Formulation and Antioxidant Evaluation of Lotion Kleinhovia Leaves Extract

Suryani, A.Eka Purnama Putri*, Rahma Sari Camalia

Abstract—Kleinhovia leaves extract from Kleinhovia hospita Linnaeus contains flavonoids. Mainly, the presence of phenolic compound such as flavonoid was applied for cosmetical application. This study was conducted to formulate lotion from Kleinhovia leaves extract and evaluate its antioxidant activity of the lotion by using DPPH (1,1-diphenyl-2-picryl-hydrazyl) method and to determine physical stability of the lotions by using cycling test method. Kleinhovia leaves extract was obtained by maceration by using 96% ethanol and purified by n-hexane. Antioxidant activity (IC50) of Kleinhovia leaves extract was measured by using DPPH. Extract was formulated into lotion with various concentrations of extract 1.IC50, 3.IC50, and 6.IC50. Stability of the lotion was carried out by cycling test method for 6 cycles. The result showed that IC50 of lotion extract with 1.IC50, 3.IC50 and 6.IC50 were 316.5 µg/ml, 247.2 µg/ml, and 161.7 µg/ml respectively. The cycling test showed that lotions have stable formulation with thickly consistency, white color, O/W of lotion type, pH lies between 6.1 to 6.6, viscosity were ranging between 370 to 586 cP, and the globule size were 0.1 to 0.8 µm.

Keywords— Kleinhovia leaves extract, lotion, physical stability, antioxidant activity, cycling test, DPPH (1,1-diphenyl-2-picryl-hydrazyl).

I. INTRODUCTION

The exposure of sunlight, and air pollution can have negative impacts on human skin. Ultraviolet (UV) from sunlight might cause adverse effects such as black spots, sunburn, skin irritation and also skin cancer (Zulkarnainia et al., 2013)(1). In addition, air pollution and bad environment can form free radicals that implicate to degenerative diseases such as heart disease, arteriosclerosis and symptoms of premature aging (Tahir, 2003)(2). Free radicals are molecules that have unpaired electrons and highly reactive. They can make binding of electrons with cell molecules. The human body normally has protective mechanism from its damages but in certain cases the body can not handle by itself, then it requires substances from outside the body such as antioxidant compound. Preparation of cosmetic formulations such us lotion that containing antioxidant substances might be one of the best solution. Kleinhovia hospita Linn. is a tropical plant that traditionally used to treat skin diseases in Malaysia, Indonesia and Papua New Guinea (Sahirin, 2010)(3); World Health Organization, 2009(4)). Previous study has revealed that bark and leaves of Kleinhovia hospita L. contains many flavonoids that have antioxidant activity (Saeufdin et al., 2013)(5). In our present research, we evaluate the antioxidant activity of Kleinhovia leaves extract using DPPH (1,1-Diphenyl-2-picrylhydrazyl). The extracts were then formulated into lotion. Furthermore the physical stability of the lotions were determined using cycling test method.

II. METHOD

A. Material

Leaves of Kleinhovia hospita Linn was obtained from Andowia Asera North Konawe Southeast Sulawesi, Ethanol, n-Hexane, and chloroform, cetyl alcohol, Propylene glycol, Lecithin, Na-carboxymethylcellulose (Na-CMC), olive oil, Glycerin, benzoic acid, green tea, aquadest, methylene blue, ammonia, andradical DPPH (1,1-Diphenyl-2-picrylhydroxyl).

B. Instruments

Spectrophotometer UV Vis (Spectronic 2D®), viscometer (Rion Viscometer VT-04F®), microscope (Leica 1CC50 Hammert), refrigerators (Sharp®), Rotary evaporator (Buchi®), Thermometer, Filler, Plate Thin Layer Chromatography (TLC) (MERCK®), Pipe capillaries, Chamber TLC, UV lamp 254 nm and 366 nm, and glass tools (Pyrex®).

C. Extract preparation

Kleinhovia leaves powder was weighed in 750 g and macerated by using ethanol 96% for 3 x 24 hours with the ratio of leaves powder and ethanol was 1: 7 (DG POM, 2000)(6). The filtrate was collected and evaporated by using rotary evaporator at 55°C. Extract partitioned by n-hexane with liquid-liquid extraction method. The undissolved hexane fraction was collected and kept in well closed container until used.

D. The quantitate of antioxidant activity (DPPH method)

Determination of antioxidant activity by DPPH method (1,1-diphenyl-2-picrylhydrazyl). 1000 ppm of DPPH reagent and sample solution was prepared. Determination of the maximum wavelength of DPPH solution by making a reference solution with 2 mL of DPPH solution was added 4

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emulsion was formed, then added into aqueous phase carefully until the stable value. According to Molyneux (2004), the extraction were dissolved in distilled water at 70°C. Oil phase was regresi linear to calculate Inhibition Concentration 50% (IC50) value. The materials such as stearic acid, cetyl alcohol, olive oil, lecithin, and benzoic acid were melted in a beaker glass on hotplate at 70°C, while the water soluble material phase including glycerin, propylene glycol, Na-CMC, and Kleinhovia leaves extract were also dissolved in 20 mL of distilled water, then part of the cathode of pH meter was immersed in the lotion that has been dissolved.

**E. The formulation of lotion**

Ingredients used for formulation of the lotion are shown in table 1 belows:

![Table 1. FORMULATION OF LOTION](https://doi.org/10.17758/EIRAI.IAE1017104)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>F0</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kleinhova extract</td>
<td>-</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Cetyl alcohol</td>
<td>6.3</td>
<td>6.3</td>
<td>6.3</td>
<td>6.3</td>
</tr>
<tr>
<td>Lecithin</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Na-CMC</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Olive oil</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Glycerin</td>
<td>2.4</td>
<td>2.4</td>
<td>2.4</td>
<td>2.4</td>
</tr>
<tr>
<td>Benzoin acid</td>
<td>12.6</td>
<td>12.6</td>
<td>12.6</td>
<td>12.6</td>
</tr>
<tr>
<td>Aquaest</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Green tea</td>
<td>Ad100</td>
<td>Ad100</td>
<td>Ad100</td>
<td>Ad100</td>
</tr>
</tbody>
</table>

The materials were separated into two phases, water soluble material and oil soluble material phase. The oil phase materials such as stearic acid, cetyl alcohol, olive oil, lecithin, and benzoic acid were melted in a beaker glass on hotplate at 70°C, while the water soluble material phase including glycerin, propylene glycol, Na-CMC, and Kleinhovia leaves extract were also dissolved in 20 mL of distilled water, then part of the cathode of pH meter was immersed in the lotion that has been dissolved.

**F. Physical stability test using cycling test method**

The method of cycling test based on ASEAN Guidelines on Stability Study of Drug Product (2005). The method of cycling tests was performed one cycle when the preparation lotion is stored at 4°C for 24 hours and then removed and placed in an oven at a temperature of 40 ± 2°C for 24 hours. This experiment was repeated for 6 cycles. The lotions were then observed for the physical characteristic.

**Organoleptic Test**

The test referred to method of Hernani et al. (2012)(9). Organoleptic test was conducted on 10 panelists. Preparations were observed regarding changes in consistency and color of visual and odor of the lotion preparations.

**Type of lotion test**

The test was performed by dropping a solution of 0.1 gram of methylene blue to the sample of lotion, the spread of methylene blue in preparation was then observed. If the methylene blue was spread homogenously, it shows that the type of lotion is oil in water (O/W), but if the color only in the form of spots, it shows that the type of lotion is a water in oil (W/O).

**pH Test**

Test the degree of acidity (pH) was performed using a pH meter with previously calibrated at pH 4 and pH 7. 2 g of lotion was dissolved in 20 mL of distilled water, then part of the cathode of pH meter was immersed in the lotion that has been dissolved.

**Viscosity test**

Viscosity test was conducted by using a Rion viscometer with spindle no. 3.

**Globules size test**

Measurement of size of the globules was done using an optical microscope with 400x of magnification (Patmarani, 2017).

**III. RESULT AND DISCUSSION**

The extraction process resulted yield value 65.6% w/w of Kleinhova leaves extract which has IC50 value of 93.5 g/mL that indicate active category. The ability of Kleinhova leaves extract in reducing free radicals was proven from the reduction of the intensity of the purple color of the DPPH solution which has been added in the sample. The reduction of intensity of the color of the DPPH solution indicates that the reaction occurs between hydrogen atoms which was released by DPPH radical molecules to form the compound of 1,1-diphenyl-2-pirilhidrazin that has yellow color. Reduction of the intensity of the purple color of DPPH solution can be calculated quantitatively from the decreasing of absorbance of the solution. The higher the concentration of the sample, the lower the absorbance value, which indicates that the activity of the sample is higher than DPPH radical. Absorbance value is the absorbance of residual DPPH which does not react with the sample solution. According to Molyneux (2004)(8), a substance which has antioxidant properties showed by the IC50 value of < 200 ppm. Based on the results of the preliminary test, the concentration of the extract used in the lotion formulation was varied into 1 IC50, 3 IC50, and 6 IC50.

Lotion formula consists of the active ingredient and the base material. The basic ingredients consist of oil phase and water phase can be mixed with the addition of emulsifiers (emulsifier) and then form base of lotion. The stability of the lotion is influenced by mechanical factors, temperature, and the process of forming the emulsion. According to Silva et al (2006)(11), emulsion droplet shape and size were influenced...
by the rate of stirring during the emulsification process. Organoleptic test conducted by examining the physical appearance of the lotion preparation. Examination was conducted to determine the consistency, smell, and the color of the lotion. Organoleptic test of the physical appearance of lotio showed that lotions of Kleinhovia leaves extract have thicky consistensy, white color, and green tea smell (figure 1). Overall lotion formulas did not show any changes after cycling test.

Lotion type was checked with the observation of the color spread of methylene blue in sample. It showed that the blue color of methylene blue was spread homogenously, it indicates the type of lotions were oil in water (O / W). The lotions did not show any changes of the after cycling test.

The pH test showed that pH of lotion was ranging from 6.1 to 6.6 (figure 2). Changes in pH during storage can be caused by external and internal factors. External factors such as temperature and humidity, while the internal factors including the characteristics of emulsifiers (stearic acid) which is relatively acidic, but pH lotio lies on the pH range of topical preparations due to the pH of the skin (5.0 to 7.0). In addition, the pH value of the lotion lies on the range pH value of the commercial skin lotion (Agnessya 2008; ASEAN Guidelines on Stability Study of Drug Product, 2005).

The Viscosity is an important parameter of emulsion stability. Viscosity makes preparations easily applied on the skin. Preparations with higher consistency will affect the application usage. The higher the viscosity of the product, the lower the rate of separation of the dispersed phase and the dispersing phase is. Viscosity data of lotions showed the increasing after the cycling test. It might be caused by several factors such as particle size, the amount of the active substance and changes in room temperature. The increasing of temperature can interfere binding of the water phase and the oil phase and also improve the movement of the dispersed phase globules. If the temperature gets higher, the viscosity decreases and the formula become watery. Conversely, if the temperature is lower, the viscosity will increase and the formula becomes thicky (Wihelmina, 2011)(13). Losio viscosity during storage was still in the range of viscosity contained in SNI-16-4399-1996 as skin moisturizers quality requirements, under 370-586 cP (figure 3).

Globule size is a major indicator of creaming or breaking in emulsion. Lotion tend to shrink during storage at low temperatures, so that the particles will tend to form bonds between the particles consequently the viscosity increased and the flow rate decreased (Zulkarnaina et al., 2013). Figure 4 shows that in all four formulas showed improvement of globule size after cycling test. Before the cycling test lotion globule size was ranging between 0.2 to 0.8 µm, whereas after the cycling test globule size increased slightly from 0.3 to 0.8 µm (Figure 4). However, the size of the globules obtained both before and after the cycling test as a whole, including good emulsion globule size which lies on 0.1-10 µm.

The viscosity and pH value of the product lie in the range of topical preparations and commercial skin lotions. The viscosity of the formula lies in the range of SNI-16-4399-1996 for skin moisturizers quality requirements. The pH value of the lotion lies in the range of the pH value of the commercial skin lotion. The viscosity and pH value of the formula are within the acceptable range for the skin application.

Note:
A1 : globul size before cycling test (lotion base)
A2 : globul size after cycling test (lotion base)
B1 : globul size after cycling test (lotion with extract 1.IC50)
B2 : globul size after cycling test (lotion with extract 3.IC50)
C1 : globul size before cycling test (lotion with extract 1蒋50)
C2 : globul size after cycling test (lotion with extract 3蒋50)
D1 : globul size before cycling test (lotion with extract 1蒋50)
D2 : globul size after cycling test (lotion with extract 3蒋50)
Antioxidant activity was conducted by using DPPH method. The decreasing of absorbance value indicates the reduction of concentration of free radical DPPH due reaction with the antioxidant compounds in the sample. It showed the reduction of DPPH molecules and accompanied by the reduction of intensity of the purple color in the DPPH solution (Molyneux, 2004)(8). IC50 value of active extracts and antioxidants with DPPH standards were listed in Table 2.

### Table II

<table>
<thead>
<tr>
<th>Lotion formula</th>
<th>IC50 (μg/mL)</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>F0</td>
<td>2483.3</td>
<td>Not active</td>
</tr>
<tr>
<td>F1</td>
<td>316.5</td>
<td>weak</td>
</tr>
<tr>
<td>F2</td>
<td>247.2</td>
<td>weak</td>
</tr>
<tr>
<td>F3</td>
<td>161.7</td>
<td>intermediate</td>
</tr>
</tbody>
</table>

**Fig 5. Antioxidant activity of lotion**

The table 2 showed that the higher concentration of the extract were added to the lotion formula, the lower the IC50 values obtained which shows that the lotions have significant activity as antioxidants. The lower of IC50 value of the sample the more effective as a radical scavenging (Putri et al., 2013)(16). Samples F1 and F2 have weak antioxidant activity while the samples F3 has intermediate antioxidant activity. Sample F0 (base) has no activity. The base was used as a negative control.

### IV. CONCLUSION

Based on the research, it can be concluded that Kleinhova leaves extract has antioxidant activity with IC50 value of 93.5 μg/mL. Lotion extract which has the highest antioxidant activity was F3 (6. IC50) which have antioxidant activity in intermediate category with IC50 value of 161.7 mg/mL.Formula of lotion Kleinhova leaves extract is stable chemically and physically including organoleptic, type of lotion, pH, viscosity and globule size.

**REFERENCES**


**Suryani**, lecturer at the department of pharmacy, faculty of pharmacy, Universitas Halu Oleo. She obtained her MSc. from Gadjah Mada University Jogjakarta, Indonesia in 2012 and bachelor of pharmacy in Indonesian Islamic University at 2006. She has 8 years experiences in teaching pharmacy especially in the field of pharmaceutical technology and drug delivery system. She has authored and co authored research paper published in journal and proceeding of conferences.