel with the addition of natural dyes derived from aloe vera flesh. Three formulations were made with the difference between the three: the amount of mangosteen peel extract added. The made gel is then evaluated for its physical properties to see its physical characteristics. The results of the physical evaluation are presented in the table below.

The physical evaluation results showed that there were several differences and similarities that appeared in each of the physical characteristics of the gel. The organoleptic of the three gels differed in the color of the gel, which was darker with the use of more aloe vera extract. In the homogeneity test, the three gels were homogeneous. In the viscosity test, formula 3 has a higher viscosity than formulas 1 and 2. In the adhesion test, the gel sticking ability has the same characteristics. The dispersion of the three gels tended to be different, especially in formula three, which had the highest dispersion. The acidity or pH of the gel formula 3 has the highest value or is close to alkaline, compared to formulas 1 and 2. The best formula is the third formula with 20% natural dye.

The aloe vera plant used came from 2 different places: South Kalimantan and West Kalimantan. Two plants from other areas are used to compare the quality of aloe vera raw materials so that high-quality raw materials can be selected. Researchers have not found studies that compare the secondary metabolite and mineral content of aloe vera plants that grow in two different places. Where to grow will affect the levels of secondary metabolites in a plant, including the mineral content of a plant. Aloe vera from South Kalimantan and West Kalimantan comes from the lowlands but at different locations, allowing differences in soil water content and nutrients contained. In the ground. Aloe vera from South Kalimantan was taken from the Pelaihari lowland area of Tanah Laut Regency. In contrast, aloe vera from West Kalimantan was taken from the lowland area of Siantan Hulu.
Table 3. Results of Evaluation of Physical Properties of Aloe Vera Gel

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Formula 1</th>
<th>Formula 2</th>
<th>Formula 3</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organoleptic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Odor</td>
<td>Typical</td>
<td>Typical</td>
<td>Typical</td>
<td>Homogeneous</td>
</tr>
<tr>
<td>Taste</td>
<td>Tasteless</td>
<td>Tasteless</td>
<td>Tasteless</td>
<td>(Suryani et al., 2019)</td>
</tr>
<tr>
<td>Form</td>
<td>Semi solid</td>
<td>Semi solid</td>
<td>Semi solid</td>
<td>2000-4000 cps</td>
</tr>
<tr>
<td>Color</td>
<td>Light Purple</td>
<td>Light Purple</td>
<td>Deep Purple</td>
<td>(Garg et al., 2002).</td>
</tr>
<tr>
<td>Homogeneity</td>
<td>Homogeneous</td>
<td>Homogeneous</td>
<td>Homogeneous</td>
<td>&gt; 4 seconds</td>
</tr>
<tr>
<td><strong>Viscosity</strong></td>
<td>3843 cps</td>
<td>3854 cps</td>
<td>3921 cps</td>
<td>(Nevi, 2006)</td>
</tr>
<tr>
<td><strong>Adhesion</strong></td>
<td>6 seconds</td>
<td>6 seconds</td>
<td>6 seconds</td>
<td>7,605 – 19,625 cm2</td>
</tr>
<tr>
<td><strong>Spreadability</strong></td>
<td>9.42 cm²</td>
<td>9.54 cm²</td>
<td>9.88 cm²</td>
<td>4.5-6.5</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>6.09</td>
<td>6.11</td>
<td>6.18</td>
<td>(Tranggono and Latifah, 2007)</td>
</tr>
</tbody>
</table>

According to Astuti et al. (2014), the location where it grows is known to influence the secondary metabolite levels. Differences in where to grow will affect differences in environmental conditions where it grows, such as the temperature around the plant, air humidity, water content in the soil, and nutrients contained in the ground.

Physically, aloe vera meat originating from South Kalimantan and West Kalimantan has a different texture. Aloe vera from West Kalimantan has a large size with a slightly stiff texture than South Kalimantan. Aloe vera meat contains 90% water 4% carbohydrates, and the rest is in the form of amino acids and minerals (Kurnianingsih, 2004). The water content in the aloe vera plant will affect the texture of the aloe vera flesh. Water content that is too low or too high is also not always suitable for plants. A water content that is too low will cause the aloe vera flesh to shrink, while a water content that is too high will cause the aloe vera meat to become too mushy. There are more than 200 different types of molecules in aloe vera. Aloe vera contains almost 98% water, while the total solids of aloe vera gel are 0.66%, and soluble solids are 0.56% (Ahlawat & Khatkar, 2011).

Analysis of mineral content in aloe vera from South Kalimantan and West Kalimantan showed differences in potassium, zinc, and iron levels (table 1). The highest levels were obtained from aloe vera from West Kalimantan. This is because aloe vera from West Kalimantan is aloe vera that comes from cultivated plants. Cultivated plants tend to come from the best seeds with environmental conditions where they grow that support these plants to grow optimally (Istanto, 2014). Aloe vera grows in fertile soil with high nutrient content in the ground. Aloe vera can also grow on calcareous soils, including soils with high acid content (Chowdhury et al., 2018).

The results of this study, when compared with similar studies, have total phenolic levels in aloe vera from West Kalimantan (0.512%), while those from South
Kalimantan have low levels (0.312%) (Table 2). In the research of Mulyanita & Setiasih (2019), it was found that the total phenolic content of aloe vera was 4.088 microgram/mg sample or equivalent to 0.40%. Another study stated that there were 0.023% quercitin compounds and 0.0344% querectin compounds, both of which are flavonoids, while the phenolic group compounds in vanillic acid were 0.0023% (Lopez et al., 2013). This result shows that aloe vera from West Kalimantan has better quality than South Kalimantan and similar studies.

Flavonoids are chemical compounds that are spread in almost all parts of plants. In this study, aloe vera from West Kalimantan had a higher total flavonoid content (1.31%) when compared to similar studies, which reached 1.23%. Aloe vera from South Kalimantan has lower levels (1.12%) than similar studies (1.23%) (Mulyanita & Setiasih, 2019). The difference in levels can be influenced by the environmental conditions and the nutrients contained in the soil where the plants grow (Astuti et al., 2014).

An anthraquinone is a group of compounds that are most responsible for the efficacy of aloe vera. Aloin compounds are anthraquinone compounds that have been isolated from the aloe vera plant. Total anthraquinone levels in aloe vera from West Kalimantan were higher (2.28%) than similar studies that determined anthraquinone levels in aloe vera from Kulonprogo (1.79%) and Semarang (1.46%) (Mutiara, 2007). Aloe vera anthraquinone from South Kalimantan was lower than similar studies. This result shows that the secondary metabolism of aloe vera from West Kalimantan is better than that from South Kalimantan.

The physical evaluation results showed that there were several differences and similarities that appeared in each of the physical characteristics of the gel. The organoleptic of the three gels differed in the color of the gel, which was darker when using more aloe vera extract. In the homogeneity test, the three gels were homogeneous. In the viscosity test, formula 3 has a higher viscosity than formulas 1 and 2. In the adhesion test, the gel sticking ability has the same characteristics. The dispersion of the three gels tended to be different, especially in formula three, which had the highest dispersion. The acidity or pH of the gel formula 3 has the highest value or is close to alkaline, compared to formulas 1 and 2 (Table 3). All physical evaluations of the gel, when compared with the literature as shown in the results table, met the requirements for the physique gel properties. According to Garg et al. (2002), the diameter of semi-solid preparations that are good for topical use is in the diameter range of 3-5 cm. Topical preparations are recommended in the skin pH range of 4.5-6.5 (Tranggono and Latifah, 2007). The viscosity value of a good gel preparation is recommended to be in the field of 2000-4000 cps (Garg et al., 2002).

This study has limitations, namely, only testing three different formulations. The tested formulation could be more to get a lot of data to enrich the research results. This research is also a preliminary study, so more studies are needed to obtain the optimal formula of aloe vera, which is expected to be a candidate disclosing agent. Researchers suggest testing the mineral content and secondary metabolites of aloe vera in various places in Indonesia to know the best aloe vera.

**CONCLUSION**

This study concludes that aloe vera has the highest mineral content from West Kalimantan (iron content 0.314 mg/g; potassium 93.42 mg/g; zinc 0.059 mg/g), and the highest secondary metabolite content also comes from West Kalimantan (phenolic content). 0.512%; flavonoids 1.31%; anthraquinone 2.28%). The best formula based on the physical properties test of the gel showed that the gel was the third formula with
20% natural dye content. Aloe vera from this research has the potential as a disclosing agent, so further research is needed at the in vivo stage and clinical trials.

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CONFLICT OF INTEREST
All of the authors have no conflict of interest

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