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Antioxidant Activity Of Cream Dosage Form Of Lime Extracts (*Citrus Aurantifolia* (Christm.)Swingle)

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ABSTRACT

Lime (*Citrus aurantifolia* (Christm.)Swingle) is a plant that used for the traditional treatment because of flavonoid and vitamin C which is believed to be an antioxidant. Antioxidant is a compound that could ward free radical, so this research used juice from *Citrus aurantifolia*(Christm.)Swinglefruit as an active compound for cream preparation. The aim of this research was made the cream preparation from juice of lime (*Citrus aurantifolia* (Christm.)Swingle) that has antioxidant activity. Cream preparation had made for 6 (six) formula which different content of juice from *Citrus aurantifolia*(Christm.)Swinglefruit ((F1:0%; F2:0,1%; F3:0,2%; F4:0,3%; F5:0,4%; F6:0,5%). Evaluation of cream preparation are cream type with colour test, pH with pHmeter, stability test used accelerated stability test and antioxidant activity test used DPPH methode with calculated IC_{50} value. Cream type from this preparation is O/W with pH 5,0-6,5. Those cream could save on room temperature for 21 days. The different IC_{50} value are obtained from those cream that decrease from juice of *Citrus aurantifolia*(Christm.)Swingle fruit before preparation made that had 38,99 µg/mL (strong activity antioxidant). The strongest activity antioxidant are obtained from F6 that had IC_{50} value 86,91 µg/mL and the weakness activity antioxidant are obtained from F1. An antioxidant activity of lime juice(Citrus aurantifolia (Christm.) Swingle). reduced if made preparations cream, and reduced again after stored 21 days at 25 o C temperatures with P>0.05 in t-paired.

Keywords: Antioxidant, Vitamin C, *Citrus aurantifolia*(Christm.)Swingle

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INTRODUCTION

The antioxidant activity of some fruits both ripe and unripehas been done in some previous researchs. Antioxidants can prevent or delay the damage the body to oxidation reaction by free radicals that enter to the body, which can prevent a variety of diseases that are believed caused of free radicals such as cancer [1,2]

The Free radicals compounds caused the skin to become dull and even can caused black spots on the skin, especially on the fac Examples sunshine. Dull skin to black spots arising as a result of free radicals that can disrupt the process of the energy formation comes from glucose and oxygen through enzymatic reactions in the mitochondria that part of the cell [3]

Lime is a fruit that contains compounds that can prevent the oxidation reaction caused by free radicals. Compounds contained by lime is vitamin C and group of flavonoid like hesperitin, naringin, tangeretin, naringenin, erioctrin, hesperidin [2,3,4,5]

Based on that,this research tried to continueof the antioxidant activity of cream dosage form of limejuices (*Citrus aurantifolia* (Christm.) Swingle).

MATERIALS AND METHODS

Plant materials:

Sampling of this researches are taken from the fruit of plants of lime from lime plantation at kumpe, Jambi province

Chemical materials:

Juice of lime (*Citrus aurantifolia* (Christm.)Swingle), adeps lanae, gliserin, Trietanolamin, Sodium metabisulfit, Stearic acid, cetyl alkohol, methyl paraben, aquadest, DPPH, methanol.

Instruments:

An analytical balance ®Shimadzu, pHmeters, waterbath, spectrophotometer UV-Vis ®Shimadz,

Methods:

Extract from fresh lime fruitshad has evaluated by phytochemical screening like flavonoid test and vitamine C test based on the researches before and antioxidant activity test from those extracts with DPPH assays. And then, those extracts has formulated with cream preparation to 6 (six) formulas which different content of juice from *Citrus aurantifolia*(Christm.)Swingle. Those formulas of cream preparations was evaluated include activity antioxidant with DPPH assays

Procedure 1:

Extracted Juice From Fresh Lime

100 grams from fresh lime fruitshad cleaned and dried. Lime peeled, squeezed and filtered in order to get water lime juice. Those samples evaluated by phytochemical screening like flavonoid test and vitamin C test based on the researches before [2]

Procedure 2:

Phytochemical Screening

Flavonoids test

40 mg of lime juice in 100 ml of boiled water for 5 minutes, then filtered. The filtrate was added 5 ml of 0.05 mg of Mg powder and 1 ml of concentrated HCl, a positive test is indicated by the formation of red, yellow or orange [6]

Vitamin C test

Lemon juice added benedict's reagent and then heated at 40 ° C for 5 minutes. Positive if the precipitation of red brick [6]

Procedure 3:

Cream Formulation

Emulsion based cream (O/W type) made from the oil phase was melted at a temperature of 70 - 75^{o}C. All water-soluble materialshad dissolved in water and named as the water phase. The water phase added to the oil phase at a temperature of 70 - 75^{o}C, formed a cream base. A Lime juice was added to the cream base until homogeneous. Formula from the cream is given from the table

Table 1. Formulation of lime juice cream

Materials	Composition (% b/b)					
	F0	F1	F2	F3	F4	F5
Juice of lime	0	0,1	0,2	0,3	0,4	0,5
Stearic acid	12	12	12	12	12	12
Cetyl Alcohol	2	2	2	2	2	2
Triethanolamin	4	4	4	4	4	4
Gliserin	23	23	23	23	23	23
Adeps Lanae	5	5	5	5	5	5
Methyl Paraben	0,18	0,18	0,18	0,18	0,18	0,18
Sodium Metabisulfit	0,01	0,01	0,01	0,01	0,01	0,01
Aquadest ad	100	100	100	100	100	100

Key :
F0 : Formula without juice of lime as a control
F1 – F5 : Formula wih juice of lime

Procedure 4:

Evaluation Preparations

Organoleptic

The examination formula include of the shape, color and smell of the preparation and homogeneity has been using two pieces of transparent glass. The preparation is said to be homogeneous if there is no longer part of the clot and no phase separation occurs [7]

Type of Cream

The Preparations had reacts by added methylene blue dye. If the preparation can be mixed with the dye, the type of cream is W/O. If could not mix the preparation includes type of cream isO/W.

pH

pH is measured by using a calibrated pHmeter

Coverage Test

0.5 grams the preparation measured diameter of distribution after being given a load of 1g, 5g, 10g and 50g with an interval of 15 seconds. This test uses a comparison dosage form of another cream dosage form from the market.

Accelerated Stability

The stability of the preparation has been done by an accelerated stability test for 21 days at 25 ^{o}C an examined pH, color, homogeneity and Test of an antioxidant activity

Procedure 5 :

Antioxidant Activities

Sub Procedure 5 :

Antioxidant Activity Juice of Lime

Prior to measuring the test solution, DPPH solution has made with a concentration of 35 ppm and then measured wavelength DPPH with UV - Vis spectrophotometry. The solution then made a series of test concentration of 10 ppm, 30 ppm, 50 ppm, 70 ppm and 90 ppm. Each series has mixed with a solution concentration of DPPH as much as 3.8 ml to 0.2 ml each test concentration. Then measured by UV-Vis spectrophotometry. Done in triplo [7,8,9]

Antioxidant activity Preparations

Juice of lime had extracted from the cream preparation by dissolving dosage cream with distilled water and filtered. The filtrate had been treated the same as the test solution with test of juice of lime solution [7,8,9]

Data Analysis

Data and Statistic analysis with t-paired : Normality with kolmogoro smirnov, Homogenity test with levene,

RESULTS AND DISCUSSION

Results and Discussion

Lime plants has identified by herbarium of Andalas University that known thespecies of lime isCitrus aurantifolia (Chistm.) Swingel. Juice obtained implies qualitative test and positive results obtained containing flavonoids and Vitamin C.

Additional materials that used for the preparation has based on the requirements according to the Handbook of Pharmaceutical excipients and the VI edition of the Indonesian Pharmacopoeia fourth edition [8,9] Formula evaluation obtained as follows:

Organoleptic test of the cream preparation is white, semi-solid form and homogeneous. pH of the dosage form in ranges 5-6 for all formulas. This indicates that pH of these preparation had the same as the skin pH, the evaluation proved those preparation could not caused skin irritation from irritation testfrom 24 volunteers, who did not happen itching, redness and even swelling. Type a cream made is the type of W/O for the methylene blue was added to the preparations, becaused Those contained use water as dispersing phase.

The test results of the covereged testfrom the preparation was obtained that its spread has a different value to the benchmark. This means that the base used has a different consistency than the preparation of cream as control. This can be seen in the table below:

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Table 2. The Covereged Test By Juice Of Lime Cream

Formula	Weight (g)			
	1	5	10	50
F0	1,55 ± 0,05	2,32 ± 0,08	2,65 ± 0,07	2,98 ± 0,04
F1	1,51 ± 0,06	2,12 ± 0,08	2,31 ± 0,03	3,01 ± 0,06
F2	2,00 ± 0,04	2,07 ± 0,06	2,21 ± 0,05	3,11 ± 0,06
F3	2,16 ± 0,05	2,44 ± 0,04	2,86 ± 0,06	3,13 ± 0,03
F4	2,17 ± 0,06	2,59 ± 0,04	2,99 ± 0,04	3,29 ± 0,05
F5	2,57 ± 0,04	2,83 ± 0,04	3,11± 0,06	3,46± 0,04
Control (+)	1,65 ± 0,03	2,77 ± 0,06	2,99 ± 0,03	3,68 ± 0,02

Key : F: Formula

The stability of the preparation was measured at temperatures of 25 °C accelerated stability. F1 –F5 in color, concistensy and homogeneity had not changed after 21 days. Its means the preparation had stabil after storage for 21 days on stability test. But in pH has changed on all formulas, but still at the same as skin pH. The test results of stability of the preparation after storage for 21 days is:

Table 3. Accelerated Stability Test At 25^{o}c

Parameters	Formula	Days			
		0	7	14	21
Colors	F0	NCC	NCC	NCC	NCC
	F1	NCC	NCC	NCC	NCC
	F2	NCC	NCC	NCC	NCC
	F3	NCC	NCC	NCC	NCC
	F4	NCC	NCC	NCC	NCC
	F5	NCC	NCC	NCC	NCC
Concistency	F0	SS	SS	SS	SS
	F1	SS	SS	SS	SS
	F2	SS	SS	SS	SS
	F3	SS	SS	SS	SS
	F4	SS	SS	SS	SS
	F5	SS	SS	SS	SS
Homogeneity	F0	H	H	H	H
	F1	H	H	H	H
	F2	H	H	H	H
	F3	H	H	H	H
	F4	H	H	H	H
	F5	H	H	H	H
pH	F0	6,50 ± 0,07	6,44 ± 0,07	6,39 ± 0,02	6,24 ± 0,06
	F1	5,69 ± 0,03	5,63 ± 0,06	5,54 ± 0,04	5,45 ± 0,03
	F2	5,56 ± 0,03	5,31 ± 0,08	5,16 ± 0,05	5,02 ± 0,01
	F3	5,60 ± 0,01	5,46 ± 0,02	5,39 ± 0,04	5,23 ± 0,04
	F4	5,77 ± 0,06	5,64 ± 0,06	5,46 ± 0,02	5,36 ± 0,01
	F5	5,48 ± 0,08	5,24 ± 0,05	5,26 ± 0,04	5,12 ± 0,05

Key : F: Formula; NCC: Not Change in Color; SS: Semi Solid; H: Homogenity;

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The antioxidant activity conducted on juice of lime and cream dosage form after 21 days at a temperature of 25°C are as follows:

Table 4. The Antioxidant Activity Conducted On Juice Of Lime After 21 Days Stored at 25°C

Formula	IC_{50} µg/mL	
	Before storage	After storage
F0	165,05	171,30
F1	126,51	128,51
F2	123,54	123,62
F3	108,58	109,49
F4	95,03	99,00
F5	88,93	92,22

Key : F : Formula

The antioxidant activity of lime juice before made preparations classified as a very strong activity of antioxidant with IC_{50} = 38.99 µg/mLcompared to vitamin C as control were IC_{50} = 57.26 µg/mL. But after being made preparations cream, a decreased in the value of IC_{50}. IC_{50} value of the lowest an activity of antioxidant on the F5 at 88.93 µg/mL with a juice of lime content of 0.5%. This is because that the effect of cream dosage form which used the temperatures up to 75°C

Data Analysis

The antioxidant activity conducted on juice of lime and cream dosage form after 21 days at a temperature of 25°C was analysis with statistically with t-paired. With Hypothesis : H_0 : before ≥ 25 degree, H_1 : before < 25 degree. Assumed : test of normality distributed data and homogeneity data.

Normality with kolmogoro smirnov; Significancy > 0.05 so both of the data was normally distribute

Table 5. Test of Normality

	VAR00001	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
VAR00002	before	.211	6	.200*	.924	6	.538
	25 degree	.225	6	.200*	.903	6	.391

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Homogenity test with levene; significancy > 0.05 both of data was homogen

Table 6. Test of Homogeneity of Variances

VAR00002

Levene Statistic	df1	df2	Sig.
.000	1	10	.998

t-paired test ; Significancy> 0.05 so H_0 rejected and H_1 accepted

Table 7. Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1 before - twentyfivedegree	-2.75000	2.24085	.91482	-5.10163	-.39837	-3.006	5	.030

Results of Antioxidant Activity

The different IC_{50} value are obtained from those cream that decrease from juice of *Citrus aurantifolia*(Christm.)Swingle fruit before preparation made that had 38,99 µg/mL (strong activity antioxidant). The strongest activity antioxidant are obtained from F6 that had IC_{50} value 86,91 µg/mL and the weakness activity antioxidant are obtained from F1. The IC_{50} value from cream preparation after storage 21 days at $25^{O}C$ decreased if compared the IC_{50} value of cream preparation before storage. It has proved from statistically with t-paired.

CONCLUSIONS

An antioxidant activity of lime juice(*Citrus aurantifolia* (Christm.) Swingle). reduced if made preparations cream, and reduced again after stored 21 days at 25 ^{o}C temperatures with P>0.05 in t-paired. An antioxidant activity of lime juice(Citrus aurantifolia (Christm.) Swingle). reduced if made preparations cream, but activity was still strong enough, so that the preparation of this cream can be used as an antioxidant cream on the skin, especially for facial trowel.

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