

## Formulation And Physical Quality Test Of Lime Peel Ethanol Extract Gel Moisturizer (*Citrus Aurantiifolia S.*) And Antioxidant Activity Test

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### Abstrak

This research was carried out as a form of preserving skin care from generation to generation using natural ingredients in the form of the lime plant (*Citrus aurantiifolia S.*), namely the skin of the fruit which contains high flavonoid compounds which act as antioxidants. This research aims to produce a gel moisturizer formulation with the active ingredient ethanol extract of lime peel (*Citrus aurantiifolia S.*). This research uses a type of experimental research conducted at the Pharmacy Laboratory, Palopo Muhammadiyah University in September 2023. In this research, sample preparation, extract making and moisturizing gel formulation were carried out. The results of the organoleptic tests of the three formulas until the fourth week did not change. The pH test results of the preparation have met the pH test requirements that are acceptable to the skin. In the homogeneity test, the three preparations had good homogeneity. The results of the spreadability and adhesiveness tests for the three preparations obtained good results. The viscosity test obtained high viscosity in F3 due to the addition of gel base to each formula. In the irritation test results, none of the respondents experienced irritation. The best results of the moisture test and antioxidant test were F3 with an IC50 value of 53 which is classified as strong. The results of the hedonic test for F3 were the formulation that respondents liked most, including aroma, color and texture.

Keywords : Lime peel; Moisturizer gel; Antioxidants; IC50

### 1. Introduction

Skin is the outermost part of the human body that is always exposed to the surrounding environment such as temperature, sunlight, and humidity. This will affect the balance of skin conditions so that skin moisture decreases and dryness [1]. One of the causes of dry skin is free radicals. The human body does not have excess antioxidant reserves, so if radicals occur, the body needs antioxidants [2].

Antioksidan adalah salah satu zat yang berperan dalam peremajaan kulit yang diperoleh diantaranya dari vitamin C dan flavonoid [3]. Jeruk nipis adalah salah satu buah dengan kandungan flavonoid dan vitamin C yang tinggi yang berkhasiat sebagai antioksidan. Flavonoid adalah metabolit sekunder paling tinggi yang terdapat pada kulit jeruk nipis [4]. Kulit jeruk nipis telah diteliti dapat berperan sebagai antioksidan dengan IC50 54,458 mg/mL [5]. Indonesia's abundant natural wealth in plant utilization has been developed as cosmetics and medicine. One plant that has potential as a cosmetic and medicine is lime (*Citrus aurantiifolia S.*) [6] Kulit jeruk nipis yang berlimpah dan mudah diperoleh menjadikan limbah tersebut tepat sebagai bahan aktif kosmetik pelembab.

Moisturizer is one type of cosmetic that functions to provide hydration to the skin by reducing the evaporation of water from the skin and drawing water from below into the stratum

corneum. ingredients that can increase hydration for the skin are ingredients that have oily occlusive properties and ingredients that can draw water into the stratum corneum which are commonly called humectants. Gels are currently more widely used and more stable which can provide better release of active substances than other semisolid preparations (Tatu et al., 2019). One of the active compounds that has an important role in moisturizing preparations and is found in plants is flavonoids [7]. Based on the above background, the researcher is interested in making a cosmetic formula in gel preparation and physical quality test and antioxidant activity test of the preparation.

## 2. Methodology

### Type of research

The type of research conducted is experimental, namely the formulation of gel moisturizer preparations and then testing the physical quality. This research was conducted in September-October 2023 at the Pharmacy Laboratory, Pharmacy Study Program, Faculty of Health Sciences, University of Muhammadiyah Palopo. The population in this study was lime fruit peel (*Citrus aurantiifolia* S.) taken from Jenne Maeja Village, South Pontrang Sub-district, Luwu District, South Sulawesi Province. The sample used in this study was lime fruit peel (*Citrus aurantiifolia*.S.).

### Tools and materials

The tools used consist of glassware such as beakers, measuring cups and object glass, blenders, analytical scales, digital scales, cutting boards, scissors, glass jars, universal pH, drop pipettes, volume pipettes, electric stoves, mortar, pestle, volumetric flasks, Petri dishes, horn spoons, stirring rods, Brookfield viscometer, gel pots, test tubes, UV-Vis spectrophotometry, and glass cuvettes. The materials used are ethanol extract of lime peel (*Citrus aurantiifolia*), TEA (triethanolamine), carbopol 940, glycerin, propylenglycol, propyl paraben, oleum citri, aquadest, 70% ethanol, PA methanol, aluminum foil, filter paper, universal pH, DPPH, vitamin C.

### Research procedures

#### Sample Collection of Lime Peel (*Citrus aurantiifolia* S.)

- a. The lime peels that have been obtained are collected then sorted and cleaned with running water to remove the dirt attached then the lime is peeled and separated from the contents.
- b. The washed lime peel is then chopped.
- c. After chopping the lime peel is then dried in the sun and covered with a black cloth.
- d. The dried lime peel sample is then pulverized using a blender until a simplisia powder is obtained [5].

#### Preparation Process of Ethanol Extract of Lime Peel (*Citrus aurantiifolia* S.)

- a. Simplisia weighed as much as 400 grams put into a glass jar, add 3000 ml of 70% ethanol then macerate for 5 days while repeatedly stirring and protected from light.
- b. After 5 days, the macerate is filtered and remacerated with 250 ml of distiller's solution for 1

day, stirred and filtered again.

- c. The obtained macerate is then evaporated until a thick extract is obtained (Ministry of Health of the Republic of Indonesia, 1986).

Process of Making Moisturizer gel Ethanol Extract of Lime Fruit Peel (*Citrus aurantiifolia* S.)

- a. Prepare the tools and materials to be used.
- b. Weigh the ingredients according to the formula.
- c. Carbopol is developed with distilled water in a mortar (mixture 1).
- d. Dissolved propyl paraben in glycerin until dissolved (mixture 2).
- e. Lime peel ethanol extract was crushed and then added some propylenglycol until the texture was homogeneous and soft (mixture 3).
- f. After carbopol expands (mixture 1) then add TEA little by little to form a gel base (mixture 4).
- g. The mixture of glycerin and propyl paraben (mixture 2) is put into the gel base (mixture 4) while being crushed until homogeneous (mixture 5).
- h. Add the remaining propylenglycol to the base mixture and blend until homogeneous. Mixture 3 is then incorporated into the gel base (mixture 5) while grinding until homogeneous.

Moisturizer Gel Testing of Ethanol Extract of Lime Peel (*Citrus aurantiifolia* S.)

Physical Quality Test of Preparations

Physical quality test of Moisturizer gel preparation was carried out for 4 weeks which included organoleptic test, homogeneity, pH, adhesion, spreadability, viscosity test.

#### 1) Organoleptical test

Organoleptical tests are carried out by observing odor, color and shape using the five human senses as the main tool to see the quality of the product being tested. Gels are usually clear with a semi-solid consistency.

#### 2) Homogeneity Test

The homogeneity test is carried out using a glass object by applying the preparation on a glass object and then observing whether there are still coarse grains. A homogeneous preparation is that no coarse particles are found in the preparation.

#### 3) pH test

The pH test is carried out using a universal pH by dipping the pH paper into the preparation and then matching it with the color listed on the universal pH container to determine the pH value obtained. Topical preparations have the same pH as the pH balance of the skin, which is 4.5-8.0. Preparations that are too acidic (pH value below 4) will cause skin irritation, while preparations that have a pH that is too basic (above 8) will cause dry skin [8].

#### 4) Adhesive Test

The adhesion test is carried out by weighing 0.25 grams of gel preparation then placed on a glass object and pressed with another glass object above the preparation. Given a load on it of 500 grams for 5 minutes (Maria et al., 2018). The adhesion of a good preparation must meet the standard, which is more than 1 second.

5) Uji Daya Sebar

Dilakukan dengan menimbang sediaan gel sebanyak 0,5 gram kemudian letakkan diatas kaca arloji. Tutup kaca arloji menggunakan kaca arloji lain, setelah itu letakkan beban 150 gram di tengah-tengah kedua kaca arloji. Catat diameter sediaan yang menyebar. Syarat daya sebar sediaan semi padat adalah 5-7 cm [9].

6) Uji Viskositas

Uji viskositas gel dilakukan dengan Viskometer Brookfield dengan spindle 64. Sampel dimasukkan ke dalam gelas ukur 50 ml kemudian diukur dengan kecepatan 50 rpm. Viskositas sediaan gel yang baik adalah antara 2000-4000 cps.

7) irritation test of preparations

Uji iritasi yang dilakukan terhadap 14 orang panelis untuk uji iritasi dan uji kelembapan dengan kriteria inklusi sebagai berikut :

1. Pria dan Wanita berbadan sehat (Mahasiswa Farmasi angkatan 2).
2. Usia antara 20-30 tahun.
3. Tidak ada penyakit yang berhubungan dengan alergi.
4. Bersedia menjadi panelis uji iritasi
5. Sukarelawan adalah orang terdekat dan sering berada disekitar, sehingga lebih mudah untuk diamatai apabila terjadi reaksi pada kulit yang sedang diuji [1].

Uji iritasi yang dilakukan adalah uji tempel terbuka atau *open patch* pada bagian lengan bawah 14 orang responden [10] dengan diameter 2 cm, lalu didiamkan hingga 2 jam kemudian amati perubahan yang terjadi berupa gatal, bengkak, kemerahan dan pengkasaran pada kulit [11].

8) moisture test

The moisture test was conducted on 14 panelists who were observed for 5 days. Before the application of the preparation on the skin of the panelist's arm, the moisture of the panelist's arm was first measured with the Skin analyzer HX-B03-005. The panelists tested did not wear other topical products at the test site before and during the study for 5 days. The preparation was then applied to the panelist's arm with a diameter of 2 x 2 cm by using it once a day after bathing. The test duration was 6 hours, after the panelist had been active for 6 hours and then measured again. Measurement with the Skin Analyzer HX-B03-005 tool uses % parameters, namely 0-29% dehydration, 30-50% normal, 51-100% hydration [11].

9) Hedonic test

The liking test or called the hedonic test was conducted on 14 respondents with the aim of knowing the level of respondents' liking for the Moisturizer gel preparation made. The test of liking is done visually by applying the preparation to the skin of the back of the hand. After that, the respondents gave an assessment of the preparation with several parameters including aroma, color and texture of the preparation. The assessment that has been made by the respondent is then categorized into 4, namely very like, like, quite like and dislike, then the percentage of the respondent's liking level is calculated.

10) Antioxidant Activity Test

Pengukuran antioksidan dengan metode DPPH diukur berdasarkan nilai IC50 (*Inhibition Concentration 50%*) yang merupakan konsentrasi efektif yang berfungsi untuk menghambat sampai 50% aktivitas radikal bebas. Semakin tinggi antioksidan maka semakin kecil nilai IC50 yang diperoleh. Nilai IC50 dapat dihitung dengan membuat kurva regresi linear [12].

### 3. Result and Discussion

#### 3.1. Result

Organoleptical test

Organoleptic test data of moisturizer gel preparation

Formula	Minggu ke	Parameter		
		Color	Odor	Form
F1	1	brownish green	typical orange	Semi solid
	2	brownish green	typical orange	Semi solid
	3	brownish green	typical orange	Semi solid
	4	brownish green	typical orange	Semi solid
F2	1	brownish green	typical orange	Semi solid
	2	brownish green	typical orange	Semi solid
	3	brownish green	typical orange	Semi solid
	4	brownish green	typical orange	Semi solid
F3	1	brownish green	typical orange	Semi solid
	2	brownish green	typical orange	Semi solid
	3	brownish green	typical orange	Semi solid
	4	brownish green	typical orange	Semi solid

pH test

pH test data of moisturizer gel preparation

Formula	pH
F1	7
F2	7
F3	7

Homogeneity test

Homogeneity test data of moisturizer gel preparation

Formula	Homogenitas
F1	Homogen
F2	Homogen
F3	Homogen

Spreadability Test (cm)

Spreadability Test (cm) data of moisturizer gel preparation

Formula	Uji Daya Sebar (cm)
F1	5,2
F2	6
F3	5,3

Test power sticky

Formula	Daya Lekat (detik)
F1	5
F2	5
F3	5

Viscosity test

Formula	Viskositas (cPs)
F1	2602
F2	2397
F3	3095

Irritation test

Reaksi	Panelis													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Gatal	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bengkak	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Kemerahan	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Kasar	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Keterangan

- : Tidak ada reaksi
- ✓ : Terjadi reaksi

Moisture test

Formulas	Responden	Condition Beginning(%)	Day To- (%)					Flat-rata(%)
			1	2	3	4	5	
F1 (15%)	14person	17.5	43.7	48.9	47.9	51.8	57	49.86
F2 (20%)	14person	19.45	46.8	50	52	55	58.7	52.5
F3 (25%)	14person	19.45	48	53.7	54.7	57.3	59.8	54.7

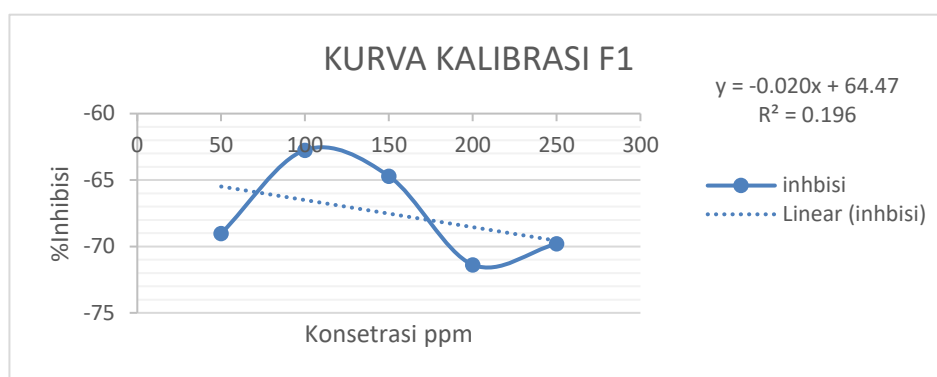
Hedonic test

Formulasi	Penilaian	Sangat suka	Suka	Cukup suka	Tidak suka
F1	Aroma	50%	42,9%	7,1%	-

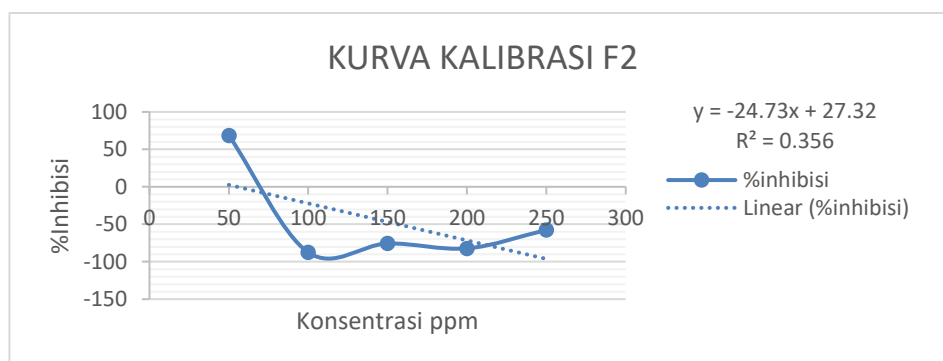
	Warna	21,4%	50%	28,6%	-
	tekstur	38,6%	61,4%	-	-
F2	Aroma	42,9%	50%	7,1%	-
	Warna	21,4%	50%	28,6%	-
	tekstur	35,7%	64,3%	-	-
F3	Aroma	57,1%	35,7%	7,1%	-
	Warna	21,4%	50%	28,6%	-
	tekstur	42,9%	57,1%	-	-

### Antioxidant Activity

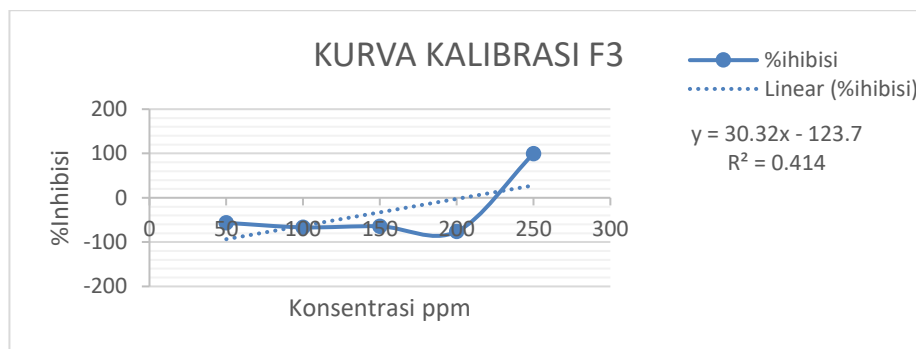
Sampel	IC50 (µg/ml)	Kategori
Vitamin C	5,28	Very strong
F1 (15%)	113.2679	Medium
F2 (20%)	51.104	Strong
F3 (25%)	45.920	Very strong



Linear regression curve graph of sample F1



Linear regression curve graph of sample F2



Linear regression curve graph of sample F3

### 3.2. Discussion

The sample used in this study is part of the plant, namely the skin of the lime plant (*Citrus aurantiifolia* S.). Lime fruit (*Citrus aurantiifolia* S.) is picked when the sun is shining and there is no remaining dew, which is at 9 am. Fruit harvested too early in the morning and still dewy will be easily dirty while harvesting done during the day the fruit will experience evaporation so that more shrinkage. Lime fruit is harvested when the fruit is old enough and close to ripe, which is when the fruit is green to yellowish green [13]. Lime fruit (*Citrus aurantiifolia* S.) used as samples in this study were obtained from Jenne Maeja Village, Luwu Regency. Samples obtained as much as 10 kg were then sorted and cleaned with running water to remove dirt that sticks after that separated from the fruit then chopped and dried for 3-5 days in the sun and covered with a black cloth to avoid evaporation too quickly which can reduce the quality of the simplisia and keep flavonoids that are not heat resistant from being easily damaged. After drying the sample, it was then blended to obtain simplisia powder [5].

Determination of drying shrinkage is carried out with the aim of knowing the range of weight reduction of simplisia in the drying process. The requirement for the drying shrinkage rate of lime peel simplisia is no more than 10% [14]. Drying shrinkage is done by weighing 2 grams of simplisia in a cup and then drying it in the oven for 30 minutes at 105 ° C. At 105 ° C the water will evaporate. At 105°C water will evaporate and compounds with low boiling points will also evaporate. The drying shrinkage test result obtained from lime peel simplisia is 9%. These results indicate that the simplisia meets the drying shrinkage requirement of no more than 10%.

The water content test was carried out with the aim of knowing the range of water content contained in the simplisia powder. The maximum range of water content of lime peel simplisia is 20%. The water content test was carried out by weighing 10 grams of simplisia in a cup and then in the oven for 5 hours at 105°C. High water content can cause simplisia to be less durable and susceptible to mold and microbial growth. The result of the water content test of lime peel simplisia powder is 11%. These results indicate that the lime peel simplisia meets the maximum moisture content requirement of 20%.

The extraction process of lime peel simplisia is carried out by maceration method because it does not require a lot of money, it is simpler because it can avoid damage to

compounds that cannot withstand heat [15]. The solvent used is 70% ethanol because it is polar with a level of polarity close to the polarity of bioactive compounds. The closer the solvent's polarity to the substances contained in the extract, the more components of the extracted substance and the increase in the extract obtained. Flavonoids are water-soluble compounds and their active compounds can be extracted with 70% ethanol. Simplisia used as much as 400 grams with a solvent of 3000 ml of 70% ethanol then macerated for 5 days. The results of maceration are then evaporated until a thick extract is obtained (Depkes RI, 1986).

Yield is a parameter in making simplisia which aims to determine the ratio of simplisia weight and raw material weight. The longer the extraction time, the higher the yield obtained. This happens because of the reaction between the material and the solvent so that the solvent penetration process into the material cells is getting better so that more compounds diffuse out of the cells. According to [14] the requirement for the yield of lime peel simplisia is not less than 15%. The results showed that the yield of lime peel extract obtained was 16.45%.

### **Physical Quality Test of Preparations**

#### **Organoleptic test**

Organoleptical tests are carried out by observing odor, color and shape using the five human senses as the main tool to see the quality of the product being tested. Gels are usually clear with a semi-solid consistency. Based on observations made for 4 weeks, there were no changes in terms of odor, color and shape. All three formulations have a distinctive smell of citrus aroma derived from the smell of the orange peel itself and the addition of a little scent. The color of the three preparations is brownish green which is obtained from the color of the extract itself with a semi-solid form.

#### **pH test**

The pH test in this study was carried out with a pH stick to determine the level of acidity of the preparation made to not cause irritation to the skin. The range of pH values that are the same as the pH balance of the skin is 4.5-8.0 [8]. Gel preparations that are too acidic will cause skin irritation while if they are too alkaline they will cause dry skin. The pH value of the gel preparations obtained from the three preparations was 7 and was stable during observation, which means that it is in the pH range that is still acceptable to the skin.

#### **Homogeneity test**

The homogeneity test aims to see that all ingredients are evenly mixed or evenly dispersed. Homogeneous preparations will produce good quality preparations so that the active substance is dispersed in the carrier evenly. The homogeneity test is carried out by applying the gel preparation to the glass object and then observing whether there are coarse grains. From the results of the homogeneity test research from the three preparations, it shows that the preparations made are homogeneous and no coarse grains are found.

#### **Spreadability test**

Spreadability is a parameter of a preparation that shows the preparation is easy to flatten and determine the ability to spread the gel on the skin. The spreadability test is carried out using a petri dish that is given a load on it. The spreadability test is carried out with the aim of seeing the speed of spread and softness of the gel preparation when applied to the skin. Good

spreadability has a diameter between 5-7 cm. From the results of the spreadability test research, the spreadability was obtained in formula 1 with a diameter of 5 cm, formula 2 with a diameter of 6 cm and formula 3 with a diameter of 5 cm. The results obtained have met the requirements of the spreadability test, which is 5-7 cm.

#### **Adhesion test**

The adhesion test is carried out by applying the preparation on a glass object and then covering it with another glass object to find out how long it takes for the two glass objects to separate. Adhesive power aims to determine the length of time the gel adheres to the skin surface. The adhesion of the gel preparation is directly proportional to the viscosity value. The higher the adhesion, the higher the viscosity. The requirement for good adhesive power is more than 1 second. From the results of the adhesion test, the three formulations have met the requirements of good adhesion.

#### **Viscosity test**

The viscosity test of the gel preparation was carried out with a brookfield viscometer using spindle 64 at a speed of 50 rpm. The viscosity test aims to determine the viscosity of a gel preparation. The viscosity of a good gel preparation is between 2000-4000 cps. From the results of the study, the highest viscosity was obtained in formula 3 with a value of 3095 cPs with a variation of 1.5% gelling agent. The higher carbopol concentration can form a gel base so as to increase viscosity [16].

#### **Irritation test**

The irritation test is carried out with the aim of seeing whether or not there are side effects after the use of the preparation on the skin. The data obtained from the three formulas with concentrations of 15%, 20% and 25% did not show any irritating reactions such as redness, itching, swelling or rough skin caused by the preparation [11]. From all the data obtained, the moisturizing gel preparation made is safe to use on the skin.

#### **Moisture test**

The moisture test in the study was carried out using the HX-B03-005 skin analyzer before and after administration of the preparation. The moisture test aims to determine the ability of a preparation to moisturize the skin. The highest percent increase in moisture of the preparation against the skin was obtained from the preparation formula F3 obtained an increase from day 1 to day 5 of 54.7% followed by formulas F1 and F2 of 52.5% and 49.86%. Formulas F3 and F2 are included in the moist or hydration category, and F1 is in the normal category according to the literature % parameters, namely 0-29% dehydration, 30-50% normal, 51-100% hydration. From the data obtained, the three formulas show a good level of moisture from day 1 to day 5 and it can be seen that the addition of extracts to the preparation affects the results of the moisture test.

#### **Hedionic test**

The liking test was conducted on 14 respondents on the aroma, color and texture of the gel moisturizer preparation of ethanol extract of lime peel (*Citrus aurantiifolia* S.). which aims to determine the level of respondents' liking for the preparation that has been made. Based on the results of the liking test of the aroma of the gel moisturizer preparation, all respondents liked

the aroma of the three preparations with the highest percentage of aroma liking level in formula 3 in the very like category, this was obtained from the distinctive aroma of citrus from ethanol extract of lime peel that respondents liked. The results of the liking test for the color of the gel moisturizer preparation, respondents liked all the colors of the preparation with the same percentage in the three formulas and the highest percentage in the like category. The results of the liking test on the texture of the gel moisturizer preparation, respondents liked all the textures of the gel moisturizer preparation of ethanol extract of lime peel (*Citrus aurantiifolia*), with the highest percentage of liking level in formula 2. Based on the liking test that has been carried out on the aroma, color and texture of the gel moisturizer preparation of ethanol extract of lime peel (*Citrus aurantiifolia*), it can be seen that F3 is the preparation that is most liked by respondents.

#### **Antioksidan Activity test**

Antioxidant measurement with DPPH method is measured based on IC50 (Inhibition Concentration 50%) value which is an effective concentration that serves to inhibit up to 50% of free radical activity. The higher the antioxidant, the smaller the IC50 value obtained. IC50 value can be calculated by making a linear regression curve [12]. In this study, Vitamin C was used as a comparison or positive control because Vitamin C has a strong antioxidant. From the test data, the IC50 value of Vitamin C is 5.28  $\mu\text{g/ml}$  with a very strong category, Formula F1 with an IC50 value of 113  $\mu\text{g/ml}$  with a medium category, F2 with an IC50 value of 51  $\mu\text{g/ml}$  with a strong category and F3 with an IC50 value of 45  $\mu\text{g/ml}$  with a very strong category. The category is in accordance with the literature [17]. IC50 value  $<50 \mu\text{g/ml}$  is very strong antioxidant, IC50 value 50-100  $\mu\text{g/ml}$  is strong antioxidant, IC50 value 100-250  $\mu\text{g/ml}$  is moderate antioxidant, IC50 value 250-500  $\mu\text{g/ml}$  is weak antioxidant and IC50 value  $>500 \mu\text{g/ml}$  is inactive antioxidant.

#### **4. Conclusion**

Based on the research that has been done on the gel moisturizer formula of ethanol extract of lime peel (*Citrus aurantiifolia* S.), it can be concluded that:

1. Gel moisturizer preparation formula with the best gel texture is found in formula 3 with 1.5% gel base concentration.
2. From the results of the physical quality test, irritation test, moisture test, and hedonic test, the gel moisturizer preparation of ethanol extract of lime peel (*Citrus aurantiifolia* S.) has different results. The physical stability test found that the best preparation was in formula 3 with 25% lime peel extract (*Citrus aurantiifolia* S.). The results of the irritation test showed no irritation reaction to 14 respondents. From the hedonic test, the most preferred preparation is F3.
3. In the antioxidant activity test, the gel moisturizer preparation of ethanol extract of lime peel (*Citrus aurantiifolia* S.) which has the best antioxidant activity is found in formula 3 with 25% extract concentration. The IC50 values obtained were F1 at 113.2679  $\mu\text{g/ml}$  (medium), F2 at 51.104  $\mu\text{g/ml}$  (strong) and F3 at 45.920  $\mu\text{g/ml}$  (very strong).

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