Physical stability test and irritation test of lotion moringa oleifera leaves ethanol extract with variation concentration

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Abstract. Leaves of *Moringa oleifera* have sunscreen activity formulated in lotion form. The stability and safety of a cosmetic preparation must be considered. This research aimed to determine the effect of variations in the concentration of *Moringa oleifera* leaves extract in lotion on the irritating power and stability of the preparation. Ethanol extract from *Moringa oleifera* leaves was obtained by maceration method with 50% ethanol solvent. The lotion preparation contains variations extract concentrations of 1% (FI), 3% (FII), 5% (FIII). The preparations were stored in a climatic chamber at 40 ± 2°C with RH 75 ± 5% for 28 days, and then evaluated for physical stability with organoleptic, pH, and viscosity parameters. Observations were made on days 1, 7, 14, 21, 28. The lotion was evaluated for irritancy using albino rabbit test animals. Data was analyzed with Two Way ANOVA statistics and Kruskal-Wallis non-parametric statistics. The results physical stability test showed an effect of extract concentration on the pH value and viscosity of the lotion (p=0.000). Increasing the extract's concentration decreased the preparation's pH value and increased its viscosity. *Moringa oleifera* leaves ethanol extract lotion with 3% concentration has better physical stability than other formulas and doesn't irritate the skin.

1 Introduction

Moringa oleifera leaves have phenolic compounds that function as sunscreens because they conjugate bonds in the benzene core. Exposure to UV light causes resonance by transferring electrons that can absorb UV A / B rays to reduce their intensity on the skin [1]. *Moringa oleifera* leaves contain bioactive compounds such as quercetin, beta carotene, phenolics, vitamin A, vitamin D, and Vitamin C, identified as antioxidants [2]. Herbal cosmetics that function as sunscreens must contain one or more active sunscreen ingredients that are antioxidant in nature to achieve the photoprotection effect [3].

In research [1], higher antioxidant activity is produced in phenolic compounds that have more hydroxyl groups in the flavonoid core, so the 50% ethanol extract of *Moringa oleifera* leaves with IC50 155.58 ± 2.21 has more potential as an antioxidant than 70% and 96% ethanol extracts of *Moringa oleifera* leaves. The existence of conjugation systems in phenolic compounds and chemical compounds that are usually contained in sunscreens causes this

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compound to have photoprotection potential. Research on the development of dosage forms in the form of lotions shows that moringa leaf extract can be applied in topical form. *Moringa oleifera* leaves have good tolerance to the skin after a patch test, so *Moringa oleifera* leaves extract can be used as a topical antioxidant formulated into a safe and appropriate topical base [4].

The development of dosage forms from *Moringa oleifera* leaves extract needs to be done so that it can be utilized optimally; that is, the properties of *Moringa oleifera* leaves can be utilized as well as possible. The lotion preparation was chosen because with a large enough water content, the lotion dosage form can be applied easily, the spread and penetration power is quite high, it doesn't give a greasy feeling, and it gives a cool effect [5]. Lotion is a semi-solid dosage form that is applied to the body, containing one or more dissolved or dispersed medicinal ingredients in a suitable base material and formulated as an emulsion of water in oil or oil in water [6].

Evaluation of the stability of physical properties and irritation test of the lotion was carried out by varying the concentration of *Moringa oleifera* leaves extract. The extract's concentration affects the preparation's effectiveness in providing efficacy. The physical stability evaluation of *Moringa oleifera* leaves extract lotion includes pH and viscosity tests. Stability tests must be carried out because they can evaluate the effect of environmental factors on the quality of the formulated product so that it can be used to predict its shelf life and determine good storage conditions [7]. In addition, every drug or cosmetic product is expected to have a high level of safety when used by consumers so it is necessary to evaluate product safety, namely by testing the potential for irritation to the skin [8]. We heighted that current study study in line with one of the agendas of Sustainable Development Goals (SDG's) which were to ensure healthy lives and promote well-being for all, at all ages.

The above description underlies the researcher to determine the effect of variations in ethanol extract concentration on physical stability with the parameters of the pH test and viscosity test and determine the irritating power of lotion *Moringa oleifera* leaves ethanol extract.

2 Material and Methods

2.1 Tools and Materials

The tools used were a set of glassware (Pyrex), porcelain cup, mortar and pestle, stirrer, pH meter, vacuum rotary evaporator (Heidolph), analytical balance (Brookfield), water bath (Memmert), viscosimeter (Rheosys merlin vr), hair shaver and hair removal machine (veet).

The materials used were *Moringa oleifera* leaves extract macerated, lotion base with pharmaceutical grade: stearic acid, cetyl alcohol, triethanolamine, glycerin, methyl paraben, propyl paraben, and distilled water. Irritation test material is healthy albino rabbits weighing approximately 2 kg and aged 4-6 months [9].

2.2 Simplisia Preparation

Moringa oleifera leaves from Bringharjo Market Yogyakarta were dried using an oven with a temperature of 60°C and a drying time of 5 hours [10]. The dried *Moringa oleifera* leaves were then pollinated [11].

2.3 Extraction of *Moringa oleifera* Leaves Simplisia

Extraction was carried out by maceration method at a powder and solvent ratio of 1:40 [1]. The extract was concentrated by rotary evaporation at 60°C [12].

2.4 Phytochemical Screening of *Moringa oleifera* Leaves Extracts

Identification of flavonoid and phenolic content was conducted qualitatively [13, 14].

2.5 Formulation of Lotion *Moringa oleifera* Leaves Extract

After obtaining a thick extract of *Moringa oleifera* leaves, the extract was then formulated in the form of lotion concerning research by [15, 16]. The lotion was made using the melting method. The formula is presented in Table 1.

Table 1. Formulation of lotion *Moringa oleifera* leaves extract [17]

Materials	F1 (%)	F2 (%)	F3 (%)	F4 (%)
Moringa extract	-	1	3	5
PEG 400	1	1	1	1
Cetyl Alcohol	0.05	0.05	0.05	0.05
Liquid Paraffin	10	10	10	10
Glycerin	10	10	10	10
Triethanolamine	1.5	1.5	1.5	1.5
Citric Acid	0.5	0.5	0.5	0.5
Stearic Acid	3	3	3	3
Propyl Paraben	0.5	0.5	0.5	0.5
Methyl Paraben	0.5	0.5	0.5	0.5
Aquadest	*Ad* 100	*Ad* 100	*Ad* 100	*Ad* 100

Description: F1 = Base lotion formula; F2 = Lotion *Moringa oleifera* leaves extract formula 1%; F3 = Lotion *Moringa oleifera* leaves extract formula 3%; F4 = Lotion *Moringa oleifera* leaves extract formula 5%.

2.6 Stability Test of Lotion

The accelerated stability test was carried out by storing the preparation in a climatic chamber at a temperature of 40 ± 2 ° C with RH 75 ± 5% for 28 days [16]. Observations were made on days 1, 7, 14, 21 and 28. The parameters observed in this test were organoleptic, pH and viscosity.

2.7 Irritation Test of Lotion

The irritation test was carried out after obtaining a certificate of ethical feasibility from the Universitas Ahmad Dahlan Research Ethics Committee with number: 0119007076. The test animals used were six male or female albino rabbits with healthy bodies and adults weighing about 2 kg. After the test animals were acclimatized for 5 days, 24 hours before testing, the hair on the rabbit's back was shaved clean. The rabbit's back was divided into six parts and then exposed to 0.5 grams of 1%, 3%, 5%, and base concentration lotion preparations in four parts, while the other two parts were kasa controls and healthy controls (without treatment). The exposure site was then covered with kasa and plastered with non-irritant plaster. The evaluation was conducted for 72 hours and examined at 1, 24, 48, and 72 hours [19, 9].

2.8 Data Analysis

Observation data were statistically analyzed using SPSS 25 with 95% confidence level.

3 Results and Discussion

3.1 The Result Identification Test of Flavonoid and Phenolic Content

The flavonoid test in this study showed positive results with the addition of HCl and Mg metal. The purpose of adding Mg and HCl metal is to reduce the benzopyrone core contained in the flavonoid structure so that a red or orange flavilium salt is formed. Flavonoids are compounds that contain two aromatic rings with more than one hydroxyl group. Phenol compounds with more hydroxyl groups have a greater solubility in water or are polar, so they can be extracted in polar solvents. Furthermore, the phenolic test, the characteristic of phenolic compounds is to form complex compounds so that blackish green color changes occur. The reaction of $FeCl_3$ with the sample makes the color formation in this test, the role of which is Fe^{3+} ions [20]. The results of phytochemical screening can be seen in Figure 1.

(a) (b)

Fig. 1. Skrining Test Results (a) Flavonoid dan (b) Phenolic in extract

3.2 The Result Stability Test of Lotion

Table 2 shows the results of organoleptical observations during 28 days of storage in a climatic chamber. The lotion did not change color, odor or consistency. This indicates that all formulas are organoleptically stable during storage.

Table 2. The result organoleptic test of lotion Moringa oleifera leaves extract lotion

Observations	Parameters	Formula			
		I	II	III	IV
Day-1	Color	-	+	++	+++
	Consistency	-	+	++	+++
	Odor	TB	K	K	K
Day-7	Color	-	+	++	+++
	Consistency	-	+	++	+++
	Odor	TB	K	K	K
Day-14	Color	-	+	++	+++
	Consistency	-	+	++	+++

	Odor	TB	K	K	K
Day-21	Color	-	+	++	+++
	Consistency	-	+	++	+++
	Odor	TB	K	K	K
Day-28	Color	-	+	++	+++
	Consistency	-	+	++	+++
	Odor	TB	K	K	K

Description: **Color**: White (-), Brownish White (+), Light brown (++), and Dark brown (+++); **odor**: Odorless (TB) and Extract characteristic (K); **consistency**: Liquid (-), Less viscous (+), Moderately viscous (++) and Viscous (+++).

3.3 The Result pH Stability Test

The results of the pH stability test are presented in Figure 2. Figure 2 shows that the length of storage affects the pH value of the four formulas, which tends to decrease in pH value. The base has an alkaline pH because the ingredients used contain ingredients that are as a bufer or buffer, namely triethanolamine. Triethanolamine is a material that is slightly alkaline with a pH of approximately 8 [21]. The active substance level of 50% ethanol extract of *Moringa oleifera* leaves in the lotion is able to reduce the pH value, which means that the increase in active substances makes the preparation more acidic. This happens because *Moringa oleifera* leaves extract contains polyphenols, flavonoids and vitamin C (acidic) so that it can reduce the pH value [4].

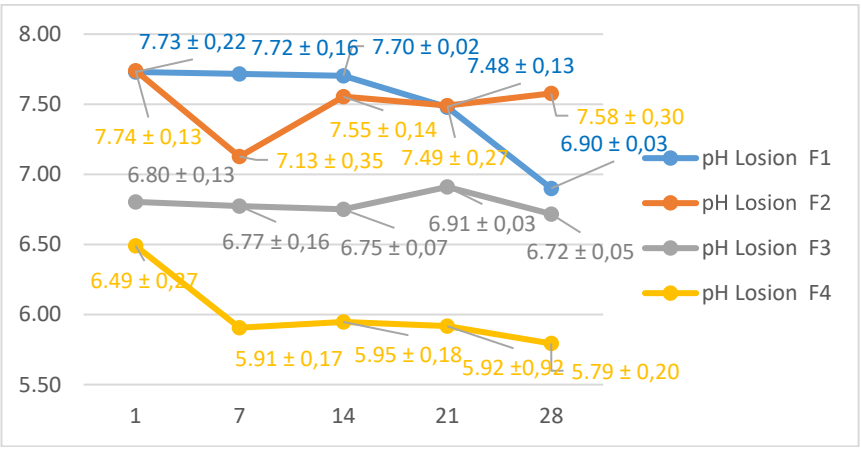

Fig. 2. Graphic of pH Test Changes

Based on the graph above, the base becomes more acidic during storage compared to other formulas; this indicates the lack of stability of the base during storage; this happens because environmental factors such as temperature, poor storage, and oxidation can cause changes in pH [22]. The decreased pH value during storage may occur because the packaging or container (ointment pot) used is not impermeable, so that air (CO2) can enter and react with the preparation. CO2 can react with water in the lotion phase and form bicarbonate acid (H2CO3) to make the preparation acidic. A sealed container is a container that can prevent air or gas from penetrating during handling, transportation, storage and sale.

$$CO_2 + H_2O \leftrightarrow H_2CO_3$$

Data analyzed statistically using two ways ANOVA using SPSS 25.0 to determine whether there was an effect of 50% ethanol extract concentration of *Moringa oleifera* leaves and storage time on the pH of the lotion from day 1 to day 28. The test results showed that storage days and variations in extract concentration had a major effect on pH values. The results of the post hoc test showed that there were statistically significant differences in pH between the four different formulas.

3.4 The Result Viscosity Test of Lotion *Moringa oleifera* Leaves Extract

Based on Figure 3, the viscosity test results show that the viscosity of each lotion every week continues to experience changes that tend to decrease. It can be seen in the data below that on the 7th day after being stored in the climatic chamber, the lotion decreased in viscosity, which means that the lotion is getting thinner, then there was a slight increase in viscosity on the 21st day, and again decreased on the 28th day. The change in viscosity is an unstable form of lotion preparation. This happens because the stability of the lotion is affected by temperature and time during storage. Most emulsions become thinner at high temperatures and become thicker when allowed to reach room temperature [23].

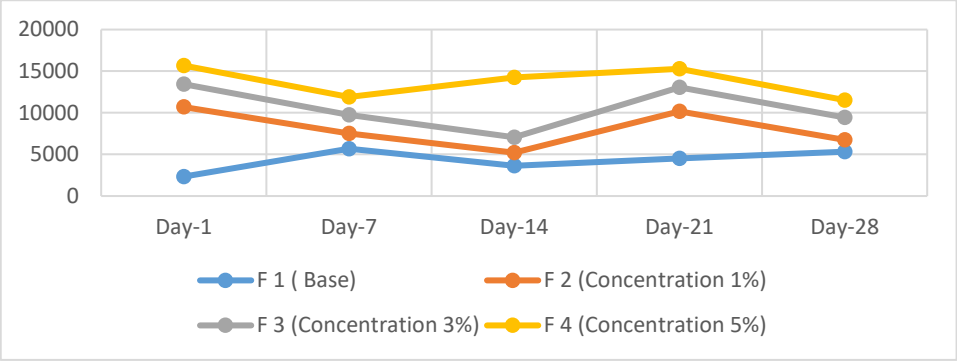

Fig. 3. Viscosity change graph of lotion every week

Statistical test results showed that the different concentrations of 50% ethanol extract of *Moringa oleifera* leaves and the length of storage gave significant differences to the lotion viscosity. Significant differences were seen between the base with 3% and 5% lotion.

3.5 The Result Irritation Test

The calculation of the irritation index is obtained by averaging the values of erythema and edema obtained from observations at 1, 24, 48 and 72 hours. The results of the irritation test can be seen in Table 3.

Table 3. Observation results of irritation test for 72 hours

Formula	Irritation Score					
	Rabbit 1	Rabbit 2	Rabbit 3	Rabbit 4	Rabbit 5	Rabbit 6
F 1 (base)	0	0	0	0	0	0
F 2 (concentration 1%)	0	0	0	0	0	0
F 3 (concentration 3%)	0	0	0	0	0	0
F 4 (concentration 5%)	0	0	0	0	0	0
Healthy control	0	0	0	0	0	0
Kasa control	0	0	0	0	0	0

Based on the table 3, the rabbit skin shows no signs of irritation or gets an irritation score of 0. Then, compared with the table of irritation response categories in rabbits, it is known that the lotion base and those added with ethanol extract *Moringa oleifera* 1%, 3% and 5% made in this study fall into the category of very mild (negligible) irritation response on rabbit skin. This study has the disadvantage of not having a positive control to ensure the irritation index in humans. Basically, the skin sensitivity of experimental animals is slightly different from human skin. Especially rabbits whose irritation level is very easy to see, the irritation index in humans still cannot be ascertained if the value in experimental animals has a mild irritation index [24].

3.6 Optimal Formula

In this study, it can be concluded that the variation of 50% ethanol extract concentration of *Moringa oleifera* in lotion affects the physical stability of lotion preparation. The concentration of 50% ethanol extract of *Moringa oleifera* in lotion does not affect the irritating power of the preparation and all formulas show a mild irritation index value in rabbits. Lotion with 3% *Moringa oleifera* leaves extract gave good and stable physical properties.

Formulas that have been made with variations in extract concentrations of 1%, 3% and 5% have been carried out stability tests with 28 days of observation. In addition, the irritation test showed no irritation for 72 hours. The results of the stability test, pH test and viscosity test showed that the 3% concentration had the most stable pH stability, the viscosity showed the most stable. Based on the stability test, the 3% concentration did not change color after being stored for 28 days in a climatic chamber.

Formula 3 with 3% extract concentration has a pH that is acceptable to human skin (pH 4.5-7.5), at the highest pH shown on day 21 with pH 6.91 ± 0.03 and at the lowest pH on day 28 with pH 6.72 ± 0.05. Formula 3 has a viscosity that is in accordance with the Indonesian National Standard on sunscreen preparations, which is 2,000-50,000 cP [25]. The stability of formula 3 is the best stability among other formulas that have been made.

4 Conclusion

In this study, it can be concluded that the variation of 50% ethanol extract concentration of *Moringa oleifera* in lotion affects the physical stability of lotion preparation. In addition, the concentration of 50% ethanol extract of *Moringa oleifera* leaves in the lotion doesn't affect the irritating power of the preparation and all formulas because it shows a mild irritation index value in rabbits. Lotion with 3% *Moringa oleifera* leaves extract give good and stable physical properties.

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