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 thicky 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 (Zulkarnaina et al., 2013)(1). In addition, air pollution and bad environment can form free radicals that implicate to degenerative diseases such as heartdisease, 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 it self, 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 (Sahrin, 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 (Saefudin 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.

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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-picrylhydrazyl).

B. Instruments

Spectrophotometer UV Vis (Spectronic 2D®), viscometer (Rion Viscometer VT-04F®), microscope (Leica 1CC50 HD®), pH meter (Jenway®), Hot plate (Stuart®), Stirrer, Mixer (Sharp®), analytical Scales (Precisa®), Oven (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 quantitave 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

mL of ethanol and shake until homogeneous, then observed absorption in the wavelength range 400-700 nm using a UV-Vis spectrophotometer. Samples of extracts were made by 4 series of concentration 50, 100, 150 and 200 ppm respectively, while samples of lotion were made with 4 series of concentration 50, 150, 250 and 350 ppm respectively . Each 4 mL of sample was added with 2 mL of DPPH were then further incubated at room temperature for 30 minutes. Uptake test performed at maximum wavelength of DPPH. Value of antioxidant activity is expressed in IC50. IC50 calculated by pattern belows (Chang ,2005)[7]:

I% = (absorption - uptake blank sample) / (absorbance of the blank) x 100 %

Value I% of each extract concentration was plotted in regresi linear to calculate Inhibition Concentration 50% (IC50) value. According to Molyneux (2004)[8], the IC50 value is the concentration of antioxidants that can inhibit 50% DPPH radical.

E. The formulation of lotion

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

TABEL 1.
FORMULATION OF LOTION

Tabel 1 Formulation of lotion

Ingredients	lotion 70 mL (%b/v)			
	F0	F1	F2	F3
Kleinhova extract	-	10 (1 IC_{50})	10 (3 IC_{50})	10 (6 IC_{50})
Stearate acid				2,5
cetil alcohol	2,5	2,5	2,5	6,3
Propilenglikol	6,3	6,3	6,3	5
Lesitin	5	5	5	1,3
Na-CMC	1,3	1,3	1,3	1,6
Olive oil	1,6	1,6	1,6	2,4
Gliserin	2,4	2,4	2,4	12,6
Benzoat acid	12,6	12,6	12,6	0,2
Aquadest	0,2	0,2	0,2	Ad
green tea	Ad 100 g.s	Ad 100 g.s	Ad 100 g.s	100 g.s

The materials were separated into two phases, water soluble material and oil soluble material phase. The oil phase materials such us 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 dissolved in distilled water at 70°C. Oil phase was then added into aqueous phase carefully until the stable emulsion was form..

F. Physical stability test using cycling test methode

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 refered 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 methylen 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 used 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 IC_{50} 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-pikrilhidrazin 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 IC_{50} 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.IC_{50}, 3.IC_{50}, and 6.IC_{50}.

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 will 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 homogenouesly, it indicates the type of lotions were oil in water (O / W). The lotions did not show any changes of the after cycling test.

Fig 1. Lotion of Kleinhovia leaves extract

(a). before cycling test (b). 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[12]; ASEAN Guidelines on Stability Study of Drug Product, 2005).

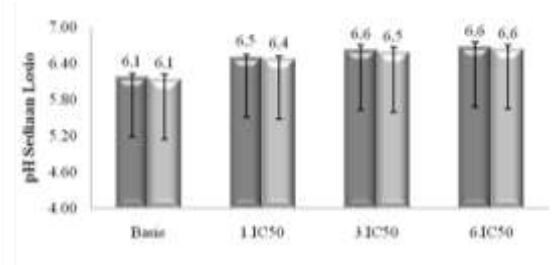

Fig2. pH losio Kleinhovia leaves extract before cycling test(■)dan after(■)cycling test.

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 us 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).

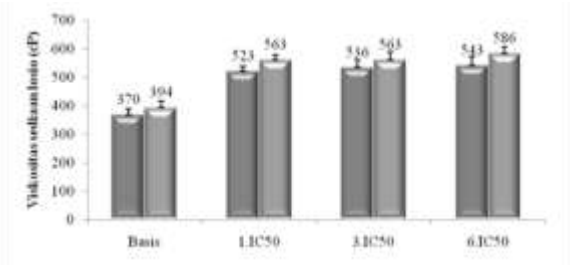

Fig 3. Viscosity of lotion Kleinhovia leaves extract before cycling test (■) and after cycling test (■)

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)[14]. 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.

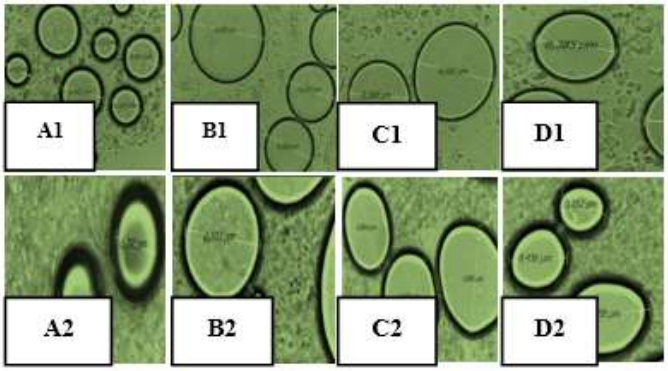

Fig 4. Globul size of lotions Kleinhova extract before and after cycling test using optic microscope with 400x of magnification.

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.IC_{50})
B2 : globul sizeafter *cycling test* (lotion withextract1.IC_{50})
C1 : globul size before *cycling test* (lotion with ekstract 3.IC_{50})
C2 : globul size after *cycling test* (lostion with extract 3.IC_{50})

D1 : globul size before *cycling test* (lotion with extract 6.IC_{50})

D2 : globul size after *cycling test*(lotion with extract 6.IC_{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
THE ANTIOXIDANT ACTIVITY OF LOTION BY USING DPPH METHOD

Losion formula	IC_{50} (µg/mL)	Activity
F0	2483,3	Not active
F1	316,5	weak
F2	247,2	weak
F3	161,7	intermediate

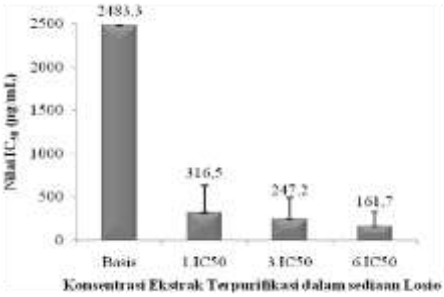

Fig 5. Antioxidant activity of lotion

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)[(14)]. 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 IC_{50} value of 93.5 ug/mL. Lotion extract which has the highest antioxidant activity was F3 (6.IC_{50}) which have antioxidant activity in intermadiate category with IC_{50} value of 161.7 mg/mL.Formula of lotion Kleinhovia leaves extract is stable chemically and physically including organoleptic, type of lotion, pH, viscosity and globule size.

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